# RESOURCE ENHANCEMENT AND SUSTAINABLE AQUACULTURE PRACTICES IN SOUTHEAST ASIA:
## Challenges in Responsible Production of Aquatic Species

*Proceedings of the International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia 2014 (RESA)*

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# RESOURCE ENHANCEMENT AND SUSTAINABLE AQUACULTURE PRACTICES IN SOUTHEAST ASIA:
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***On the cover***
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# FOREWORD

We are all aware of the increasing pressure on our coastal resources to supply food and income to many people living in the coastal areas in the region. Signs of over-exploitation of many important marine resources are evident. The fishermen's daily catch has dwindled and the sizes of their catch have become smaller; they now have to go farther and spend a longer time at sea to get some catch. This means that the rate of extracting the natural resources is faster than their capability to reproduce and replenish themselves. This imbalance of resource extraction and natural recruitment will ultimately result in the extinction of many of these important species. Management measures need to be properly implemented to allow the resource to recover and continue to support future generations. In addition, aquaculture practices that take into consideration the health of the environment need to be promoted to ensure the sustainability of the activity and maximize benefits for all in the long term.

With financial support from the Government of Japan Trust Fund, experts and representatives from SEAFDEC member countries were gathered in a workshop to share experiences and exchange ideas on resource enhancement and sustainable aquaculture practices. The proceedings of the workshop are contained in this publication. This will be helpful as a guide and as an additional source of information to those who are embarking on or engaged in resource enhancement and aquaculture activities.

**Felix G. Ayson, DSc.**
Chief, SEAFDEC Aquaculture Department

# MESSAGE

It is with pleasure that we launch the proceedings of the "International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia", held in Iloilo City from 5 to 7 March 2014.

This international workshop aimed to promote and augment regional initiatives on resource enhancement and sustainable aquaculture practices, and to contribute to poverty alleviation, livelihood and food security. A total of 149 participants attended the workshop, composed of aquaculture and resource enhancement experts, scientists, representatives and observers from 11 Member Countries of SEAFDEC. It also featured 42 oral presentations and 15 poster papers. This meeting was organized through the funding support of the Government of Japan.

The relationship of resource enhancement, sustainable aquaculture and healthy environment is vital to the future development and sustainability of fisheries and aquaculture. For sustainable aquaculture, we have to consider not only fish biomass for feeds, and broodstock for seeds in natural resources, but also the healthy environment for their habitats. Stock releases in the wild for enhancing depleted aquatic populations, are one of the effective ways to increase our natural resources.

I hope that this proceedings will be utilized for the dissemination and promotion of environment-friendly resource enhancement and sustainable aquaculture in the Southeast Asian region.

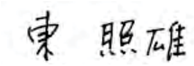
**Takuro Shibuno, Ph.D.**
Deputy Chief and GOJ Trust Fund Co-Manager

# MESSAGE

The Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) implemented regional programs on Resource Enhancement of Internationally Threatened and Over-exploited Species in Southeast Asia through Stock Release (or Resource Enhancement) and Promotion of Sustainable and Region-Oriented Aquaculture Practices (Sustainable Aquaculture), these being important programs under the Fisheries Consultative Group of the ASEAN-SEAFDEC Strategic Partnership (FCG/ASSP) Mechanism. These R&D programs along with other two regional programs on Fish Health and Food Safety, were conducted from 2010 to 2014 with financial support from the Government of Japan (GOJ-Trust Fund). In March 2014, SEAFDEC/AQD, organized an international workshop on Resource Enhancement and Sustainable Aquaculture (RESA) Practices in Southeast Asia at Iloilo City, Philippines, as the milestone of these aforementioned regional programs. The workshop gathered several distinguished local and international speakers and about 150 participants from 14 countries comprising of national and local government officers, academicians, as well as industry stakeholders. It was a great opportunity for the participants to exchange timely information and discuss research gaps and issues on broad topics under RESA practices in the Southeast Asia to reduce poverty and secure livelihood in the region through further development of fisheries and aquaculture.

As the program manager who launched these regional programs in 2010 and the chairperson of the said international workshop, I am so happy that the invaluable information included in this proceedings with full papers orally presented at the workshop, have become available for use as reference by students, fisherfolks, aqua-farmers, practitioners, researchers, policy makers, etc. Taking this opportunity, please allow me to say thank you very much to the authors for their respective contribution to this proceedings. I would also like to express my sincerest acknowledgements to Dr. Ma. Rowena R. Eguia, Dr. Fe Dolores Parado-Estepa, Dr. Nerissa Salayo, Dr. Junemie Lebata-Ramos and other editorial staff of this proceedings for their hard work to launch this issue after I left Iloilo as I ended my term in March 2014.

Finally, I hope for the further development of RESA practices in Southeast Asia, and I wish to believe that this proceedings will make a great contribution to the pursuit of that aim.



**Teruo Azuma, Ph. D.**
Former Deputy Chief, SEAFDEC/AQD
Director, Fisheries Technology Division, National Research Institute of Fisheries Engineering, Fisheries Research Agency of Japan

# KEYNOTE ADDRESS

*By Dr. Chumnarn Pongsri, SEAFDEC Secretary-General*
*At the International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia, 5-7 March 2014, Iloilo City, Philippines*

This Workshop is important as it reiterates the critical fisheries scenario faced by the Southeast Asian region as well as at the global level where the supply of fish and fishery products has been limited by the deterioration of fishery resources and habitats; however, it should be noted that the demand side has continued to increase over the years. The situation on deterioration of fishery resources has called for improved utilization of fishery resources in a more responsible and sustainable manner. Several initiatives were undertaken by countries in the Southeast Asian region toward this, both on the aspect of capture fisheries and aquaculture. For aquaculture, initiatives undertaken in the region cover ranges of R&D to come up with appropriate aquaculture technologies; aquatic animal health management including disease surveillance and control; safe use of chemicals and antibiotics in aquaculture; research on alternative protein sources for aquaculture; and development and application of appropriate quality assurance systems in order to comply with requirements of importing markets, etc. In addition to direct production of aquaculture commodities, aquaculture technologies have also been developed and applied to support resources enhancement.

SEAFDEC has also undertaken activities to support sustainable utilization of aquatic species under international concerns, including scientific studies on the status of fishery resources in order to serve as a basis for their conservation and management. SEAFDEC also has programs and projects that aim to develop practical aquaculture technologies for species under international concerns, including appropriate releasing strategies for resources enhancement. In addition, SEAFDEC also takes leading roles in providing discussion forum among countries in the region to come up with common conservation and management approaches for important species; and the available scientific information and evidences on initiatives undertaken by the region are envisaged to provide strong justification during further discussion at relevant international fora in order to safeguard the fisheries sector of the region. As SEAFDEC Secretary General, let me therefore encourage active inputs from the Workshop participants in order to come up with fruitful results.

# PREFACE

The Southeast Asian region has highly diverse marine flora and fauna. Many of these aquatic species have been utilized for human food and trade, and yet continuously over-exploited for decades. As a consequence, many species in the region have become threatened or endangered. Public concern in environmental protection and marine resource conservation has also been heightened. Immediate action towards replenishment of the over-exploited species is needed to maintain and secure a wholesome ecosystem, which also supports sustainable fisheries for food security and livelihood in the region.

Aquaculture is undoubtedly a practical way to reduce fishing pressure on wild aquatic species and is an effective measure to fulfill man's demand for food fish without harming wild resources. Release programs, that actively repopulate local stocks, ascribe to the development of seed production technologies in aquaculture. Moreover, aquaculture has continuously been addressing the issues of food security and widespread poverty in the Southeast Asian region. However, broodstock and fry of not a few aquaculture species, particularly high value species, still depend on wild resources. Hence, fishing pressure on these species has seriously affected the sustainability of the coastal resources.

Endeavors toward resource enhancement and sustainable aquaculture practices are complement efforts in securing livelihood and decreasing poverty in Southeast Asia. With regard to resource enhancement, stocking through release programs together with appropriate fishing regulations are recognized as effective means to restore the population of overexploited aquatic species. Although an array of seed production technologies and hatchery practices have been developed for numerous species in several countries, thus far, practical information and skills which could be utilized for stock enhancement are still lacking in most Southeast Asian countries. Similarly, promotion of sustainable and environment-friendly aquaculture practices must be pursued through the development of region-oriented technologies and knowledge.

In order to promote and augment regional initiatives on resource enhancement and sustainable aquaculture practices, and to contribute to poverty alleviation, livelihood and food security, the SEAFDEC Aquaculture Department held the *International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia* in 2014. Papers from two plenary speakers, reports from SEAFDEC member countries' representatives as well as contributed papers on sustainable aquaculture and resource enhancement in the region were presented and are now compiled in this conference proceedings. Finally, SEAFDEC/AQD acknowledges the Government of Japan for fully supporting the workshop and this publication.

# ACKNOWLEDGEMENTS

The Government of Japan through the Trust Fund Programs of the SEAFDEC Secretariat provided financial support for the conduct of the *International Workshop on Resource Enhancement and Sustainable Aquaculture practices in Southeast Asia* in March 2014 in Iloilo, Philippines, as well as, the publication of this proceedings.

The editors also acknowledge the efforts of the Publication Review Committee of SEAFDEC/AQD especially Dr. Relicardo M. Coloso, Dr. Maria Lourdes Cuvin-Aralar, Dr. Evelyn Grace de Jesus-Ayson, Dr. Rolando Pakingking and Dr. Myrna B. Teruel for reviewing the draft of the proceedings prior to its publication. Thanks are also due Ms. Imee S. Hacla for the copy-editing and layout.

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# Is Small-hold Tropical Aquaculture in a Genetic Plunge Towards Extinction?

*Roger W. Doyle*

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## *Abstract*

Tropical shrimp aquaculture is in a disease-induced crisis of lost production. The response to this crisis currently focuses on microbiology and pathology, quarantine, and transboundary transfer of shrimp. The crisis also involves an interaction between shrimp genetics and various human interests including protection of intellectual property. Breeders of high-quality strains generally employ (and are encouraged to employ) some form of "breeder lock" that generates inbreeding when broodstocks are "copied". Smaller hatcheries sell these copied, inbred shrimp to farmers, who thereby increase the likelihood of losing their crops to disease. The joint behavior of breeders, hatcheries and farmers causes inbreeding to accumulate in tropical regions.

The depressive effect of inbreeding on disease resistance is exceptionally strong in shrimp, as shown in a re-analysis of published field and experimental data. Inbreeding increases the severity and frequency of disease through a variety of mechanisms. We have relatively few, marker-based estimates of accumulated inbreeding in any non-pedigreed shrimp aquaculture system. Simulation shows, however, that locked post larvae (PLs) can be distinguished from copies in broodstocks and farm ponds, given appropriate analysis of genetic markers.

Culture of stocks certified to be free of specified pathogens (specific pathogen free or SPF stocks) is strongly recommended and only SPF stocks can now be legally imported into most jurisdictions. These recommendations are appropriate, beneficial and necessary. But insofar as they increase the commercial value of proprietary genetic strains, such regulations may also increase the likelihood of copying, and thus inbreeding at farm level and ever-increasing susceptibility to disease and climate stress (Doyle, 2014a).

The intellectual property value of disease-resistant strains will be extremely high and intellectual property rights are fundamental to science-based economic innovation. Breeders will, and must, continue to protect their genetic improvement programs with genetic locks, especially in regions where judicial sanctions are ineffective. The regulatory objective should be to encourage biosecurity and genetic progress while discouraging copying and consequent inbreeding.

The current consensus that inbreeding is unimportant may therefore be out of date. Inbreeding may be amplifying the severity of diseases (including the major current threats: white spot syndrome virus or WSSV, infectious hypodermal and hematopoietic necrosis virus or IHHNV and early mortality syndrome or EMS (acute hepatopancreatic necrosis disease or AHPND). Continuing to ignore the interaction between inbreeding and disease may become a fatal error for tropical shrimp aquaculture.

**Keywords:** tropical shrimp aquaculture, inbreeding, disease resistance, biosecurity, genetic progress

## Introduction

Shrimp production in Asian farm ponds rose continuously from 1992 until 2010, when 2.5 million metric tons were harvested and 45 million people employed (FAO, 2013b). In 2011, a sudden increase in losses from disease caused production to fall, and in 2012, it fell again (Anderson and Valderrama, 2013). In 2012, disease was ranked as the greatest challenge in a global survey of the aquaculture industry (Anderson and Valderrama, 2013) and as much as 40% of tropical shrimp production was thought to be lost to disease (Stentiford *et al.*, 2012).

Social and economic fall-out from this crisis is described in an FAO newsletter (Reantaso, 2012) as, *devastating impacts including direct production losses, therefore loss of food availability; direct and indirect impacts on income and livelihoods/ employment; increased operating costs; restrictions on trade; impacts on biodiversity; loss of market share or investment; loss of consumer confidence, and in some cases, collapse of the sector*.

To date, discussion of the shrimp diseases has focused on the microbiology of aquaculture pathogens and the regulations needed to limit their spread locally and across national boundaries (FAO, 2008; Reantaso, 2012; Jones, 2012; Lightner, 2012). In this essay, I take a wider perspective, that of a geneticist and evolutionary biologist. I argue that the ultimate cause of the crisis is an agro-economic system that locks shrimp breeders, hatcheries and farmers into behavior that induces critical levels of inbreeding at farm level. The inbreeding manifests itself as increased susceptibility to disease and frequency of epidemics over vast areas of Asia, Central and South America, Africa and the Middle East. This global disease crisis may therefore continue to get worse until its roots in human behavior are addressed.

## Agro-economic system that leads to inbreeding in farm shrimp populations

### Interactions between breeders, hatcheries and farmers

The (aquacultural) agro-economic system of tropical shrimp farming comprises a transfer of genetic material, in the form of adult spawners, juvenile shrimp and post-larvae (PLs), through a network of interconnected transactions between breeders, hatcheries and farmers (Doyle, 2014b). These relationships must be described here in some detail because, while they are central to the proposed mechanism that links agro-economics, genetics and an agricultural disease crisis, they may be unfamiliar to many readers. Figure 1 shows the relationships in a diagram that describes the essential aspect of relationships in many parts of the world.

At the top of the schematic diagram in Figure 1 is a breeder, either private or public sector, who maintains a broodstock with due attention to genetic improvement and minimization of inbreeding ("family breeding program" (Gjedrem *et al.*, 2012)). The breeder provides broodstock animals as juvenile or adult spawners to a hatchery, which produces the young animals (nauplii or post-larvae) sold to farmers for grow-out.

Spawners sent by breeders to hatcheries generally represent only a fraction of the total allelic diversity in the breeder's own broodstock (Gjedrem *et al.*, 2012; Rye, 2012). Often, the subset supplied to a hatchery comprises only two full-sib families of spawners, each containing thousands of brothers and sisters. The intention of the breeder is that the hatchery will produce post-larvae (PLs) by mating these animals according to instructions that specify which spawners to mate together to produce high-quality offspring for sale.

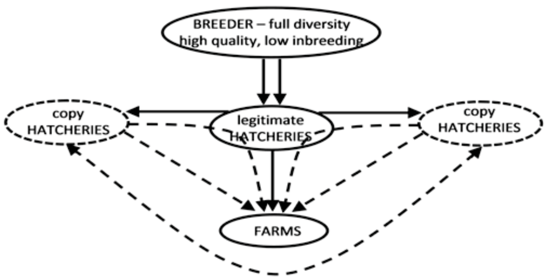


Figure 1. Gene flow through the lock-copy interactions among breeders, hatcheries and farms.

PLs flow onwards from hatcheries to farmers along two channels, only one of which is optimized by mating instructions from the breeders. Called here "legitimate" and shown as solid arrows in Figure 1, PLs in this distribution channel are intended to have maximal uniformity and minimal inbreeding. The flow of genetic material along the solid arrows in Figure 1 is similar to that recommended as good practice by Ponzoni *et al.,* (2012) in their Figure 3.

The "copy" distribution channel shown as dashed arrows in Figure 1 carries PLs or spawners that are diverted from the legitimate channel – either by the hatchery itself or by farmers – and grown to maturity as broodstock in "copy hatcheries". The offspring of these copy spawners will be inbred to varying degrees depending on the genetic composition of the legitimate channel at the point where diversion takes place. Moreover, hatcheries tend to spawn as few shrimp as possible due to the fecundity of shrimp, and often use a highly skewed sex ratio because this enables them to maintain fewer brood shrimp (FAO, 2008). The inbreeding level in the copy channel is expected to range from 0.125 to 0.25 among PLs in the first generation in copy farms and as high as 0.375 in the second (Figure 2) and Doyle (2014b).

### *The "breeder lock" that generates inbreeding*

The primary objective of the legitimate channel (solid arrows) in Figure 1 is to provide highly uniform, non-inbred seed. Aan important secondary objective is to protect the breeder's intellectual property because breeding programs are expensive and breeders protect their investment in various ways, both contractual (Ogden and Weigel, 2007) and biological. The most widely used biological defense against copiers is the "breeder lock", a mating scheme that produces highly inbred offspring in the copy channel (Doyle, 2014a, 2014b). There are many possible types of breeder lock (Sellars and Preston, 2008; Janhunen *et al.*, 2012) but the simplest is probably the one illustrated in Figure 2, which has been reproduced with some changes from Doyle *et al.* (2006). Batches of many millions of seed animals are frequently descended from just two pairs of grandparents, or four full-sib families of grandparents, as shown in Figure 2. PLs from hatcheries that propagate seed according to instructions provided by the breeder normally give good results. However, seed produced by copiers are, as the breeders intend them to be, inbred and give poor growth and survival (Doyle *et al.*, 2006; Sellars and Preston, 2008; Gjedrem *et al.*, 2012; Janhunen *et al.*, 2012; Ponzoni *et al.*, 2012).

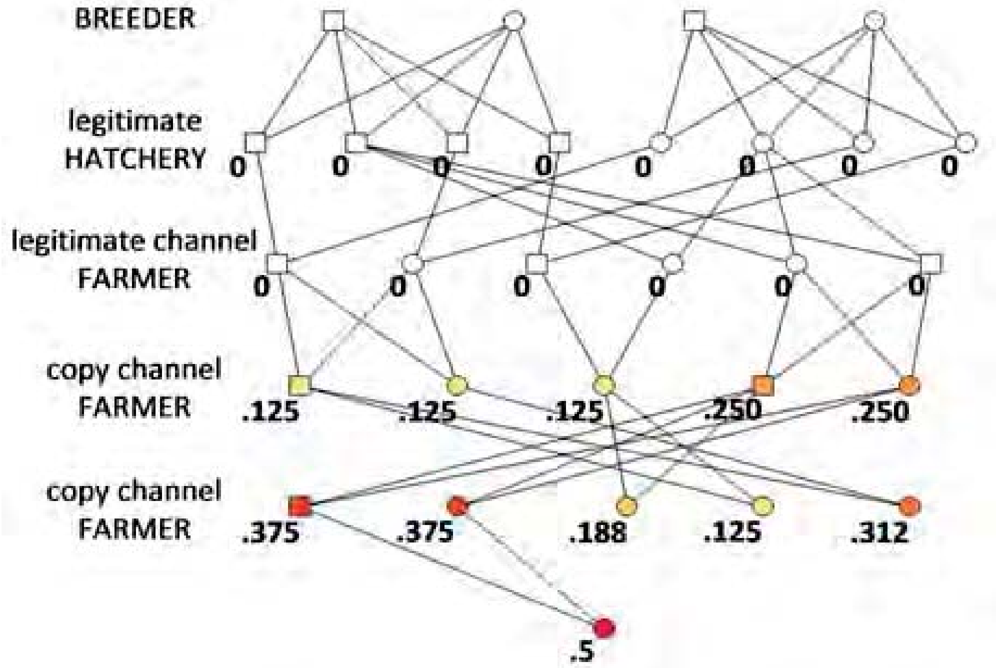


Figure 2. Increase of inbreeding in copied PLs. At the top of the diagram are two pairs of grandparents in the source broodstock. Kinship between these pairs (source kinship) is unspecified but breeders strive to keep it low. The numbers show inbreeding (F), additional to that from source kinship, at successive generations along lines of descent to hatcheries and then to farmers. F=0.125, equivalent to offspring of double first cousins; F=0.25, full sibs; F=0.5, self-fertilization. The diagram is merely a schematic: actual levels obtained by locking a real broodstock will depend on other operational factors such as the number of offspring in each family, sex ratios etc.

The breeder lock in one form or another is widely used, defended and recommended for protecting the intellectual property of breeders (Doyle *et al.*, 2006; Ponzoni *et al.*, 2012).

### *Copy hatcheries disseminate inbred shrimp*

In the world of tropical aquaculture, an improved strain is generally copied shortly after it appears. Due to the high reproductive capacity of fish and crustaceans, unauthorized reproduction and use of improved stocks tend to be widespread for many species (Rye, 2012). Other hatcheries propagate the strain and sell later generations to farmers. They also mix inbred, copied animals with animals in the legitimate distribution channel and sell the mix to unsuspecting farmers, as illustrated in Figure 1. These activities have been grouped with poor broodstock management as “malpractice” (Ponzoni *et al.*, 2012). When hatcheries copy from breeders or other hatcheries, there is an immediate, large decrease in genotypic diversity and a large increase in inbreeding. Copying hatcheries receive only a fraction of the genetic diversity possessed by breeders even when there is no lock. This is ultimately due to the high and variable fecundity of shrimp, which allows very few females to produce enough offspring to stock a farm or provide the next generation of breeders (FAO, 2008). Cumulative loss of genetic diversity over time and during transfers is well documented in shrimp (Benzie, 2009) and other aquacultural species (Doyle *et al.*, 2001).

Although hatcheries in the copy channel may try to circumvent the locks by mating males and females from different hatcheries in the legitimate channel (Fig 1), this tactic is mostly futile. There are generally very few breeder companies – often only one – supplying the legitimate channel with non-inbred stock in any one aquacultural region (Ponzoni *et al.*, 2010; Ponzoni *et al.*, 2012). The breeder usually tries to supply all its client hatcheries with spawners from the same limited group of broodstock families. Again, this helps protect intellectual property as well as supply PLs from top-quality broodstock families.

### *Estimates of the global extent of copying*

Most production of penaeids now depends on domesticated stocks (Stentiford *et al.*, 2012). As *Penaeus vannamei* is an exotic species in Asia, Africa and the Middle East, it is entirely dependent on domesticated broodstocks in those regions.

Rye (Rye, 2012; Gjedrem *et al.*, 2012) estimates that production from uncontrolled breeding programs constitutes more than 90% of worldwide hatchery production from all species. There is as yet no individual estimate for any shrimp species, but people directly involved in the tropical shrimp industry believe copying to be substantial. The following "guesstimates" have been offered as personal communications with permission to cite the source by name: Thailand, conservatively, 50% copied (Mr. Robins McIntosh); Ecuador > 90% copied, Honduras ≈ 50% copied, Mexico > 90% copied, Nicaragua ≈ 50% copied, Panama < 10% copied, Venezuela > 90% copied (Mr. José B. Martinez, Panama.) These estimates are in general agreement with consensus estimates developed during a recent international workshop on the possible connection between inbreeding and shrimp disease (NACA, 2014).

The designation "copied" in the preceding paragraph by no means implies that all copying involves a breeder lock following the highly non-random gene flow in Figure 1. It includes any broodstock that was initiated with restricted genetic diversity and propagated thereafter without a pedigreed family structure to limit inbreeding. A study of genetic erosion in wild and cultivated populations of *Penaeus monodon* here in the Philippines (Xu *et al.*, 2001) provides an exceptionally clear demonstration of this process. Preliminary though it is, the information given above is all we currently have on the extent of copying in shrimp broodstocks. It may be taken as informative to an order of magnitude – that is to say, when properly estimated, production from copied broodstock is likely to be closer to 70% than 7% of the total.

### *Estimating degree of inbreeding*

The obvious way to estimate inbreeding is through surveys of microsatellite diversity in farms receiving PLs through the legitimate and copy channels. The difficulty of doing this might surprise those unfamiliar with the practice of shrimp aquaculture. In idealized, large populations where mating is random the relationship between observed heterozygosity and various definitions of inbreeding is predictable from simple combinatorial rules (Halliburton, 2004). The structure of aquacultural populations is too complex for this approach.

In terrestrial agricultural populations that are divided into sub-groups (breeds, farms, herds etc.), estimates of inbreeding derived from the ratio of observed to expected heterozygosity are often an artifact of unrecognized heterogeneity within samples (Hedrick, 2012; Hedrick, 2013) and works cited therein. Even within a single batch, individuals could on average be either highly inbred or highly outbred, relative to random-mating expectations based on neutral marker data from the same batch, if the batch is part of a breeder locking protocol, kinship-minimizing protocol or some other mating scheme other than haphazard.

Another technical problem is that field estimates from microsatellite markers are usually close to zero, and often negative. Furthermore, low as they are, such estimates are biased upwards by null alleles and unrecognized population substructure. These technical caveats provide another reason for non-specialists to conclude that the impact of inbreeding is likely to be small (Doyle, 2014b).

Although there are notable exceptions (Bierne *et al.*, 2000), most microsatellite estimates actually provide no direct information about inbreeding in aquacultural broodstocks. There are two related difficulties with these estimates. Firstly, the indicators of inbreeding most often reported are deviation from Hardy-Weinberg equilibrium and/or the fixation index, Fis. Secondly, Analysis of Molecular Variance (AMOVA) and simpler procedures for estimating H-W and Fis are usually based on allele frequencies in the same set of samples for which the estimate is made.

The fixation index, Fis, is an indicator of non-randomness in the mating system and thus indicates a potential cause of inbreeding but does not directly measure inbreeding (Templeton and Read, 1994; Waples, 2015). Deliberate non-random mating is rare in aquaculture except in the lock-copy situation shown in Figure 1. Instead, inbreeding in aquaculture broodstocks nearly always accumulates owing to bottlenecks, small population sizes, unequal fecundity and other random processes, rather than deliberate consanguineous mating. “In finite populations, some individuals mate with biological relatives just by chance and inbreeding in the pedigree sense is the result” (Templeton and Read, 1994). The resulting inbreeding will not produce a significantly positive Fis so long as mating is random. Even the offspring of a population of full-sib brothers and sisters, Fped = 0.25, shows neither H-W deviation nor positive Fis if the siblings mated at random.

The second difficulty with Fis and related indicators of inbreeding/non-random mating arises when allele frequencies are calculated from the samples for which estimates are to be made. “Fis is defined with respect to the populations that are included in the sample, either through population-specific estimates or through the average of those estimates” (Holsinger and Weir, 2009). The practical and conceptual difficulty arising from this is beautifully explained by Wang (2014), whose paper should be studied by anyone seeking further information. “Frequently genotype or allele frequency data are unavailable from an ancestral population, and allele frequencies used in calculating relatedness have to be estimated from the current sample in which relatedness between individuals is being calculated. This practice effectively uses the current population (sample) as reference, and an estimator conforming to the correlation concept of relatedness should give an average estimate of zero. This is true regardless of the actual relatedness among individuals in the sample, as shown by simulation (Doyle, 2014b) and analytical results in Wang's 2014 study."

This may surprise non-specialists who believe that inbreeding is likely to be unimportant when in fact we have hardly any direct evidence concerning inbreeding in hatcheries that lack pedigree data.

The obvious solution to both difficulties is to use maximum likelihood estimators such as Wang's trioML rather than Fis, and base inbreeding estimates on reference allele frequencies from an earlier generation, as in the trioML(B) estimator of Figure 2. An appropriate reference is the generation that would be called the founder of the pedigree had pedigree records been kept. A survey of *P. vannamei* broodstocks in Mexico (Perez-Enriquez *et al.*, 2009) is a good example of this approach. For their study of *P. stylirostris* in New Caledonia, Bierne *et al.,* (2000) used published data from wild populations of other penaeid species – a daring move.

It is also possible to estimate inbreeding – inaccurately, for many reasons – from estimates of gene diversity or heterozygosity in the reference generation and the harmonic mean of the effective population number over the intervening generations if these quantities happen to be known, e.g. in Coombs *et al.,* (2009). If data from two or more generations can be obtained, a variety of inbreeding estimators are available e.g. in Hoehn *et al.,* (2012), Waples (2015) and references therein. The reference should, ideally, include only animals that actually contribute to subsequent broodstock generations, and not all the animals in the reference population, many of which may not have reproduced (Lacy, 1995).

At levels of population structure higher than the batch, e.g. farm, hatchery and geographical region, it is actually more useful to ignore observed heterozygosity (as an uninterpretable artifact) and instead pay attention only to the biogeographically and temporal structure of allele number (Jost, 2008; Gregorius, 2010). As generations follow one another there will be a monotonic, inverse relationship between allele number and accumulated inbreeding in an aquacultural region. The correlation will be mainly due to the passage of time and the ineluctable, irreversible loss of genetic material caused by too-small broodstocks, artificial and natural selection, variable reproductive success, serial transfers of subsets of broodstock from hatchery to hatchery, irrational or careless breeding and deliberate breeder locks. The correlation will grow stronger as time passes, and declining allele diversity will reflect the cumulative erosion of the regional genetic environment for aquaculture.

Microsatellites are not the only markers available. The technology of high-throughput sequencing is developing rapidly and it may soon be possible use high-density genomic data to routinely estimate the inbreeding of individuals with an accuracy close to that obtainable with pedigree data (Li *et al.*, 2011). With a sufficient number of genome-wide markers, the relationship of any pair of individuals can be inferred by estimating their kinship coefficient, independent of sample composition or hidden population structure (Manichaikul *et al.*, 2010).

The most straight-forward – and immediately available – way to estimate inbreeding depression (as distinct from pedigree inbreeding) would probably be to measure it directly, by hybridizing samples of hatchery spawners with an unrelated tester strain or strains. Inbreeding depression would then be evaluated by comparing fitness traits of inbreds and outbreds in standardized stress tests, disease challenges etc.

### *Inbreeding increases susceptibility to disease and other stresses*

Inbreeding depression is the decrease in growth and other traits, most importantly reproductive success and survival that is seen in inbred relative to outbred animals and populations (Lynch and Walsh, 1998). Inbreeding depression is especially severe in environments where survival is low, even in outbred populations, owing to poor nutrition, extreme temperatures, the presence of pathogens or a myriad of other possible stressors alone or in combination (Frankham *et al.*, 2002; Liao and Reed, 2009; Bijlsma and Loeschcke, 2012; Cheptou and Donohue, 2011; Cheptou and Donohue, 2013; Enders and Nunney, 2012; Reed *et al.*, 2012).

Remarkably, data from many plant and animal taxa and many different kinds of natural and artificial stressors can be fitted to a common regression line of inbreeding depression against stress (Fox and Reed, 2010). Stress was defined in Fox and Reed's (2010) meta-analysis as the proportional decrease in survival of outbred individuals in a stressful environment compared to a benign environment. Shrimp, like other animals, are affected more strongly by stress when inbred. Inbred and outbred experimental populations of the mysid shrimp *Americamysis bahia* differed greatly in their survival under low salinity stress, and genetic load was much higher in stressful environments for several fitness indices (Markert *et al.*, 2010). The authors note that this is actually an underestimate of the amplification of genetic load by stress because many of their inbred lines did not survive long enough to be tested.

The fit of *P. vannamei* and other shrimp species to the meta-analysis regression of Fox *et al.,* (Fox and Reed, 2010) cannot simply be assumed. Several aquacultural species, like *P. vannamei* and oysters, have exceptionally high fecundities and "huge" inbreeding loads (Bierne *et al.*, 2000). Inbreeding depression in oysters, which have fecundities on the order of 106 offspring per spawn, has been studied in considerable detail (Bierne *et al.*, 1998; Launey and Hedgecock, 2001; Plough, 2012) and found to be high. The shrimp *P. vannamei* has a fecundity on the order of $10^5$ offspring per spawn.

### *Inbreeding increases mortality from viral disease in P. vannamei*

Two viruses, white spot syndrome virus (WSSV) and Taura syndrome virus (TSV), bore most of the responsibility for the global economic loss from disease in shrimp as of 2011 (Stentiford *et al.*, 2012). A new disease, Early Mortality Syndrome disease, EMS/AHPND, has recently become the most serious disease problem facing tropical shrimp aquaculture. EMS appears to be a septicaemic vibriosis involving at least two *Vibrio* species infected by a bacteriophage (Lightner *et al.*, 2013; FAO, 2013a).

*Penaeus vannamei* is by far the dominant shrimp species in aquaculture (FAO, 2013b; Anderson and Valderrama, 2013). Mortality induced by exposure to both of these viruses has been examined at various levels of inbreeding in a population of *P. vannamei* at the Oceanic Institute, in Hawaii (Moss *et al.*, 2008; Moss *et al.*, 2007). Re-analysis of the Oceanic Institute data reveals that the interaction between inbreeding load in *P. vannamei* and disease stress is significantly stronger than the regression meta-analysis of Fox *et al.,* (Fox and Reed, 2010). High as it is, *P vannamei* inbreeding loads under disease stress are comparable to loads in oysters (Bierne *et al.*, 1998; Launey and Hedgecock, 2001; Plough, 2012).

An important and possibly unique field study shows the effect of inbreeding mortality from disease in the farmed shrimp *Penaeus stylirostris* in New Caledonia (Bierne *et al.*, 2000; Goyard *et al.*, 2008). Both components of yield, mortality and growth, were depressed by inbreeding that accumulated slowly over many generations (not rapidly, as in the lock-copy system described here). Inbreeding depression was especially evident in years when the environment was poor and overall yields low. This work is particularly relevant at this time because the disease affecting *P. stylirostris* was a septicaemia caused by a species of *Vibrio*, the bacterium which has recently been implicated (Lightner *et al.*, 2013; FAO, 2013a) in the current EMS (or AHPND) disease crisis.

Inbred and outbred experimental populations of the mysid shrimp *Americamysis bahia* differed greatly in their survival under low salinity stress, and genetic load was much higher in stressful environments for several fitness indices (Markert *et al.*, 2010). The authors note that this is actually an underestimate of the amplification of genetic load by stress because many of their inbred lines did not survive long enough to be tested.

There appears to be no published experimental data relating inbreeding to disease or any other stress in aquacultural shrimp species, other than the data from Moss *et al.,* (Moss *et al.*, 2008; Moss *et al.*, 2007) and the *P. stylirostris* study in New Caledonia (Bierne *et al.*, 2000; Goyard *et al.*, 2008). Shrimp are routinely challenged for a variety of diseases and other stresses in breeding programs. Usable data must therefore exist unexamined, or at any rate unpublished, in the files of many family breeding program that keep pedigree and mortality records.

### *Monoculture and the incidence of epidemics*

Epidemiological theory (Lively, 2010; Keesing *et al.*, 2006; Keesing *et al.*, 2010; King and Lively, 2012) and observation suggest that the incidence of epidemics is inversely proportional to the genotypic diversity of the host population, a relationship called the *dilution effect or monoculture effect* (reviewed by Ostfeld and Keesing 2012). Increases in the prevalence of infection in the wild are associated with genetic bottlenecking and inbreeding induced by founder effects and/or mating systems (King and Lively, 2012). Small, genotypically uniform populations of endangered species are especially prone to epidemics, as are populations at the edge of a recent range expansion (instances cited in King and Lively, 2012).

The breeder lock illustrated in Figure 2 leads to low levels of genotypic diversity within farms and farming regions. The legitimate and copy distribution channels both contribute to a restriction in genotypic diversity but it is particularly true in the copy distribution channel. Hatcheries that copy from other hatcheries start with founder populations that will, in many cases, have been deliberately locked. In such cases the low genotypic diversity results both from random founder effects (small sample of available allele diversity) and mating system (founders deliberately related by descent, e.g. double first cousins).

It appears from modelling exercises that small increases in genotypic diversity can cause dramatic reductions in the likelihood of an epidemic outbreak (Lively, 2010). The effect is distinct from increased disease susceptibility through inbreeding depression, although in practice inbreeding is usually associated with low genetic diversity, as explained above. In Lively`s theoretical model (Lively, 2010) the incidence of epidemics is inversely proportional to genotypic diversity in the host population, and epidemics are less severe and die out more quickly in genetically diverse populations. Under the assumptions of some models, small increases in allelic diversity dramatically reduces pathogen load even in very large host populations (King and Lively, 2012). The effect is expected to be greater when the pathogen is not host – specific (Ostfeld and Keesing, 2012). It is therefore worth noting that more than 93 species of arthropods are reported to be hosts of WSSV (Moss *et al.*, 2012), one of the worst shrimp disease viruses.

### *Verifiable information might persuade farmers to avoid the copy channel*

Intellectual property rights are fundamental to science-based economic innovation. Breeders will continue to protect their genetic improvement programs with breeder locks that generate inbreeding when copied, especially in regions where judicial sanctions are ineffective. Farmers are well aware of inbreeding depression, as previously mentioned, and may have a good notion of how broodstocks are managed by copy hatcheries in their local areas. However, farmers often cannot be sure the seed animals they purchase are not inbred even when they buy from supposedly legitimate hatcheries. Their puzzling reluctance to pay more for genetically superior aquaculture stock (Ponzoni *et al.*, 2009; Gjedrem *et al.*, 2012) could be due in part to lack of credible information. This possibility has already been noted (Ponzoni *et al.*, 2012).

If verifiable information by legitimate breeders and hatcheries are available, for example through a national broodstock information exchange network (Doyle, 2015), farmers could more easily choose to avoid the copy distribution channel shown in Figure 1. "Certificates of authenticity" have been offered by some breeders, but this strategy fails when the certificates too are copied. Certificates offered to date have been missing the essential element of verifiability. Verification is technically easy in principle. A certificate from the breeder attesting that a particular batch is 100% heterozygous for two particular alleles at a particular locus (both specified in the certificate) would be sufficient to verify that the batch is a first-generation hybrid, i.e. minimally inbred.

Could breeders be persuaded to provide verifiable genetic information to farmers? Some individual breeders might see a marketing advantage in doing so. As a group, breeders as well as farmers might come to realize the pernicious, cumulative effect of the collective behavior of breeders, hatcheries and farmers on the whole aquaculture sector and therefore on themselves. In disease-ridden environments even the most modern, isolated and bio-secure breeding facilities are at risk.

### *Unintended consequences of disease-control policies that ignore genetic side effects*

International organizations concerned with aquaculture, including the Food and Agriculture Organization of the United Nations (FAO), are responding vigorously to the disease crisis by developing regulations and codes of practice for transferring aquacultural stocks between and within regions, and also by promoting standard and guidelines for disease detection, identification and surveillance (FAO, 2008; Reantaso, 2012). The culture of stocks that are certified to be free of specified pathogens (SPF stocks) is strongly recommended, and only SPF stocks can now be legally imported into most jurisdictions. These recommendations are appropriate and beneficial from a strictly microbiological perspective.

It appears that those concerned with disease control are not yet thinking of the genetic consequences of their policies and recommendations. Neither the word "inbreeding", nor the phrases "genetic erosion" or "host diversity" (pertaining to monoculture effect) appear in any of the discussions of aquaculture diseases and disease-related regulations that I found published in 2012 or in the first half of 2013, totalling more than 300 pages (Murray, 2013; Stentiford *et al.*, 2012; Lightner, 2012; Moss *et al.*, 2012; FAO, 2013b; Chamberlain, 2013; Flegel, 2012; Jones, 2012; Kibenge *et al.*, 2012; Reantaso, 2012; NACA, 2013). Although farmers themselves often blame inbreeding for poor yields from their ponds their concerns have been deprecated and dismissed (FAO, 2008, p. 13).

In this review I suggest that disease crises in tropical shrimp aquaculture may be growing more severe and more frequent owing to an agro-economic system that generates genetic erosion at farm level. Genetic erosion results from a pattern of human behavior in which breeders protect intellectual property through breeder locks (expressed only when broodstock is "copied"), copying hatcheries sell inbred offspring, and farmers stock their ponds with seed animals they are unable to evaluate. The resulting inbreeding and low genotypic diversity increase susceptibility to disease, which leads to more infected individuals and farms and thus, by standard epidemiological reasoning, increases the frequency and severity of epidemics.

The hypothesis is not that inbreeding triggers shrimp diseases – which have myriad environmental and other immediate causes – but that inbreeding increases the prevalence and severity of disease, and that inbreeding is accumulating on regional scales. We may be making a dangerous mistake in treating the torrent of shrimp diseases of the past decade as isolated events with independent, microbiological causes, describable with some (unknown but invariant) distribution of risk. The distribution of risk may be shifting towards higher values in a farming system experiencing genetic erosion.

Culture of stocks certified to be free of specified pathogens (SPF stocks) is strongly recommended and only SPF stocks can now be legally imported into most jurisdictions. These recommendations are appropriate, beneficial and necessary.

But insofar as they increase the value of proprietary, high-quality SPF strains, such regulations may also increase the use of breeder locks and the likelihood of copying, and thus inbreeding at farm level (Doyle, 2014a, 2014b). Intellectual property rights are fundamental to science-based economic innovation. Breeders will, and should, continue to protect their genetic improvement programs with breeder locks that generate inbreeding when copied, especially in regions where judicial sanctions are ineffective. The intellectual property value of disease-resistant strains will be extremely high.

The current consensus that inbreeding is unimportant may therefore be out of date. Inbreeding may be amplifying the severity of diseases, including the major current threats: WSSV, IHHNV and EMS (or AHPND). The regulatory objective should be to encourage biosecurity and genetic progress while at the same time discouraging copying and consequent inbreeding.

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# Rapid Adaptation to a New Environment: Is it Reversible?

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## *Abstract*

Accumulating evidence suggests rapid adaptation of fish populations when they are exposed to artificial hatchery environments. However, little is known if rapidly-adapted populations can readapt to their original, natural environment at the same rate. Here, I review recent studies on salmonid fish that address this issue. They indeed suggest rapid adaptation of hatchery populations, in which reproductive fitness under a natural environment became much lower than that in the wild population after only 1-2 generations of captive breeding. However, the reproductive fitness did not recover after one generation of natural rearing, implying that rapid adaptation to a new environment was not reversible at the same rate. I discuss potential consequences of the irreversible fitness reduction in extensively stocked fish species. Understanding the mechanism behind the irreversible rapid adaptation in fish populations will help us figure out a better, nature-friendly, and hence sustainable means of hatchery operations for human welfare.

**Keywords:** fish stocking, hatchery, rapid adaptation, reproductive fitness, salmonid species

## *Introduction*

While fish has been recognized as our most important food resource long since ancient days, we keep in captivity, many fish species for personal and public viewing to ultimately enjoy their biodiversity. For example, fish catches have been around 90 million tons since 1990s, and aquaculture production has reached 60 million tons in 2011 (FAO, 2012). More than 80% of them were sold in fish markets and utilized for human consumption. While aquaculture has been developing rapidly, there is no other industry that depends so much on 'natural' resources at the moment. In addition, we have more than 400 public aquariums worldwide, and c.a. 500 thousands of people visit them each year in Japan alone. On the one hand, they make us very familiar with fish species. On the other hand, wild fish populations often became overexploited, vulnerable to environmental disturbances, and endangered worldwide. Despite the popularity of the fish species, however, our knowledge on ecology and evolution of fish in the wild is very limited. Efficient means of conservation and sustainable management of wild fish stock is yet to be established.

Salmonid species are one of such species. Although they are recognized as economically and socially important species, ecology of salmonid species in the wild is largely uncertain. In this review, I briefly summarize our knowledge on the ecology and adaptive capability of salmonid species, followed by an introduction of related information from my own research and that of my colleagues. It is hoped that this review would contribute to broad discussions on better, sustainable uses of fish for our future generations.

### *Ecology of salmonid species*

Salmonid species are often characterized by their nature of large-scale migration and of homing behavior (Quinn, 2005). However, their life histories are very diverse among individuals, populations and species (Groot and Margolis, 1991). In brief, they are born in cold freshwater, typically in 4-10°C. After a few months from hatching, some already start their migration. Majority of Pacific salmonids, for example, have short freshwater residence as juvenile, whereas rainbow trout (*Oncorhynchus mykiss*, also a part of Pacific salmonids) and the other 'trout' species can live their whole life in freshwater. In fact, some species have multiple life history forms, typically male-biased. O. mykiss is one of them, and its sea-run form is called steelhead. Brown trout (*Salmo trutta* L.), one of the two species in Atlantic salmonids, also has a sea-run form called sea trout. Just like other salmonid species, they grow fast in the ocean and come back to their natal rivers for reproduction. The basis of their life history differentiation is still unclear, although it is most likely determined through genetic-environmental interactions.

Ocean migration takes one to a few years. Pink salmon (*O. gorbuscha*) is unique in this context because they have a strict two-year life cycle. Salmon migration range covers whole of the North Pacific for Pacific salmonid species and a northern part of the Atlantic Ocean for Atlantic salmon (*S. salar*). The time for salmon runs to the river for reproduction varies among species and among populations in the same species. If any, resident fish can spawn together with sea-run fish in the same spawning ground. Resident males often use 'sneaking' behavior for their reproductive success with sea-run females. This is part of the reasons why multiple paternity is common in salmonid species. Although majority of salmonid fish die after the first spawning, trout species and a few sea-run species can repeat the migration and reproduce multiple times in their life (e.g., Atlantic salmon).

### *Rapid adaptation to hatchery environments and its downside*

Due to an increasing demand for salmonid species as a food resource, hatchery and domestication programs have been very popular worldwide. Hatcheries and programs were first established in the late 19$^{th}$ century. The rearing technique has been developed and improved for many species, most notably for Atlantic salmon. Together with developments in refrigerated cargo transportation systems, full-life cycle aquaculture enabled us to find this species in fish markets worldwide today. For majority of sea-run Pacific salmonid species however, full-life cycle aquaculture system is either not established yet or unrealistic due to economic reasons. This is why we still depend heavily on fish stocking for salmonid species, which utilizes hatcheries for juvenile development from fertilization to parr or fingerling, typically for <1 year, and releases juveniles into the wild with a hope for their successful return as adults.

One question is whether the hatchery-born fish can survive well in the wild and return to the point of release so that fisheries can gain from the hatchery fish stocking. Even more profound question is then whether adults that have returned (but not caught) can spawn naturally and reproduce successful progenies. Both questions are important but the latter one is even more critical for conservation and self-sustainable stock management. To answer this question, we used molecular genetic markers to identify individuals and the pedigree of steelhead *Oncorhynchus mykiss* in the Hood River, Oregon, USA (Araki *et al*., 2007a, 2007b). The DNA-based parentage assignment method, together with highly polymorphic genetic markers (called 'microsatellite'), provided a powerful means of identifying parent(s)-offspring pairs in the field samples of >15,000 returning adults. We found that 'old' hatchery stock performed poor in the system, suggesting only 10-30% of successful natural reproduction compared with wild-born fish that spawned in the same river in the same year (Araki *et al.,* 2007a). The 'old' hatchery stock might have suffered, having come from a non-local origin and from multi-generation captive rearing with the surviving stock becoming forcibly adapted to the artificial rearing environment. The first generation of 'new' hatchery stock, which was designed for conservation, performed much better. Nevertheless, they still showed significantly lower reproductive success than wild fish in the wild (Araki *et al.,* 2007a). On average, the relative reproductive success of the first generation was 0.848, suggesting that they reproduced 15% less than their wild counterpart in the river. The most interesting part of the study was on the second generation of the 'new' hatchery stock – those who had a returning hatchery-born parent and they themselves were also reared in a hatchery. Their relative reproductive success to their wild counterpart was on average 0.379, which was rather close to that of the 'old' stock above (Araki *et al*., 2007b). Together with other studies on reproductive fitness of hatchery-born salmonids, we concluded that c.a. 38% of natural reproductive fitness can be lost per captive-reared generation. This result suggests rapid adaptation of fish to the new, artificial environment coinciding with maladaptation to the original, natural environment once they are released (see also Christie *et al*., 2012).

How general is it? Currently, there are a limited number of comparable studies, and they are all on salmonid species (Araki *et al*., 2008). The reduced reproductive fitness of hatchery-reared fish was also suggested in Chinook salmon (*O. tshawytscha*) and coho salmon (*O. kisutch*) (Williamson *et al*., 2010; Thériault *et al*., 2011 but see also Hess *et al*., 2012). In addition, there is little evidence for hatchery fish releases helping local wild stock enhancement, together with a few exceptions as a hope for better fish stock management (Araki and Schmid, 2010).

***Re-adaptation to the original environment?***

The next question is whether offspring of the hatchery-born yet naturally-spawned fish can reproduce well in the wild. Note that the offspring themselves are born, reared and reproduced in the wild. Therefore, if they can re-adapt to their original environment at the same rate as their adaptation rate to the captive environment, we can expect a rapid recovery of reproductive fitness. However, this was not the case for the Hood River steelhead, where we found the rapid decline of reproductive fitness of hatchery-born fish in the wild (Araki *et al.*, 2009). Among wild-born offspring of hatchery-born parent, fish from two hatchery-born parents had the lowest reproductive fitness. On average, they had 63% lower reproductive success than that of fish from two wild-born parents. Reproductive success of fish from a hatchery-born parent and a wild-born parent was intermediate (on average 13% lower), but the estimate was not significantly different from that of two wild-born parents. These results indicate that even after stopping the fish stocking, remaining wild populations can still suffer from the carry-over effect of past fish stocking (also known as "genetic pollution"). Indeed, we estimated that eight percent of the wild population might have been lost due to the carry-over effect in the case above (Araki *et al.*, 2009). This value strongly depends on the proportion of offspring from two hatchery-born parents, and hence it is most likely sensitive to the amount of fish stocking. If 50% of the mature fish were hatchery-born fish in the wild, for instance, loss of the wild population in the next generation due to the carry-over effect could be >20% in the case above.

### *Conclusion: For better stock management*

We have seen that reproductive fitness of hatchery-born fish reduces very rapidly and that they are suffering from the reduced fitness after being stocked in the wild. And it is likely that this process is not reversible at the same rate. However, there are many questions left. Firstly, it is not entirely clear why reproductive fitness can be reduced so rapidly in captive environment but not in natural environment. The most likely reason is very strong domestication selection reducing not only the reproductive fitness but also genetic variations in the loci under selection. Once the genetic variation is lost from the population, the selection potential for re-adaptation to natural environment will also be lost. In fact, reduction of neutral genetic variation in hatchery stocks has been reported in many species, suggesting small selection potential left for these stocks (Araki and Schmid, 2010). Most importantly, however, we should identify the trait under domestication selection first because neutral genetic variation does not necessarily reflect the selection potential for re-adaptation to the original environment. This is one of the main reasons why genomic study becomes increasingly important for fishery science. Secondly, we do not know the consequences of coexistence of wild-born and hatchery-born individuals in the wild very well. Theoretical predictions suggest that stocking of hatchery fish with maladapted genetic background can have serious demographic consequences when they interbreed with their wild counterparts (Ford, 2002; Lorenzen, 2008). Using a modeling approach, Satake and Araki (2012) also suggested that intermediate levels of hatchery fish stocking can cause not only reduction in population size in the long term but also local gene pool replacement. But empirical evidence for linking hatchery fish stocking and its outcome in the status of wild stock is scarce at best. Lastly but not least importantly, social responses to the fishery activities are not well documented and predicted. We should bring and keep politicians, stakeholders and local societies in the discussions over the better stock management, so that the risk and potential advantages of fish stocking can be shared among them. It is eventually them who decide what kinds of fish and fisheries should be accepted in the societies, and we are responsible for informing them to find the best solution.

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# COUNTRY PAPERS

# Current Status of Sustainable Aquaculture in Cambodia

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***Abstract***

In Cambodia, the extension of technologies in fish aquaculture is a vital activity that contributes to improving the daily livelihood of the rural poor farmer communities. Technology extension was introduced since 1994 through a project of the Asian Institute of Technology (AIT) and other local non-government organizations (NGOs) or international organizations (IOs) in some fish production deficient provinces. Prior to the introduction of such activities, wild fish were still abundant. From then to date, aquaculture extension is being done under the Freshwater Aquaculture Improvement and Extension Project Phase II of Japan International Cooperation Agency (FAIEXII-JICA), and Department for International Development/Danish International Development Agency (DFID/DANIDA) Projects.

Recently, aquaculture extension is one of the national policies under the National Rectangular Strategy Policies of the Government. There are several different freshwater aquaculture systems including floating cage/pen culture, earthen pond culture and rice-fish culture, and other fish culture in smallwater bodies or aquaculture-based fisheries in Cambodia as practiced in over 20 provinces and cities, with less development focused on coastal aquaculture.

Freshwater aquaculture production continued to grow over the past two decades and increased from 1,610 tons in 1984 to 20,760 tons in 2004, representing 11.9 times increase or growth of 16.3% per year This further increased to 74,000 tons in 2012, representing 11.9 times increase or a growth rate of 15% per year. However, aquaculture development in Cambodia is in its infancy stage compared to other countries in the region. It has encountered some problems and constraints during its development, which include inadequate and unreliable supply of good quality seed; lack of capital, fund or credit for aquaculture investment; inadequate knowledge of aquaculture technology; inadequate manpower for aquaculture extension service; and climate change, which have adversely impacted aquaculture development in Cambodia.

In order to achieve the goal of supplying the nation's future fishery requirements through aquaculture, the Cambodia Fisheries Administration (FiA) published the Strategic Planning Framework (SPF) for Fisheries (2010-2019). Within this framework, the scenarios for future fish demand-supply for 2019 suggest that aquaculture production will increase by 15% per year to 185,000 tons by the end of 2019.

**Keywords:** Cambodia, freshwater fish species, aquaculture extension, constraints, aquaculture development

## Introduction

Cambodia is located in Southeast Asia between latitudes 10° and 15°N and longitudes 102° and 108°E, and has a mainland area of 181,035 km$^2$ extending approximately 580 km from east to west and 450 km from north to south with a total population estimated at about 14.1 million people in 2006 and a population growth rate of 2.4% per annum, reported to be the highest in Asia. Cambodia's coastal zone, which is located in the southwest of the country, has a total length of approximately 435 km.

Cambodia's climate is characterized by two major seasons: a dry season from mid-November to mid-May and a rainy season from mid-May to mid-November. The annual average temperature is 27°C, and rises to a maximum of 38°C in April or May and falls to a minimum of 14°C in December or January.

Agriculture is the major occupation for about 85% of the population, which can provide both rice and fish, which are the basic diet of Cambodian people. Fish is the most important source of animal protein for Cambodians, providing around 75% of total animal protein intake for the population. Moreover, fish not only plays a major role in the daily diet, but also in the economy of the people. Based on the National Statistics of the country, the average fish consumption of Cambodian people is 52.4 kg/person/year while an average household consumes between 60-66 kg/person/year, and households around Tonle Sap Lake consume between 67-80 kg/person/year. In recent years, an annual estimate of freshwater capture fisheries production ranges from 405,000 to 445,000 tons in 2010 and 2011. The change in productivity in freshwater capture fisheries is closely related to the change in flooding level that occurs on an annual basis. Meanwhile, marine capture fisheries production is about 60,000 tons annually.

Since 2000 when Cambodia adopted reforms in the fisheries sector, inland fisheries took off rapidly and freshwater aquaculture production continued to show growth over the past two decades and increased from 1,610 in 1984 to 20,760 tons in 2004, representing a 12-fold increase or 16.3% increase per year. Production continued to increase to 74,000 tons in 2012, also representing a 12 times increase or a growth rate of 15% per year. Therefore, Cambodian aquaculture has expanded, diversified and intensified. Its contribution to aquatic food production has increased gradually. It is highly diverse and consists of a broad spectrum of systems, practices and operations, ranging from simple backyard small household pond systems to large-scale, highly intensive, commercially-oriented practices. However, aquaculture development in Cambodia is in its infant stage of development compared to other countries in the region. It has some problems and constraints encountered in development including: (a) inadequate and unreliable supply of good quality seed; lack of capital, fund or credit for aquaculture investment; (b) inadequate knowledge of aquaculture technology; (c) inadequate manpower for aquaculture extension service; and (d) climate change. All of these have impacted aquaculture development in Cambodia.

Recently, aquaculture extension is one of the national policies under the National Rectangular Strategy Policies of the government. There are several different freshwater aquaculture systems including floating cage/pen culture, earthen pond culture and rice-fish culture, and other fish culture in small water bodies or aquaculture-based fisheries which have been practiced in Cambodia in over 20 provinces and cities, while there is less development of marine aquaculture.

In order to achieve the goal of aquaculture fish production and to supply the nation's future fish requirements, the Cambodia Fisheries Administration (FiA) has already prepared its 10-year Strategic Planning Framework (SPF) for the Fisheries Sector (2010-2019). Within this framework, the scenarios for future fish demand-supply for year 2019 suggested that aquaculture production will increase by 15% per year to 185,000 tons by the end of 2019.

The main objectives of this study aims to review the existing literature and combine this with primary data to understand the evolution, current situation and potential of freshwater and marine aquaculture development, and to identify problems/ constraints, issues, gaps and opportunities in aquaculture development in Cambodia.

***Methodology***

The methodology used by the authors for this study combined a review of secondary data with primary research data with focus on interviews with key stakeholders.

***Results and Discussion***

***Implementing the SPF for the Aquaculture Sub-sector***

Aquaculture offers enormous long-term potential for Cambodia. However, the starting level is fairly low (only 50,000 tons was produced in 2009, mostly from small-scale operations). In order to achieve immediate growth whilst also maintaining a pro-poor focus, the main interventions will be to support small and family-scale development, primarily through training, the provision of fingerlings, and establishing risk management systems. Targets include:

- At least 85,000 trained fish farmers actively engaged in aquaculture by the end of 2019
- Fish seed production is increased to 250,000,000 per year by the end of 2019
- A surveillance, monitoring and control system for fish disease outbreaks is developed and implemented by the end of 2014
- Research and development to identify commercially viable production of indigenous species in cooperation with regional organizations, i.e. Mekong River Commission (MRC)

In order to facilitate the general growth of aquaculture, the FiA will also develop comprehensive regulations and technical standards under the Law on Fisheries that are specifically designed to support the ability of the aquaculture sector to reach the targets set out for it.

***Current Status of Aquaculture Technology***

Aquaculture activities have been categorized at different types, especially in terms of scale and intensity. In practice these various "types" are continually evolving, and individual farmers and the sector as a whole operate on a continuous spectrum of scale and intensity depending on resources, skills and market/economic incentives. Farmers may also shift species depending on input costs and market conditions. We have therefore found it more useful to recognize certain basic technologies, all of which may be applied at different scales and levels of intensity, and for different species (Table 1).

***Freshwater Cage Culture***

Freshwater aquaculture, especially of snakehead, has been undertaken in Cambodia since the 10th century – partly as a means of storing and fattening fish to reduce the seasonal surplus noted above, and partly as a means of converting low-value fish into high-value fish. Historically, both Chinese and Vietnamese have taken a significant role in this activity. In the 1960s production of fish in cage already stood at around 4,000-6,000 tons/year and during the 1980s comprised 80-90% of total aquaculture production and 70-80% during the 1990s. Since 2005, however, cage culture of snakehead has been banned because of concerns about over-exploitation of wild fingerlings – for both stocking and feeding – and the overall level of cage culture has declined, though it is still thought to comprise more than 50% of all aquaculture production.

Most cage culture takes place in the Tonle Sap, Mekong and Brassac rivers, and in the Great Lake. Apart from illegal rearing of snakehead (mainly *Channa micropeltes*), the main species stocked are *Pangasionodon hypopthalmus*, *Hemibagrus wyckioides* (redtail catfish) and *Oreochromis niloticus* (tilapia). *Clarias* (catfish), *Puntius* (silver barb), *Oxyeleotris* (sand goby), and *Leptobarbus hoevennii* (Hovens carp) may also be fattened or stored over a few months to exploit seasonal price variations. Cage culture probably remains the most important type of aquaculture, at least in terms of the number of enterprises.

Cage sizes vary from 48 to 540 $m^3$ for Pangas catfish culture, and 18-180 $m^3$ for snakehead culture, and are usually made from bamboo or wood, though net cages are becoming more common. Sometimes these are large boat-shaped structures with accommodation and sometimes pig sties on board. For Pangas, the average yield is between 28 and 90 kg/$m^3$; and for snakehead 75-150 kg/ $m^3$.

Overall trends are unclear other than the decline in snakehead farming. However, it seems likely there has been a shift in favor of Pangas (perhaps related to seed availability) and redtail catfish.

***Freshwater Pond Culture - Smallholder***

Pond aquaculture has not been a traditional activity in Cambodia, probably because of the abundance of wild fish. However, since the 1990s there have been substantial efforts by donors and NGOs to promote pond based fish culture – on individual farms, in community ponds and in rice fields. These initiatives have been accompanied by introduction of a range of Chinese and Indian carps, tilapia, and hybrid catfish for which breeding technology is well developed. While initially, care was taken to keep these exotic introductions away from the Great Lake and Mekong river system, they have since been introduced widely across the country, and there is some evidence that some species, such as common carp (*Cyprinus carpio*), are breeding in the wild. Native species, including silver barb (*B. gonionotus*), walking catfish (*Clarias batrachus*), river catfish (*Pangasianodon gigas*) and occasionally L*eptobarbus hoeveni*,

Table 1: Fish species cultured in Cambodia

| No. | Scientific name | Common name / Khmer name | Culture method | Source of seed | Culture area |
|---|---|---|---|---|---|
| ***Indigenous species*** | | | | | |
| 1 | *Pangasianodon hypophthalmus* | Striped catfish/ Trey Pra Thom | FC, P | HS, WS | Great Lake, Tonle Sap, Mekong |
| 2 | *Pangasianodon bocourti* | Basa catfish/ Trey PraKe | FC | WS | Great Lake, Tonle Sap |
| 3 | *Pangasianodon larnaudii* | Spotted-ear catfish/ Trey Po | FC | WS | Great Lake, Tonle Sap, Mekong |
| 4 | *Pangasianodon conchophilus* | Snail eating catfish/ Trey PraKchao | FC | WS | Great Lake, Tonle Sap, Mekong |
| 5 | *Channa micropeltes* | Giant snakehead/ Trey Chhdor | FC | WS | Great Lake, Mekong, Tonle Sap rivers |
| 6 | *Channa striata* | Snakehead/ Trey Ros | FC | WS | Great Lake, Mekong, Tonle Sap rivers |
| 7 | *Barbodes gonionotus* | Silver barb/ Trey chhpenprak | FC, P, RF | HS, WS | Whole country |
| 8 | *Leptobarbus hoeveni* | Saltan fish/ Trey praloung | FC, P | HS, WS | Mekong, Tonle Sap, Great Lake, Takeo, SvayRieng, Prey Veng |
| 9 | *Hemibagrus wyckiode* | Redtail catfish/ Trey kyakrahom | FC | WS | Mekong river, Tonle Sap, Great Lake |
| 10 | *Oxyeleotris marmorata* | Marble goby/ Trey Domrey | FC, P | WS | Mekong, Tonle Sap, |
| 11 | *Anabas testudineus* | Climbing perch/ Trey kranh | P | HS | Whole country |
| 12 | *Barbodes altus* | Red tailed tinfoil/ Trey kahekrahom | P, RF | HS, WS | Whole country |
| 13 | *Epinephelus* spp. | Grouper | FC | WS, Imported | Coastal, Koh Kong, Sihanoukville |
| 14 | *Lates calcarifer* | Seabass | FC | WS, Imported | Coastal, Koh Kong, Sihanoukville |
| 15 | *Lutjanus malabaricus* | Snapper | FC | WS, Imported | Coastal, Koh Kong, Sihanoukville |
| 16 | *Penaeus monodon* | Tiger Shrimp | P | WS, Imported | Coastal, Koh Kong, Kampot |
| 17 | *Eucheuma cottonii* | Seaweed | Open water | Imported | Coastal, Kampot |
| ***Exotic species*** | | | | | |
| 1 | *Oreochromis niloticus* | Nile tilapia | P, RF, FC | HS | Whole country |
| 2 | *Hypophthalmichthys molitrix* | Silver carp | P | HS | Whole country |
| 3 | *Cyprinus carpio* | Common carp | P, RF | HS | Whole country |
| 4 | *Aristichtys nobilis* | Bighead carp | P | HS | Whole country |
| 5 | *Ctenopharyngodon idella* | Grass carp | P | HS | Whole country |
| 6 | *Cirrhina mrigal* | Mrigal | P, RF | HS | Whole country |
| 7 | *Clarias* spp. | Hybrid catfish | P | HS | Whole country |
| Note: P: pond; FC: floating cage; RF: rice field; WS: wild seed; HS: hatchery seed | | | | | |

*Barbichthys altus* and climbing perch (*Anabas testudineus*) are also grown.

These systems range from relatively low input systems using on-farm products (rice bran, duckweed, termites, morning glory, pumpkin, etc.) yielding less than 1 kg/m$^2$ (mostly 0.25-0.5 kg/m$^2$) for 6 months of culture (May to October), to systems using rice bran mixed with small-sized fish or fish powder and/or commercial pelleted feed, giving yields from 1.5 to 20 kg/m$^2$ (15-200 t/ha) with high quality feed. Lower input systems tend to be dominated by carps and tilapia; higher input systems are typically carp-dominated polyculture with tilapia, pangasius-dominated polyculture, or pangasius or tilapia monoculture. Ponds may be as small as 100 m$^2$, although high-input smallholder systems are usually 0.1 to 0.2 hectares.

There are currently two significant projects facilitating development of small-scale freshwater pond aquaculture – the USAID "Harvest" project and the JICA-funded FAIEX project. JICA is promoting small-scale hatchery production as a key element in increasing seed supply, encouraging farmer networks and supporting lead farmers. They do not subsidize inputs. The Harvest project is concentrating on extension and farm business development, encouraging farmers to understand the costs and returns associated with different levels of intensification, as well as offering intensive regular extension advice to focus on farmers. They also assist farmers with input purchase on a declining trajectory over six production cycles. The number of ponds reportedly used for fish culture increased from 3,455 (239 ha) in 1993 to 56,234 (962 ha) in 2009-2010, operated by 40,500 farmers.

### ***Freshwater Pond Culture - Small and Medium-Sized Enterprises***

More recently, intensive Pangas (*P. hypophthalmus* and hybrid catfish) farming in ponds has increased significantly, driven in part by expatriate Vietnamese farmers seeking better water quality, biosecurity, lower wages and cheaper feed ingredients (especially "trash" fish). Productivity is very high (several hundred tons/ha/yr) based on intensive feeding with pellets (usually at start and end of production cycle), rice bran, trash fish and a variety of other ingredients. Most farmers mix and prepare their own feed mixture, producing home-made pellets. Low-value fresh fish may be purchased in bulk and stored with rice bran as a semi-fermented product for several months. This type of aquaculture takes place mainly around Phnom Penh and Kandal. In addition, there are now significant areas of nursing ponds – primarily for imported Pangas seed – for example to the north of Phnom Penh.

Pond sizes in intensive culture systems may range from a few hundred square meters to 10,000 m$^2$ (average 2,400 m$^2$), with depth of 2 to 3 meters, and permanent access to a water source. The culture period is 8-12 months for Pangasius and 2.5-3 month cycle for hybrid catfish culture with an average of 3 cycles per year. Some of these Pangas farmers are diversifying to *Clarias* catfish, *Anabas* (climbing perch), sand goby, and a variety of other species such as featherback since the price for pangas is low. Most farmers would like to be able to rear snakehead.

### *Rice-fish Culture*

Rice-fish culture is typically based on stocking rice fields and/or small connecting ponds and channels mainly with fish species such as tilapia, or common carp, *Pangasius* catfish, and silver barb (*Barbonymus gonionotus*). Fish are stocked at a density of 0.03-0.45 individual/$m^2$ in concurrent rice-fish systems with an average area of 0.4 ha, harvesting yield between 100 and 300 kg per hectare. The growth cycle lasts around 4 months.

Development of this type of aquaculture, while highly desirable from a food security perspective, is perhaps the most challenging, since many factors, including intensification of rice production, chemical use, short production cycle, losses to theft, flood and migration may all constrain further growth.

### *Freshwater Prawn and Shrimp Farming*

Traditional extensive shrimp farming is practiced on a small scale in Kampot Province. This relies mainly on natural seed and feed, and productivity is less than 100 kg/ha/year. More intensive shrimp farming began in the 1990s and its production rose to a peak of over 700 tons/year in 1995. Tiger shrimp *Penaeus monodon* and banana shrimp *Penaeus merguensis* were the main cultured species. Unfortunately, shrimp farming suffered from serious disease and collapsed. Production has been less than 100 tons in recent years.

More recently a new medium-scale enterprise has restarted in *Koh Kong* province in ponds previously used for fish culture.

Technology for the production of *Macrobrachium* seed has been developed at two government hatcheries, and there is some limited farming of this species in inland waters, but this has not become a significant activity as yet.

### *Marine Cage Culture*

Marine finfish culture began in the late 80s, early 90s in both Kompot and Koh Kong. Unfortunately this collapsed in 1993 due to freshwater runoff after heavy rain. Marine cage culture restarted in the early 2000s and there is now probably around 1,000 tons production from marine cage culture production in Sihanouk, Kompot and Koh Kong. Species cultured are mainly seabass (60-70%) and grouper. Seeds are sourced from the wild, from Thailand (seabass) or from Indonesia and Taiwan (grouper). Feed is exclusively trash fish sourced locally. This sub-sector is currently suffering from chronic disease problems (up to 50% losses), which seem to be endemic throughout the region.

### *Crocodile Farming*

The farming of the indigenous species, *Crocodylus siamensis* has been undertaken since the early 20$^{th}$ Century. Crocodile farming has increased quite rapidly in recent years, from 4,816 heads in 1993 to 230,000 heads in 2011. This involves mainly production of 30 cm long juveniles that are mainly exported.

### *Seaweed Culture*

*Eucheuma cottonii* was cultured in Kampot province by a Malaysian company in the mid 2000s, with production reaching 18,500 tons in 2005. However, no production of farmed seaweed has been reported since 2006.

### *Other Potential Species for Aquaculture*

In Cambodia, some intensive production of frog and soft-shelled turtle has been found. There have been some initiatives with oysters and green mussels (*Perna viridis*), though it appears that these activities have now ceased due to financial difficulties.

### *Fish Seed Supply*

There are four primary sources of seed (fry or fingerlings) for aquaculture:

- Imports from Viet Nam and Thailand (freshwater); and from Indonesia, Taiwan (marine)
- Fingerlings caught in the wild
- Private sector hatcheries (small and medium scale)
- Government hatcheries

### *Fish Feed Supply*

Cambodia has significant resources of low-value fish from both marine and freshwater sources that can be used directly as fish feed, or converted into fishmeal and mixed with other ingredients to produce home-made or commercial compound feeds. Indeed, the seasonal excess of low-value freshwater fish underpins the long history of intensive aquaculture in Cambodia. While this is strength in many ways, increasing demand from aquaculture is putting pressure on both freshwater and marine resources.

No recent studies of the species composition of "trash" or low-value fish, and its allocation and value for different uses (fresh for human consumption, fresh for aquaculture, pigs, and ducks, fish paste, fish sauce, dried fish, fish meal, exports in all these forms). This is an important strategic issue for both the aquaculture and fisheries sector.

At present, Cambodia has one fishmeal plant that uses a proportion of this excess, as well as low-value marine species. The country also produces significant quantities of other basic feed ingredients including rice bran, broken rice, corn, cassava, and some soy. Traditionally, various vegetables have been used in the absence of higher quality feeds, including *Lemna*, *Azolla*, morning glory and household waste.

As yet, there is no commercial manufacturer of dedicated fish feeds. As a result a large quantity of fish feeds are imported, mainly from Viet Nam and Thailand. Discussions with suppliers suggest this market is currently around 20,000 t/yr, growing at around 10%/year, and supplied mainly by Proconco (Viet Nam), CP (Thailand) and several other smaller producers from Viet Nam.

The lack of Cambodian production of fish feeds is widely regarded as a constraint, and there is a lot of complaints from farmers about feed cost and quality. However, it should be recognized that Cambodia is fortunate in being able to access reasonably priced and well-formulated fish feeds from both Viet Nam and Thailand. While there may be some quality issues, this is probably partly related to unwillingness to pay the necessary (international) price for high quality feed. There are some interest in investing in a feed plant (both foreign and Cambodian), but demand remains low to justify the significant investment required. However, once demand reaches a sufficient level (probably around 50,000 t/yr) it is highly likely that such an investment will be made.

Limited demand for pelleted feeds relates both to the limited scale of aquaculture production, and to the wide availability of trash fish in Cambodia. Stricter control and management of low-value fish fisheries is desirable from the perspective of biodiversity and fishery sustainability; it would also lead to increased demand for pelleted feed which would ultimately justify investment in feed plant in Cambodia sooner rather than later.

### *Fish Diseases*

Disease is a significant problem in aquaculture globally. Epizootic fish disease has been a periodic problem for freshwater fish culture and indeed wild fish, and spread widely across Southeast Asia in the 1980s. Shrimp farming has suffered from chronic viral and bacterial disease problems throughout the region, and this was the primary cause of collapse of the industry in Cambodia in the 1990s.

Marine finfish farming is currently suffering chronic disease problems across the region. While these diseases occur naturally, the severity of outbreak tends to be exacerbated by intensive culture conditions and over concentration of development.

### *Aquaculture system*

In general, the aquaculture system in Cambodia is commonly known and categorized into two types: extensive/semi-intensive system and intensive system. The proportion of intensive aquaculture system contributes about 75% to the total aquaculture production, whereas 25% from extensive/semi-intensive system (Figure 2). The main purpose of extensive aquaculture system is additionally to support family fish consumption. The promotion of small fish culture activities was presented in 1986 by the UNICEF's Family Food Programme and then from 1990s by many NGOs/IOs and Projects: AIT Aquaculture and Aquatic Resources Management (AIT-AARM), MRC, FAO, Partnership for Development in Kampuchea (PADEK), etc. Recently, aquaculture activities that have grown rapidly are those that are supported by JICA, DFID/DANIDA, USAID-HARVEST, Ayuda Intercambio y Desarollo (AIDA), FAO/EU, etc.

### *Aquaculture Production*

Aquaculture in Cambodia has undoubtedly grown in recent years – from less than 14,600 tons in 2002 to 74,000 tons in 2012. The production trends for total aquaculture production are shown in Figure 3. In the last ten years, the contribution of aquaculture activities to total fisheries production has been increasing dramatically. Aquaculture development has become a "national moment", as noted in the speech of the Cambodian Prime Minister in 2008.

### *Economic and social value*

Pangasius and snakehead species dominate the aquaculture sector in terms of gross revenue with more than USD 30 million generated for both species. Surprisingly, marine cages represent more than USD 7 million, while its contribution to the total aquaculture production is only about 1.2%. In terms of employment, it has been estimated that there are some 27,000 people involved in aquaculture in Cambodia.

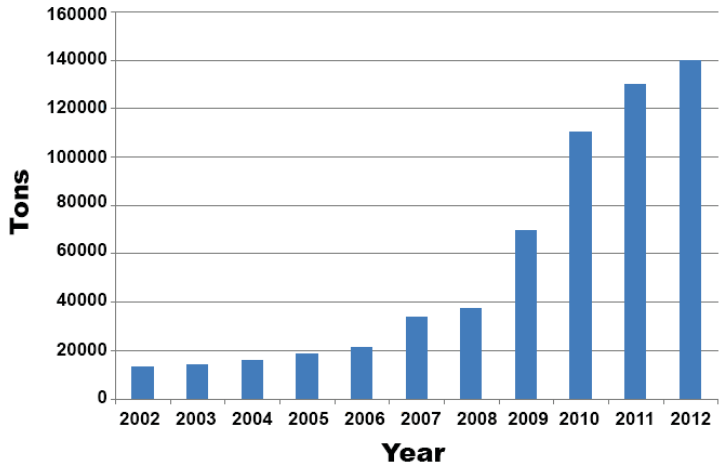


Figure 1. Fish seed production (in thousands) in Cambodia in 2002-2012.

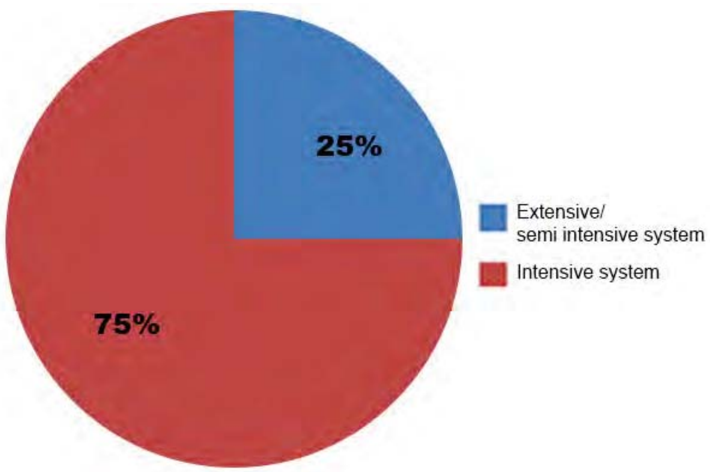


Figure 2. Fish production in pond culture systems (Source: Aquaculture Development Department).

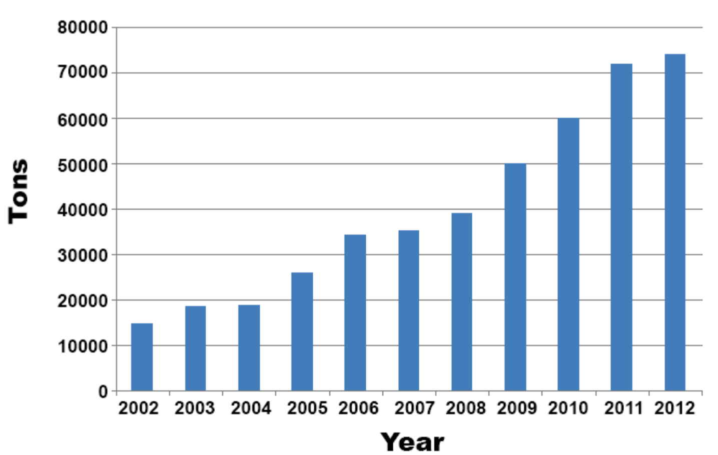


Figure 3. Aquaculture production in Cambodia in 2002-2012.

### *Constraints in Aquaculture Development*

In general, aquaculture activities in Cambodia are mainly on small-scale operations targeting the improvement of nutrition of local people. During the process of fish culture, some constraints were usually reported such as lack of technical assistance, lack of water supply, lack of seed and feed supply, and limited awareness of fish culture technologies among fish farmers. The following key issues are commonly noted in Cambodian aquaculture:

* Problems in cage culture
  - High mortality rates of fishes during summer months, particularly from March-May when the water temperature is high and the water flow is reduced
  - Large amount of waste being discharged, causing deterioration of water quality
  - The seed used for cage culture are collected from the wild and may have significant impact on wild stock
  - For Pangasids and other species, the sharp decline of wild seed supply led to shortage of seed for stocking, while hatchery-produced seed have not been available
  - There is heavy reliance on catching or buying low-value fish/trash fish for feed

* Problems in shrimp culture
  - Unavailability of hatchery-produced shrimp post-larvae
  - Reliance on imported post-larvae from neighboring countries
  - Diseases
  - Lack of special extension programs focusing on shrimp farming

* Problems in pond culture
  - Inadequate water supply is a serious constraint
  - Since most pond water is stagnant, fish kills occur during the summer season
  - Unavailable hatchery-produced seed of high-value culture species.
  - Poor knowledge of farmers about feeds and feeding technology
  - Imported commercial feeds (pellet feed) are expensive
  - There is a heavy reliance on catching or buying trash fish for feed
  - Aquaculture cooperative or society does not function well resulting in a lack of communication to exchange ideas or techniques

* Marine Aquaculture
  - Mariculture is less developed as compared to the neighboring countries
  - Common cultured species: groupers and snappers (wild seed and imported)
  - In 2011, there were about 800 cages.
  - Reliance on wild seed and imports.
  - Unavailability of hatchery-produced seed
  - Unavailability of commercial feeds.
  - Lack of training and extension programs focusing on marine aquaculture
  - Investment for marine aquaculture is quite high (i.e. cage construction, feed cost)

### *Opportunity for Aquaculture Development*

The main points of opportunities for aquaculture development in Cambodia are as follows:

- Remarkable genetic resource in the form of more than 500 freshwater species and a similar number in marine species in the MRC research/breeding programme
- Many potential aquaculture species have high nutritional value
- Many indigenous species have higher price than mainstream aquaculture species
- Region-wide there is an excellent range of high-quality breeds of exotic species in public and private sector
- A wide range of species is suitable for marine and brackishwater aquaculture: filter-feeding shellfish; marine finfish, seaweed. There are also opportunities for the production of tilapia and catfish in brackishwater
- At least 10 species grow well in rice fields and are easy to sell
- Production and consumption of even a small quantity of fish can generate significant benefits and income to the rural poor people in society
- Small-scale fish farmer can reduce costs of inputs, increase price of product, exchange knowledge and experience, and reduce share risk by forming cooperatives, associations and networks
- Small-scale hatchery or nursery production can generate substantial revenue from modest land/water resources – far higher than from rice production
- Successful small-scale fish farmer can expand to medium-scale and large-scale, and land can also be rented
- Aquaculture enterprises at all scales can generate employment for both men and women
- Efficient industrial scale production of easily grown species typically results in widely available low-cost fish, which will benefit the poor, especially in urban areas, etc.

***Conclusions and Recommendations***

Aquaculture in Cambodia plays an important role in contributing to the improvement of the daily livelihood of the rural poor farmer community. Fish is the most important source of animal protein in Cambodian people's daily diet. There is enormous potential in fish culture production from floating cage culture, earthen pond culture and rice-fish culture and other fish culture activities in small water bodies or aquaculture-based fisheries in Cambodia. Moreover, fish does not only play a major role in the daily diet, but also in the employment, economy of the rural poor farmer and improvement of women's role in aquaculture.

Recognizing the potential role of aquaculture in subsistence farming, NGOs and other IOs have been contributing a significant role towards the development of aquaculture and in the management of aquatic resources in Cambodia. The FiA has been taking a number of steps to promote aquaculture in all potential areas in partnership with various NGOs, IOs and other agencies involved with rural development projects. The FiA, in collaboration with diverse NGOs and IOs, have established public sector hatcheries in different provinces and also assisted in establishing private sector hatcheries in many rural areas to produce seed to supply seeds to family-scale fish farming operations, as there is a need for good quality seed available all year round. Aside from the establishment of hatcheries and due to shortage of trained human resource to carry out extension activities, all collaborating NGOs/IOs have assisted in capacity building for the fisheries staff and farmers through short-term training course, supported in producing aquaculture extension materials for distribution to farmers and in some cases research activities were conducted in the fish seed production stations and in the fields. However, there are some constraints facing the development of aquaculture such as lack of capital for pond/cage construction, lack of credit system or access to credit is poor as no subsidy exists, unavailability of good quality seed, seasonal nature of pond, competition for the farm resources from other agricultural operation, and lack of aquaculture extension systems and aquaculture research center. The following recommendations are therefore proposed. The Royal Government of Cambodia through the MAFF, particularly the FiA and other development partners such as the NGOs, IOs and other institutions concerned in aquaculture development should address and support the following activities towards aquaculture development:

- To produce good-quality broodstock in government hatcheries or Center for Aquaculture Research and Development to be distributed to farmer-managed hatcheries or private hatcheries in order to produce good-quality fish seed for aquaculture farming;
- To establish sub-research center for aquaculture development and extension service in all regions that have a good potential for aquaculture development in Cambodia;
- To strengthen capacity of existing fish seed producer farmer networks and establish more farmer hatcheries in all provinces or so-called fish seed production decentralization in Cambodia; and
- To strengthen the existing guideline for good aquaculture practice (GAP) and law/ regulation performance in aquaculture farming and fish marketing.

### *Acknowledgements*

First of all, I would like to thank H.E. Prof. Nao Thuok, PhD (Delegate of the Royal Government of Cambodia, Director General of Fisheries Administration), Dr. Hav Viseth (Director of Aquaculture Development Department) and Mr. Pich Sereywath (Former Deputy-Chief of Aquaculture Division) for providing useful information and data for this report preparation. And also I would like to thank the fish farmers who participated in the interviews and provided information and invaluable feedback on their experiences in fish culture.

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# Status of Resource Enhancement and Sustainable Aquaculture Practices in Japan

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## *Abstract*

Contrary to the rapid increase in the world aquaculture production, fish production in Japan has been decreasing slightly due to the decreasing trend in seafood consumption of Japanese. Aquaculture production is approximately 20% in terms of yield, and 30% in terms of market value, of the country's total fisheries production. In Japan, about 80 species are targeted for release for sea ranching and resource enhancement purposes. The local governments (prefectures) are the main driving force in resource enhancement programs. Chum salmon, *Oncorhynchus keta*, and scallop *Mizuhopecten yessoensisis* are examples of successful resource enhancement in Japan. Japanese flounder, *Paralichthys olivaceus*, and red seabream, *Pagrus major*, represent intensely released fish species in Japan, and around 10% of the total catch of those species are estimated as released fish. The low price of products and increasing costs of production, such as costs of fuel and fish meal, are the major pressing issues in coastal fisheries and aquaculture in Japan. For aquaculture, the guarantee of food safety, minimization of environmental impact, and management of natural stock populations are highly necessary in order to achieve the sustainability of the industry. For resource enhancement, budget constraint is the major issue, and possible impact on natural stocks caused by released fish should also be considered. The Government of Japan (GOJ) is implementing some measures to rectify unstable business practices of aquaculture and to improve production techniques in aquaculture. For resource enhancement, the GOJ encourages cooperation among local governments (prefectures) for seed production and release of certain targeted species in order to reduce the cost and improve the efficiency of stock enhancement. In Japan, traditionally, the purpose for release was mainly sea ranching, namely harvesting all released animals. Nowadays, actual resource enhancement, i.e. the integrated release program including resource management and development of suitable nursery for released fish, is encouraged by the government. The evaluation and counter measures for the negative impact of stocked fish on genetic diversity of the wild population are also implemented. Recently, marked progress was achieved in seed production technologies of two important tropical fish species, namely coral trout, *Plectropomus leopardus*, and humphead wrasse, *Cheilinus undulatus*. These technologies are expected to contribute to the advancement of the aquaculture industry in the South East Asian region.

**Keywords:** resource enhancement, aquaculture practices, Japan, sea ranching, integrated release program

### Introduction

Fisheries and aquaculture production in Japan have been decreasing in recent years (Figures 1 and 2). There are two major reasons for the decrease, namely the decreasing trend of the consumption of seafood by the Japanese and the reduction of fish price. Figure 3 shows changes in average daily consumption of seafood and meat by Japanese; consumption of seafood has been decreasing continuously, while meat consumption is increasing. Japanese who prefer meat to seafood is increasing because meat is easier to cook and eat, lasts longer than seafood and the price is comparable. The decreasing demand for seafood caused a reduction in price. Figure 4 indicates the changes in the price of Japanese flounder, *Paralichthys olivaceus*, and the red seabream, *Pagrus major* at the Tokyo Metropolitan Central Wholesale Market in Japan from 1993 to 2012. Prices are continuously decreasing. Therefore, the situation in fisheries and aquaculture in Japan is very tough these days; Japanese fishermen and aquaculturists have encountered difficulties in their business practices. The low price of products and increasing costs of production, such as costs of fuel and fish meal, have been the major pressing issues in coastal fisheries and aquaculture. In addition, for aquaculture, the guarantee of food safety, minimization of environmental impact, and management of natural stock populations are highly necessary in order to achieve the sustainability of the industry.

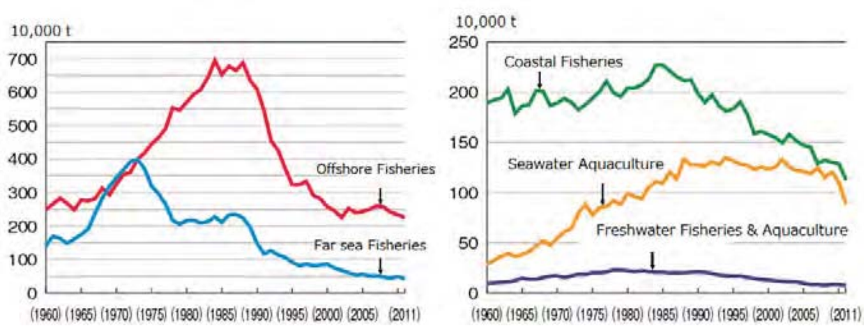


Figure 1. Changes in annual production of fisheries and aquaculture in Japan from 1960 to 2011. Aquaculture production in 2011 was influenced temporarily by the earthquake disaster. Figures are based on Fisheries and Aquaculture Production Statistics (2013), Ministry of Agriculture, Forestry and Fisheries.

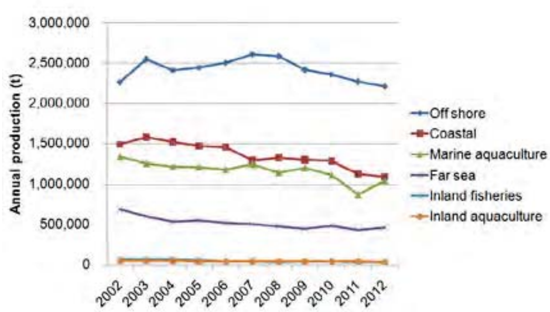


Figure 2. Changes in the annual production of fisheries and aquaculture from 2002 to 2012 in Japan. Marine aquaculture production in 2011 was influenced temporarily by the earthquake disaster. The figure was based on Fisheries and Aquaculture Production Statistics (2013), Ministry of Agriculture, Forestry and Fisheries.

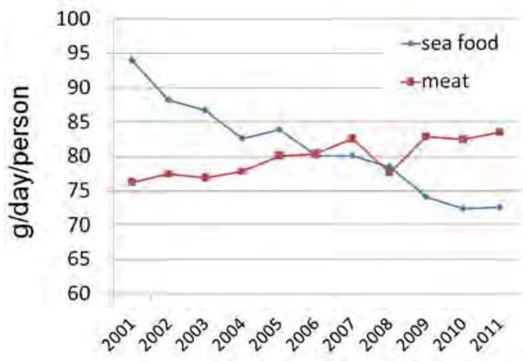


Figure 3. Changes in the consumption of seafood and meat per day per person in Japan from 2001 to 2011. Data was obtained from the Investigation for the Nutrition of Japanese Citizens, Ministry of Health, Labour and Welfare.

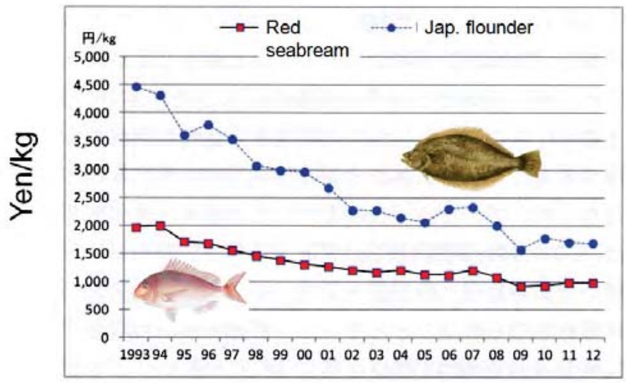


Figure 4. Changes in the annual average price of fresh red seabream (red squares) and Japanese flounder (blue dots) at Tokyo Metropolitan Central Wholesale Market in Japan.

## Aquaculture in Japan

### *Present status*

Contrary to the rapid increase in the world aquaculture production, Japan's aquaculture production has been slightly decreasing these days. Figures 1 and 2 indicate changes in the production of fisheries and aquaculture in recent decade; all productions are decreasing. In Japan marine aquaculture production is much bigger than inland aquaculture and marine aquaculture production is comparable to coastal fisheries. Aquaculture industry has contributed to compensate the decrease in fisheries production in Japan; the aquaculture production accounts for approximately 20% in terms of yield, and 30% in terms of market value, of the country's total fisheries production.

Figure 5 shows both marine and inland aquaculture production of each commodity in Japan in 2012. In marine fish culture, yellow tail is the most important commodity. Actually, two closely related species, namely, yellow tail, *Seriola quinqueradiata* and greater amberjack, *S. dumerili*, are included in this amount of production (160,215 t). The red seabream is the second, followed by coho salmon, *Oncorhynchus kisutsh* and bluefin tuna, *Thunnus orientalis*. In freshwater, eel, *Anguilla japonica*, accounts for half of the production and followed by ayu, *Plecoglossus altivelis*, rainbow trout, *Oncorhynchus mykiss*, and carp, *Cyprinus carpio*, followed.

In shellfish, the scallops, *Mizuhopecten yessoensisis* and the oyster, *Crassostrea gigas*, are the major commodities, and the laver, *Porphyra yezoensis*, dominates the seaweed aquaculture (Figure 6). The prawn culture industry is rather small in Japan and kuruma prawn, *Penaeus (Marsupenaeus) japonicus*, production is the highest (1,596 t in 2012).

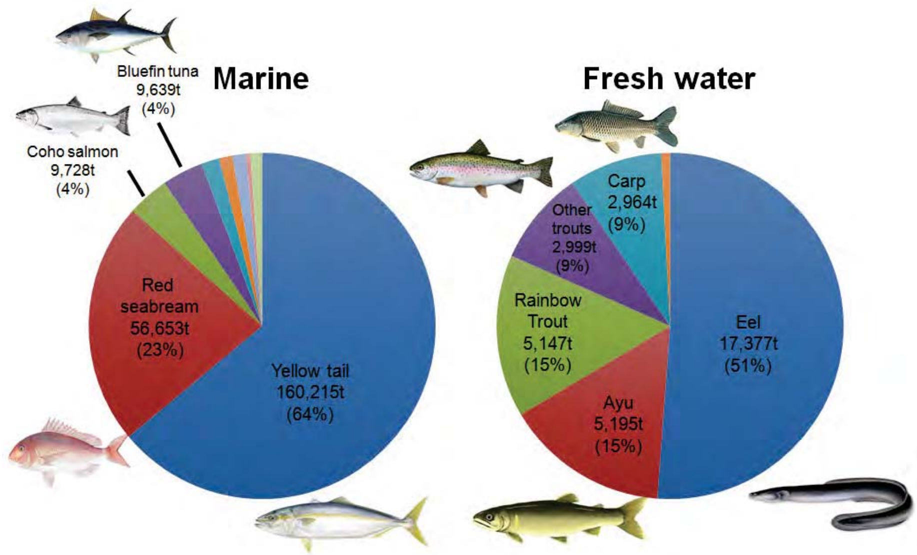


Figure 5. Marine (left) and freshwater (right) finfish aquaculture production in Japan in 2012. Figures were made from Fisheries and Aquaculture Production Statistics (2013), Ministry of Agriculture, Forestry and Fisheries.

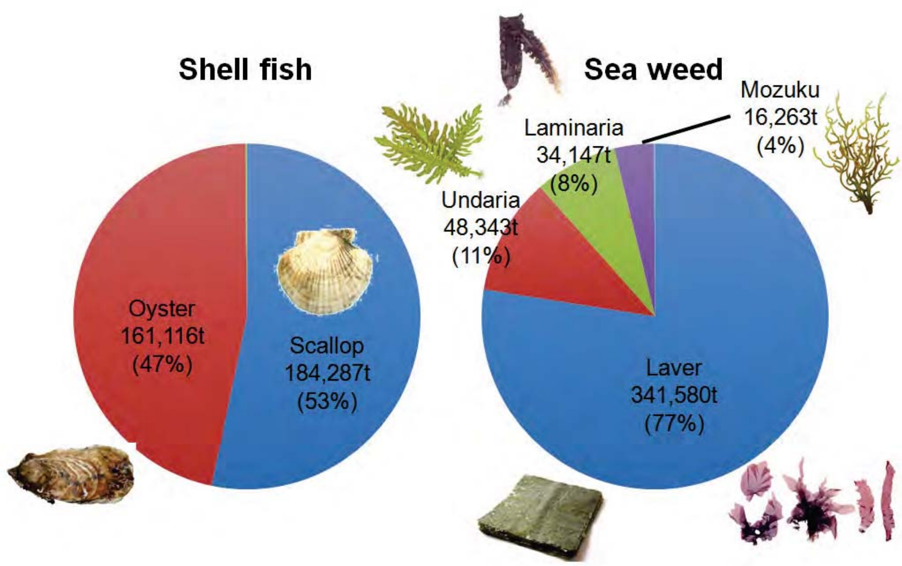


Figure 6. Marine shellfish (left) and seaweed (right) aquaculture production in Japan in 2012. Figures were from Fisheries and Aquaculture Production Statistics (2013), Ministry of Agriculture, Forestry and Fisheries.

### *Pressing issues in Japanese aquaculture*

In Japan, most aquaculture farms are small in size, and their business practices are unstable. There are several reasons for the instability. The prices of aquaculture products are rather low, because of overproduction and/or oligopoly on price determined by the supply chain. High cost of production due to increases in the cost of fish meal, mandated environmental management, dependence of seed on wild populations and/or the risk of disease outbreak are also the issues. Increasing demands by consumers for guaranteed food safety of the products is another issue to be tackled.

### *Possible strategies for aquaculture*

#### *Measures to improve unstable business practices in aquaculture*

There are three major policies implemented by the Government of Japan (GOJ) or Japan Fisheries Agency to improve unstable business practices of aquaculture in Japan. First, the expansion of mutual-aid systems to support business practices of fishermen and aquaculture farmers, including compensation systems for increases in feed and oil costs. Second, promotion of planned production in correlation with supply-demand balance. Japan Fisheries Agency set the guideline for aquaculture production every year, for example the ideal amount of production for yellow tail + greater amberjack and red seabream in 2014 were determined as 140,000 and 72,000 tons, respectively. Third, enhancement of added value to aquaculture products and promotion of exportation are recommended.

#### *Measures to improve production techniques*

Measures to improve production techniques include four aspects: 1) food safety, 2) conservation of the environment, 3) management of natural resources, and 4) new technologies for aquaculture.

##### Food safety

Food safety is an essential issue for the aquaculture industry. Management systems for food safety and information technologies for communication between producers and consumers are necessary. R&D for management systems of product quality assurance, e.g., global Good Aquaculture Practice (GAP) and traceability systems, should be further promoted. R&D for vaccines to prevent infectious diseases and the dissemination of such vaccines are strongly required. Risk management is also important for food safety.

##### Conservation of the environment

To achieve sustainable aquaculture, wastes such as uneaten feed and excretions should be minimized in order to maintain an appropriate environment and prevent red tide from occurring around the culture cages. For feeding-type aquaculture, the drawing up of plans for conserving the environment and using low-emission feeds are strongly recommended. For non-feeding aquaculture such as that of seaweeds and bivalves, monitoring of the coastal environment is necessary in order to prevent harmful environmental changes such as red tide, poor oxygen content, and high temperatures.

## Management of natural resources

Aquaculture activities often impact natural resources. For example, wild juveniles of bluefin tuna, eel or yellow tail are used for aquaculture. Aquaculture feed also depends on the use of natural stocks of sardine and anchovy. Therefore, R&D to produce sufficient quantities of artificial seeds and the development of breeding technology is of high importance. Also, R&D to develop assorted feeds using alternative sources of protein having high quality is necessary.

## New technologies for aquaculture

To reduce the cost and promote productivity, R&D for new aquaculture technologies are implemented. This includes 1) development of feeds with low fish meal content or inclusion level and determination of appropriate feeding amounts, 2) determination of appropriate fish density in order to avoid pathogen infections, 3) development of submersible net cages for offshore aquaculture to minimize environmental emissions, 4) development of breeding technologies to produce fish strains adaptable to various aquaculture conditions, and 5) development of enclosed recirculating aquaculture systems.

## Resource enhancement

### *Present status of resource enhancement in Japan*

In Japan, about 80 species are targeted for release for sea ranching and resource enhancement. Figure 7 shows the top 10 species released for resource enhancement in Japan. The local governments (prefectures) are the main driving force in resource enhancement programs. Chum salmon, *Oncorhynchus keta*, is an example of successful resource enhancement in Japan; around 1.7 billion fry are released and 50-70 million salmons return (recovery rate is 2-3%) every year. Sea ranching of the scallop is another success story, about 3 billion spats are released and 300,000 t scallops are harvested per year. The production of chum salmon (129,000 t) and scallop (302,000 t) accounts for about 40% of the total production from coastal fisheries (1,129,000 t) in Japan in 2011. Japanese flounder and red seabream are the representatives for intensely released commodities; around 15 and 12 million juveniles respectively were released in 2011 (Figure 8). Around 10% of total catch of these species are estimated as released fish.

### *Pressing issues in resource enhancement in Japan*

There are several pressing issues in resource enhancement in Japan. Among them, budget constraint is the biggest one. Due to the long period of economic depression in Japan, most local governments, or prefectures, in Japan have suffered from severe budget constraint, and total budget for resources enhancement programs of all prefectures decreased from 7.5 billion yen in 2002 to 5.1 billion yen in 2012. In addition, budget from the GOJ drastically decreased since 2005.

Formerly, the GOJ had intensely encouraged stock enhancement programs and utilized sufficient amount of the budget for the stock enhancement when the economy was in good condition. However, the GOJ had changed the policy against resource enhancement and the roles of the GOJ in resource enhancement had decreased since 2005, and prefectures are

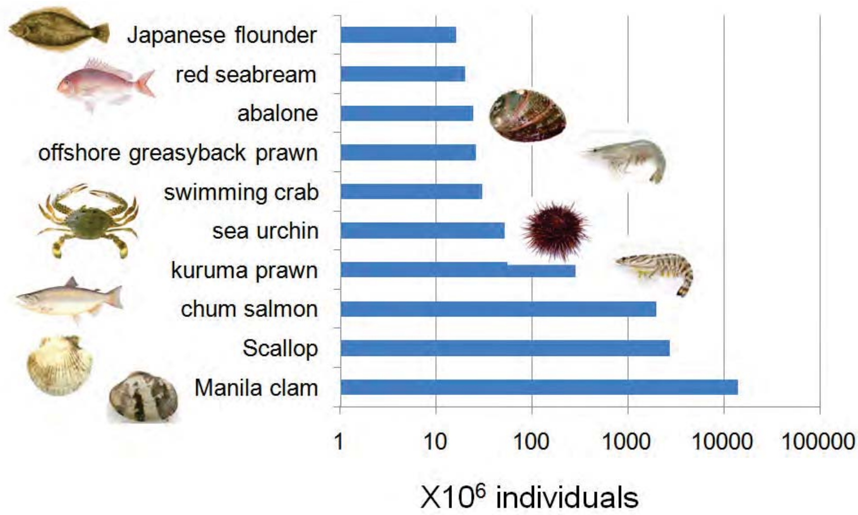


Figure 7. Annual release of top 10 species for resource enhancement in Japan. The numbers are the averages from 1983 to 2000. The figure was based on data in the Achievement of Seed Production and Release of Stock Enhancement in Japan.

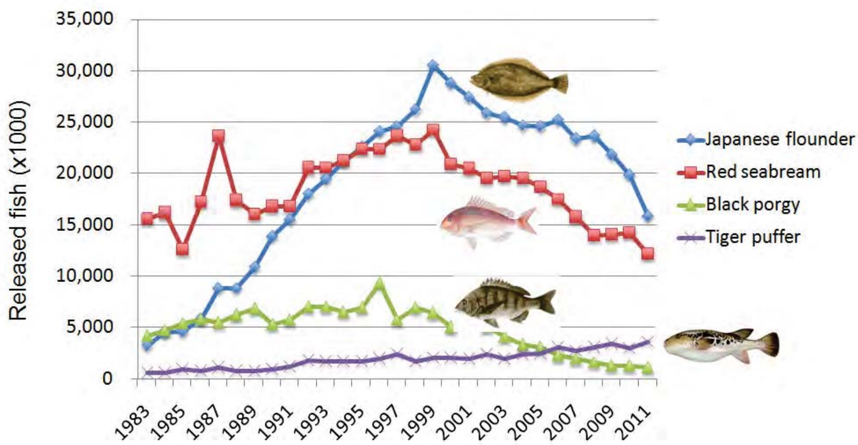


Figure 8. Changes in total numbers of released representative marine fish species aimed for resource enhancement from 1983 to 2011in Japan.

designated as the main driving force for resource enhancement programs. The GOJ allocated the budget for the resource enhancement that was formerly used by GOJ to the prefectures. However, because of the budget constraints in prefectures, they did not use all allocated budget for the resource enhancement activities but divert it to some other purpose. Taken together, the total budget for resources enhancement in Japan had shrunk, and this resulted in the decrease in the number of released fish, which is shown in Figure 8.

Another pressing issue is the impact on natural stocks caused by released fish, in other words reduction of genetic diversities. Several studies have been implemented to clarify the impact (Kitada *et al.,* 2009).

***Possible strategies***

To overcome the decreased budget problem, a strategy for the reduction of the cost for both seed production and release is being implemented in Japan. The GOJ encourages prefectural governments to cooperate with each other in seed production and release for migratory species, such as Japanese flounder and tiger puffer. For this purpose, the "Regional Council for the Promotion of Stock Enhancement" have been established in six coastal areas (Northern Pacific, Northern Japan Sea, Southern Pacific, Mid-west Japan Sea, Seto Inland Sea and Kyusyu) in Japan, which is composed of prefectural government, fisheries cooperatives, public-service corporation for seed production and other stake folders.

In Japan, traditionally, the purpose for release was mainly sea ranching, namely harvesting all released animals. Nowadays, actual resource enhancement, i.e. the integrated release program including resource management and development of suitable nursery for released fish, is encouraged by the GOJ. To mitigate possible negative impact on wild populations, the evaluation and counter measures for the impact of stocked fish on genetic diversity of the wild population is implemented by the Fisheries Research Agency of Japan (FRA) and universities and prefectural research centers. And the GOJ will set guidelines about this issue in the near future.

***Recent progress in the seed production technologies of tropical fish species in Japan***

The FRA recently achieved much progress in the seed production of two commercially important tropical fish species, namely coral trout, *Plectropomus leopardus* and humphead wrasse, *Cheilinus undulatus*.

***Success in mass seed production of coral trout***

Recently, the FRA achieved marked progress in seed production of coral trout (Takebe *et al.*, 2011). The seed production of this species was very difficult and unstable. The key for our success is to provide the ideal environment for rearing larva. Figure 9 and 10 show a horizontal and a lateral views of the rearing tank of coral trout that was developed by Takebe *et al.* (2011). We named this system as "new pump method". In this method, rearing water is circulating within a tank powered by an underwater pump. The pump placed in the center of the tank sucks water, then the water is discharged from the bottom of the tank (Figures 9 and 10). On the other hand, in the conventional method, water was supplied only in one side of the tank (Figure 9). With this new pump method, survival rate until Day 10 has drastically increased (Figure 11). Also, the survival rate until juvenile and total production drastically increased after employing the new pump method (Figure 12).

***Success in seed production of humphead wrasse***

Humphead wrasse, or Napoleon wrasse, is the largest labrid distributed around the coral reefs of the Indo-Pacific (Sadovy *et al.*, 2003). They are known as proterogynous species: all fish initially mature as females, and later change sex to be male. The age at maturity is relatively later than other labrids (Choat *et al.*, 2006), and this is a reason for the difficulty in the conservation of this species.

Humphead wrasse is an important fishery resource, especially in Hong Kong, Mainland China, and Southeast Asian countries. This fish is very popular as luxury species hence the wild resource decreased in the last two decades due to the heavy exploitation (Sadovy *et al.*, 2003). We also have local fishing in Ryukyu Islands, Okinawa, Japan with spear fishing, but this fish is not expensive in Okinawa, and mostly consumed locally or transferred to some luxurious Chinese restaurants in Chinatown in Yokohama.

Efforts to manage this resource has been made since this species was listed in CITES Appendix II in 2004. After that, international trade has been limited, or some countries in Southeast Asia or Oceania banned the fishing of this species.

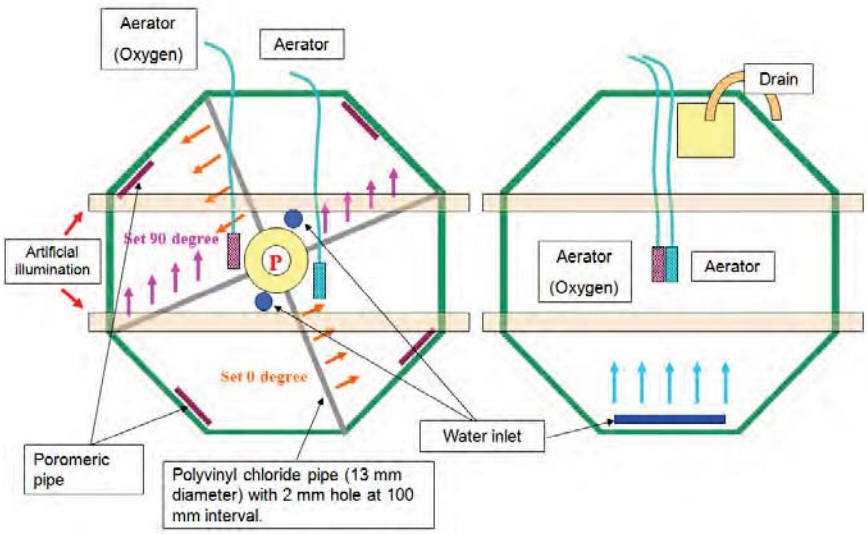


Figure 9. Horizontal view of the conventional (right) and new pump method (left) rearing 60 kL tanks for coral trout, *Plectropomus leopardus*, at Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Fisheries Research Agency of Japan (redrawn after Takebe *et al.*, 2011). The arrows indicate the directions of the discharged rearing water.

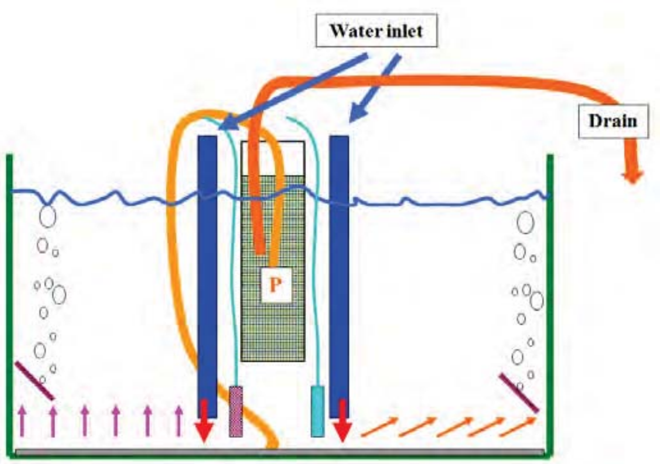


Figure 10. Lateral view of 60 kL rearing tanks with the new pump method for coral trout, *Plectropomus leopardus*, at Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Fisheries Research Agency of Japan (redrawn after Takebe *et al.*, 2011). The arrows indicate the directions of the discharged rearing water.

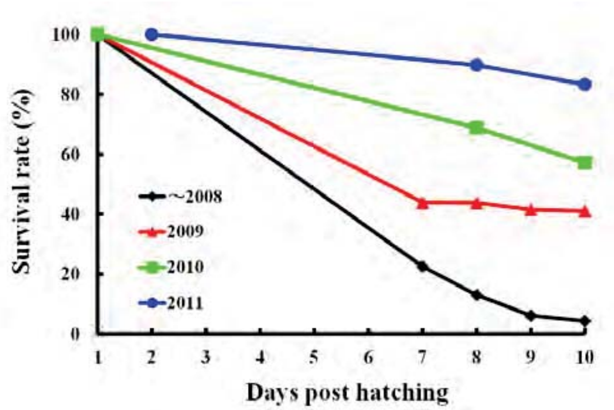


Figure 11. Changes in the survival rate with conventional (until 2008) or new pump method (from 2009 to 2011) until 10 day post hatching. Black line indicates survival rate with conventional method, and other colors indicate the result with new pump method.

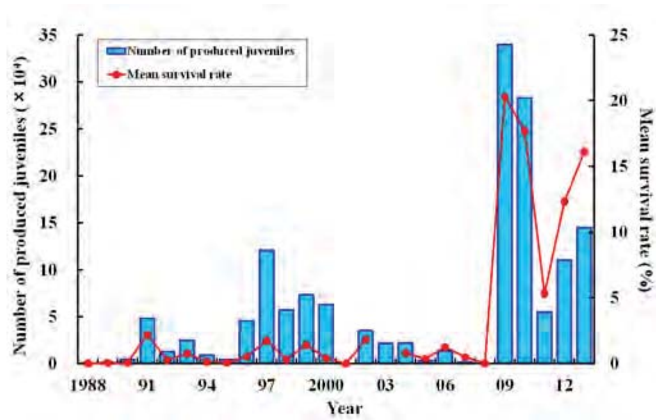


Figure 12. Change in mean survival rate (red dots and lines) and total number of coral trout juveniles (blue bars) produced annually in the Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Fisheries Research Agency of Japan.

On the other hand, the R&D on broodstock management and seed production for sustainable aquaculture of this species has been tried. However, the artificial seed production of this species is very difficult, and there was only one success in seed production recorded in Indonesia in 2003 (Slamet and Hutapea, 2005).

### *R&D for induced spawning*

We collected wild humphead wrasse in Yaeyama islands in Okinawa and reared them in indoor octagonal 60kL tanks (maximum diagonal 5.8 m, depth 2.5 m) at the Yaeyama Laboratory, Research Center for Subtropical Fisheries, Seikai National Research Institute, FRA. We found that humphead wrasses spawn spontaneously in the tanks from June to September when water temperature exceeds 28°C in Ishigaki island (Hirai *et al.,* 2012). They spawn mainly at the onset of the new moon (one week before and one week after new moon). However, fertilization rate was less than 25%. Therefore, some intervention to induce spawning was required. By observing the behavior of the broodstock, we found that the male chased females when the water level was low, and that mating behavior could be induced when the water level is reduced. After each mating episode, we always obtained the fertilized eggs. There has been no failure for fertilization with this method in the three years of research. Thus, draining water in the rearing tank to a low level can induce mating behavior and fertilization of the humphead wrasse.

### *Study for larval rearing*

Eggs of the humphead wrasse are rather small (egg diameter: 620-660 µm) and the larva hatch out in very short hours (16 hours at 28°C) and they are very small (total length is 2 mm). Accordingly, the mouth is also very small: the diameter is 154 µm and the width is 133 µm at first feeding. Thus, it was predicted that they could not eat the rotifers, *Brachionus rotundiformis* that are usually used as the first feed for most fish larva. Therefore, we tried to feed more minute rotifer *Proales similis* collected from Ishigaki island (Figure 13). This rotifer is smaller than SS type rotifer, the body mass is only 10% of SS-type rotifer. This proalid was tested in seven band grouper and larvae were found to feed on this monogonant (Wullur *et al.,* 2011). Moreover, in previous study (Hirai *et al.,* 2012), this proalid can be enriched by fatty acids, so it was considered to be a suitable candidate for initial feed for humphead wrasse larva.

The rearing conditions for humphead wrasse was studied (Hirai *et al.,* 2012), and the study revealed that the addition of oil prevents death on the surface (Figure 14) and that too much aeration caused the mortality of this species (Figure 15). After this study, using the improved rearing method and *P. similis* as initial live food, 22 and 537 juveniles of humphead wrasse were produced in August (survival rate 0.25%, mean TL: 9.0 mm), and September (survival rate 10.7%, mean TL: 9.1 mm) in 2011, respectively. The dietary sequence to raise juveniles was *P. similis* from 2-11 day post hatch (DPH), SS-type rotifers from 6-29 DPH, and S-type rotifers from 28-50 DPH. Humphead wrasse juveniles accepted *Artemia nauplii* after 50 DPH.

Figure 13. A photograph showing a *Proales similis* collected from Ishigaki island, Okinawa, Japan. Scale bar = 50 µm.

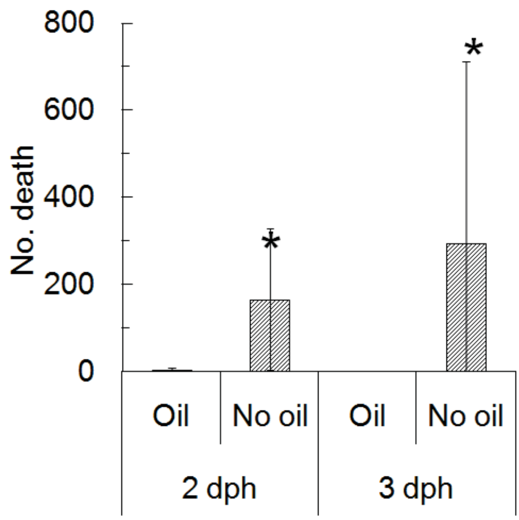


Figure 14. Effect of addition of oil to the rearing tank of humphead wrasse on the surface death observed at 2 and 3 days post hatch (redrawn after Hirai *et al.*, 2012). Values are means ± standard deviation. Asterisks indicate statistical differences ($p<0.05$) between oil and no oil groups.

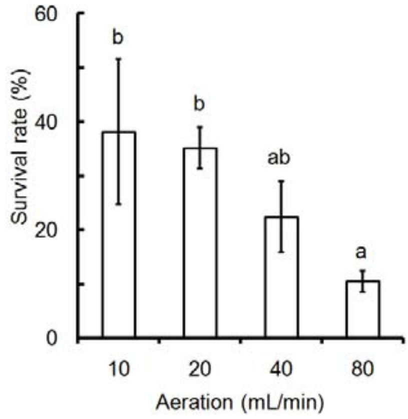


Figure 15. Effect of aeration on the survival rate at 4 days post hatch of humphead wrasse (redrawn after Hirai *et al.*, 2012). Values are means ± standard deviation. Values are statistically different ($p<0.05$) if there is no common.

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### *Suggested Readings*

Hirai N, Koiso M, Teruya K, Kobayashi M, Takebe T, Sato T, Okuzawa K and Hagiwara A. 2013. Success of seed production of humphead wrasse *Cheilinus undulatus* with improvement of spawning induction, feeding, and rearing conditions. In: Rust M, Olin P, Bagwill A and Fujitani M (eds). Hatchery Technology for High Quality Juvenile Production: Proceedings of The 40th U.S.-Japan Aquaculture Panel Symposium. Honolulu, Hawaii, October 22-23, 2012, U.S. Dept. Commerce, NOAA Tech. Memo. NMFS-F/SPO-136.

# Status of Resource Management and Aquaculture in Malaysia

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***Abstract***

Malaysia is a maritime nation and its fishing industry is a source of income for 134,000 fishermen. In 2012, the fisheries sector produced 1.7 million tons of fish valued at RM10.8 billion and generated trade worth RM6 billion. The landings from capture fisheries are expected to increase from 1.32 million tons in 2010 to 1.76 million tons in 2020 at an annual growth rate of 2.9%. In 2012, 65% of total catch was contributed by the coastal fisheries as compared to 35% from deep sea fishing. Landing from deep sea fishing is expected to rise from 381,000 tons in 2012 to 620,000 tons in 2020. Deep sea fishing has been identified for its potential to contribute to the increase in the country's fish production. With a growing population and an increasing preference for fish as a healthy source of animal protein, the National Agro-food Policy (2011-2020) estimated that the annual demand for fish will increase to 1.93 million tons by the year 2020. The Department of Fisheries (DOF) has developed the Capture Fisheries Strategic Management Plan (2011-2020) based on three main documents i.e.; National Agro-food Policy (NAP, 2011-2020), Department of Fisheries Strategic Management Plan (2011-2020), and Malaysia National Plan of Action on Sustainable Fisheries for Food Security towards 2020.

Aquaculture is now being promoted in Malaysia as an important engine of growth and eventually to become the mainstay of the nation's economy. Situated in a region with abundant supply of land and water, two determinant factors for aquaculture activities, Malaysia has always strived to ensure that this sector is not sidelined in their development efforts. With a growing population and an increasing preference for fish as a healthy source of animal protein, it has been estimated that the annual demand for fish will increase to 1.7 million tons in 2011 and further to 1.93 million tons by 2020. From the present annual aquaculture production of about 525,000 tons, this output would need to be raised to 790,000 tons to meet the projected demand by 2020. In a move to develop the aquaculture industry, the DOF, has initiated the Aquaculture Industrial Zone (AIZ) Program involving the development of 49 zones, located across Malaysia, which will be used for culture of various types of high value aquatic species. The DOF has identified several strategic areas that would be developed for downstream activities such as fish seed production, feed mills, fish processing plants, and other supporting industries. Aquaculture is also currently listed amongst the 16 Agro-food's Entry Point Projects (EPP) of the National Key Economic Area (NKEA). The government aims to double the Agro-food sector's contribution to Gross National Income (GNI) from Malaysian Ringgit (RM) 20.2 billion in the year 2010 to RM49.1 billion by 2020, or an increase of RM28.9 billion.

**Keywords** : Malaysia, resource, aquaculture

## Country Profile

Malaysia is a federal constitutional monarchy in Southeast Asia. It consists of thirteen states and three federal territories and has a total landmass of 329,847 sq km separated by the South China Sea into two similarly sized regions, Peninsular Malaysia and Malaysian Borneo. Land borders are shared with Thailand, Indonesia, and Brunei, and maritime borders exist with Singapore, Viet Nam, and the Philippines. From the total land area, 1,200 sq km or 0.37% is made up of water such as lakes, rivers, or other inland waters. Malaysia has a total coastline of 4,675 km, whereby Peninsular Malaysia has 2,068 km, while East Malaysia has 2,607 km of coastline. The capital city is Kuala Lumpur, while Putrajaya is the seat of the federal government. In 2012 the population was 28.6 million, with 22.6 million living in the Peninsula. Since independence, Malaysia has had one of the best economic records in Asia, with GDP growing on average at 6.5% for almost 50 years. The economy has traditionally been fueled by its natural resources, but is expanding in the sectors of science, tourism, commerce and medical tourism.


Figure 1. Map of Malaysia.

## Overview of the Fish Industry

### Trend in Fish Demand

Malaysia is surrounded by sea and blessed with rivers and lakes. These fundamental ecosystems provide natural sources of fish and other aquatic resources for its inhabitants. Hence, fish has been in the daily diet of Malaysians and a main protein source. Until now the trend does not indicate much change despite the availability of many other animal protein sources at competitive prices. There is indeed a preference for fish among Malaysians and it seems it has no replacement. This is well indicated in the household spending on fish and consumption pattern. An average family spends about 20 percent of their food expenditure on fish. Fish consumption index increased from 53.1 kg in 2011 and is expected to be 61.1 kg in 2020. This has put Malaysia among the highest consumers of fish in the world. Given this situation and along with an increasing population, the supply of fish indeed needs to be outsourced. Current records indicate that domestic landing supplies only about 85% of the demand for its 28.9 million population plus another 2 to 4 million foreign workers in the country.

### *Fish Resources*

Fish landed in Malaysia mainly comes from the sea. Freshwater fish at the moment comprises only less than 5 percent of the total landed volume (DoF, 2012) while catch from the sea in 2012 contributed about 1.6 to 1.8 million metric ton annually. The pattern will not improve further as most of the catch are from coastal zone which indicated declining trend. At this stage, the government encourages and provides incentives for deep-sea fishing venture and emphasizes the need to expand aquaculture activities.

Fish resources in Malaysian waters do not only provide food supply to its population but small percentages are being exported for income and foreign exchange. The fish species for export consisted mainly of high-value fish such as grouper, snapper, shrimp and few species of molluscs. At present, exports brought in significant income. In fact, the return always indicate surplus even after taking into account the expenditures to import other fish species to supplement domestic needs. Fish commodity indeed continues to cushion Malaysian deficit in agricultural food product for some time and also during global economic crisis such as in 1997.

### *Fisheries Profile*

In 2012, the fisheries sector comprised of food fish and non-food fish contributed RM11,440 million to the economy. The food fish sector which is comprised of the marine capture fisheries, inland fisheries and aquaculture (excluding seaweed) produced 1,780,168 tonnes with a value of RM10,598 million. For the non-food fish sector, seaweeds, ornamental fish and aquatic plants contributed RM843 million. Fish production from the fisheries sector contributed 1.1% to the GDP in 2012.

The marine capture fisheries sub-sector which is comprised of inshore and deep-sea fisheries is still the major contributor, producing 1,472,240 tonnes (82.70%), valued at RM7,982 million (73.98%). On the other hand, aquaculture sub-sector produced 302,886 tonnes (17.01%) of fish valued at RM2,599 million (24.15%). The total contribution from both sub-sectors have exceeded the target of 1,356,600 tonnes and RM6,805 million set under the National Agro-Food Policy for 2012.

In the non-food fish sector, ornamental fish sub-sector is the major contributor with a value of RM632 million (74.94%), followed by seaweed at RM 199 million (23.61%) and aquatic plants at RM12.3 million (1.45%). The total workforce of the fisheries sector consisted of 136,514 fishermen working on licensed fishing vessels and 29,494 fish culturists engaged in various aquaculture systems.

Table 1. Production and Value of the Fisheries Sector, Malaysia, 2012.

| Fisheries Sector | | 2012 | |
|---|---|---|---|
| | | Quantity Tonnes | Value (RM Million) |
| Food Fish | Marine Capture Fisheries | 1,472,240 | 7,981.66 |
| | Inshore | 1,136,182 | 6,310.05 |
| | Deep Sea | 336,057 | 1,671.61 |
| | Aquaculture | 302,886.32 | 2,559.17 |
| | Freshwater | 163,756.81 | 992.39 |
| | Brackishwater | 139,129.51 | 1,566.78 |
| | Public Water Bodies | 5,041.90 | 56.78 |
| | Total Food Fish | 1,780,168.22 | 10,597.60 |
| Non-Food Fish | Seaweed | 331,490.06 | 198.94 |
| | Ornamental Fish | 376,679,177 | 631.51 |
| | Aquatic Plants | 114,453,668 | 12.26 |
| | Total Non-Food Fish | na | 842.71 |
| **Grand Total** | | **na** | **11,440.31** |

***Aquaculture Production***

In 2012, a total of 29,494 fish farmers and culturists were involved in the aquaculture industry. The majority (77.23%) of the 22,779 workforce were involved in the freshwater aquaculture sub-sector. The remaining 22.77% or 6,715 fish farmers/culturists were involved in the brackishwater aquaculture industry.

In 2012, freshwater aquaculture contributed 163,757 tonnes valued at RM992 million. The main cultured species were freshwater catfish (*Clarias* sp.), black and red tilapia (*Oreochromis* spp.), riverine catfish (*Pangasius* sp.), and freshwater giant prawn (*Macrobrachium rosenbergii*). Brackishwater aquaculture production in 2012 contributed 139,129.51 tonnes valued at RM1,566.78 million. The main cultured species were marine prawns (*P. monodon* and *P. vannamei*), cockles (A*nadara granosa*), marine finfish, mussels (*Perna viridis*), other crustaceans and other species.

The seaweed (wet weight) production in 2012 was 331,490 tonnes valued at RM198.94 million. The total area for seaweed culture is 12,897 hectares. The production of ornamental fish in 2012 was 376,679,177 pieces valued at RM550.41 million.

In 2012, a total of 9,658 million pieces of freshwater and brackishwater fish hatchlings/fries were produced by government and private hatcheries. Meanwhile, the total production of brackishwater and freshwater prawn nauplii/fries produced by government and private hatcheries was 12,518 million pieces.

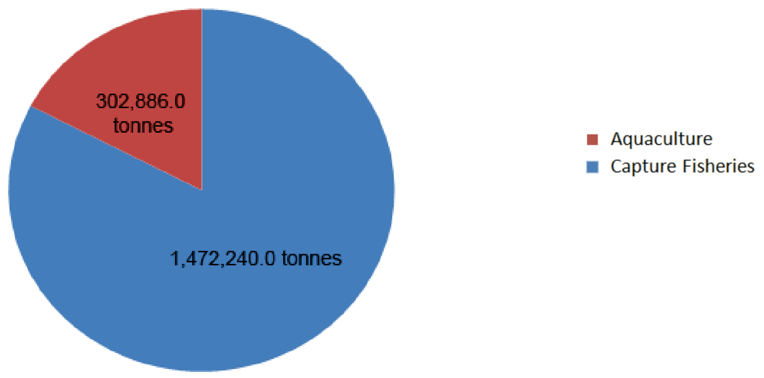


Figure 2. Production of food fish in Malaysia in 2012.

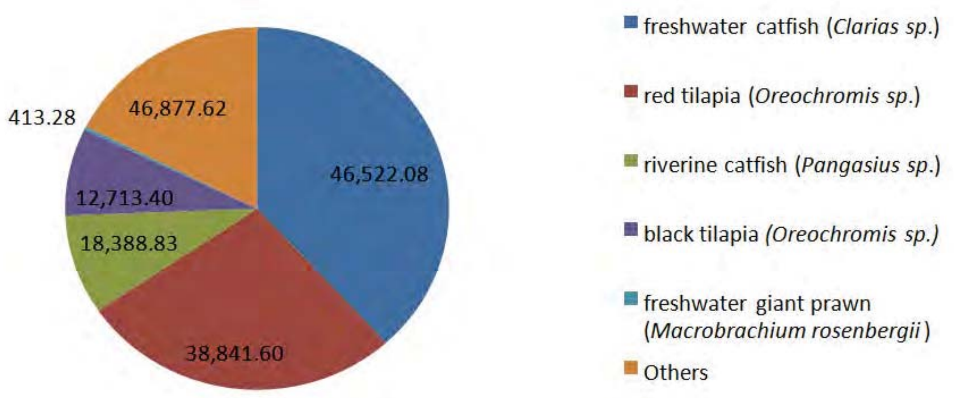


Figure 3. Production of freshwater species (tonnes) in Malaysia in 2012.

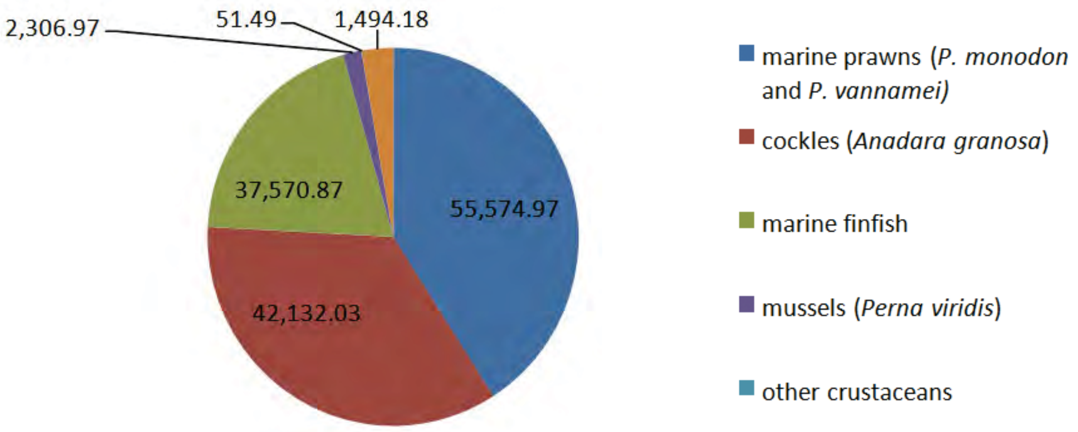


Figure 4. Production of brackishwater species (tonnes) in Malaysia in 2012.

## Status of Aquaculture Development

### Production System

The aquaculture production system in Malaysia has recently undergone moderate transformation. The industry adapted the traditional and conventional systems to meet the requirements of the bio-secure indoor recirculating aquaculture system (RAS) to cater to modern needs and for productivity improvement.

Within the freshwater environment, there are six common categories of production systems employed. These are ponds, used-mining pools, tanks, cages and pen culture systems (Table 2). The pond system is traditional and still the preferred system used to produce aquaculture commodities in freshwater. The next preferred system is the floating cages. The modern polyethylene cages are getting popular in many aquaculture operations in lake environment. The other common production system is tank, mainly made of cement, followed by canvas and polyethylene materials. The production from this sector has gained impact mainly in catfish production.

In the brackishwater/marine environment the production systems employed are ponds, cages, and raft system for mussel and oyster, bottom culture for cockle and long line for seaweed. One good aspect of pond operation in brackishwater environment is quick to respond to innovation and changes. The common system that is used next to ponds are floating cages and rafts. Floating cages as well has undergone kind of transformation. Poly cages material is now commonly found in cage farming areas. Fish are mainly produced in cages. Tank system is fast getting popular for indoor fish production. Imported modules and system from overseas and varieties of modules introduced by locals are being used extensively now. Raft is another system applied in marine aquaculture production. It is used for mussel and oyster production. Another system employed is the seaweed long line intended for propagation and cultivation.

Table 2. Aquaculture production systems in Malaysia (2012).

| Brackishwater | Area | Culturists (No.) | Freshwater | Area | Culturists (No.) |
|---|---|---|---|---|---|
| Ponds (ha) | 7,525.43 | 1,174 | Ponds (ha) | 5,642.31 | 18,875 |
| Cages ($m^2$x1000) | 2,374.8 | 1,984 | Cages ($m^2$ x 1000) | 404.0 | 1,357 |
| Bottom culture (ha) | 10,740.2 | 1,004 | Ex-mining pools | 1,794.34 | 211 |
| Raft culture ($m^2$ x 1000) | 65.4 | 793 | Tanks | 430.5 | 1,485 |
| Long line (ha) | 1259 | 12,896.8 | Pen culture | 28.36 | 205 |

## *Current Development in Aquaculture Production and Technology*

### *Commodity of Interest*

Similar with many countries in the region, aquaculture in Malaysia serves to provide supplementary fish for national food security and production of high value fish for foreign exchange earnings. For freshwater or inland aquaculture, the priority species are tilapia, catfish, clarias and carps. For coastal and marine aquaculture, the priority species are giant seabass, grouper, snapper, shrimp/crustaceans (*Penaeus vannamei* and *P. monodon*), bivalves (cockle, mussel, oyster) and seaweed.

Aquaculture food production in the country continues to show an increasing trend. Again, the trend (Figure 5) highlighted the freshwater sector as a major contributor. This is expected following incentives and encouragement by the government towards production for export market, monetary gain and food security. Based on these production trends and guided by improved technology and disease management control, Malaysia will be able to produce more fish through aquaculture within the next few years.

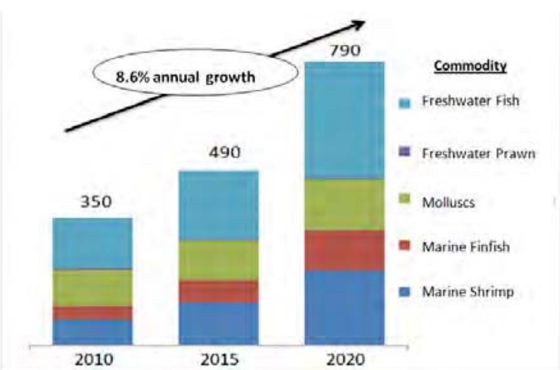


Figure 5. Aquaculture targeted production for 2010-2020 ('000 mt).

## *Fisheries Development Policy*

In conjunction with the 10th Malaysian Plan (2011-2015), various policies were introduced to promote and develop aquaculture. One of the policies is to create a pro-business environment with various specific initiatives such as tax exemption. This will encourage more investment from local and foreign companies. Next is to upgrade the delivery system and support services of government agencies such as basic infrastructure, training and capacity building, marketing and distribution, as well as research and development institutions to facilitate in developing a sustainable aquaculture. The third policy is to identify and zoning of strategic areas or activities for aquaculture and deep sea fishing, downstream processing, halal food hub and agro-biotechnology. With specified zoning, planning for developing a project is easier, faster and more focused. The fourth policy is to ensure food security in the country. Under the National Agro-food Policy (NAP 2011-2020), various steps such as enhancing the production capacity, is taken to reduce import and increase self-sufficiency level. The NAP strategic directions are: 1) ensure adequate food safety and security, 2) increase the contribution of agro-food industry, 3) empower human capital, 4) strengthen R&D activities, innovation and use of technology, 5) create the environment for private sector-led businesses, and 6) strengthen the delivery system.

The three-pronged objectives of the Fisheries Development Policy are: 1) change the mindset of traditional farmers to adopt a commercial approach and operate as business entity that minimize cost and maximize profits to be more viable and competitive in the industry; 2) attract graduates to become modern farmers and remove the stigma that associate agriculture with poverty by highlighting that agriculture is a business venture like any other market-driven businesses; and 3) attract local entrepreneurs and investors to invest and develop the agro-food and downstream processing commercially.

Meanwhile, the following are the strategies to promote private investments: 1) macro planning for sectoral development such as establishment of Aquaculture Industrial Zones; 2) provide infrastructure and common facilities for cluster development projects in rural areas; 3) research and development support; 4) training and human resource development; 5) market access including international promotions by involving and promoting the product in international arena; 6) international and domestic market standards and certification such as the Malaysian Aquaculture Farm Certification Scheme (SPLAM) and Good Aquaculture Practices (GAqP); 7) credit facilities from local bank for industry players to expand their business; and 8) technical support services and good regulatory frameworks for the industry.

## Prospectus of Aquaculture in Malaysia

### Aquaculture Master Plan

In 1997, following the Asian economic turmoil, the Malaysian government saw the important contribution of fish in the national economy. The commodity was never given a focus in any of the country's earlier development plans. Following the outcomes, the commodity was prioritized and set in place in the Third Agricultural Development Plan (NAP3) 1998-2010 (MOA, 1999). This is highlighted in Article 32 of NAP3 master plan (NAP3, 2006). The plan mentions the need to increase production of fish through deep sea fishing and aquaculture.

During the implementation of the ninth Malaysian Plan (RMK9) (2006-2010), the Ministry of Agriculture came out with figures and strategies to increase fish production. In the list, coastal landing is to be maintained at 900,000 metric tonnes, deep sea landing was targeted to land 500,000 metric tonnes and aquaculture to produce 508,000 metric tonnes annually after the year 2010 (Othman, 2008) (Table 3). Aside from food security, the target is set out with the aim to create an income of RM3.4 billion to cushion the deficit in trading of agricultural food-based products. The portion from aquaculture is RM0.32 billion. The strategy aims to strengthen aquaculture production along with the development of the Aquaculture Industry Zone (AIZ) and the Corridor Economic Region Plan.

### Corridor Economic Region (CER) Plan

The CER development strategy aims to expand the economic contribution from selected economic activities where the region has the potential to excel. Accordingly, the key economic sectors identified for promotion are agriculture, manufacturing, tourism and logistic services. The government, through its link companies (GLC), will initiate and promote the development of these sectors. Toward this initiative, GLCs will execute by providing incentives for private sector investment, identifying anchor investor and addressing key enablers that would create a conducive business environment including developing the required human capital, furnishing technologies, and enhancing infrastructure. In the transformation of the agricultural sector, financial incentives will be given to encourage corporations to coordinate the local communities to achieve the optimum level of scale. CER has the vision to become a modern food zone for Malaysia. It will assist the nation to increase its food production. CER will encourage commercial scale farming which finally increase productivity and embark in downstream agriculture activities.

### *The Aquaculture Industrial Zone (AIZ)*

AIZ or Aquaculture Industrial Zone is a zoning programme of identified suitable lands and water bodies to be developed at commercial scales dedicated for aquaculture projects with the purpose of increasing the output of fishes under the NAP3. The programme is part and parcel of the government initiative through the Department of Fisheries Malaysia to develop aquaculture per se into a massive industry in line with overall government vision to transplant agriculture sector to become the third engine of economic growth.

The importance of the formation of AIZ ventures is to address several key problems or issues encountered by the aquaculture sector as a whole, namely, the continuous increasing demand for fish consumption, reduction in supply of fish from catches due to 'overfishing' and to drive the economy in achieving positive balance of trade or surplus of payment of agricultural food based commodity. All of these will need the AIZ so that aquaculture can produce 217,000 metric tonnes valued at RM2.07 billion to supplement existing production from traditional areas. In total, the target set was 508,000 metric tonnes valued at RM 3.3 billion to be achieved annually by 2010. In order to come close to the production target, DOF has allocated 39 sites under AIZ with selected aquatic species to be cultured. These are species which are at present high in demand and high in market value. The 39 sites and projects are listed as high impact projects (HIP). The portion allocated for cultivation of designated species and income expected to be generated following the full scale operation are indicated in Table 4.

The establishment of AIZ and high impact projects is a long-term production plan and involves the set-up of approaches that will finally offer necessary incentives and support. In the beginning it involves the roll-out of specific development strategies on AIZ sites by both the federal and state governments. Upon approval, the land or water bodies are then offered on site to the private sectors.

The establishment of AIZ is one aspect of strategy to realize the government objective towards food security, balance of trade surplus, income earnings and job opportunities (Table 5). Details of the business prospectus are available now at the Department of Fisheries.

Table 3. Projected figures for aquaculture production under the Third Agricultural Development Plan (NAP3).

| Commodity | Production (mt) | (RM million) |
|---|---|---|
| Freshwater fish/prawn | 230,000 | 863 |
| Marine shrimp | 120,000 | 4,500 |
| Marine finfish | 100,000 | 860 |
| Bivalves | 100,000 | 102 |
| TOTAL | 508,000 | 6,325 |

Table 4. Area and figures projected within AIZ, 2009.

| Subject | Shrimp pond | Grouper cage | Seabass cage | Snapper cage | Mussel ropes | Seaweed raft |
|---|---|---|---|---|---|---|
| Area (ha) | 5,428 | 693 | 693 | 693 | 55 | 5,600 |
| Volume (mt) | 52,923 | 92,252 | 208,000 | 183,456 | 1,833 | 153,216 |
| RM' billions | 0.95 | 3.69 | 2.70 | 2.75 | 0.004 | 0.38 |

| Subject | Tilapia cage | Patin cage | Keli tank | Arowana aquarium | Discus aquarium | Goldfish aquarium |
|---|---|---|---|---|---|---|
| Area (ha) | 1561 | 1561 | 1561 | 135 | 135 | 135 |
| Volume (mt) | 699,179 | 1,098,709 | 126,363 | 23 | 27 | 72,720 |
| RM' billions | 3.50 | 3.85 | 0.57 | 0.02 | 0.00011 | 0.02 |

Table 5. Summary of objectives on the development of AIZ and HIP, 2008.

| Item | Objective | Description |
|---|---|---|
| 1. | Contribution to gross domestic product, GDP | Increase the output of fishes and raw material used in processing of fish products |
| 2. | Balance of Trade, BOT | Increase exports of fishes and high-value fish products; and reduce import of low-value fish for consumption and raw materials used in processing of fish products |
| 3. | Private sector involvement | Increase investments from both local and foreign companies |
| 4. | Increasing the income of aquaculture farmers and entrepreneurs | Increase income of aquaculture farmers/ entrepreneurs to a minimum of RM3,000/month and create new business opportunities and employment |
| 5. | Innovation and technology capability | Introduce new technologies suited for the aquaculture industry |
| 6 | Enhancing the value chain | Establish new hatcheries, livestock field, farm food factory, processing factory and effective marketing systems to support the value chain of the aquaculture industry |
| 7 | Efficient aquaculture development | Certification of farms in accordance with SPLAM / SAAB |

### *Factors that Promote the Growth of the Aquaculture Industry in Malaysia*

According to survey conducted after the compilation of the business prospectus for HIP in AIZ (Anon., 2009), the market drivers are as tabulated in Table 6. These drivers likely contribute to the development of aquaculture business in Malaysia from the marketing perspective.

#### *Continuous Government Support*

The government of Malaysia realized the importance of aquaculture as a food security sector and for generating income to balance out the deficit in the agro-food sector. Accordingly, the government mapped out the development of the aquaculture industry in its 9MP (2006-2010) and NAP3 (1998-2010) with the target to spur fish production to 1.93 million tonnes or more than RM9.36 billion in revenue.

#### *Declining Level of Wild Fish Catch*

The pressure on the global demand for fish caused mainly by China and India which are undergoing economic improvement has resulted to severe depletion of landings from wild. Another factor is the contribution of pollution and the impact of climate change; wherein close environments such as lagoons and lakes are more susceptible.

#### *Population Growth*

The demand for food is directly linked with the increase in population and the demand further increase due to the growing affluence in developing countries such as in the Asia-Pacific region where fish is a food of choice. These driving factors will end up with growing pressure to step up production of fish worldwide. Malaysia which still has vast resources to develop should capture this opportunity for future economic gain.

#### *Growing Consumers Health Consciousness*

The move towards consumption of fish for healthier lifestyle is prevalent in developed countries only, but it has become a global concern. In Malaysia, the trend is presented in yearly per capita consumption and fish production. With more scientific findings and consensus that fish is the better food for the future, it will likely play a significant role in driving the growth of aquaculture.

Table 6. Market-driven factors that contribute to aquaculture development in Malaysia.

| Rank | Market Drivers | Impact (years) | | |
|---|---|---|---|---|
| | | 1-2 | 3-4 | 5-7 |
| 1. | Continuous government support | High | High | High |
| 2. | Declining level of wild fish catch | Medium | High | High |
| 3. | Population growth | Medium | Medium | Medium |
| 4. | Growing intra-regional trade | Medium | Medium | Medium |
| 5. | Growing consumer health consciousness | Medium | Medium | Medium |
| 6. | Cultural significance of consuming seafood-marine fish | Medium | Medium | Medium |
| 7. | Innovation in seafood products | Medium | Medium | Medium |

### *Growing Intra-Regional Trade*

Aquaculture in the region in the coming years will not only cater to the traditional market but also supply the domestic and intra-regional needs following the improved standard of living of the population. This will allow bigger aquaculture production.

### *Cultural Significance of Consuming Fish*

Fish consuming countries and ethnicity such as China and other countries in the Asia Pacific region is projected to achieve better economic development. Hence, these countries are expected to be a big market for fish especially for shrimp, grouper and other high-value seafood. Although fisheries and aquaculture are highly developed in these countries, the demand for seafood consistently outstrip supply.

### *Conclusion*

Various policies and plans are established for the development of Malaysia's aquaculture industry. With strategic planning, the Department of Fisheries hopes that the aquaculture industry will be more competitive in the local and international market.

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# Inland Fisheries Resource Enhancement and Conservation Practices in Myanmar

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## *Abstract*

Myanmar has impressive freshwater capture fisheries. Inland freshwater bodies cover 8.1 million ha of which 1.3 million ha are permanent while the rest are seasonally inundated floodplains. There are repeated references to the crucial importance of fish and fish products in the nutrition of the Myanmar people. Over the past few decades, inland fisheries resources have increased pressure from overfishing, use of destructive fishing gear/methods, pollution and environment changes. In order to make a sustainable inland capture fisheries and conservation of aquatic biodiversity as well as nutritional security and improved rural livelihoods, fisheries resource enhancement and conservation measures have long been adopted in Myanmar since 1967, initiated through a seed replenishment program in natural waters, such rivers, lake, dams, even rice fields, etc. However, the institutional, policy, legislative and financial environments under which enhancement and capture fisheries regimes exist are not conducive to the interests of the fishers. Strong tools for valuation of ecosystem goods and services, enabling governance arrangements and estimation of environmental flows are needed. Fishing communities need to be organized into strong co-management/participatory/community regimes in order to ensure that all stakeholders take part in decision-making process and the benefits accrued are shared equitably by all.

**Keywords:** inland fisheries, seed replenishment program, conservation

## *Introduction*

Myanmar is divided into seven major topographical regions, namely; the Northern Hills, Western Hills, Shan Plateau, Central Belt, Lower Myanmar Delta, Rakhine Coastal Region and the Tanintharyi Coastal Strip. Overall, Myanmar possesses a wide range of inland water resources, the major resources being associated with the three river systems, Ayeyarwaddy (2,170 km long), Chindwin (960 km) and Sittaung, and their vast flood plains and deltaic areas. In addition, there are three large natural lakes: Lake Inle in Shan Plateau, Indawgyi in Kachin State and Indaw in Katha with approximate water areas of 15,500 ha, 12,000 ha and 2,850 ha, respectively. Fish is a very important component of the diet of the people of Myanmar, with an estimated consumption of 43 kg/person/year in 2008-2009, which is one of the highest in the region. Fish is consumed fresh and in various processed forms apart from fermented fish, being a staple part of the daily diet of most people.

All inland waters, except reservoirs, are utilized for inland fish production. However, most remains artisanal. Stock enhancement practices of varying forms were employed since 1967 to increase inland fish production, which currently stands at around 899,430 tonnes. The inland waters of Myanmar also possess a high biological diversity, particularly of finfish. For example, the fish fauna of inland natural lakes exhibits a high degree of endemicity, and actions have been launched under the National Fisheries Development Plan and National Resource Management Policy to conserve the biodiversity of inland waters. This review attempts to address the stock enhancement practices in inland waters of Myanmar and the actions taken to conserve biodiversity in the inland waters.

### *Current status of inland fisheries in Myanmar*

In 2012-2013, the total fish production in Myanmar was around 4,716 thousand metric tonnes of which 1,246 thousand metric tonnes is from inland fish and accounting for approximately 26.4% of the total (Table 1). Over the years, the contribution of inland fish production to the total, as in the case of aquaculture, has gradually increased (Table 1) and consequently become an important means of food fish supply to the population. These increases in fish production have been achieved through the introduction of several measures, one of which is stock enhancement and other measures relevant to biodiversity conservation. The main forms of inland fisheries in Myanmar are open water fisheries and leasable fisheries. Inland fisheries are all regulated by provisions in the Freshwater Fisheries Law (1991).

### *Leasable fisheries*

There are currently 3,717 leasable fisheries in Myanmar of which 3,409 are still exploitable and the licenses are issued by Department of Fisheries (DOF), Myanmar in 2012-2013. Leasable fisheries are key fishing grounds on floodplains which are cordoned off by barrage fences and fished using various methods. The peak fishing season involves capturing fishes migrating out of the floodplain as the water level recedes. This is referred to locally as the "Inn" fishery in Myanmar language. The

Table 1. Trends in fisheries production (in x 1000 tonnes) from 2003-2004 to 2012-2013 in Myanmar.

| Year | Total | Culture | Leasable | Open | Marine |
|---|---|---|---|---|---|
| 2003-2004 | 1,986.96 | 400.36 | 122.28 | 331.98 | 1,132.34 |
| 2004-2005 | 2,217.47 | 485.22 | 136.79 | 366.75 | 1,228.71 |
| 2005-2006 | 2,581.78 | 574.99 | 152.69 | 478.43 | 1,375.67 |
| 2006-2007 | 2,859.86 | 616.35 | 170.10 | 548.09 | 1,525.32 |
| 2007-2008 | 3,193.92 | 687.67 | 191.05 | 625.44 | 1,689.76 |
| 2008-2009 | 3,542.19 | 775.25 | 209.72 | 689.71 | 1,867.51 |
| 2009-2010 | 3,921.97 | 858.76 | 237.46 | 764.97 | 2,060.78 |
| 2010-2011 | 4,163.46 | 830.48 | 250.04 | 913.12 | 2,169.82 |
| 2011-2012 | 4,478.21 | 898.96 | 282.64 | 963.82 | 2,332.79 |
| 2012-2013 | 4,716.20 | 929.36 | 290.00 | 1,012.97 | 2,483.87 |

Note that leasable and open fisheries are the main forms of inland fisheries.

leases are auctioned yearly, but DOF has extended the lease period up to nine years to promote improved long-term management (3 years x 3 lease terms). The management systems of leasable fisheries are normally handled by the DOF, mainly through the auctions which are conducted in conjunction with townships and regional authorities.

In this leasable fishery, the lessee has the obligation and the right to exploit all the fish resources, using any form of gear. The lessee is obliged to adopt stock enhancement practices, often provided by the DOF. The peak fishing season is August to October, when the flood waters recede. The production from leasable fisheries have increased, albeit gradually, through the year. Leasable fisheries could vary in intensity, from the management and production view point, some being treated in a manner similar to large fish ponds or small reservoirs, and taking the form of culture-based fisheries. For example, the leasable fishery of Kan Daw Gyi (300 ha; permanent water body in Mandalay Division) has adopted an exclusive stocking (2-3 million fingerlings of major carps per year) and recapture 500 to 600 thousand full grown fish every year, averaging approximately 4,200 kg/ha/yr (FAO-NACA, 2003). In contrast, the leasable fishery of Thaung-Tha-Man (600 ha; in Mandalay township), 60% of the yield is of the exotic Nile tilapia *Oreochromis niloticus* and the rest being of stocked species such as rohu, mrigal, etc., with an overall average yield of 2,800 kg/ha/yr (FAO-NACA, 2003).

### *Open water fisheries*

Open water fisheries in Myanmar refer to all forms of inland fisheries, except the leasable ones and reservoirs. Almost all open water fisheries in inland waters are artisanal, and fishing is often conducted using non-motorized, traditional wooden crafts. The permit or right to fish license is issued by DOF, Myanmar. All fishing gears require a respective implementation license. There is a set fee for most licensees. Some of the larger gears such as "stow net" set in rivers is allocated by tender system. Fees are variable between locations according to the production levels and capacities. License fees for small gears are low. All gear licensees are expected to report the daily catches to DOF. In some of the lakes, such as in Inle Lake, the gears that are used are unique to that body of water; for instance the use of a conical bamboo device surrounded by a moveable and maneuverable small-meshed net is typically used to catch fish by driving it to the bottom and lifting it gradually while closing the net.

### *Social dimensions of inland fisheries in Myanmar*

The great bulk of open water fisheries in Myanmar are artisanal and subjected to a licensing system for use of any form of gear. However, there is an increasing tendency to auction the fishing rights of selected areas of lakes and such open waters, in a manner comparable to that of lease fisheries of flood plain areas. In general, the leasable fisheries, though in existence for over five decades, tend to marginalize the use of the water bodies by the community, as often the more productive areas being leased are held on an almost continuous basis by the richer more powerful segments of the society. This situation will be further exacerbated by the new plans to increase the lease period up to nine years. On the other hand, a long-term lease will induce the leasees to improve the production of the water bodies, adhere to more productive measures of stock enhancement, encourage more people be engaged in day-to-day management, harvesting, marketing and other activities.

### *Biodiversity of inland waters*

The biodiversity aspects of inland waters in Myanmar is best documented with respect of its three large natural lakes, Inle, Indawgyi and Indaw. Perhaps, the best documentation among these being that of Lake Inle. Early studies (Annandale, 1917) reported 23 to 42 species are found in Lake Inle and its inflows and outflows, which included two endemic cyprinid genera, *Inlecypris* and *Sawbwa*. More recent data indicated that there are 36 species of which 16 are endemic to the Lake (Table 2), as well as seven species have been introduced into it. The most extensive survey of the fishes to date in Lake Indawgyi is by Prashad and Mukerji (1929) in which 43 finfish species were recorded. They considered that three of these, *Barbus sewelli* (redescribed as *Puntius orphoides*), *Burbas myitkyinae* (redescribed as *Hypsibarbus myitkyinae* and *Indostomus paradoxus* were endemic to the lake. However, all three of these species have also been found in other localities. A total of 67 species were recorded in the Indawgyi Lake basin when inflowing streams and marshy areas were included. The endemic species found in the lake (after further surveys and taxonomy changes) was the catfish *Aky prashadi*. However, there are several endemics that Prashad and Mukerjin recorded from pools and streams in the Indawgyi lake basin: *Gudusia variegata* (Clupeidae) which is mainly found in rivers in Myanmar, *Esomus altus* (Cyprinidae) and *Salmostoma sladoni* (Cyprinidae).

### *Stock enhancement practices of inland waters in Myanmar*

Stock enhancement of inland waters has been conducted since 1967, some of which are obligatory for certain fisheries. For example, in leasable fisheries, the lessees are obliged to stock seeds as recommended by the government. However, these are often provided by the government, consisting of both suitable indigenous species to augment the natural recruitment and alien species which are fast growing and capable of utilizing the food resources in the leased area. The latter species primarily consist of Indian and Chinese major carps, and in specific instances even tilapia (FAO-NACA, 2003). Stock enhancement of rivers is regularly conducted using mainly rohu, *Labeo rohita*, fingerlings of 7 to 10 cm in length. Such enhancement is conducted on an annual basis, and in certain instances, required fingerlings are provided at a subsidized price to private owners of water bodies. The water bodies where the activities are mostly implemented are the main rivers viz: Ayeyarwaddy, Chindwin and some other river locations. In Kachin State, stock enhancement is mainly conducted in reservoirs and lakes. The fingerling requirements for stock enhancement purposes are produced in 27 government-owned hatcheries spread across the country in different water sheds (Table 3). The fish release program is also linked to a program of replenishment of broodstock of the major cultured species, in particular rohu and mrigal, *Cirrhinus cirrhosus*. In addition, other species are also used for stock enhancement purposes of open waters in Myanmar, these being *Cyprinus carpio*, *Catla catla*, *Ctenopharyngodon idella*, *Hypophthalmicthys molitrix*, *Tilapia*

Table 2. Fish species list of Inle Lake, Myanmar.

| Non-endemics | Endemics | Introduced or Status uncertain |
|---|---|---|
| *Notopterus notoptopterus* | *Cyprinus carpio intha* | *Colisa labiosa* |
| *Clarias batrathus* | *Neolissochilus nigrovittatus* | *Parambassis* sp. |
| *Monopterus cuchia* | *Cirrhinus lu* | *Parambassis lala* |
| *Monopterus albus* | *Physoschistura brunneana* | *Labeo rohita* |
| *Channa striata* | *Physoschistura shanensis* | *Ctenopharyngodon idellus* |
| *Ophicephalus butleri* | *Yunnanilus brevis* | *Glossogobius* sp. |
| *Chaudhuria caudate* | *Sawbwa resplendens* | *Trichogaster pectoralis* |
| *Lepidocephalichthys berdmorei* | *Microrasbora rubescens* | *Clarias garipinus* |
| *Acanthocobitis botia* | *Microrasbora erythromicron* | |
| *Physoschistura rivulicola* | *Barilius auropurpureus* | |
| *Puntius stoliczcanus* | *Danio erythromicron* | |
| *Amphipnous cuchia* | *Inlecypris auropurpurea* | |
| *Lepidocephalus berdmorei* | *Poropuntius schanicus* | |
| | *Poropuntius* sp. | |
| | *Percocypris compressiformis* | |
| | *Gerra gravely* | |
| | *Silurus burmanensis* | |
| | *Channa harcourtbutleri* | |
| | *Macrognathus caudiocellatus* | |
| | *Nemachilus brevis* | |
| | *Nemachilus brunncanus* | |
| | *Discognasthus lamta* | |
| | *Cirrhina latia* | |
| | *Barbus sarana caudimarginatus* | |
| | *Barbus scnanicus* | |
| | *Barbus stedmanensis* | |

spp., *Puntius* spp., *Pangasius hypothalamus*, etc. Overall, large numbers of seeds have been stocked over the years to enhance fisheries of open inland waters in Myanmar (Table 4). In areas where seed is released, such as along the Ayeyarwaddy River and associated floodplains, there is an agreement that fishers should in turn provide to the hatcheries certain number of potential broodstock candidates of major stocked species such as rohu, mrigal, etc., to partially replace poorly performing broodstocks with frequency of replacement ranging from every one to five years

Table 3. Finfish hatcheries in states and divisions in Myanmar and the production of seed stock used for stock enhancement in 2012-2013.

| Location of hatcheries | Numbers | Production (Millions) |
|---|---|---|
| Yangon division | 3 | 1,487.75 |
| Bago division | 3 | 595.49 |
| Mandalay division | 5 | 2,327.25 |
| Ayeyarwaddy division | 5 | 954.14 |
| Magwe division | 2 | 22.82 |
| Kachin state | 2 | 71.00 |
| Sagaing division | 3 | 187.07 |
| Mon state | 1 | 27.98 |
| Shan state | 2 | 19.40 |
| Kayin state | 1 | 6.04 |

depending on the hatcheries (Aung *et al.*, 2010). Often, brood stocks of seven or more years old become less productive and are discarded, and the younger broodstocks are recruited periodically based on this practice. The practices described above, however, have been undertaken without detailed understanding of the genetic structure of the species and the impacts of the practices on wild and cultured stocks remain unknown. This process, a practical experience and welcome strategy, though open to science-based improvement, has avoided inbreeding of stocks and maintenance of genetic diversity to a very large degree (Aung *et al.*, 2010).

***Other enhancement practices***

In addition to stock enhancement through the release of seed stocks there are other measures that are adopted for stock enhancement of inland waters. The main such measure is the implementation of closed seasons. All open fisheries are generally closed during June, July and August to permit spawning and recruitment. However, in a specific geographic area, closure could be different during the above period. This means that a closed season can be enforced in selected areas during spawning periods, through the prohibition of fishing in certain areas. These closed season provisions are enacted under the Fisheries Law of 1991. The Freshwater Fisheries Law also prohibits some types of destructive fishing and activities which may have adverse impacts on fish stocks. Specifically, use of explosives and poisons are banned all together as well as some unspecified methods and equipment. Within a fishing area, it is prohibited to cut undergrowth or light a fire, to alter the natural flow of water or to cause pollution. The Law also states that "No one shall cultivate agricultural crops within the boundary of a fisheries creek."

***Impacts of major enhancement and conservation activities***

Impact assessment studies on stock enhancement have not been undertaken in Myanmar. However, at least so far, there was no evidence of negative impacts on the natural fish populations because of stock

Table 4. The number of seeds stocked (in millions) in different inland waters of Myanmar, 2003-2013 (Source: DOF Myanmar).

| Years | Numbers of seeds stocked | | | | | |
|---|---|---|---|---|---|---|
| | Dams | | Natural rivers and streams | Ponds | Rice-Fish culture | Total |
| | Number of dams | Number of seeds stocked | | | | |
| 2003-2004 | 105 | 1,100 | 600 | 430 | 33 | 2,163 |
| 2004-2005 | 164 | 1,087 | 633 | 598 | 48 | 2,366 |
| 2005-2006 | 218 | 1,178 | 562 | 255 | 62 | 2,057 |
| 2006-2007 | 228 | 860 | 444 | 60 | 65 | 1,429 |
| 2007-2008 | 219 | 906 | 804 | 32 | 70 | 1,812 |
| 2008-2009 | 228 | 1,032 | 917 | 34 | 71 | 2,054 |
| 2009-2010 | 240 | 1,229 | 824 | 25 | 80 | 2,158 |
| 2010-2011 | 249 | 1,880 | 673 | 27 | 81 | 2,661 |
| 2011-2012 | 236 | 1,185 | 1,182 | 228 | 89 | 2,684 |
| 2012-2013 | 197 | 397 | 538 | 204 | 74 | 1,213 |

replenishment programs in the nation. On the positive side, there are indications of higher catch rates by artisanal fishers in the villages near rivers. For instance in Inle Lake, grass carp are released on a regular basis to prevent the spread of aquatic macrophytes, which in turn also serve as feed for grass carp pond culture in the surrounding areas.

***Biodiversity conservation***

Myanmar has been actively engaged in biodiversity conservation practices in inland waters. The leasable fisheries in flood plain areas are productive. In the same manner, these also are crucial to biodiversity conservation as these habitats, being the nursery grounds for maintaining the viable populations of indigenous wild stocks. The government realized the importance of some leasable fisheries to biodiversity conservation and has promulgated protective measures for these fisheries and transformed these areas to fish sanctuaries. Myanmar remains one of the few, if not the only country, in the region that does not have a reservoir fishery. This decision is based on the fact that development of reservoir fisheries will impact the reservoir catchment biodiversity, the catchments being under the jurisdiction of the Ministry of Agriculture and Irrigation. Myanmar also has been active regarding introductions and the movement and use of alien species in fishery and aquaculture activities. For example, there is a complete ban on the use of the African catfish *Clarias gariepinus* including its use in aquaculture and even its sale in popular markets. Areas in selected waters are being designated as conservation areas and the habitats thereof are often improved to provide favorable nursery and spawning grounds for selected indigenous species. In addition specific notifications are enforced for conservation purposes. For example:

### *Notification 2/92*

This notification prohibits the catching or keeping in captivity of spawners, breeders, and fingerlings of freshwater fishes in the months of May, June, July and August without permission of the Director General of DOF.

### *Notification 2/95 and 3/95*

It prohibits the catching, for any purpose, of spawners and fingerlings of the freshwater prawn *Macrobrachium rosenbergii*, and *M. malcolmsonii* in the months of May, June and July, unless permitted by the Director General of DOF, Myanmar. If caught accidentally, these should be released immediately.

### ***Constraints and problems***

The main constraints encountered in stock enhancement programs in Myanmar are the limitations in seed stock availability, and particularly for stocking in remote places which are far from the hatcheries. These constraints are also associated with the cost of transportation and materials needed for effective transportation. In addition, hatcheries may not be able to function at full capacity, particularly when electricity supply is interrupted. The situation with regard to fry and fingerling availability is further exacerbated by the demand of the aquaculture sector, which perhaps at present is witnessing one of the fastest growth rates in the region. Although not a direct constraint, it is important to improve public perceptions on the benefits of stock enhancement and the associated stocking programs, particularly at the implementation or release sites. In this regard, there is a need to educate communities on the long-term advantages of stock enhancement, and the basis of implementation of other strategies such as closed seasons, conservation areas, etc.

### ***Recommendation***

Much technological advancement is needed to place stock enhancement programs in inland waters in Myanmar on a firmer footing. For example, a variety of techniques, ranging from culture to support capture fisheries, to intensive aquaculture can be used to compensate for decline in the productivity of the fisheries due to overfishing, environmental changes or inadequacies in the natural ecosystem (Welcomme and Bartley, 1998) and some of these have to be adopted in Myanmar. Introduction of the new species to exploit underutilized niches of the food chain and to compensate for loss of species due to environmental disturbance is needed.

Equally, there is need for engineering of the environment to improve levels of reproduction, shelter, food resources and vital habitats of the major species in the inland fisheries, as well as eliminate unwanted species that either compete with or predate upon target species. So far, there is no evidence to support that stock enhancement strategies have brought about a reduction in genetic diversity of the wild stocks. There is a need for constant and regular monitoring of this aspect using modern molecular genetic tools. However, it should be noted that the current practices adopted in Myanmar in respect of replenishment of broodstocks, though not conducted strictly on a scientific basis, has been lauded as a good interim strategy which could be improved upon relatively easily with the application of modern scientific tools and approaches (Aung *et al.*, 2010).

There is an urgent need for improvement of operation and impact assessments in relation to stock enhancement in inland waters of Myanmar, which has been lagging behind most countries in the region.

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# The Philippine National Aquasilviculture Program

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## *Abstract*

The Philippine National Aquasilviculture Program (PNAP) is a banner program of the Department of Agriculture (DA) being implemented by the Bureau of Fisheries and Aquatic Resources (BFAR). To implement the PNAP, a Memorandum of Agreement (MOA) was executed between BFAR and the Commission on Higher Education (CHED). The program concept is primarily mangrove resource rehabilitation and livelihood provision to help address climate change, food security and poverty among municipal/artisanal coastal fisherfolks. To achieve its goals and objectives, the BFAR identified three strategic interventions, such as: (1) replanting of destroyed mangrove resources; (2) establishment of community-based multi-species hatcheries (CBMSH), and (3) provision of aquasilviculture livelihood projects to fisherfolk-beneficiaries throughout the country. As envisioned, the BFAR shall provide support funds for the establishment, operation and management of the PNAP while CHED shall provide logistical support during program implementation. The program covers at least 71 state universities and colleges (SUCs) and 61 provinces throughout the country. Potential areas targeted by the PNAP are abandoned, undeveloped and underutilized (AUUs) fishpond lease agreements (FLAs) and the Department of Environment and Natural Resources (DENR) identified areas (*Key Biodiversity Areas, reforestation areas and co-management agreement areas*) from BFAR coastal Regions 1 to 13 and the Autonomous Region of Muslim Mindanao (ARMM). Participating agencies are DA-BFAR Regional Fisheries Offices (RFOs) and Provincial Fisheries Offices (PFOs), CHED (SUCs), DENR Provincial Environment and Natural Resources Offices (PENRO) and Community Environment and Natural Resources Offices (CENRO), and the local government units (LGUs) in the provinces and municipalities. Target beneficiaries for the aquasilviculture livelihood projects are at least 1,000 coastal fisherfolks and for the community-based multi-species hatcheries are 64 SUCs who were signatories to the MOA. For mangrove rehabilitation, the PNAP will involve the coastal fisherfolks in the planting of 100 million propagules for the next 3-4 years. Funding support from BFAR are PhP 6.00 per surviving propagule, PhP 1.2 million per SUC for the establishment and operation of CBMSH and PhP 65,000 per aquasilviculture project. As part of the over-all management strategy, a National Steering Committee (NSC) was formed to formulate policy guidelines of the PNAP while Regional Steering Committees (RSCs) were created to oversee policy implementation in the regions. Program Management Offices (PMOs) were formed to implement and supervise program implementation in the provinces. Community Organizers (COs) were hired in each province to assist in the implementation of daily activities. The approved PNAP implementing guideline details the procedures to follow, both relating to the technical and administrative operations of the program.

**Keywords:** PNAP, mangrove, rehabilitation, aquasilviculture livelihood projects

## Introduction

The Philippines is an archipelago of more than 7,100 islands with a marine habitat hosting one of the world's richest aquatic biodiversity. It has a total land area of 300,782 square kilometers representing only one-seventh of its total territorial water area (*including the Philippines Exclusive Economic Zone, EEZ*) of 2.2 million square kilometers, excluding inland aquatic resources estimated at 496,000 hectares (Figure 1). The Philippine coastline stretches to around 36,000 kilometers (BFAR, 2011).

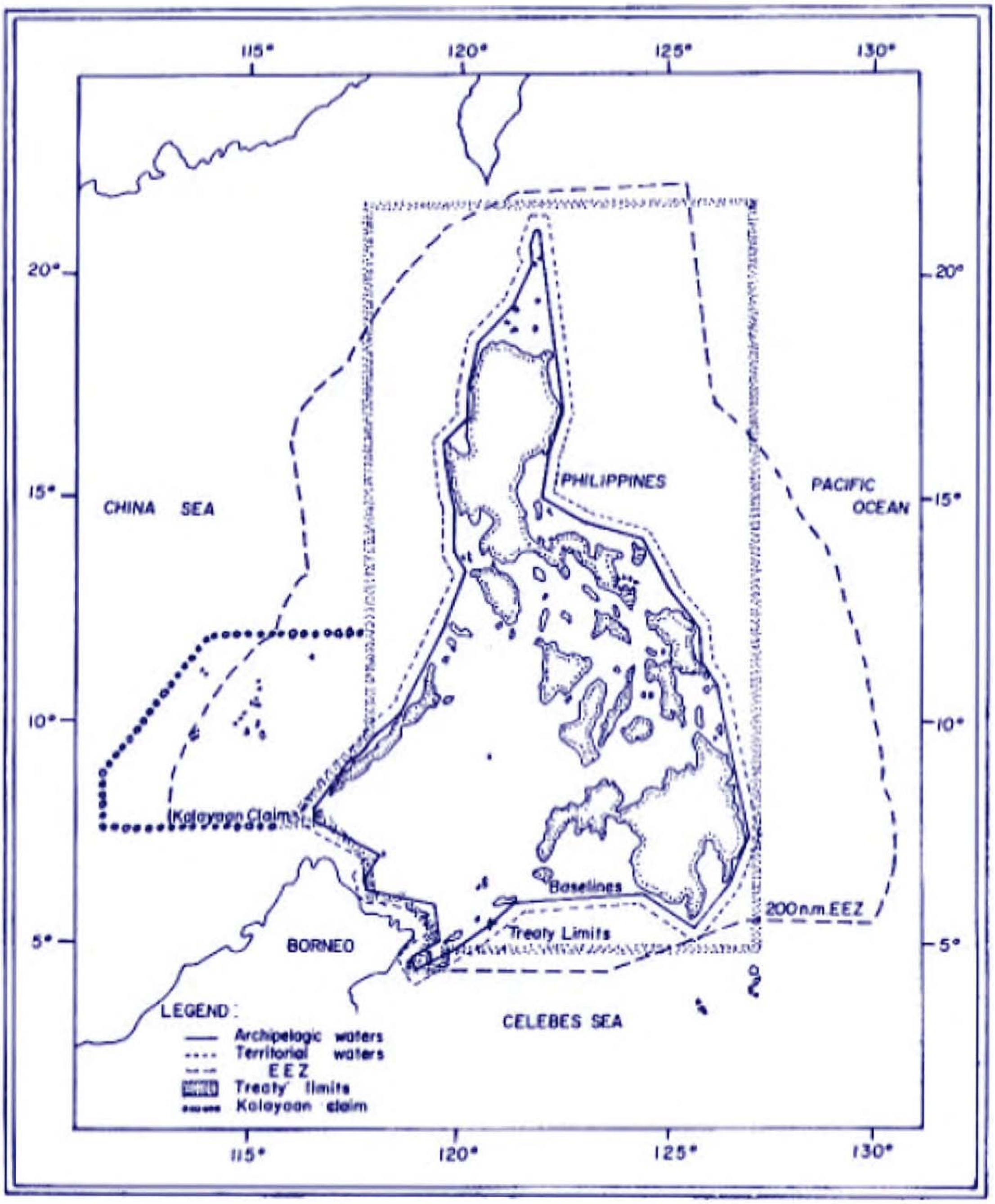


Figure 1. Map of the Philippines showing the limits of archipelagic, territorial waters treaty limits Exclusive Economic Zone (200 N.M. EEZ) and Kalayaan claim.

The Bureau of Fisheries and Aquatic Resources (BFAR) of the Department of Agriculture (DA) is the government agency mandated to ensure the development, management and conservation of the country's fisheries and aquatic resources. It is also committed to (a) contribute in achieving food security for the Filipino people and improve quality of life of fisherfolks through rational and equitable utilization of fisheries and aquatic resources; (b) empower fisheries stakeholders enabling them to adapt to changing environmental conditions and global trade and regional fisheries management regimes; and (c) improve productivity of fisheries and aquaculture within ecological limits. Therefore, one of the strategies to realize these missions is the implementation of the Philippine National Aquasilviculture Program (PNAP), a fishery livelihood and conservation program. The PNAP is a banner program of DA-BFAR jointly undertaken with the Commission on Higher Education (CHED) through a Memorandum of Agreement (MOA) signed on December 16, 2012. The program covers the 15 coastal regions of the country. The participating agencies include the BFAR, with its Regional and Provincial Fishery Offices; CHED, and its participating State Universities and Colleges (SUCs); the Department of Natural Resources (DENR), with its Provincial Environment and Natural Resources Offices (PENRO) and Community Environment and Natural Resources Offices (CENRO); and the Local Government Units (LGU). The fisherfolks are the primary beneficiaries of the resource rehabilitation and protection and aquasilviculture projects while the participating SUCs are the beneficiaries of the community-based multi-species hatcheries.

***The Program***

The concept of PNAP is to come up with self-sufficient fisherfolk families who are advocates of fisheries resource protection through mangrove habitat rehabilitation, promotion of aquasilviculture and the establishment of community-based multi-species hatcheries that will produce fry for restocking in natural waters.

The PNAP has three (3) components, namely: (1) mangrove resource rehabilitation and protection; (2) provision of aquasilviculture livelihood projects and (3) establishment of community-based multi-species hatcheries (CBMSH). The projects are being implemented under the guidance of the BFAR-National Brackishwater Fisheries Technology Center (NBFTC) in Pagbilao, Quezon that serves as the National Program Secretariat, with assistance from resource persons and technical consultants from BFAR, DENR-Forest Management Bureau (FMB) and Protected Areas and Wildlife Bureau (PAWB).

***1. Mangrove resource rehabilitation and protection***

Mangroves are valuable sources of forest products and aquatic resources. Both offshore and inshore fisheries depend on mangroves as natural habitats. Melana and Courtney (2000) reported that parallel with the decline in the mangrove areas of the Philippines is the significant reduction of fishery resources. The loss of mangrove forests in the Philippines is also correlated with decreasing fisheries production in municipal waters and the depletion of larval and juvenile stages of shrimps and milkfish which are seed sources for pond aquaculture (Camacho and Malig 1988 as cited in ADB 1990).

In 1918, Brown and Fischer estimated the mangrove forest to be as much as 400,000 – 500,000 ha. However, the mangrove areas were indiscriminately alienated for other uses such as conversion to fishponds during the 1960s and 1970s, reclamation for residential and industrial development, over-harvesting of mangrove trees for charcoal or fuel wood and urbanization. In 1994-1995, mangrove forest was estimated at 120,000 ha (Primavera and Esteban, 2008). Long and Giri (2011) conducted the latest study on the aerial extent and spatial distribution of Philippines' mangrove forest. They estimated that the total area of mangrove forest of the Philippines was 256,185 ha circa 2000.

The rapid decline of mangrove forest is alarming considering the ill effects that may be brought about by climate change in archipelagic countries, like the Philippines, with little mangrove cover. Thus, restoration of mangrove forest is essential to mitigate or build the country's resiliency to climate change. To achieve this, BFAR has targeted to plant 100 M mangrove trees in 3-4 years to bring back health to its degraded coastal cover. Potential areas targeted by the PNAP are abandoned, undeveloped and underutilized (AUUs) fishpond lease agreements (FLAs) and the DENR identified areas (key biodiversity areas, reforestation areas and co-management agreement areas) from BFAR coastal regions 1 to 13 and ARMM. Participating agencies are DA-BFAR Regional Fisheries Offices (RFOs) and Provincial Fisheries Offices (PFOs), CHED (SUCs), DENR Provincial Environment and Natural Resources Offices (PENRO) and Community Environment and Natural Resources Offices (CENRO), and the Local Government Units (LGUs) in the provinces and municipalities. The coastal fisherfolks will be encouraged to collect, plant and nurture mangrove propagules. As an incentive, a farmer will be paid P 1.50 for every propagule collected, P 2.00 for every propagule planted and P 2.50 for every fully-grown plant. With this management scheme more coastal fisherfolks will participate and will be motivated to nurture and protect each propagule planted.

### *2. Aquasilviculture*

Aquasilviculture is a multi-purpose production system that allows production of fish in a mangrove reforestation project. It is a mangrove-friendly aquaculture technique of producing fish in a watered area enclosed with net but does not allow cutting of mangrove trees. A model of aquasilviculture is showcased at the BFAR-NBFTC Pagbilao, Quezon. The design for the project follows a 70:30 ratio of mangrove to water canal area. This system provides a source of additional income and at the same time increases fish production that is easily adaptable for municipal/artisanal fisherfolks. The fisherfolk-beneficiary who participated in the resource rehabilitation activity shall be the primary beneficiary of the aquasilviculture project. The BFAR shall provide P 65,000 for each aquasilviculture project of fisherfolk beneficiary as input assistance in the form of fry/fingerlings, supplemental feed and nets. Target beneficiaries for aquasilviculture livelihood projects are at least 1,000 coastal fisherfolk.

### *3. Establishment of community-based multi-species hatchery*

The community-based multi-species hatchery (CBMSH) is a facility for spawning gravid fish or crustacean, such as blue crab, caught in the wild to save its offspring that might otherwise be lost due to misuse. The hatchery will produce the fry for stock enhancement and eventually become source of fingerlings and seed stock for aquasilviculture and other aquaculture projects. Moreover, the CBMSH will serve as a working laboratory of fisheries students of the participating State Universities and Colleges. CBMSH may be land-based or holding cages ("lying-in") for gravid, ready to spawn crabs. Funding support from BFAR is P 1.2 million per SUC for the establishment and operation of CBMSH. Target beneficiaries for the CBMSH are 64 SUCs who were signatories to the MOA.

Figure 2. Four-hectare aquasilviculture model at BFAR-NBFTC Pagbilao, Quezon.

## Strategies

### *Implementing Guidelines*

A comprehensive implementing guideline was prepared and approved by the National Steering Committee (NSC) to ensure the success of the implementation of the PNAP. It defined the organizational structure and strategies of implementation of the program.

The NSC was created to provide over-all policy directions and guidelines. The convenors of the NSC are the DA Secretary and CHED Chairperson; Co-chaired by the BFAR Director and CHED Commissioner; and members composed of 3 BFAR Regional Directors and 3 SUC Presidents representing Luzon, Visayas and Mindanao; BFAR-Assistant Director; DENR-FMB Director and PAWB Director. The activities of the NSC are being managed and coordinated by the BFAR-NBFTC-based National Program Secretariat.

At the regional level, a Regional Steering Committee (RSC) was created to supervise policy implementation and oversee the Program Management Office (PMO). It is composed of the BFAR Regional Director and SUC Presidents. The PMO was also created to oversee the operations and implementation of the program in the province. The BFAR Provincial Fisheries Officer (PFO) heads the PMO as over-all Project Coordinator. The members of the PMO are the authorized representative of the SUC President, PENRO and the Provincial Agriculturist. In addition, the PMO engaged the services of a Community Organizer (CO) who directly implements the program in the field.

### *Capacity Building*

BFAR and SUC coordinators, PFOs, focal persons, COs and fisherfolk beneficiaries were given technical training on the three components (mangrove resource rehabilitation and protection, aquasilviculture and CBMSH) as well as constituency building, value formation and leadership development. Training of implementers was done at BFAR-NBFTC while that of the beneficiaries was done at the BFAR Regional Fisheries Training Centers (RFTCs). CBMSH training was done at BFAR Guiuan Station in Guiuan, Samar. Resource persons from BFAR, DENR, the private sector and non-government organizations were invited to discuss specific subjects relating to their line of expertise.

### *Participation of other Relevant Government Agencies*

The NSC and RSCs may enter into agreements and partnerships with other relevant national, regional and local government agencies in the implementation of the program. Such agreements and partnerships may cover: (a) joint funding and counter-parting; (b) conduct of training and technology transfer; (c) research, development and extension; (d) market development and credit facilitation; and (e) other relevant support for the implementation of the program.

### ***Status of Implementation***

The PNAP is in its third year (FY 2014) of implementation. Technical training has been completed for all components. Mangrove rehabilitation has started in FY 2012 and still continuing (Figure 3). Report of mangrove propagule planting as of September 2013 indicated that around 31,000,000 out of 36,000,000 target for the year has been planted (85% accomplished) covering more or less 10,000 ha throughout the country. Almost 32,000 fisherfolk participated in the activity. For aquasilviculture, 76% has been attained benefitting almost 1,900 fisherfolk throughout the country (Figure 4). For CBMSH, almost 20% of participating SUCs had completed establishment, while still continuing for the others (Figure 5).

Figure 3. Planted *Rhizophora* in various sites.

Figure 4. Aquasilviculture pond stocked with mangrove crabs.


Figure 5. Community-based multi-species hatcheries (CBMSH) established by SUCs.

### *Future Interventions*

The government is committed to achieve food security for the Filipino people, increase fish production and improve standard of living of coastal fisherfolks. Therefore, there should be continuing rehabilitation of denuded mangrove areas, particularly AUUs and make the coastal fisherfolks sustainably productive through aquasilviculture. Mangrove areas reported as rehabilitated should be validated and assessed in terms of surviving propagules planted, as well as to the extent of cover. Fisherfolk communities should be continuously organized and empowered through training and information dissemination to conserve and protect mangrove areas. Stock assessment studies should be conducted in areas with established CBMSH to determine if there is improvement in catch of fisheries products.

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# Good Aquaculture Practices (VietGAP) and Sustainable Aquaculture Development in Viet Nam

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***Abstract***

The shrimp (black tiger and white leg shrimp) and catfish industries in Viet Nam continue to experience increasing growth due to rapid aquaculture development. However, disease outbreaks become a major issue. Moreover, seafood consumers at present are likely to be more concerned about how the products are produced and how to control/manage aquatic animal health instead of treatment. Hence, the main objective of this abstract is to focus on one of the solutions to address these problems/issues and ensure sustainable aquaculture development in Viet Nam.

**Keywords:** Viet Nam, disease outbreaks, aquatic animal health, sustainable aquaculture

## *Current status of aquaculture in Viet Nam*

During the last decade, fisheries production in Viet Nam has significantly increased in volume (Figure 1) and export value. In 2011, total fisheries production from aquaculture and capture fisheries combined reached 5.25 million tons and the export value was at USD 6.18 billion. In 2012, production from aquaculture reached 5.8 million MT while and capture fisheries recorded 6.05 million MT. The export values were 6.13 and USD 6.8 billion, respectively, contributing 4% to the gross domestic product (GDP). Total fisheries production also accounted for about 40% of the country's animal protein production, and created approximately 4 million jobs. In 2013, aquaculture accounted for the majority of the fisheries output (3.34 million MT), 2% higher compared with 2012. Thus, the fisheries sector plays an important role in the national economy and rural development.

In Viet Nam, the most important aquaculture species by volume is Pangasius (*Pangasianodon hypophthalmus*) (38%), followed by traditional freshwater fish species (28%) and brackishwater shrimp (16%) (*Penaeus monodon* and *P. vannamei*). Freshwater species are cultured for domestic consumption while shrimp and pangasid catfish are processed mainly for export. Figures 2 and 3 show the annual culture area and production of black tiger shrimp and white leg shrimp. Table 1 shows the annual data on the culture areas and production of Tra catfish.

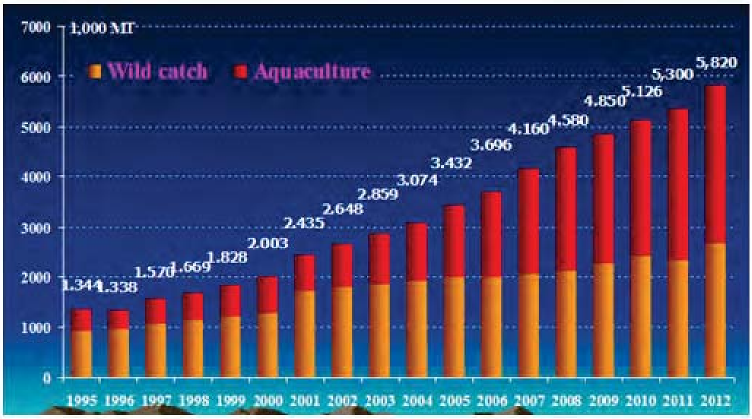


Figure 1. Development of fisheries sector in Viet Nam.

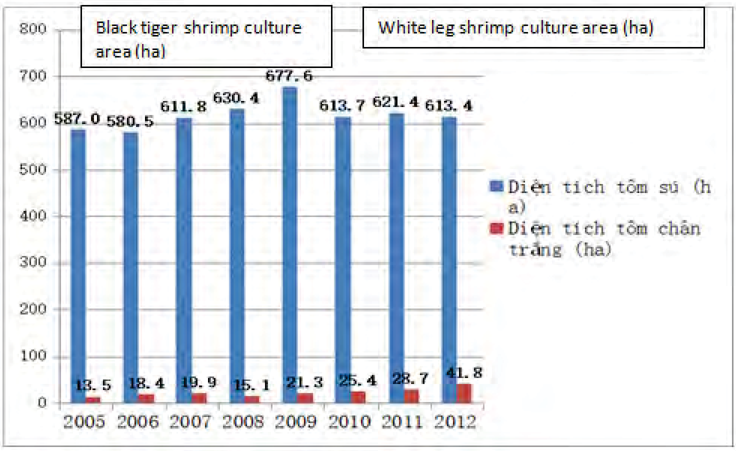


Figure 2. Culture area for black tiger and white leg shrimp in Viet Nam, 2005-2012 (Source: Directorate of Fisheries, Ministry of Agriculture and Rural Development).

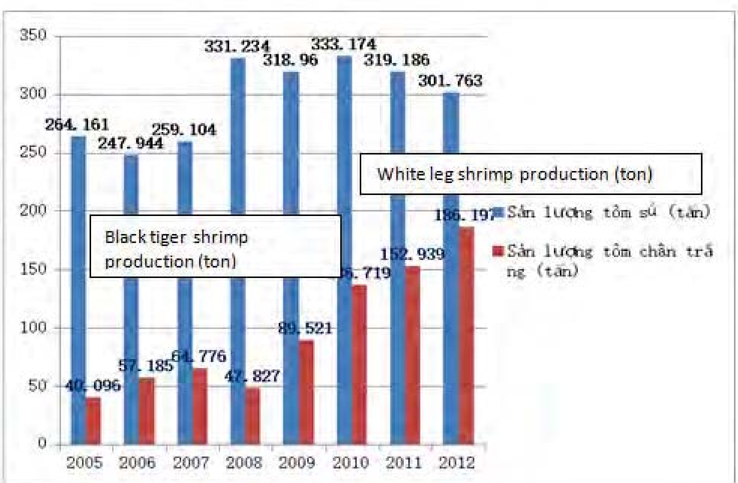


Figure 3: Production of black tiger shrimp and white leg shrimp in Viet Nam, 2005-2012 (Source: Directorate of Fisheries, Ministry of Agriculture and Rural Development).

**Table 1.** Culture area of Tra catfish, Viet Nam, 2010-2013 (Source: Directorate of Fisheries, Ministry of Agriculture and Rural Development).

| | 2010 | 2011 | 2012 | 2013 |
|---|---|---|---|---|
| Area (ha) | 5,420 | 5,430 | 5,910 | 5,735.3 |
| Production (tons) | 1,141,000 | 1,195,000 | 1280,500 | 1,093,718 |
| Productivity (tons/ha) | 211 | 220 | 274 | 191 |

Tra/catfish were originally raised in a family's pond for daily consumption. When Viet Nam opened its doors to the world market, Tra/catfish became a commercial product and is now exported around the world. People in the Mekong Delta have shifted the business of raising Tra from small to large scale. Many farms and special areas of hundreds of hectares have been established for the production of Tra. Scientific and technological applications in raising Tra have become popular in each farm.

In 2001, the Tra output in the Mekong Delta was only 100,000 tons. The number increased to over 1 million tons in 2009, yielding a turnover of USD 1.4 billion. Recent data in 2014 note that yield is more than 1.17 million tons providing USD 1.8 billion of export turn over. Many fish farmers have become rich because of farming Tra. The pictures below depict the key activities in the culture and processing of Tra for domestic and export consumption.

***Disease***

In 2012, a total of 657,523 ha of brackishwater surface area were used for aquaculture, but after a maximum of 2 months of culture, around one-sixth (100,776 ha) of the total area suffered serious losses due to infectious diseases called early mortality syndrome (EMS). The use of veterinary drugs and disinfectant in aquaculture for disease prevention, and the intensification of production led to the emergence of resistant strains of bacteria.

Figure 4. Tra catfish are carefully selected before reproduction.

Figure 5. Tra catfish farming is widely adopted in Cuu Long River Delta in southern Viet Nam.

Figure 6. Tra catfish being harvested.

Figure 7. Harvesting and packaging of catfish in farms.

Figure 8. Processing catfish for export at IDI Aqua-products Processing Factory in An Giang Province.

Figure 9. Processing catfish fillets before freezing.

Figure 10. Packing catfish for export.

Thus, research on the use of probiotics for aquaculture has increased with the demand for environment–friendly sustainable aquaculture. The benefits of such supplements include improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, anti-mutagenic and anti-carcinogenic activity, and increased immune response. These probiotics are harmless bacteria that help the well-being of the host animal and contribute, directly or indirectly, to protect the host animal against harmful bacterial pathogens. Last year, Dr Lightner (Arizona University) announced the pathogenic agent for shrimp as the early mortality syndrome (EMS). Viet Nam successfully controlled the EMS disease in shrimp. Areas affected by disease infestation in shrimp from 2010-2013 are as follows:

Table 2: Shrimp culture areas affected by diseases, Viet Nam, 2010-2013.

| Year | Total area affected by disease (ha) | Black tiger shrimp culture area affected by disease (ha) | White leg shrimp area affected by disease (ha) | Note |
|---|---|---|---|---|
| 2010 | 60,000 | 57,150 | 2,850 | |
| 2011 | 96,610 | 92,576 | 3,590 | |
| 2012 | 100,776 | 93,708 | 7,068 | EMS/AHPNS account for 46% |
| 1st 6 months of 2013 | 33,039 | 28,556 | 4,483 | |

For catfish, there are no significant records on disease outbreaks.

## *Aquaculture legislation and management*

Viet Nam has shown a large potential for aquaculture. In 1999, production was 480,810 tons while in 2013, it reached 3.34 million and thus higher than capture fisheries output. The government gives aquaculture development a priority as it has a very positive impact on food security and development of rural communities. The Government of Viet Nam has made many laws, decrees, decisions, circulars as well as national technical standards which more or less directly support and regulate the Pangasius industry. In response to the recent crisis of the Pangasius industry, the Ministry of Agriculture and Rural Development (MARD) of Viet Nam drafted in 2013 a decree on the management of production and consumption of Pangasius. This draft decree focused on the regulation of the whole value chain of production, processing and export, to avoid the imbalance in supply and demand and to improve production. The draft decree included clear regulations on aquaculture zoning, conditions for seed, feed and grow-out production, as well as for processing plans and export. The lowest regulated or floor price for pangasius export products, policies for credit and investment were also mentioned. However, during the approval hearing, more emphasis on the management of production and export has been noted as these have recently been shown to be a more relevant issue that needs to be addressed. A revised version of the decree is expected to be re-submitted during the first quarter of 2014.

Aside from the Good Aquaculture Practice in Viet Nam (called VietGAP) that created a major recent change in aquaculture performance and in the coming period, the Agriculture Restructuring Plan will focus on sustainable development and value chain improvement. The details will be presented in the next section of this paper.

**VietGAP – solution for sustainable aquaculture development in Viet Nam**

In order to ensure the sustainable development of aquaculture in general, and shrimp and Tra catfish raising in particular, many solutions have to be considered as follows:

- Master plan development: local authorities need to develop a master plan for the local fisheries industry;
- Infrastructure investment: develop the irrigation canal (separate inlets and outlets) in congested aquaculture area;
- Aquaculture restructuring plan: link farmers, suppliers, processing enterprises;
- VietGAP: promote the adoption of VietGAP in aquaculture
- Technology enhancement: prioritize new, clean and environment-friendly technologies; create new value-added products;
- Administrative reform
- Trade: marketing promotion

VietGAP was introduced for the following reasons, namely: (a) pollution issues and disease outbreaks caused by the fast growing aquaculture sector; (b) food safety for consumers; (c) introduction of many international standards causing confusion and neglecting the small-scale producers; and (d) sustainability of the industry. Hence, the concept of VietGAP for aquaculture emerged and became a national standard applied in the aquaculture industry from the grow-out stage to the postharvest stage.

Under the Government's policy on VietGap development in aquaculture, the country would foster its application in breeding key export commodities like Tra catfish, tiger prawn, and white-leg shrimp. The VietGAP standard is a single aquaculture module which complies with existing legislation and allows the application of VietGAP standard to different species.

The structure of VietGAP for Aquaculture is divided into five parts. These five parts have a total of 68 critical points that should be met before VietGAP is achieved. These five parts are: 1) general requirements, 2) food safety and quality; 3) animal health and welfare; 4) environmental integrity; and 5) socioeconomic aspects.

VietGAP for Aquaculture was based on the Code of Conduct for Responsible Fisheries of the Food and Agriculture Organization (CCRF of FAO). The general principle, technical guideline on aquaculture certification (FAO - Feb, 2011); general principal, structure and certification regulation as stipulated in the ASEAN GAP for shrimp; and structure with 4 main components. There are other recognized standards such as GlobalGAP, Aquaculture Stewardship Council or ASC, Global Food Safety Initiative or GFSI, ISO and Codex.

VietGAP for aquaculture is supported by a number of government regulations including:

a) Prime Minister Decision No. 01/2012/QĐ-TTg: Issue the Policies on encouraging the application of Good Agricultural Practices in Agriculture, Forestry and Aquaculture
b) Minister decision No. 1503/QĐ-BNN-TCTS: Issue the National Standard on Good Aquaculture Practices in Viet Nam (VietGAP)
c) Minister decision No. 1617/QĐ-BNN-TCTS: Issue Guidelines for the application of VietGAP standards for pangasius (*P. hypophthalmus*), tiger shrimp (*P. monodon*) and white leg shrimp (*P. vannamei*); and
d) Circular No 48/2012/TT-BNNPTNT on Regulations on the certification of aquaculture, crops and livestock products produced in accordance with application of good agricultural practice

When farmers adopt VietGAP, they can easily upgrade to other certificates required by importing countries and gain wider acceptance in both domestic and international markets. Government agencies are mandated to look for more markets that accept VietGap-certified products.

### Conclusions

With the principle that states "prevention is better than cure", sustainable aquaculture development connects closely with market-oriented development. VietGAP is a comprehensive solution for controlling the quality of input materials and water, maintaining good health of aquatic animals, ensuring better life for laborers or farmers, as well as maintaining the integrity of the environment.

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# Country Status on Sustainable Aquaculture in Lao PDR

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## *Abstract*

Capture fisheries and aquaculture in Lao PDR are based on water resource ecosystems which consist mainly of rivers and streams, hydropower and irrigation reservoirs, diversion weirs, small water bodies, flood plains and wet-season rice-fields. The total area of water resources for capture fisheries is believed to be more than 1.2 million ha. The estimated consumption of inland fish in Lao PDR is approximately 167,922 tonnes per year while consumption of other aquatic animals is estimated at 40,581 tonnes per year. Most of the consumption is from internal production (i.e. imports are of minor importance), so these figures represent approximate catches or yield from fisheries. These estimated yields are conservatively valued at almost US$150 million per year.

The people of Lao PDR, especially in the rural communities that account for more than 75 per cent of the population, still depend upon the country's fish and other aquatic animals as their most reliable sources of animal protein. The estimate of actual fish consumption per capita (kg/capita/year) of inland fish is 24.5 kg, while other aquatic animals account for about 4.1 kg and marine products around 0.4 kg, to make a total of 29 kg of fish and aquatic products consumed per capita per year.

As aquaculture in Lao PDR expands, many forms of production systems are being developed, for example pond culture, communal ponds, rice-cum-fish culture and cage culture. Most fish culture systems in Lao PDR are small-scale. Such forms of production systems are divided into sub-categories depending on the nature and main activity of the producers. According to the Department of Livestock and Fisheries, aquaculture production in 2007 accounted for 54,750 tonnes in an area of more than 42,000 ha, including cage culture in the Mekong and some tributaries.

There has been a significant increase in intensive tilapia production in recent years in Lao PDR (MRC Technical Paper No. 5 April 2002) based on tilapia cage culture in the Mekong river and irrigation reservoirs. In last two years, an enterprising farmer has established about 360 cages .

Constraints in the large-scale development of tilapia cage culture are the lack of technical support (e.g. extension services) to the farmers and insufficient supply of advanced fingerlings. Morever, tilapia cage culture in the Mekong river system is perceived to be difficult to sustain because of environmental factors such as river flooding and strong currents during the rainy season and the lack of water during the dry season.

It can be concluded that sustainable intensification of aquaculture in Lao PDR, still requires building the capacity of both extension workers and farmers with assistance from other SEAFDEC member countries. The training should cover the following:

- Selection of suitable aquaculture systems, and the promotion of appropriate technologies that can be adopted by farmers in Laos.
- Fish seed production, especially local production of sufficient and good quality seeds.
- Promotion and development of technologies on the propagation and culture of local fish species with aquaculture potential.
- Adoption of environment friendly feeding practices and production of aquaculture feeds.

**Keywords:** production systems, local fish species, capacity-building

# Current Status of Aquaculture in Singapore

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## *Abstract*

Singapore is a small country state with a demographic profile of over 5 million in population. With limited land for agricultural purposes and sea space available for fish farming, Singapore depends heavily on importation of fresh seafood. Even so, Singapore has a small but thriving and increasingly important food fish farming industry which accounts for about 6% of local food fish consumption.

The main bulk of local food fish production comes from coastal farming in floating netcages along the northern coast of Singapore. Popular species of marine food fish cultured include seabass, pompano, groupers, mullets and milkfish. There are also a few land-based fish farms culturing species like tilapia, marble goby and snakehead.

The ornamental fish farming industry is concentrated mainly in Agrotechnology Parks and there are about 75 fish farms producing ornamental fishes with an approximate value of $76.7 million that is exported to over 80 countries.

The Agri-Food and Veterinary Authority of Singapore (AVA) is the national authority for aquaculture development in Singapore and manages aquaculture farms through the issuance of fish farming licenses. For marine food fish farms, the farm licensee has to abide by good farm management guidelines to maintain the farm in good condition and ensure that the farm does not engage in activities that would impact the farming environment. For land-based farms, there are also guidelines that address infrastructure layout, farming system and water treatment facilities. The latter requires that sedimentation ponds, reservoir ponds/tanks, supply/drainage systems and trade effluent treatment plant are included in the farm set-up.

There are several challenges and issues faced by the aquaculture industry in Singapore. One of these is the consistent supply of good quality fish fry as farmers have to source for fish fry from overseas sources that may not be consistent or readily available.

Issues of fish health and farm management are other challenges faced by our fish farms. These factors affect farm productivity and the sustainability of farming operations.

The AVA has established the Marine Aquaculture Centre (MAC) on St John's Island to address the needs of aquaculture development for Singapore through development of fish reproduction and seed production as well as large-scale fish farming technology. At present, the fish reproduction technology research work involves closing the reproductive cycles of key marine food fish species and also fry production at a commercial scale level. Closing the reproductive cycle will help to reduce the reliance on imported fry. Good quality brooders are selected,

maintained and bred to produce quality fry, which would translate to better growth performance and shorter culture period. This, together with good farm management practices, will optimize the usage of fish feeds during the culture cycle.

To fill the gap in production and supply of good quality fish seeds for local fish farms, AVA shares information on hatchery technology development with local commercial hatcheries.

The AVA collaborates with research institutes and local fish farms in the development of vaccines to boost the survival rate of fish fry and fingerlings. This will improve survivability, thus increase the production of the farms and reduce the reliance on prophylactic drugs that may have negative consequences from prolonged use.

The AVA also renders technical assistance to the farmers to formulate viable production plans to improve production. By leveraging on the use of technology and good farm practices, such as implementation of fish health, fish nutrition and feeding protocols, it is possible to reduce production costs and improve productivity. The introduction of the Good Aquaculture Practice scheme for food fish farming will help improve the standards of the local aquaculture industry and sustainability through responsible management practices.

**Keywords:** Singapore, coastal farming, ornamental fish

## Sustainable Aquaculture and Resources Enhancement in Indonesia

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### *Abstract*

With a human population of 230 million and a huge potential for marine and fisheries resources development, Indonesia promotes aquaculture as a major sector to accelerate economic growth for rural communities. There are recent initiatives to improve the country's legal framework to mitigate the adverse impacts of aquaculture and make the aquaculture more sustainable.

The Directorate General of Aquaculture under the Ministry of Marine Affairs and Fisheries (MMAF) has the mandate to develop the aquaculture sector of Indonesia. Aquaculture has an important role in the development of its national economy and play a key role in rural development. As aquaculture production expands, there is also a growing concern over the impacts on sustainability of aquaculture and resource enhancement as well as food quality and safety requirements of fish products.

For this reason, there is a need to improve aquaculture technology and its management system in Indonesia to address the need for eco-friendly production process and food safety concerns while maintaining the sustainability of the country's aquaculture sector. The Indonesian Fisheries Act No. 31 (2004) Amendment No. 45 (2009) mentioned that, among others, the Indonesian fisheries management strategies should include the creation job opportunities, improvement of the welfare of fishers and their communities, and ensuring the sustainability of the country's fishery resources and aquatic environment.

The most critical factors to achieve sustainable aquaculture in Indonesia are the availability of good quality seed, good practice in grow-out systems, healthy aquaculture environment, fish health management, good-quality products, strategic marketing, and improving marketing and stock enhancement. In addition, it is also a concern that the products from aquaculture should meet the quality standard and product safety. This paper presents a review of Indonesian aquaculture in relation to sustainable practices and management schemes to preserve the aquaculture environment, food safety requirements for aquaculture products, food security and to enhance the biodiversity of fishery resources. A policy that was recently established is the development of the marine and fisheries sector based on the principles of the Blue Economy program of the Indonesian government.

**Keywords:** sustainable aquaculture, food safety traceability, resource enhancement, Blue Economy

# Milkfish: New Choice for Aquaculture in Thailand

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***Abstract***

Milkfish is an economically important fish cultured in many countries in Asia. In Thailand, milkfish culture has not been given much attention and has not as been developed as in the other Asian countries because in the past the farmers prefer to grow shrimps and other high value fishes. Nowadays, environmental changes and degradation can affect water resources as well as the important aquaculture species that thrive in them hence the Thai Department of Fisheries recognizes the importance of developing aquaculture that is environment-friendly. This includes milkfish in particular because milkfish meat tastes good, easy to manage on farm, grows rapidly and can be grown in sea water, brackish or even freshwater. Milkfish farming is a low cost operation because milkfish feed mainly on algae and organic matter and these are natural food produced from other types of aquaculture activities. Milkfish can therefore be co-cultured with other species and are capable of reducing the amount of organic material from the process of aquaculture before entering the environment. In 2002, milkfish was first bred successfully through hormone injection and later broodstock mated naturally in Thailand. At present, production of the 1-inch milkfish has reached 1,000,000 per year. The culture sites are in the southern and eastern parts of the country, in brackish and salty areas. Culture methods are either monoculture or polyculture with other species such as shrimp, mussel etc. Milkfish culture in reservoirs last from 6 to 12 months when fish size is about 500 g or two pieces to a kg. and the price is about 50 baht/kg. On the other hand, milkfish that are 600-1,000 g can sell at 65-90 baht/kg. Apart from culture, processing as well as marketing promotion of milkfish has also started in Thailand. Milkfish processing training is being conducted at least 2 times a year. As for the marketing initiatives, there is a move for the milkfish to be declared the symbol of Prachuap Khiri Khan Province since it was here that the fish was first found naturally in Thailand. This, apart from the plan to promote milkfish in the festivals throughout the country. Although found promising, some problems in the Thai milkfish industry are also recognized. Such issues notwithstanding, the Thai Department of Fisheries is coming up with guidelines for milkfish aquaculture as it is optimistic that this commodity shall open the doors to a new alternative industry in Thailand.

**Keywords:** milkfish, culture, processing, alternative industry

# CONTRIBUTED PAPERS

# Assessment of Humphead Wrasse (*Cheilinus undulatus*), Spawning Aggregations and Declaration of Marine Protected Area as Strategy for Enhancement of Wild Stocks

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## *Abstract*

Humphead wrasse, known as the Napoleon fish (*Cheilinus undulatus*), is the largest living member of the family Labridae. It is slow growing but can grow to a maximum size exceeding 2 m and 190 kg. This species was the first commercially important coral reef food fish to be listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II in 2004 because of its vulnerable status and the ongoing threat to its conservation from international trade. Like many coral reef fishes, the humphead wrasse, *Cheilinus undulatus*, aggregate in reef areas when they spawn and this spawning behaviour makes them highly vulnerable to overfishing. Assessment of the spawning aggregations of this species was conducted in the municipalities of Sibutu and Sitangkai in the province of Tawi-Tawi, Philippines. Key informant interviews (KII) with fishermen, mariculturists, and other stakeholders and focus group discussions (FGD) with local government leaders, Fisheries and Aquatic Resources Management (FARMC) members, mariculturists, and exporters were conducted. Guided by the results of these KII and FGDs, underwater visual census of mameng (local common name for Napoleon wrasse) populations (juvenile and mature) were conducted to document spawning aggregation sites. Since there was no photo-documentation of actual spawning aggregations of *mameng* in the reef areas, indirect measures were used. Result of the KII and FGD indicated that the Baligtang Reef in Sipangkot and Tando Owak are major sources of spawners. Anecdotal accounts of Bajau fishermen showed that Dungun Dungon, Baligtang reef, Tando Owak and Tugalan are traditional fishing grounds for *mameng* spawning aggregations. From the length-frequency analysis of *mameng* caught by hook and line and fish pot in the Baligtang Reef in Sipangkot, the estimated length at maturity of this species was found to be 25-35 cm. There were 134 individuals caught within this size range so they are considered potential spawners. Another indirect proof used was the underwater documentation of juvenile humphead wrasse which were regularly observed and photographed in association with seagrass beds and branching coral reefs in Baligtang Reef in Sipangkot, Sitangkai. Gonadal study also indicated that the *mameng* caught in this area had mature and ripe gonads but the number of mature fish depends on the season. These were the basis of declaring Spawning Aggregation Sites in Tando Owak and Dungun Dungun in Sibutu and Baligtang Reef, Sipangkot and Tugalan in Sitangkai. These were declared as marine protected areas by ordinance of the municipal *Sangguniang Bayan* of the two municipalities. Management and enforcement plans have been developed and *Bantay Dagat* have been trained to protect the spawning aggregations and this strategy aims to protect the wild stocks of humphead wrasse. Protecting the spawners would ensure that there would be enough recruits, prevent recruitment overfishing and enhance the wild stocks.

Keywords: humphead wrasse, assessment, spawning aggregations, management

## Introduction

The Sulu Archipelago Reef complex, an ecologically important component of the Coral Triangle, is known to be one of the main sources of the live food fish trade particularly groupers (red grouper) and humphead wrasse or Napoleon wrasse (*Cheilinus undulatus*). It constitutes about 25% of the coral reef cover of the Philippines. Fisheries and agriculture contribute 17% to the Gross Domestic Product and is the primary contributor to the national diet. Since these also provide nearly two-thirds of the population's animal protein (FAO, 2000) requirement, there is a need to assess the small-scale fisheries which produce about 95% of total marine fisheries production of the country. It was estimated that more than 80% of the small-scale fishers and their families have net incomes below the national threshold poverty level (FAO, 2000).

Sibutu and Sitangkai in the province of Tawi-Tawi, Philippines are mariculture centers in the area. A study of Romero and Injani (2010) indicated that humphead wrasse is the primary species cultured in more than 350 cages of different sizes and number per cluster of cages surveyed. This constitutes about 76% of the cultured species while others hold various species of groupers, caranx, siganids and lobsters. From these pens, a total of 31,071 humphead wrasse fingerlings (0.2-0.5g) were counted. There were 6,914 under sized (0.6-0.8g) and 4,675 marketable size (1.0kg-1.4 kg) humphead wrasse. However, in 2004 the Napoleon fish has been listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Appendix II provides that all species, although not necessarily threatened with extinction at the present time, may become so unless trade in specimens of such species is subject to strict regulation in order to avoid utilization incompatible with their survival. Section 97 of RA 8550 otherwise known as the Fisheries Code declares it unlawful to fish or take threatened or endangered species as listed in the CITES. Likewise, Section 11 of RA 9147, otherwise known as Wildlife Act also provides for the implementation of regulations in international trade of endangered species of wild fauna and flora. Despite these laws, mariculture of this Napoleon wrasse is still being done using juveniles harvested from the wild.

Francisco *et al.* (2009) recommended that "sustaining profitable, yet responsible mariculture micro-enterprises for poor fishing communities participating in fisheries management programs can be a two-pronged strategy: to increase rural income and foster broader motivation and vigilance for the protection of habitats and resources." The sheer number of individual Napoleon wrasse stocked in pens and cages shows that the fishermen are still able to catch fingerlings and immature wrasse from the wild and many believe that this species is not endangered or threatened.

Reef fish aggregations are groups of fish gathered for either spawning, feeding or shelter. By definition, a spawning aggregation is a group of con-specific individuals grouped together in densities three times higher than those found during non-reproductive periods. Species that periodically and predictably congregate on land or in the sea can be extremely vulnerable to over-exploitation. It is common practice that fishermen target these spawning aggregations sometimes with the use of dynamite. This practice could possibly result in growth overfishing of this species. Growth overfishing results in the loss in biomass that is greater than the biomass gained through growth, and is usually due to high fishing rate or to collection of stocks before they have time to grow. Humphead wrasse, like other reef fishes, is known to spawn in aggregations consistently at the same specific period of the year at a specific area. Unlike the groupers which have large spawning aggregations, humphead wrasse has small aggregations and the sites are usually on the outer reef slopes, in reef channels, or at drop-offs. This study is done to determine the occurrence, history, and management of spawning aggregations of this species.

A good understanding of the effects of fishing on reef fish spawning aggregations, gained through sound scientific study, is the key to their successful management and conservation. This is in line with the 2011 Coral Triangle Support Partnership (CTSP) Program that targets to contribute to 350 hectares of new MPAs in Tawi-Tawi and Palawan. However, the assessment of the fishery and biological parameters and spawning aggregation sites needs to be done to increase awareness among communities of their ecological significance and vulnerability and to adopt a policy of protecting these spawning aggregations as a strategy to enhance the wild stocks like declaring these spawning aggregations as marine protected areas.

***Objectives of the Study***

1. To gather baseline information about Napoleon wrasse and their spawning aggregation using KII with fishermen, mariculturists and FGD with other stakeholders;
2. To document spawning aggregation sites in Sibutu and Sitangkai areas and determine the seasonality of spawning activity by conducting underwater visual census;
3. To establish baseline data on spawning aggregations of Napoleon wrasse in terms of sizes, places and time of spawning; and
4. To adopt policies for the protection of spawning aggregation sites and their management by recommending relevant ordinance/s.

***Review of Literature***

Humphead wrasse (*Cheilinus undulatus*) is the largest living member of the family Labridae, It is slow growing but can grow to a maximum size exceeding 2 m and 190 kg. Like many coral reef fishes, humphead wrasse form spawning aggregations. Spawning is the term used to describe the reproductive act of some aquatic animals such as fish, mollusks, and crustaceans. Spawning occurs when sperm and eggs are released for fertilization into the water column or deposited onto the seabed. According to Mancilla and Ponce Taylor (http://scuba.about.com/od/ConservationandDiving/p/Fish-Spawning-Aggregations.html), the sperm and eggs must be expelled at around the same time and at the same place for this form of external fertilization to work. This species is a protogynous hermaphrodite (i.e. they mature first as females and later change to males), has low productivity and occurs in naturally low densities in reef-associated areas throughout its geographical range in the Indo-Pacific (Sadovy *et al.*, 2003). In recent years, this species has been very much in demand in the live fish trade and has become one of the most vulnerable species to the impacts of fishing in reef fish assemblages. Substantial declines in local abundance have been observed in many locations within the species' range due to several factors, but most prominently because of trade-driven overfishing (Sadovy *et al.*, 2003).

Species such as giant grouper and humphead wrasse are particularly susceptible to overfishing because of their slow growth and long development to sexual maturity. Due to over fishing, humphead wrasse (*Cheilinus undulatus*) is the first reef fish now listed in Appendix II of the Convention on International Trade in Endangered Species (CITES) and must now be strictly regulated by importing and exporting countries. Under CITES regulations, countries exporting Appendix II species are required to demonstrate that export quotas are derived from legal fisheries and that such exports will not be detrimental to the survival of the species or its role in the ecosystem. However, there is paucity of data for the humphead wrasse on the levels of exploitation of the stock from the fisheries. Data limitations in small-scale capture fisheries pose a very big constraint in decision-making for the management of this fisheries. It also recognizes the development of approaches for fisheries assessments in data-poor situations as one of the key areas of action for improving information on status and trends of marine capture fisheries (FAO, 2003). From the point of view of responsible fisheries management, a non-detrimental finding (NDF) implies defining and enforcing a catch level that maintains the abundance of the stock above a state where it would be considered overfished or depleted and that would have a negative impact on the ecosystem. The problem is that information about the targeted resources is often so poor for many fisheries that it is very difficult to make inferences about their status or about sustainable catches.

Trade in live reef fish represents an important commodity in Asia and the Pacific and includes fish caught, shipped, and exported live to the consumers like the live reef food fish trade and ornamental fish caught for the aquarium hobby industry. The live reef food fish trade primarily targets grouper species and other reef species like the humphead wrasse for the markets of Hong Kong and southern China. Southeast Asia and Australia are the major suppliers of this trade; yet, operators are increasingly seeking fish in more remote parts of the Western Pacific including Papua New Guinea and the Solomon Islands. The centre for the live reef food fish trade is located in Hong Kong. According to a report by TRAFFIC International-East Asia, a wildlife trade monitoring network hosted by World Wildlife Fund (WWF)-Hong Kong, the total annual value of live reef food fish imported into Hong Kong is estimated to be over US $400 million (40% of the estimated $1billion value of global trade). Total imports flowing into Hong Kong included 10,153 metric tons, of which 30% was re-exported to mainland China. Other major markets include Singapore and Taiwan.

Reef fishes are significant socially, nutritionally and economically, yet biologically they are vulnerable to both over-exploitation and degradation of their habitat. Their importance in the tropics for living conditions, human health, food security and economic development is enormous, with millions of people and hundreds of thousands of communities directly dependent, and many more indirectly so. Reef fish fisheries are also critical safety valves in times of economic or social hardship or disturbance, and are more efficient, less wasteful and support far more livelihoods per tonne produced than industrial scale fisheries.

Spawning aggregations are best documented with direct evidences - observations of actual spawning or documenting the presence of hydrated oocytes within gonads of females on the site (www.scrfa.org). If direct evidence is not available, indirect evidences include density increases, spawning-specific color changes and behaviours, swollen abdomens and increases in the gonadosomatic index.  A combination of indirect observations increases the likelihood that the aggregation is actually a spawning aggregation. Reef fish spawning aggregations are predictable in space and time. Two different types of spawning aggregations have been defined ("resident" and "transient") using three criteria, i.e. the frequency of aggregations, the longevity of aggregations, and the distance travelled by fish to the aggregation. Spawning in resident aggregations is common to most rabbitfish (siganids), wrasses (labrids) and angelfish (acanthurids). In this type of aggregation, spawning is brief (often 1-2 hours), occurs frequently (often daily) and involves migration over short distances to the spawning site. Spawning in transient aggregations, by contrast, is the strategy used by most grouper (serranids), snapper (lutjanids), and jacks (carangids), along with several other families.  Accounts of reproductive activity in the field reveal that, depending on location, this species spawns between several and all months of the year, in small or large groupings, that spawning coincides with certain phases of the tidal cycle and that groups of spawning fish can form daily, at a range of different reef types (Colin, Choat, Hamilton and Oakley, pers. comm.).

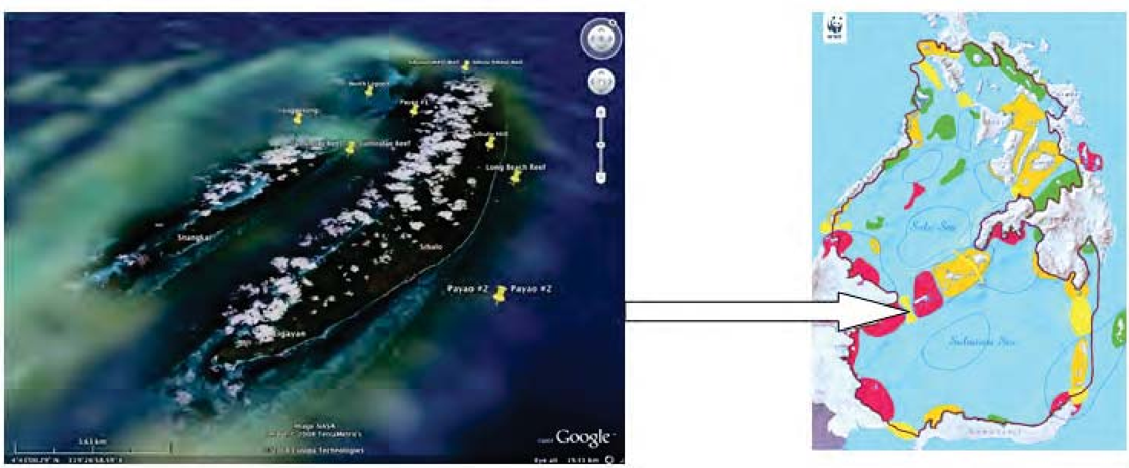


Figure 1. Location map of Sitangkai and Sibutu.

## Materials and Methods

### *Study Sites*

Sitangkai is a remote island municipality located at the southernmost portion of Tawi-Tawi province. It is one of the areas covered by the CTSP Program in the Sulu-Sulawesi Marine Eco-region in Tawi-Tawi assisted by the World Wide Fund for Nature – Philippines (WWF-Philippines). It is bounded in the east by Tumindao Channel, in the south by Celebes Sea and in the north by Sabah, Malaysia. It is a 5$^{th}$ class municipality composed of 14 barangays.

Sibutu is a newly created municipality out of the Sitangkai municipality by virtue of MMAA No. 197, which was subsequently ratified in a plebiscite held on October 21, 2006. It is a sixth class municipality in the province of Tawi-Tawi. It lies about 14 km east of the coast of Sabah, Malaysia. It is comprised of Sibutu Island and four more small and uninhabited islands 3.4 to 5.9 km south of the main island.

### *Key Informant Interviews (KII) and Focus Group Discussions (FGD)*

Gathering of information about the fishery and spawning aggregations of the fish species included in this study was done through the conduct of KII with fishermen, mariculturists, and other stakeholders. An interview instrument was formulated and administered by the enumerators in the two municipalities. A total of 40 fishermen and 50 mariculturists were randomly selected for the KII. Interview instruments were designed to get anecdotal accounts or actual information about fishing practices and spawning sites of the humphead wrasse. The best means of obtaining information is to compile traditional knowledge from resource users. Traditional fishers were particularly valuable source of information as they provided a temporal perspective on given spawning aggregation sites. KII results were validated through FGD with local government leaders (municipal and barangay levels), FARMC members, MPA management board members, mariculturists and exporters.

### *Underwater Visual Surveys*

Underwater Visual Surveys (UVS) using SCUBA were conducted by laying a 100 meter transect line in each of the stations. The laying of the transect lines were in the reef slope parallel to the shoreline and this was repeated five times in each station. Observations were conducted 5m to the right and to the left of the transect line using two SCUBA divers. All these were recorded on slates. The area covered for each transect line was 200 m$^2$. The total area swathed by the five transect lines was approximately 1,000 m$^2$. An underwater photo-documentation was done using an underwater camera and the depth of the observation areas varied from 60 to 160 ft. This activity documented the presence of juveniles and mature humphead wrasse species and hoped to encounter a spawning aggregation. Using GPS, the coordinates of the stations for the underwater visual surveys, were documented during the first, second, third and fourth quarters of the year. The maximum depth reached was also documented. A total of 24 dive sites were surveyed.

### Physical Measurements at Spawning Sites

#### *Surface currents*

The current system in the two study sites are predominantly influenced by tidal currents and is observed to be semi-diurnal, two high tides and two low tides. The surface currents were measured quantitatively with a GPS and a current drogue. The GPS distance and bearings functions and the data sheet were used to plot the speed and direction of the surface currents.

#### *Tides and Winds*

Information on tides used the local tide charts for 2012 issued by the National Mapping and Resource Information Authority (NAMRIA). The wind direction was assessed by a hand-held compass. For the wind patterns, although there are observed localized winds the monsoon winds were used for this study. April and May (SW monsoon) locally known as *Satan*, August (Inter-monsoon) and November (NE monsoon) locally known as *Uttara* were used as the wind indicators. Winds and currents are considered to be important factors that affect the fate of eggs and their dispersal.

#### *Effort and Length Frequency Analysis*

Catch from different kinds of gears and catch per unit effort (CPUE) were monitored as one relative index of the status of the fishery. Life history data size frequency distribution was used to analyse the size (and potential age) structure of the population, and to gather information on the size at reproductive maturity. The best place to collect CPUE data is at fishery landing site, while fishermen are capturing, cleaning and processing freshly caught fish.

For size frequency analysis, the following information were recorded on the data sheet:

- Fork length - length in cm from tip of snout to middle rays of caudal fin
- Weight - gutted or whole weight in grams
- Sex - male or female; maturity stage, i.e. immature, early development, late development, ripe and running, or spent
- Hours and/or number of days spent fishing. This is also defined as “soak time” or the actual number of hours fishing. For traps, it is the number of hours the baited traps are kept underwater before they are hauled. For handlines, this is the number of hours of fishing on site.

#### *Reproduction and Gonadal Assessment*

One indirect way of assessment of spawning aggregations is to gather samples of mature *mameng* species from the landed catch and get gonad samples of mature species to determine their level of maturity. The fishes were brought to the MSU laboratory and dissected and their gonads examined under the microscope. Depending on the state of the oocytes, they were classified as inactive, immature, resting, or developing. Presence of ovulatory follicles was also noted. Anecdotal information from fishermen, and behavioural observations were also used to provide a preliminary understanding of the reproductive biology of the humphead wrasse. This is another indirect way to determine possible areas of spawning aggregations.

## Results and Discussion

### *History of the Fishery and Profile*

The results of the KIIs with fishermen, mariculturists and other stakeholders show that fishing for humphead wrasse is not considered a traditional targeted fishery. It was more of an accidental catch of other reef fishery according to 85% of the respondent-fishermen. The 95% of the key informants said that this became a targeted fishery in 1987 due to local demand for live fish from Chinese businessmen in Bongao. Fishing for this species intensified when demand for live reef fish, especially for humphead wrasse, grew among the live fish restaurants in Sabah, Malaysia. As demand and the need for ready supply increased, fishermen started to grow them out in pens in 2000 on a trial and error basis. Demand for this species increased in Hong Kong and Taiwan and because of the success of their experimental grow-out system, more fish farmers followed suit.

Humphead wrasse is locally known as *mameng*. When it is sexually mature, the locals call it *Pehakan* (referring to sexually mature female). It is considered as protogynous hermaphrodite meaning the adults can change sex from female to male. Large male species are called by the natives as *langkawit* and they are characterized by a prominent bump in the head when it has reached a very large size. It has a low productivity.

The grow-out cages are primarily dependent on fingerlings or immature fish caught from the wild for stocking. Fishing for fingerlings is limited to shallow reef edges or in the mixed seagrass beds with coralline substratum. The larger *mameng* are caught in deeper areas especially in reef channels, which the natives call *takot* or on the edges of the fringing reefs. Other species which are typically associated with the live reef food fish trade (LRFT) like groupers, caranx, and lobsters are also grown out in these pens.

Fishermen describe the fishing grounds to be characteristic of fringing reef areas, reef channels and lagoons where branching, table and massive corals are located. They claim that humphead wrasse are found in areas where there are drop offs at various depths and varying slope inclinations. Juvenile *mameng* are found in mixed seagrass and coralline substratum but the larger ones are found in deeper reef areas. These were validated in the underwater visual census and the focus group discussions.

Figure 2 shows the location of the fishing grounds for humphead wrasse in Sitangkai and in Sibutu. The areas which are marked by circles are the major fishing grounds and include sites e Cebuggal, Serunga, Ligayan Halo, Tumindao and the North Lagoon, particularly near Sipangkot Island.

The fishing crafts used are non-motorized and motorized boats. The most dominant non-motorized boats according to majority of the informants are the dug-out boats locally called as the *beggong*. This is used to fish near the edge of the reef area or within the lagoon near the community and usually catches fingerlings only. This is also sometimes used to retrieve the *lambunan* or fish traps which are set near the community. The *papet/tariret* is a small and slender type of motorized boat with a more tapered bow and powered by 7.5 hp to 13 hp engines. This is a faster craft and is predominantly used for multiple troll line for tuna fishing. The *tempel/kurikong* is bigger with a more bulging hull. This is a motorized boat powered with 10 to 16 hp engines. Some use double engines. This is used to fish in far reef areas. The *jung-jung/katigan* is an out-triggered boat usually with 10 to 18 hp engines. This is used to reach outer reef areas near the Semporna area.

For the fishing gears, hook and line is the most common traditional gear used in catching humphead wrasse and other live reef fish. Baits of fresh pelagic fish like big-eyed scad or hermit crab are hooked to attract the fish. This is the least efficient among the gears. The *lakud* is a type of modified beach seine which uses fine mesh nets and also utilizes divers with scare lines to drive the fishes to the net. This gear can catch 10 to 20 reef fishes and this can include humphead wrasse depending on the season and the fishing grounds. This is used by about 35% of the fishermen. The *linggih lakoran* is modified gill net usually 50 m long, 1.3 m wide and mesh size of 2 inches. This is usually hung vertically in the water column and fish that gets entangled in the mesh net. This is usually weighted so that it can catch fish in mid-water or at the bottom and used by 30% of the fishermen interviewed. *Bungsud* or *angpas* (fish corral) is a stationary trap designed to intercept and capture fish. This is usually set perpendicular to the shoreline. This fencelike structure consists of rows of bamboo stakes, plastic nets, and other materials with a wide entrance leading to a catching chamber, bag or purse where the fish are caught or trapped. This is used by 20% of the fishermen. *Lambunan* is a fish trap usually made of bamboo frame with hard green plastic net wrapped into a rectangular basket with dimension 6 inches x 24 inches x 40 inches. The bottom part of the trap is loosely fastened on one side so that the trapped fish can be easily removed. On one side of the trap is a conical opening usually about 6 inches in diameter to serve as entry point of the fish and this would not allow the fish to escape. This is not usually baited but is covered with coral stones to simulate a coral formation and catches 1-5 fingerlings depending on the season. Cyanide with compressor diving is also used. Although majority of the fishermen interviewed do not admit directly using cyanide in catching humphead wrasse, use of this destructive method is still prevalent. The mortality of humphead wrasse in the earlier stage of grow-out in pens is an indication of shock due to cyanide exposure.

Eighty-six percent of the fishermen also claimed that they usually have a good catch during the southwest monsoon from April to June and during full moons. However, nowadays there has been a decreasing trend in their catch and they associate this to the sheer number of fishermen fishing for this species. Some say that there may already be less *mameng* that could be caught in the wild. Majority of the respondents also say that they spend longer time in the fishing grounds; have to go farther to catch fish than in the past; catch has not been stable and they catch smaller humphead wrasse. These were validated in the FGDs.

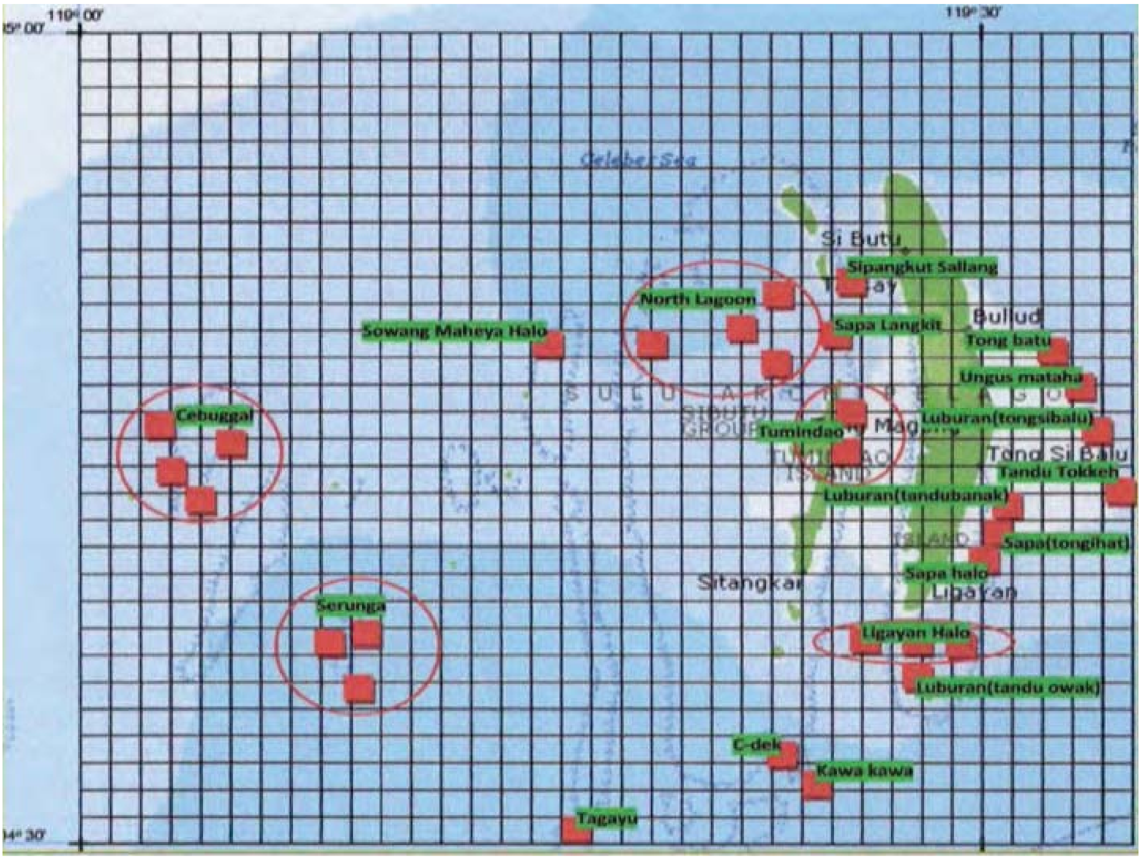


Figure 2. Map of the fishing grounds.

### *Mariculture Practices*

As the demand for the humphead wrasse and groupers grew and considering that the price of live *mameng* is 250% more than that of fresh dead fish, more and more pens were constructed as grow-out for these live reef fishes in the eastern coastline of Sibutu Island. Now the grow-out cages are found in many areas of the North Lagoon, in the reef edges of Sitangkai and from Tandubanak to Tando Owak in Sibutu within the lagoon extending to about 10 kms along the shoreline. Figure 3 shows the distribution of the grow-out cages in Sibutu and Sitangkai and Figure 4 shows the mariculture cages of *mameng*.

#### *Spawning Aggregation Sites (from interviews)*

Based on the KII results, the respondents indicated that spawning aggregation sites are the reef areas in Dungun Dungun up to Tong Bakkaan, Ligayan Halo and Tando Owak, Tando Tokkeh and Tahing in Sibutu. In Sitangkai the respondents pointed to the reef areas in Sipangkot and Baligtang Reef, Cebuggal, Tagayu and Tugalan. From the anecdotal accounts of Badjao fishermen, this phenomenon is known to them as *Nabo* – a season when this spawning aggregation occurs.

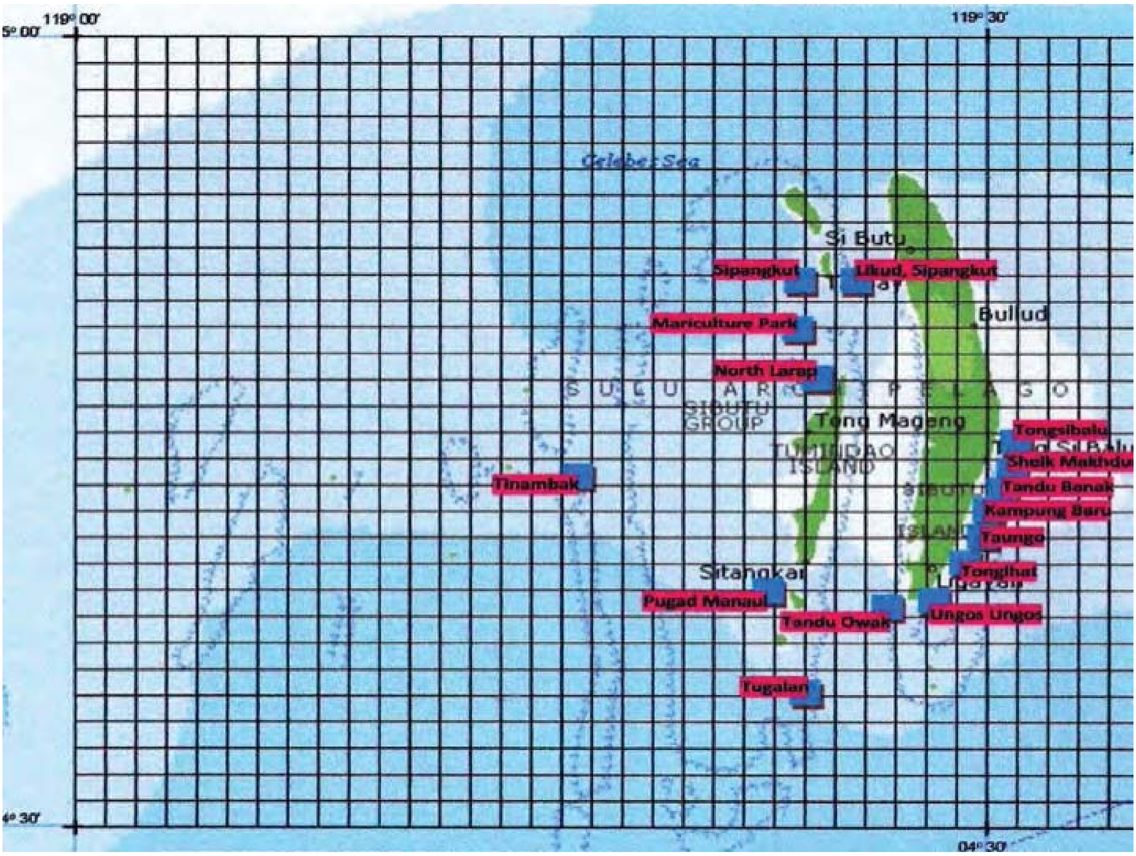


Figure 3. Location of the mariculture pens and cages.

Figure 4. Mariculture cages in Tandubanak Lagoon, and humphead wrasse species in grow-out cages.

### *Underwater Visual Surveys*

Table 1 shows the distribution and estimated abundance of the juveniles, males and females in the fifteen (15) study sites for a total of 45 transect lines. These sites were identified by local fishermen and traditional *bajau* fishermen who served as guides since they use these sites as their fishing ground for the *C. undulatus* species. Underwater surveys were conducted to document density and distribution of humphead wrasse and have an estimate of the current size of the population. The lunar phase was also noted and this is associated with the amount of catch. The fish density, expressed as the number of individuals seen in 1,000m$^2$ area, ranges from a low 0.006/1000m$^2$ to a high of 0.027 /1000m$^2$ and an average of 0.016/1,000m$^2$. These results confirm the behavior of this species to be solitary and confined to specific areas in the reef. This also indicates high fishing pressure.

Table 1. Estimated distribution and abundance of *Cheilinus undulatus* juveniles, males and females and fish density/1,000 m$^2$.

| Name of Site | Abundance | Juveniles | Mature Females | Immature Females | Date | Lunar phase* | Fish Density (no./1,000m$^2$) |
|---|---|---|---|---|---|---|---|
| *Tando Owak* | 6 | 2 | 1 | 3 | 5/13/2012 | NM | 0.006 |
| *Tando Tukkih* | 23 | 21 | 1 | 1 | 7/25/2012 | NM | 0.023 |
| *Langkawet Cave* | 6 | 2 | 2 | 2 | 7/25/2012 | NM | 0.006 |
| *Tongehat luuran* | 27 | 24 | 0 | 3 | 7/25/2012 | NM | 0.027 |
| *Taoungo Luuran* | 33 | 31 | 0 | 2 | 7/25/2012 | NM | 0.033 |
| *Tahing* | 7 | 5 | 1 | 1 | 7/25/2012 | NM | 0.007 |
| *Pamilikan* | 11 | 8 | 1 | 2 | 7/25/2012 | NM | 0.011 |
| *Dungon-dungon* | 27 | 23 | 1 | 3 | 10/10/2012 | LQ | 0.027 |
| *Baligtang* | 23 | 21 | 0 | 2 | 10/13/2012 | LQ | 0.023 |
| *Last ball* | 9 | 9 | 0 | 0 | 10/14/2012 | LQ | 0.009 |
| *Buton, Sibutu* | 4 | 1 | 0 | 3 | 12/10/2012 | LQ | 0.004 |
| *Tong Bakaan, Sibutu* | 24 | 22 | 0 | 2 | 12/10/2012 | LQ | 0.024 |
| *Serunga, Sitangkai* | 23 | 23 | 0 | 2 | 3/3/2013 | NM | 0.023 |
| *Sebabbal, Sitangkai* | 10 | 9 | 0 | 1 | 3/3/2013 | NM | 0.01 |
| *Sebuggal, Sitangkai* | 12 | 12 | 0 | 0 | 3/3/2013 | NM | 0.012 |
| *Suwang maheya, Sibutu* | 13 | 13 | 0 | 0 | 3/3/2013 | NM | 0.013 |
| **Total** | **258** | **226** | **7** | **27** | | | |

From the Underwater Visual Census, there were 27 immature and 7 mature females encountered in the dives, although they were evenly distributed, very few males with only 27 individuals were encountered in the whole stretch of the dive surveys. However, there were more juveniles encountered especially in shallow reef areas or a mix of seagrass beds with corals in areas near what fishermen say are spawning aggregation areas. Figure 5 shows the underwater shots of the juvenile humphead wrasse associated with seagrass beds and branching corals. Likewise this shows an underwater shot of the immature and mature female wrasse.

During the sampling periods, the catch of the fishermen were monitored. The catch from hook-and-line and *lambunan* (fish pots) the tail length (TL) in centimeters and weight (kg) of every individual catch were measured. The biomass of the fish caught was also determined. The catch per unit of effort (CPUE) was also computed based on the total catch and the number of hours spent fishing, which is generally 6 hrs.

***Reproduction and Gonadal Assessment***

Anecdotal information from fishermen, behavioural observations and gonad samples provide a preliminary understanding of the reproductive biology of the humphead wrasse. Throughout the study, a total of 51 fish samples of various sizes ranging from 20 mm to 105 mm were collected from the catch of the fishermen.

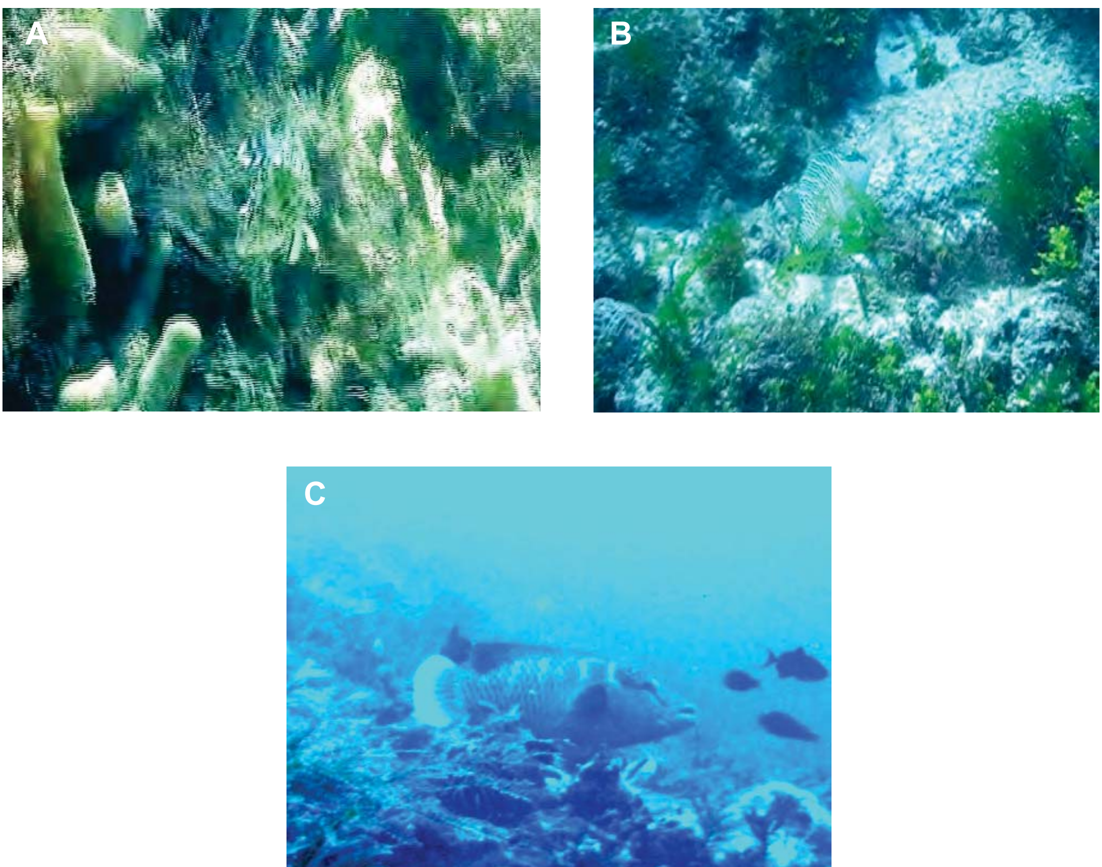


Figure 5. Pictures of juvenile (A), immature (B) and mature (C) female humphead wrasse *Cheilinus undulatus* taken during the underwater visual census.

The gonads were examined macroscopically, as shown in Figure 6, then under a stereo microscope in the laboratory. Inactive females had previtellogenic oocytes and the classification of gonads included immature and resting or developing females; the latter may or may not have spawned previously. Mature or ripe females were those with vitellogenic (i.e., yolky) or hydrated oocytes or with clear indications of spawning activity, such as post-ovulatory follicles. The presence of both features together in some individuals suggests that individual females spawn on multiple occasions during a reproductive season. Based on the Von Bertallanfy growth curve and the data on length frequency distribution, along with field observations that Napoleon fish do not reach maturity before 35 cm TL, the length at maturity was determined. Specifically, data on the proportion of fish larger than 35 cm that are mature by 5 cm size-class were used to estimate the parameters related to fecundity. The 51 female samples taken from the fishermen were in varying stages of maturity, from immature to mature but none had fully developed eggs. Thus, the egg count as a measure of fecundity could not be done. The smallest female (immature) sampled was 24 cm and the smallest mature, ripe female was 55 cm TL. The smallest immature male was 29.5 cm TL, while the smallest mature, ripe male was 55.0 cm TL. Few large fish were sampled, partly for practical and economic reasons (i.e., hard to catch or expensive), but also because they were rarely available; if larger fish are more likely to be male, then the apparent heavy female may create bias in our samples.

### *Options in the Management and Protection of Spawning Aggregation Sites*

Some of the management options for the conservation and protection of aggregating species include: declaration of spawning sites as marine protected area; temporary closure of spawning site to fishing; seasonal ban on fishing during spawning season and conventional management such as quota, and size limits, etc. throughout the year.

Figure 6. Macroscopic examination of the gonads of humphead wrasse. Mameng - 1.05 kg; gonad - 1.5 g.

Some of the indirect types of evidences of spawning aggregation sites from the landing sites include the number of gravid females, gonadosomatic index (GSI), and observed color changes in the fish species. As shown in Figure 7, out of the 299 individual fish species landed in Sitangkai, 134 or 45% were gravid females. These came mostly from the fishing grounds of Sipangkot and Tugalan and these recommended declaration of these areas as marine protected areas.

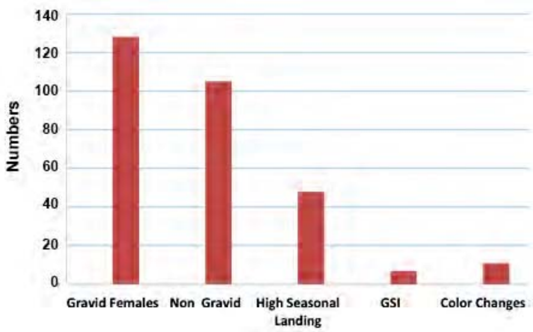


Figure 7. Indirect evidence of spawning aggregations from landed catch (n=299) in Sitangkai.

Similarly, indirect evidences such as the number of gravid females, gonadosomatic index (GSI), and observed color changes in the fish species were used in the selection of spawning aggregation sites from the landing sites in Sibutu. As shown in Figure 8, out of the 312 individual fish species landed in Sitangkai, 138 or 44% were gravid females and these came mostly from the fishing grounds of Dungun Dungon and Tando Owak. So these two sites as shown in Figure 10 were recommended for selection of spawning aggregation protected area.

Ripe adults are the capital and spawning aggregations produce interest (eggs) and enhance the wild stock. They are the source of fish for the future spawning aggregations that naturally generate larvae and juvenile humphead wrasse that would soon recruit to the fishery, contributing to food and

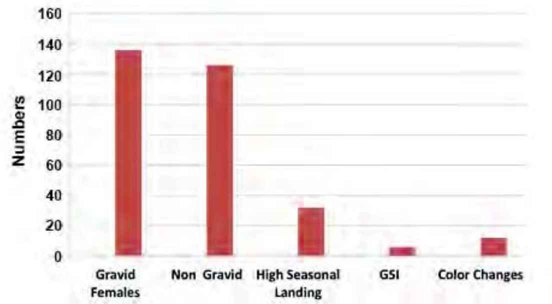


Figure 8. Indirect evidence of spawning aggregations from landed catch (n=312) in Sibutu.

livelihoods and increased income to the fishermen.

During this study, more of the anecdotal accounts from the fishermen especially the *Badjao* fishermen were relied upon in the identification of spawning aggregation sites. The initial sites identified in the KII were used in the underwater visual surveys in gathering the length weight frequency analysis and in the gonadal assessment of the levels of maturity of the humphead wrasse species. From the length and weight analysis of *mameng* species caught by hook and line and fish pot in the Sibutu Reef, there were 134 individuals caught from which the length at maturity of this species was estimated at 25 to 35cm. So potentially they would be spawning soon. In Sitangkai Reef, data from 243 individuals showed that TL of mature humphead wrasse were 26 to 35cm. Another indirect proof used was the underwater documentation of juvenile/small humphead wrasse which were regularly observed in association with seagrass beds and branching coral reefs in Tongehat Halo in Sibutu, Baligtang Reef in Sipangkot, Sitangkai. There were some accounts of juveniles found also in Tahing and Tando Owak Sibutu. The larval dispersion pattern was not determined in the study but concentrations of juveniles along the reef areas in Tongehat Halo, Sipangkot island indicate that they were spawned in nearby reef areas of Tando Owak and Baligtang Reef.

A series of stakeholder workshops were conducted with the communities to give feedback on the results of the *mameng* fishery study to the local government units, barangay officials, mariculturists, fishermen and traders. It also aims to discuss with these stakeholders the recommendations made in the study. This was also done to validate with the local government units, barangay officials, mariculturists, fishermen, traders the results of the spawning aggregation study; validate the identified spawning aggregation sites and the sites where the juveniles converge in the shallow coralline areas and seagrass beds; and to discuss the passage of an ordinance declaring these spawning aggregation sites as protected areas. All these information were presented to the communities in a series of stakeholder's workshops and the municipal council locally called Sangguniang Bayan, passed these ordinances. For Sibutu, the Municipal Ordinance No. 3 Series of 2013, An Ordinance Establishing *Mameng* Spawning Aggregations Marine Protected Area in Tando Owak, Dungun Dungon and Tong Bakkaan and Tahing Ungus Mataha in the Municipal Waters of Sibutu, Province of Tawi-Tawi, was passed providing management thereof, and for other purposes. For the municipality of Sitangkai, a similar ordinance was passed, Municipal Ordinance No. 3, Series of 2013, An Ordinance Establishing *Mameng* Spawning Aggregations Marine Protected Area in Sipangkot and Tugalan Reef Area in the Municipal Waters of Sitangkai, Province of Tawi-Tawi, providing management interventions thereof, and for other purposes. Figure 9 shows the locations of the Spawning Aggregation Marine Protected Areas.

The common provision of these ordinances defines the boundaries of the Spawning Aggregation MPA and had the following regulations:

- Prohibitions of all kinds and forms of illegal fishing, such as but not limited to the use of poisonous substances/chemicals (e.g. sodium cyanide and toxic plants), explosives (e.g. dynamites), and fine mesh nets, compressor fishing and other illegal forms of fishing as defined by the Muslim Mindanao Autonomy Act 1986 (MMAA86) and other existing local ordinances;
- Catching, gathering, collecting of berried and spawning fish specifically during the spawning period;
- Closure to any fishing activities during the spawning season of the humphead wrasse which is from January to April of every year; and
- Any other acts or activities that will destroy or will place/pose imminent danger or potential harm to the area are strictly prohibited, and other illegal activities as defined by MMAA86.

It also provided for the creation of a Management Board under the supervision of the Municipal LGU from among the representatives from municipal and barangay LGU, FARMC, academe, youth/women and religious groups in close coordination with the Philippine National Police (PNP) Maritime Group, PNP, DA-BFAR and other enforcement units.

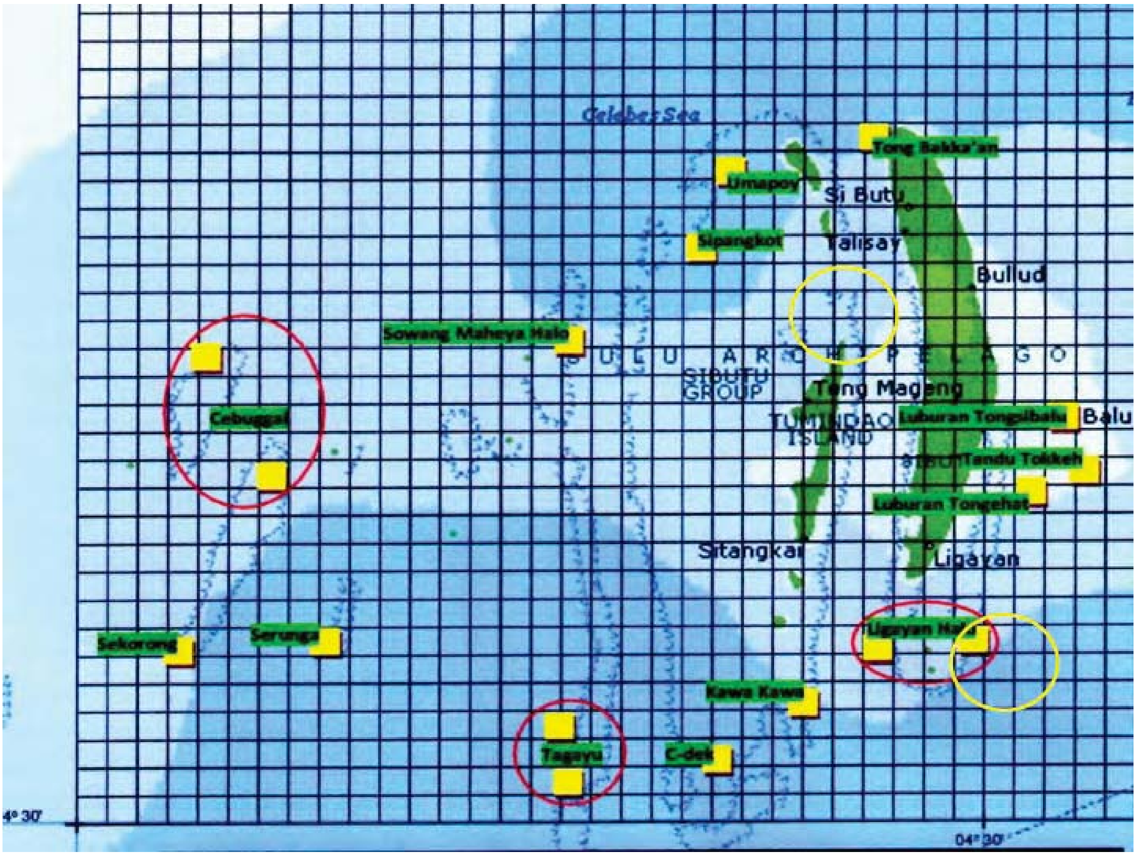


Figure 9. Spawning aggregation recommended for declaration as protected areas based on indirect evidences in Sitangkai and Sibutu.

The Council among others is responsible for management, protection, conservation, and development as well as overseeing the operations of the marine protected area. Within 90 days from the approval of this ordinance, the Management Council shall formulate its internal rules and regulations governing its operations and management. An MPA management Plan has been adopted. Subsequently, the Coastal Law Enforcement Training, Creation of the Municipal Coastal Law Enforcement Team, Training of the *Bantay Dagat* (Sea Guards), and the Development of the Mariculture areas in Sibutu and Sitangkai as Community-Managed Areas were some of the follow-up activities.

### *Summary and Conclusions*

Using both anecdotal accounts and indirect evidences, this assessment has provided sufficient information for the communities in Sitangkai and Sibutu in Tawi-Tawi province. Consequently, these municipalities were able to decide on the declaration of the Spawning Aggregation Sites in Tando Owak, Dungun Dungon and Tong Bakkaan and Tahing Ungus Mataha in the Sibutu, Tawi-Tawi, and in Sipangkot and Tugalan Reef Area in Sitangkai, Tawi-Tawi. This also provided management interventions and regulations for a Marine Protected Area, a strategy for enhancement of wild stocks and for ensuring that the health of the stock would be sustained, so as to continue to provide source of sustainable livelihood for the people of these island communities.

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# Stock Assessment of Christian Crabs (*Charybdis feriatus*, Linnaeus, 1758) in San Miguel Bay

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## *Abstract*

Assessment of the status of swimming crab fisheries in San Miguel Bay with focus on Christian or Crucifix crab, *Charybdis feriatus*, was undertaken from November 2011 to January 2013. An analytical length-based fish stock assessment was employed using the FISAT (version 1.2.2). A total of 7,679 length frequencies (3,612 *C. feriatus* and 4,067 *Portunus pelagicus*) were used in the analysis. About 15 and 14 percent gravid females were harvested monthly for both species that may contribute to recruitment overfishing. Population parameters showed exploitation rate (E) for *P. pelagicus* and *C. feriatus* exceeded the optimum exploitation (E0.5) implying excessive fishing effort and heavily exploited stocks. Size at maturity of *C. feriatus* and *P. pelagicus* in San Miguel Bay is 8.3 cm and 8.5 cm, respectively. Doable options for resources conservation and management strategies are proposed and supported by local government units (LGUs) including the Integrated Fisheries and Aquatic Resource Management Council.

**Keywords:** stock assessment, San Miguel Bay, resource conservation and management, *Charybdis feriatus*

## *Introduction*

San Miguel Bay (SMB) is a large fishing ground in the southeastern part of Luzon and northeastern part of the Bicol Region along the Pacific Coast. The bay is bounded in the west by the coastal municipalities of Mercedes and Basud of Camarines Norte province and in the southeast, by the coastal province of Camarines Sur with 5 municipalities: Calabanga, Cabusao, Tinambac, Sipocot and Siruma. It is enclosed in the north by an imaginary line drawn from Butauanan Island of Siruma, Camarines Sur to Canimog Island and Poblacion of Mercedes, Camarines Norte (Figure 1).

SMB has a total surface area of 1,115 sq. km. and a coastline length of approximately 240 km. The inner bay and its outer periphery (Mercedes and Siruma area) are consistently and heavily utilized by various fisheries with about 7,033 fishers. This translates to about 1 fisher per 34 m of coastline or 1 fisher per 16 ha of water. About 67 percent of the fishers are from Mercedes, Calabanga and Siruma, 27 percent from Tinambac and Cabusao and the rest from Sipocot and Basud. Thus, fishing is a way of life and human activities associated with aquatic resources are basically fishing and fishing-associated economic activities.


Figure 1. Map of San Miguel Bay (Source: Google Earth).

The fishery in the bay is generally multi-species, and fishers utilize multiple gears predominantly gill-net (32.8%), hook and lines (31.0%), fish traps (7.8%), seine nets (7.8%), squid jiggers (6.7%) and the rest are other gears. Based on post-RSA of SMB gear inventory, there are 6,712 gear units distributed to 33 distinct gear types where 66 percent of these gears are distributed in Siruma, Mercedes and Calabanga, 29 percent in Tinambac and Cabusao and 5 percent in Sipocot and Basud. The 10 most numerous gears include ordinary gill net, crab gill net, shrimp gill net, mini-trawl, fish gill net, hook and line, push net, long line, spear gun and filter nets which account to about 80 percent of the total number of gears in SMB.

Catch composition in SMB consists of more than 250 aquatic species distributed in 70 families; the most diverse are the scads, mackerels, slip mouths, sardines and herrings, and crabs. There is seasonal variation in catch composition and a large proportion of the catch using trawl is jellyfish. In terms of stock status, 14 of the 17 species caught are exploited below the size at first sexual maturity and 11 species are exploited at sizes below 10 cm. Current estimates of the stock density of demersal fish in the SMB decreased 60 fold since 1947. Similarly, the present stock density is about 11 times less than it was 9 years ago. In terms of the changes in the number of fishing gears used over time, post-RSA result showed an increase by 40 percent between 1980 and 1992 and by 41 percent between 1992 and 2001. The largest increases were recorded for mini-trawlers and gill nets.

For the period 1992-1995, the average municipal production was about 11,103 mt, with baby trawl contributing 9.2 percent. Several assessments (BFAR-FSP, REA SMB) indicate that the Bay is heavily exploited or, even, overfished with catch rates significantly declining. In Calabanga, the volumes and sizes of major catch showed a decreasing trend particularly in gill net, fish corral and trawl. The main catch composition includes blue crabs, croakers, anchovies, and shrimps. Similar decreasing trend was also noted in Mercedes.

In addition, resource use conflicts are also impacting the fisheries in the SMB. These include: (1) access issues in fisheries and other coastal resource; (2) enforcement issues in fisheries and other coastal resources; (3) conflict between the fishery users (small vs. large scale fishers); (4) conflict between the fishers and other resource users (i.e. tourism vs. conservation vs. industrial development) and (5) conflict between fishers and non-fishery issues (i.e. corruption, politics).

Access issues in fisheries and other coastal resources are clearly defined by the continuously increasing number of fishers and gears. This made the entire bay exploited with an overlap in the use patterns of multiplicity of gears, hence the need for resource enhancement. Destructive fishing methods (i.e. use of fine mesh net, cyanide fishing, and dynamite fishing) and capture of endangered species are among the enforcement issues. Conflict between the fishery users are better explained by the intrusion of other municipal fishers and the incursion of commercial fishers in municipal fishing grounds. Entry of some fishers in Marine Protected Areas (MPA) and the illegal conversion of mangrove areas into brackishwater fishponds are examples of conflicting uses. The rapid development of tourism along the coastal zones and the construction of ports also created conflict of varying degrees. Noteworthy to mention is the observation that in municipalities bordering SMB, fishers and other stakeholders openly blame corruption and politics as one of the most critical problems associated with conflicts between fishers and non-fishery issues.

Today, fishing in SMB still represents the largest extractive use of aquatic resources with demand exceeding the supply. As a matter of fact, during the *"Strengthening Governance and Sustainability of Small-scale Fisheries Management in the Philippines: An Ecosystem-based Fisheries Management Approach Seminar-workshop"* held in the Agri-Science Village, San Bernardino, Calabanga, Camarines Sur on February 21-22, 2011 and attended by representatives from the seven local government units (LGU's) bordering SMB, all are in agreement that the system of resource extraction is expected to continue as the population increases in the next ten years and fisheries and aquaculture will be crucial sources of income and livelihood for hundreds of thousands of people in the SMB. The trading and export of high value species like live grouper, wrasses and crustaceans is now considered a lucrative business among fishers because of the booming export market for these species. For instance, Christian crab (*Charybdis feriatus*) is exported mostly in East Asia where it commands a premium price of US$8 to US$15/kg. As such, the intensity of fishing for high-value species at all cost has put immense pressure on the wild population which when kept unregulated may lead to over-fishing and resource depletion. Unfortunately, very little work has been carried out to manage the wild stock. Given the unregulated resource extraction coupled by the alarming impacts of climate change, the fate of this species is extremely threatened.

Considering its importance and value, it is imperative that efforts toward the development of resource enhancement measures to sustain an appropriate number of parent stock and/or breeder to maintain its population, is wanting. The Project Christian crab is an attempt to provide solutions or key strategies for resource enhancement to sustain the future of natural stocks. The output derived from the project is expected to provide doable options to enhance natural stocks for sustainable resource conservation and management and possibly the development of aquaculture technology for the species in the future. This will likewise assist decision-makers and planners in the respective LGU's in SMB to formulate management strategies for this highly valuable resource at sustained levels. This in turn would mean long-term fishing livelihood for fishermen. Without proactive moves to domesticate and propagate this crustacean species, Christian crabs will be highly vulnerable and the chance of its wild population to collapse is not far from reality. In addition, the impacts of climate change will put immense pressure, stress and ecological/environmental modification that can threaten the resources systems and their habitat, which in turn might cause species modifications, biodiversity loss and eventual extinction. Acting now to save the species while it is still manageable is an urgent growing need to focus on because of the vital contribution of the fishery to poverty alleviation and food security.

***Objectives***

The purpose of this project was primarily to assess the status of swimming crab fisheries, particularly *Charybdis feriatus*, Linnaeus, 1758 in the SMB with the hope of helping fishers and LGUs formulate an implementable crab fishery resource conservation and management strategy. Specifically, the project sought to:

1. Assess the status of Christian crab (*Charybdis feriatus*) fisheries in the SMB in terms of:
    - population structure;
    - growth and mortality by species;
    - fishing gears used;
    - annual production and species composition;
    - catch per unit effort (CPUE) and exploitation rates by species; and
    - existing market and channels of distribution.
2. Develop a sustainable and implementable Christian crab fishery resource conservation and management strategy.

***Materials and Methods***

Monthly measurements of about 300 specimens per species were conducted using vernier caliper (SE 781BC Stainless Steel, 0.1 cm) from November 2011 to October 2012. Carapace width (CW) was measured as the distance from tip to tip of the last antero-lateral teeth. Carapace length (CL) was measured as the distance from the tip of the frontal teeth to the posterior end of the carapace. Total body weight (TW) was measured using digital weighing scale (Ohauz, Model CL 501T, capacity 500 g x 0.1 g). Almost all length-frequency measures were from landings and market measurements in Tinambac, Calabanga, and Cabusao in Camarines Sur and Mercedes in Camarines Norte.

Descriptive statistics and analysis of length frequencies such as normality test and generation of total length frequency distributions (histograms with constant class interval) were done using a commercial spreadsheet program. The analytical length-based fish stock assessment was employed using the FAO ICLARM Stock Assessment Tools (version 1.2.2). Several studies were already reported documenting the usage of the method on invertebrates (see Soliman and Dioneda (1998), Ingles and Bkaum (1989) for blue crab and Dineshbabu (2011) for *Charybdis feriatus*). The length-weight relationship was estimated using the equation: $W = aL^b$, where **W** is the weight, **a** is the intercept, **L** is carapace width and **b** is the slope (Pauly, 1983). The value of **a** and **b** were computed from the log transformed values of length and weight. The co-efficient of determination ($r^2$) was used as an indicator of the quality of the linear regression.

## Results and Discussion

### Population Structure

Three species of swimming crabs were identified as: *Charybdis feriatus*, *Portunus pelagicus*, *Portunus sanguinolentus*. Two mud crab species, namely, *Scylla oceanica* (?), and *Scylla serrata* were also noted. Genus *Thalamita* which are usually collected by gleaners were also observed in stocks sold in the local market. However, the study focused on *Charybdis feriatus* and *Portunus pelagicus*.

A total of 7,679 marine crabs were collected (Figure 2) comprising of 3,612 *C. feriatus* and 4,067 *P. pelagicus* with mean CW of (mean±s.d.) 12.50±2.20 cm and 11.20±3.00 cm, respectively. This result is higher than the maturity size of 8.30 cm and 10.50 cm. However, the capture of 15 percent gravid *C. feriatus* and 14 percent *P. pelagicus* from the monthly samples could be a factor contributory to recruitment overfishing. This means that the adult population was caught heavily that the number and size of the spawning biomass has been reduced to the point that it will not have the reproductive capacity to replenish the fishery. On the other hand, the mean weight (MW) obtained showed 375±207 grams for *C. feriatus* and 77.6±41.5 grams for *P. pelagicus*.

Regression equations for the carapace width-weight relationship of *P. pelagicus* and *C. feriatus* revealed high correlation (Table 2). The exponent 'b' value estimated for these species were below 3 indicating the allometric pattern of growth.

### Growth and Mortality

Population parameters of the stock extracted using the FiSAT (version 3.2) are presented in Table 3. The ELEFAN-1 (Electronic Length Frequency Analysis) program estimated asymptotic length ($L\infty$) and growth co-efficient (K) of the Von Bertalanffy Growth Formula (VBGF) for *C. feriatus* and *P. pelagicus* were noted at 26.76 cm (0.63 $year^{-1}$) and 21.36 cm (0.87 year-1), respectively. The resulting exploitation rate (E) for *P. pelagicus* and *C. feriatus* show overexploitation (E>0.50) of the species (Gulland, 1971). Also, the resulting E optima from the Y'-PR (Yield-per-Recruit) indicates that exploitation rate of *C. feriatus* (35%) and *P. pelagicus* (10%) exceeded beyond the optimum exploitation ($E_{0.5}$), implying excess fishing effort. This finding affirms the assessments result obtained during the Fishery Sector Program (FSP) and SMB-Resource and Ecological Assessment (REA) which indicated that the fishery resources in the Bay have been heavily exploited or overfished with catch

Figure 2. Main species, *Portunus pelagicus* (left) and *Charybdis feriatus*, exploited in San Miguel Bay.

Table 2. Carapace width-weight relationship of *P. pelagicus* and *C. feriatus* in San Miguel Bay.

| Crab | Sex | Carapace Width-Weight Equation | $R^2$ |
|---|---|---|---|
| *Portunus pelagicus* | Male | $W=1.17*CW^{2.91}$ | 0.80 |
| *Portunus pelagicus* | Female | $W=1.25*CW^{2.97}$ | 0.83 |
| Mean | | $\mathbf{W=1.21*CW^{2.94}}$ | **0.82** |
| *Charybdis feriata* | Male | $W=0.59*CW^{2.83}$ | 0.79 |
| *Charybdis feriata* | Female | $W=0.49*CW^{2.73}$ | 0.79 |
| Mean | | $\mathbf{W=0.54*CW^{2.78}}$ | **0.79** |

Table 3. Population parameters of *C. feriatus* and *P. pelagicus* studied.

| species | $L\infty$ (cm) | K (/yr) | Z (/yr) | M (/yr) | F (/yr) | $E_{curr}$ (/yr) | $L_{50}$ (cm) | Sampling period |
|---|---|---|---|---|---|---|---|---|
| *Charybdis feriata* | 26.76 | 0.63 | 3.70 | 1.41 | 2.29 | 0.62 | 9.62 | Dec.2011-Nov.2012 |
| *Portunus pelagicus* | 21.36 | 0.87 | 4.66 | 1.85 | 2.81 | 0.60 | 10.53 | Nov.2011-Oct.2012 |

rates significantly declining. Ingles and Flores (2000) also reported that fishing effort of swimming crabs has been well above sustainable levels since 1999.

The $LC_{50}$ statistics which is the population length at which 50% of the population is harvested (the other 50% remains) when subjected to fisheries showed a computed $LC_{50}$ = 9.62 cm for *C. feriatus* and 10.53 cm for *P. pelagicus*. These values are slightly higher compared to the size at first maturity of 8.3 cm and 10.50 cm for *C. feriatus* and *P. pelagicus*, respectively. However, this may also contribute to recruitment overfishing wherein the adult population was caught so heavily that the number and size of the adult population (spawning biomass) has been reduced to the point that it will not have the reproductive capacity to replenish the fishery.

### *Fishing gears, CPUE, Annual Production and Species Composition*

Crabbing is a multi-gear fishery (Ingles, 2004). In Tinambac, Calabanga, and Cabusao, Camarines Sur, the majority of the gears used are crab gillnet or CGN (~220 units). In Mercedes, Camarines Norte, crab pots (CP= ~59 units) are commonly used. CGN locally known as "hikot" has an average length of 1,000 meters (~40 *banata*, 25 meters/*banata*) and a mesh size of 5 cm, wherein baits are not needed. Soaking duration per set is 15-16 hours. Meanwhile, CP locally known as "bobo" is an enticing device with a length of 69 centimeters, 30 centimeters width and a depth of 18 centimeters. The frame is made of 8 mm steel bar with bamboo attachment to form the pot and polyethylene webbings of 3 centimeters mesh size with an opening at both ends. The steel bar served as sinkers of the pot. An average of 160 pots connected with a main line is being deployed per fishing boat. Trash fish are used as baits. Setting and hauling of the gear usually last for 16 to 36 hours. It is mostly recommended as environmental and social-friendly gear because it allows the fishermen to return gravid and juvenile crabs to the sea which encourage sustainable fishing. In contrast, the CGN is a non-selective fishing method that does not allow for the return to sea of juvenile crabs that have not yet reached sexual maturity or gravid crabs that have not yet spawned.

The average catch for CGN is 7.25±2.41 kg/trip (CPUE=0.48) and 11.09±7.93 kg/trip (CPUE=0.69) for CP. Highest catch for CP was observed during December (CPUE=2.11), whereas in CGN in February and September (CPUE=0.63) (Figure 4). Capture of immature crabs was observed as a factor to growth overfishing (Ingles, 2004). Noticeably, about 1 – 7 percent (Figure 5) of the *C. feriatus* are captured undersized (CW=<8.3 cm.). The catch composition for CGN consists of 58 - 99 percent P. pelagicus, 1-24 percent *C. feriatus*, and 1 - 23 percent by-catch (Table 5). On the other hand, catch composition for CP is 48 - 92 percent *C. feriatus*, 21 - 27 percent *P. pelagicus* (Figure 6b), and 8 - 25 percent by-catch. Baby trawl fishing gears were also noted operating in Cabusao and Mercedes. An annual production of 524.90 mt of crabs was estimated in the SMB (Table 4). The production constitutes 3.11 percent of the 16,879 mt fisheries annual productions in SMB (Soliman and Dioneda, 1997). Catches in the Philippines have been around 34,000 mt in recent years and evidence has shown the abundance of swimming crabs has declined (www.fao.org).

Table 4. Estimated annual production in San Miguel Bay (metric ton).

| Area | Crab gill net | Crab pot | total |
|---|---|---|---|
| Tinambak (Sogod) | 134.78 | 10.80 | 145.58 |
| Calabanga (Sabang) | 154.43 | 1.14 | 155.57 |
| Cabusao (Castillo) | 121.50 | - | 121.50 |
| Mercedes (Manguisoc & Mambungalon) | - | 102.24 | 102.24 |
| total | 410.72 | 114.18 | **524.90** |

Figure 3. Gears used in crab fishing (left: crab gill net; right: crab pot).

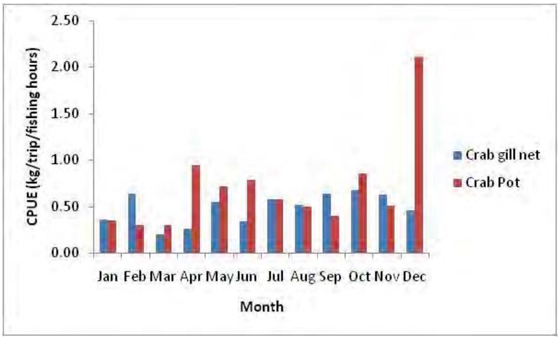


Figure 4. Monthly CPUE (crab gill net and crab pot) in San Miguel Bay.

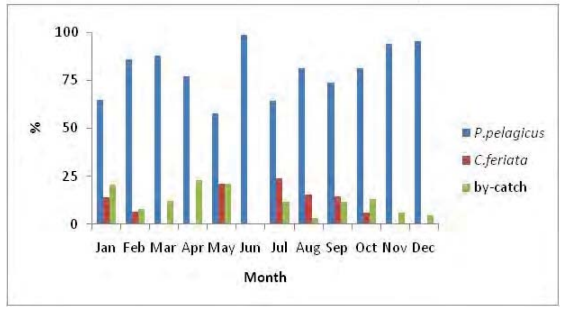


Figure 6a. Catch compositions caught by crab gillnet in San Miguel Bay.

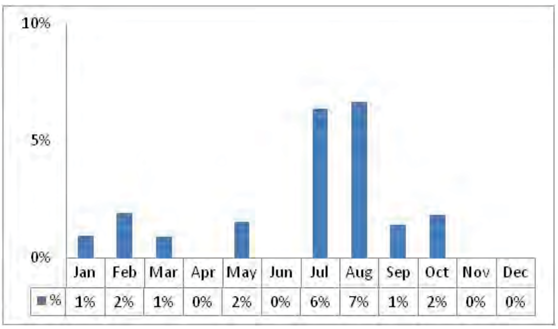


Figure 5. Percentage catch of “undersized” (<8.3 cm CW or CL) *C. feriatus* in San Miguel Bay.

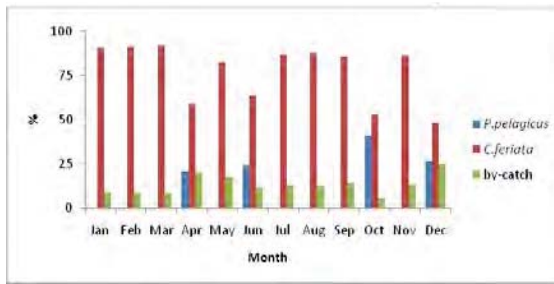


Figure 6b. Catch compositions caught by crab pot in San Miguel Bay.

Table 5. Catch composition of other species (by-catch) using crab gillnets and crab pot in San Miguel Bay.

| Common Name | Family | Catch Composition (%) Crab Gillnet | Crab Pot |
|---|---|---|---|
| Croaker | *Scianidae* | 7.65 | |
| Catfish | *Arridae* | 0.46 | |
| Grouper | *Serranidae* | 0.15 | 11.11 |
| Scad | *Carangidae* | 1.68 | |
| Trevally | *Carangidae* | 2.45 | |
| Mullet | *Mugilidae* | 0.15 | |
| Catfish | *Plotosidae* | 2.45 | |
| Sillago | *Sillaginidae* | 1.99 | |
| Toungefish | *Cynoglossidae* | 25.08 | |
| Spider conch | *Strombidae* | 0.76 | |
| Cockle | *Arcidae* | 3.06 | |
| Mantis shrimp | *Gonodactylidae* | 52.75 | 74.08 |
| Shrimp | *Penaeidae* | 0.15 | 14.81 |
| Prawn | *Penaeidae* | 0.76 | |
| Mudcrab | *Portunidae* | 0.46 | |
| | Total | 100 | 100 |

## *Market and Channel of Distribution*

Marketing and distribution in SMB is characterized by competitive market structure where there are many sellers and buyers with free trading of aquatic products. The most common practice is from crab fishers to traders ("*factorador*") or local crab meat processors ("*beneficiador*"). According to key informants, these are delivered to Manila or Cebu for domestic market or exported to China, Hongkong and Taiwan as live crabs, fresh frozen or processed crab meat. Another market player is the broker who sells crab to the trader or street/market vendor ("*regaton*"). The "regaton" sells directly to the consumers in their places (Figure 7). The number of crab fishers, *factorador*, and *beneficiador* are presented in Table 6.

Crab pot fishers usually sell 75 percent of their catch to "*factorador*", 15 percent to "*beneficiador*" and 10 percent to "regaton". On the other hand, crab gill net fishers sold 90 percent of their catch to "*beneficiador*" and the remaining 10 percent to "*factorador*"/"*regaton*". Selling price of crabs sold to "*beneficiador*" ranges from Php100.00 to Php 120.00/kg whereas rejected crabs (e.g. molting, small sizes, no longer alive, low weight, pinchers removed, etc.) are either consumed for food or brought to "*factorador*"/"*regaton*" and sold at Php 50.00. For live crabs, prices range from Php 300.00 to as high as Php 2,000.00/kg depending on the size and species of crab.

"*Suki*" system is a prevalent practice among fishers in SMB. "*Suki*" is the local term used to refer to a special relationship between two individuals wherein one provides credit for fishing input or family expenses in exchange to the exclusive right to purchase their catch at a lower price. In terms of market structure, aside from having a competitive market, there are barriers to fish trading business, which include legal (i.e. license, permit, taxes, limited operation of brokers), financial (i.e. lack of capital, limited credit and high interest rates) and market information limited to the middlemen.

Table 6. Number of crab fisher, factorador, and beneficiador in sampling areas.

| Area | Crab fisher | Factorador | Beneficiador |
|---|---|---|---|
| Tinambak (Sogod) | 152 | - | 3 |
| Calabanga (Sabang) | 198 | - | 3 |
| Cabusao (Castillo) | 250 | 10 | 15 |
| Mercedes (Manguisoc & Mambungalon) | 165 | 3 | - |

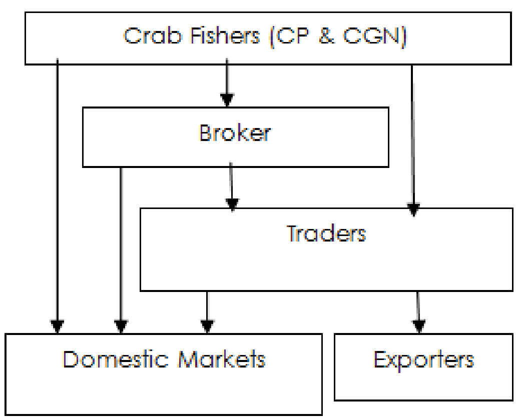


Figure 7. Marketing channels for swimming crabs in San Miguel Bay.

### *Sustainable and Implementable Christian Crab Fishery Resource Conservation and Management Strategy*

To promote the idea of crab resource conservation and management using the recent biological research findings as basis, the project co-sponsored an action planning meeting designed to update SMB-LGU's Coastal Resource Management Plans (CRMP) held February 16, 2012 at Regent Hotel, Naga City which was attended by 42 representatives. Among the major agreements were the inclusion of crab fisheries in the CRMP and the total banning of active fishing gear in SMB to sustain crab fisheries particularly *C. feriatus* and other swimming crabs.

A set of resource management options based on the biological and fisheries information generated were also prepared and presented during the *"Stakeholders Consultation and Presentation of Resource Enhancement Plan for Marine Crab in San Miguel Bay"* held at BFAR-RFFC, Bula, Camarines Sur last January 30, 2013 attended by DOST-V, BFAR-V, IFARMC and LGU's officials bordering SMB. The same option was presented and deliberated during the Integrated Fisheries and Aquatic Resource Management Council (IFAMRC) of SMB meeting held at Mercedes School of Fisheries last January 18, 2013 where all LGUs in SMB was represented.

From these exercises, it appears that scientific findings may not necessarily be the best solution should one consider the socio-economic implications of such option to livelihood and food security of the people. While poverty cannot certainly be used as an excuse for sound judgment, at least the sentiments of resource users are considered prior to decision-making and implementation. For instance, considering the reduction of fishing effort proportional to excess exploitation level through mesh size regulation (use of >12 cm stretched mesh size), catch regulation (minimum of 9 cm for *C. feriatus* and 11cm for *P. pelagicus*, based on size at maturity) and regulating fishing intensity (e.g. shifting to deeper ground, reduced fishing time, etc., LGU representatives and fisherfolks argued that such option is not realistically acceptable to fisherfolks since it will affect their only means of livelihood. In addition, regulating mesh size will not work because the crabs are entangled to the net beside that will entail procurement of another fishing gear to comply; making it very difficult for resource-poor fisherfolk. Regulating fishing intensity by shifting to deeper ground is also risky and expensive in terms of fuel cost.

It is interesting to note that LGUs and fisherfolk groups expressed unanimous acceptance to the declaration of a closed season as an option during periods of peak reproductive activity for *C. feriatus* particularly in December and January where higher percentages of GSI, mature, and gravid crabs were observed. Apparently, the underlying reason for agreement follows that the months of December and January coincide with rough sea and occurrence of bad weather making fishing risky; hence, for the fisherfolk, closed season does not matter much since they have nothing to lose. On the other hand, for the LGUs, there will be less opposition making it easier to pass a resolution for a close season but it does not make sense in as much as the decision is merely based on convenience instead of scientific basis most appropriate to the resource.

Table 7. List of management options and its implications to science and socio-economics of crab resource management in San Miguel Bay.

| Management option (Research-based options) | Scientific implication (Based-on R&D Findings) | Socio-economic implications (LGU-Fisher's perspectives) |
|---|---|---|
| 1. Reduction of fishing effort proportional to excess exploitation level. | | |
| a. mesh size regulation (use of >12 cm stretched mesh size) | Better chances for mature crabs to breed at least during their size at 1st maturity (*C. feriatus* - 8.3 cm and *P. pelagicus* - 10.50 cm) | Would mean a reduction of 14% and 15% from the catch and income. Besides, the claws may still be entangled even with stretched mesh. |
| b. catch regulation (minimum of 9 cm for *C. feriatus* and 11cm for *P. pelagicus*, based on size at maturity) | Better chances for mature crabs to breed at least during their size at 1st maturity (*C. feriatus* - 8.3 cm and *P. pelagicus* - 10.50 cm) | Would mean a reduction of 14% and 15% from the catch of fishers and income but at least no deprivation of the fisher's livelihood |
| c. regulating fishing intensity (e.g. shifting to deeper ground, lessen fishing time, etc. | Lessen fishing pressure on wild population | Unacceptable to fishers as it implies increased cost of fuel, longer fishing time and more risky |
| d. Closed season during periods of peak reproductive activity for *C. feriatus* particularly in December and January where higher percentage of gonado-somatic index or GSI, mature, and gravid crabs were observed. | Does not have much impact on the resource but 14% and 15% gravid female crabs are given the chance to propagate without much fishing pressure | Acceptable to fishers since at this point in time fishing is not favorable due to bad weather. Fishers have nothing to lose after all it has been a practice. |
| 2. No extraction of egg-bearing swimming crabs. Gravid crabs caught will be released in fishing ground immediately after caught. | Better chances of breeding biomass to reproduce to sustain the fishery | Unacceptable to fishers as they perceived it as a reduction to their CPUE and income |
| 3. Egg-bearing swimming crabs will be held in a spawning tank and monitored until it hatches. Larvae will be returned in fishing ground, or in marine protected areas. | No scientific evidence of success but releasing larvae in fishing ground or in MPA's is a sound resource conservation practice | Unacceptable to fishers as they perceived it as reduction to their CPUE and income. The practice may soon be a habit for resource conservation and management; Doable and acceptable to LGU's in collaboration with the buyers |
| 4. Increase the number of protected areas and widen the functional and well-managed protected areas in the bay. | Very essential not only to swimming but other species as nursery, feeding, and spawning grounds. | More MPAs more "no fishing zone" from the fisher's perspective |
| 5. Diversification of alternative livelihood, especially those not related to fisheries. | Will lessen fishing pressure | Acceptable to fishers with very few success stories |

Another accepted option is the holding of egg-bearing crabs in a spawning tank to spawn and the larvae released in fishing ground or MPAs. Unfortunately, no scientific evidence relative to its success is available but the practice may eventually be the basis for future sound management practice for responsible fisheries.
From the economic perspectives, the above options can be explained by simply understanding the fact that catching the breeders or small crabs is not an efficient way of using the resource rather it will just cause its depletion. A case in point is that female *C. feriatus* with CW from 8.3 cm-15 cm has a fecundity of about 1,513,660 to 6,357,133 eggs. Assuming that only 10 percent of these eggs hatched and survived to juvenile stage, some 151,366 to 635,713 juveniles may be produced. Assuming further that only 1.0 percent reach marketable size, 1,513 to 6,357 crabs is expected per female. At 8 pieces per kg, 189.13kgs to 792.13kgs of crabs can potentially be harvested. Catching gravid female crab therefore represents an economic loss of approximately Php 56.739.00 – Php 237,639.00 at a selling price of Php 300.00 per kg or Php 226,956.00 to Php 950,556.00 at a live weight price of Php 1,200 per kg. Moreover, from the reported annual production of 524.90 MT of crabs in SMB, 5.25- 126 mt are *C. feriatus* (1-24 percent), from these, 15 percent are gravid females which mean some 0.7875- 18.9 mt are not given the chance to reproduce and replenish natural population, hence may lead to resource depletion.

The last identified option is the diversification of alternative livelihood, especially those that are non-fisheries related. This is has been the usual quick answer to most coastal resource management project (CRMP), unfortunately with stories of few successes and more failures as experienced in many CRMP in the country.

Finally, a follow-up collaborative research implemented with the concerned LGUs, fisherfolks and IFARMC to translate the option into realistic action plans and that outcomes are monitored, evaluated and scaled-up for replication in other areas.

### *Conclusion and Recommendation*

Population parameters using the FiSAT (version 3.2) showed exploitation rate (E) for *P. pelagicus* and *C. feriatus* exceeded the optimum exploitation (E0.5) implying excessive fishing effort or heavily exploited fisheries. Harvest data also showed 15 percent and 14 percent of the catch are gravid *C. feriatus* and *P. pelagicus* which indicates potential recruitment overfishing. Doable options for resources conservation and management strategies supported by LGU's in SMB including the IFARMC include (1) declaration of closed seasons during periods of peak reproductive activity for *C. feriatus* particularly in December and January where higher percentages of GSI, mature, and gravid crabs were observed , (2) the collection of egg-bearing swimming crabs which will be held in a spawning tank, hatched and the larvae returned in fishing ground or in marine protected areas (MPA) and (3) diversification of alternative livelihood, especially those non-fisheries related. It is recommended that a follow-up research effort be implemented collaboratively with the concern LGU's and IFARMC to ensure that the options identified were translated into realistic action plans and that outcomes are monitored and evaluated.

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# Abalone Aquaculture for Stock Enhancement and Community Livelihood Project in Northern Palawan, Philippines

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## *Abstract*

One of the interventions to feed the poorest of the poor fisheries sector in the country is the provision of livelihood in the form of mariculture of high value marine species. In the Philippines, livelihood in rural areas is largely linked to resource depletion, hence it is wise not only to provide livelihood to the community but also to encourage them to conserve and enhance the resources. As part of the revised R&D program, the Western Philippines University partnered with NGO and existing projects to embark on a community-based environment-concerned livelihood project, using hatchery bred abalone, although top shell was also considered for stock enhancement. This is in an on-going project thus, preliminary phases such as abalone production and cage-based grow-out as well as subsequent project plans will be discussed. The objectives of this study were to: (a) share the implementing experiences in this project, (b) identify success and failure drivers of the project, (c) explain the conceptual framework for the MPA-based stock enhancement to be used in this project, and (d) give recommendations to improve the implementation and ensure the success of the project.

The following activities have thus far been conducted: (a) development of criteria for cage micro-site selection; (b) writing of proposal and provision of financial assistance for hatchery juvenile production through a partnership MOA; (c) presentation of site survey results to beneficiaries and stake holders; (d) conduct of trainings on abalone grow –out culture to POs; (e) development and improvement of training module; (f) signing of conservation agreement; (g) giving of cage materials and juveniles to people's organizations; (h) on site coaching; and (i) partial monitoring. The next activities include improvement in juvenile production, conduct of researches on abalone nutrition, and development of market and value chain flow analysis. The conceptual framework for community-managed stock enhancement will follow that of the Department of Environment and Natural Resources-ICRMP, of which the stock enhancement project is anchored on the management of marine protected areas or MPAs.

The steps in all the activities were documented and while the project was in progress, performance of the participants in training were measured, the training module was improved, the training approaches were revised according to needs, and the growth and survival of juvenile abalone were monitored. The problems identified were low production of juveniles, insufficient food for grow-out, political squabbles, social preparation, and delay in implementation schedule. Recommendations to improve or resolve the problems encountered were also presented in this paper.

**Keywords:** abalone, community-managed stock enhancement, training

### Introduction

It is common knowledge that fishermen are the poorest in this country, followed by farmers and children. Thus, aside from the Western Philippines University's (WPU) academic concerns, the University's R and D plan (2014-2018), focuses on fishermen and farmers of Palawan as primary beneficiaries.

One of the interventions to feed the poorest of the poor among the coastal fisheries sector in the country is to provide them livelihood in the form of culture of high value marine species. Success through this mode is manifested in the fact that their engagement in seaweed farming has given them supplemental incomes. However, shell fish culture can be more economically viable and stable. Moreover, apart from this being a promising mariculture product for fisherfolk, such commodities are more resilient to diseases and climate change.

The abalone *Haliotis asinina* called locally as "*sobra-sobra*" or "*kapinan*" has become a popular marine commodity both to fishermen and marine product traders for the past decades. This is primarily due to its high market price and demand. It costs 350 pesos per kilogram in the local market of Palawan, 400 pesos in the Southeast Asian Fisheries Development Center (SEAFDEC) and as high as 3,000 pesos per serving at restaurants in Ongpin, Metro Manila. Abalone meat is known as a delicacy served in prestigious restaurants and hotels in Asia. It has a soft delicious taste, while the shell is used in many decorative items and ornaments. The demand for this shell has become so great that it was overextracted from the wild, resulting to a situation where the abalone's natural process of reproduction could not cope up with the stock's decline. Thus, breeding local abalone is one of the strategies pursued to conserve the species and/or population(s).

Abalone is number three in the priority list of marine stock enhancement species for the Philippines (Gonzales, 2005). In Palawan, coral reefs have been devastated by gleaners and shell collectors turning coral reefs "upside down" in search for abalones. The abalone population has remarkably dropped, in a manner that fishermen have been pressured not to do massive collections since 2012. According to traders Palawan's production of wild abalone has drastically decreased from 100 metric tons in 1997 to 2 metric tons in 2011 (Pagliawan, pers. comm.), (Figure 1).

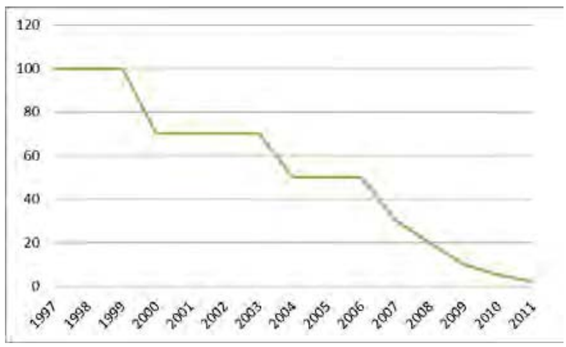


Figure 1. Production (tons) of wild abalone in Palawan for 15 years (1997-2011).

The graph suggests that Palawan alone can produce 100 tons of abalone a year, while in 2008, only 200 mt was exported from the country which was less than half of the average annual production between 1900 and 2005 (Baobao and Roslinda, 2013). Hongkong was said to be the largest buyer of abalone meat. The decrease in production was attributed to over harvest of the wild population of abalone and destruction of their habitats.

Dwindling wild abalone populations can be restored through stock enhancement. The barangay-based stock enhancement has been tested in Honda Bay (Gonzales *et al.*, 2006). In the Philippines, livelihood in rural areas is sometimes linked to resource depletion, it is wise not only to provide livelihood to the community, but also to encourage them to conserve and enhance the resources. Stock enhancement is a coastal and fisheries management tool to increase and sustain the biomass of certain population, especially in cases where the population is depleted or over exploited. Stock enhancement is also sometimes referred to as restocking (Gonzales, 2005). It is a recommendable follow through project for Marine Protected Areas (MPAs).

***Why Abalone?***

Since abalone has high market demand both locally and internationally, it has become a potential source of livelihood for fish farmers. In addition, the presence of an abalone hatchery facility and the breeding technology capability in WPU ensures the continuous production of abalones. This shall likewise make the propagation and survival of the wild species amidst threats from human activities.

Abalone reared in cages is a viable livelihood for coastal fishers. The survival and growth of abalone shells will not require costly feeding by the farmers since it is fed with algae from the wild, opening a window for another source of income for the fishermen, which is seaweed production.

Cage culture of abalones is essentially producing organic abalones which could support the food security apart from the prospects of presenting this as a delicacy for tourists.

Abalones graze on algae attached on the surface of dead corals. Dead corals devoid of algae have greater chances of recovery since new coral polyps could readily attach and grow on clean surfaces of the dead reefs. Additionally, abalone is a good candidate for stock enhancement since it is less mobile, and could readily be introduced to the other island provinces of Region 4B (MIMAROPA).

Abalone mariculture projects could readily involve women as well as indigenous peoples with great potential for project partnerships with the private sector (Private Public Partnership).

This paper presents the on-going experiences in the implementation of the community-managed abalone project. As such, the objectives of this study are to:

1. identify problems encountered by the project,
2. explain the conceptual framework for the MPA-based stock enhancement, and
3. give recommendations to improve the implementation and success of the project

***Materials and Methods***

***Main Project Activities:***

Fund sourcing - WPU entered into a Memorandum of Agreement on the implementation of the abalone project with the Malampaya Foundation Incorporated (MFI) that funded part of the production expenses, training and grow-out of abalone. WPU also partnered with the LGUs and POs for their cage grow-out culture. Other MOAs between POs, LGUs, and MFI were forged regarding environmental conservation, while pursuing livelihood projects.

Site selection – Since northern Palawan is a heavily fished area, the situation affects the income of fishermen, thus northern Palawan coastal communities were given priority by the project. The other criteria for selection of project sites were the suitability of the site for high abalone survival, growth and dispersal apart fromthe successful reproduction of abalone in the area. Considering the priorities of the partner agency (MFI) to serve the communities along the Malampaya gas project, sites selected were Coron, Culion, Linapacan, El Nido, and Taytay.. Social preparations of the beneficiaries were done by NGOs and LGUs. Orientation of the project was done during the site survey in the area. The survey covered interviews with fishermen/coastal dwellers regarding species and locations for mariculture, stock enhancement, and or marine protected areas (MPAs). A combination of key focal person and focus group discussion interviews were conducted on site as the situation permits.

Presentation of site survey results to beneficiaries and stakeholders - Results of the survey were presented in a forum in Barangay sessions, PAMB, and municipal SB sessions (Figure 2).


Figure 2. Survey team facilitating mapping and zoning of Brgy. Bulalacao MPA at Bulalacao Barangay Hall.

Figure 3. Training on construction of floating cage for abalone culture conducted in Linapacan, Palawan from September 16-21, 2013.

***Training on cage construction and grow-out culture to communities*** *(Figure. 3)*

Two SEAFDEC lecturers in abalone aquaculture were invited by WPU to conduct enhancement training and sharetechnologies on abalone cage culture with WPU faculty members and technicians from April 12 to13, 2013. Subsequently, WPU trainors conducted a series of training for community members in selected project sites in 2013 (Figure 3). Training sessions were conducted in three mariculture sites, two in Taytay; and one in Linapacan. Around 30 trainees were trained per site. The main part of the training was the construction of baskets/cage and a demonstration on how-to grow the abalone in cages, to marketable size. Participants were also taught how to pack, transport, and market their abalone products. In addition, participants were trained to manage their income from the project.

The selection of the trainees was based on the following criteria:

1. must be an identified beneficiary of the project,
2. must be a legitimate residence of target municipality/barangay.
3. MFI coordinators in identified municipalities,
4. Academic institution on-site (if qualified)


Figure 4. MOA signing on environment conservation among LGUs, POs, and MFI.

The primary objective of the community training was to provide the participants basic knowledge on CRM and how to carry-out abalone mariculture livelihood. At the end of the course, the participants will be able to:

1. describe the objectives, principles, components, and dynamics of CRM;
2. demonstrate the construction operation and maintenance of a floating cage, and
3. explain the grow-out system of the abalone culture.

The Course had the following main modules: Module I -Introduction to CRM; Module II- Biology, ecology, and hatchery of abalone; Module III-Grow-out of abalone; Module IV-Floating cage construction, Cage buoyancy, Sinker/anchorage; Module V-Harvest, package, and transport of products, and Module VI-Marketing and book keeping.

## *Results and Discussion*

### *Monitoring of abalone growth and survival in cages*

The monitoring of abalone growth and survival for the project was erratic. Some of the fishers were not able to properly monitor the abalone in cages. They depended on WPU and its project staff during monitoring. Hence, although abalones were raised in cages in August 2013 the first organized monitoring was done in November 2013.

**Breeders from the wild:** As of 29 January 2014, a total of 40 breeders were gathered from the wild. These were placed in a floating cage in Canique, Taytay, Palawan in August 2013. No mortality among the shells was observed until January 2014. Shell length ranged from 46 to 90 mm and the weight ranged from 10 to 149 grams. The average shell length of the abalone was 73 mm, while the average weight was 87.5 grams. No gonad was observed in the abalones during the January 2014 sampling.

With their sizes which are over the ideal spawning size of 50-60 mm shell length, these individuals may not spawn anymore or have poor spawning performance. It is recommended that they be sold and the proceeds used to buy younger adult individuals with more spawning vigor.

**Juveniles from WPU:** The shell length of juveniles sampled in August 2013 ranged from 18-28 mm. After five months of rearing in cages, the length ranged from 33 to 46 mm (Figure 5). The average shell length of WPU juvenile abalones increased from 23.3 in August 2013 to 39.4 mm in January 2014. The average shell length of juveniles had an increment of 16.13 mm within five months of culture, having a growth rate of 3.25 mm per month. Among 15 individuals, one male (37.5 mm shell length) and one female (37.0 mm shell length) were with ripe gonads. The average weight of the juveniles was 13.3 grams.

Juveniles in basket number 2 consisted of 80 three-month old individuals with shell lengths ranging from 10 to 21 mm. with an average of 13.9 mm in shell length. These abalones were deployed for grow-out in Pamantolon on January 28, 2014.The average weight of each shell was 1.25 grams.

**SEAFDEC juveniles:** The shell lengths of juveniles deployed in November 2013 ranged from 8.5 to 18.5 mm, while after

Figure 5. WPU abalone, above and SEAFDEC abalone, below; showing the original size (white line) from deployment till January 2014. Note the meat of abalones above and below.

two months of rearing in cage the length ranged from 15.9 to 33.5 mm in January 2014. The average shell length of SEAFDEC juvenile abalones increased from 14.5 mm in November 2013 to 25.0 mm in January 2014. The average shell length of juveniles has an increment of 10.54 mm within two months of culture, having a growth rate of 5.27 mm per month. However, the average weight of the juveniles was only 3.7 grams, much lower than WPU abalones. Mortality was 10 individuals.

**General information:** SEAFDEC abalones have faster growth rate (5.27 mm/ month) than WPU abalones (3.25 mm/ month). However, the meat of juvenile abalones from SEAFDEC was lean/slim compared to WPU's. SEAFDEC abalones have less meat with relatively larger shell, while WPU has relatively more meat. This may be because SEAFDEC abalones are still in the process of adjusting with its environment and food since they came from Guimaras Island and ate different species of algae. However, the genetic profile/ pool of the respective abalone populations should be investigated.

The next monitoring should be in March 2014. Since the newly delivered abalones are the smallest in size, size grading should be done

***Conceptual Framework for MPA-based Stock Enhancement Initiative***

Stock enhancement is a coastal and fisheries management tool to increase and sustain the biomass of certain populations, especially in cases where the population is depleted or over exploited. Stock enhancement is also sometimes referred to as restocking (Gonzales, 2005). It is a recommendable follow through project for Marine Protected Areas (MPAs). It answers the question on what happens after the establishment of MPA and MPA network. Most of the coastal management projects usually conclude with the enforcement of MPAs and network laws.

Stock enhancement focuses on the restoration of species and resources in primary coastal habitats like mangrove swamps, seagrass beds, coral reef, etc., which were overexploited. It is a proactive conservation effort wherein while waiting for the spillover effect of MPA core zone, community members may be given specific areas for stock enhancement/livelihood (Figure 6), which they can manage, harvest, and derive income from. This way, while protecting and enhancing biodiversity, POs can have a quicker benefit from MPA.

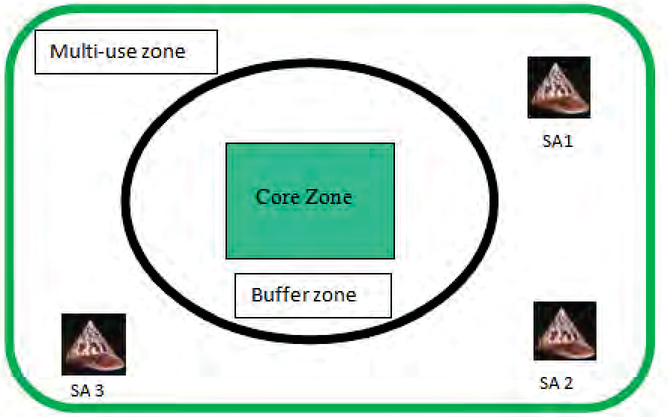


Figure 6. MPA with zoning, showing potential areas for community livelihood/stock enhancement (SA) in the multi-use zone.

After MPAs were established and zoned, the time lag between the establishment and the spill-over effect of protection becomes a challenge to MPA managers and community implementers, particularly in enforcing MPA rules and regulations. This concept aims at hastening the enhancement and restoration of depleted resources in primary coastal habitats. It is a proactive conservation effort that keeps MPA implementers and supporters actively and continuously involved while waiting for the spillover effect from the MPA core zone.

Since MPA-based stock enhancement will restore marine resources faster, it is more attractive to community members because they expect it to bring quicker and more direct benefits to them. This intervention is relatively unique to ICM in the sense that it is seldom applied as an integral part of MPA management.

Considering the importance of resource conservation project/livelihood to MPAs, a guide was developed on how to plan, implement, and manage resource conservation/stock enhancement/livelihood projects in MPAs. The document brings directions on how to proceed with MPA – based resource conservation projects/stock enhancement, including criteria for project proposal evaluation in selecting and screening proposals from communities.

***Summary of benefits from the MPA-based stock enhancement approach***

1. It can be a follow through project for MPAs and its network;
2. It aims to restore depleted population of target species in identified coastal habitat (e.g., coral, reef, seagrass beds, mangrove swamps), and at the same time extend livelihood to communities;
3. It helps in promoting greater impact to the communities by having their own area of restocking, while waiting for the MPA spill-over effect. Restocking will be done inside and outside MPAs, as well as identified areas for restocking to be managed and owned by POs or Family Groups.
4. It can respond to issues and problems indicated in the ICM or MPA plans. (less income, open access, capability building, etc.)

5. It provides a potential solution to low income, poaching, open access, limited management, and technical capability, identified in many ICM and MPA initiatives, and
6. It protects the habitat while enhancing the stock and generate income.

### *Problems Encountered*

1. Limited production of juvenile abalone;
2. Inconsistent food supply for cage grow-out;
3. No IEC materials;
4. Weak marketing strategy; and
5. Weak monitoring

### *Conclusion*

Although equipped with hatchery, technology, manpower, there were outstanding technological problems particularly in juvenile production and food consistency in the grow-out. There were unexpected problems that have emerged in the process. With the above concerns, the project is still hopeful to continue, by attempting to provide solutions to the identified problems.

### *Recommendations to Problems Encountered*

1. Limited production of juvenile abalone
   a. Improve spawning performance of abalone breeders;
   b. Explore/study spawners from other areas, especially from Taytay Bay and Liminangcong;
   c. Seek assistance from SEAFDEC;
   d. Build capability of technicians. Training in SEAFDEC (include on-site trainings); and
   e. Expand and improve the facilities and human resources.
2. Inconsistent food supply for cage grow-out
   a. Conduct experiments/ studies on Gracilaria culture;
   b. Resource mapping of brood stock and algal food from the wild;
   c. Conduct characterization of host habitat to potential algal food for juvenile shells; and
   d. Investigate the discrepancies in growth rate of SEAFDEC and WPU juveniles. Consult SEAFDEC.
3. No IEC materials
   a. Produce/print IEC materials (Training Manual and Livelihood brochure); and
   b. Compendium of potential wild algal food for abalone grow-out system.
4. Weak packaging/processing technology identified
   a. Identify potential packaging/ processing for the product.
5. Weak marketing strategy
   a. Develop value chain analysis for abalone from Palawan.
6. Weak monitoring
   a. Continued skill development to fish farmers.

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# Social Preparations Towards Community-based Approach to Stock Enhancement in Sagay Marine Reserve, Philippines

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## *Abstract*

Stock enhancement involves a set of management approaches which include the release of hatchery-produced aquatic organisms to enhance or restore fisheries. Stock enhancement of various species has a long history in developed countries and it showed that releases have the potential to yield substantial benefits for various fishery stakeholders. While the biological objectives of stock enhancement were often successfully achieved in most of these enhancement initiatives, some results showed that actual social gains in terms of yields, distribution of benefits and institutional sustainability are often inconclusive. The high cost of stocking accrues to the government which means these are supported by public funds. Meanwhile, benefits are dissipated across various stakeholders, some of them did not at all contribute and participate in the stocking program. In such government-initiated and publicly-funded stock enhancement programs, the lack of sense of stewardship among direct fishery stakeholders was observed to have contributed to a vicious cycle of excessive extraction of fishery resources for individual economic benefits.

Developing countries such as the Philippines would be confronted by budgetary limitations if it has to adopt the stocking strategies applied in developed countries. Thus, with reference to the success of co-management approaches for managing fishery resources in the Philippines, a community-based strategy for enhancement of fishery stocks was explored. SEAFDEC/AQD, with support from the Government of Japan Trust Fund, initiated a community-based approach to stock enhancement in Molocaboc, an island *barangay* or village within the Sagay Marine Reserve (SMR). The initiative aims to ensure that its goals and strategies are within the social milieu of local stakeholders, i.e. fisherfolks are without financial assets to contribute or pay for the enhancement of the fishery and stock enhancement is often not a priority approach to address fishery resource depletion for most local governments. However, the social assets of fishing communities could be explored to implement stock enhancement. This paper describes the social preparation executed from 2007 to 2011 in order to orient a fishing community such as Molocaboc towards a successful enhancement of overfished species. Initially, the project focused on donkey's ear abalone *Haliotis asinina* to provide an example for other species. Abalone or '*kapinan*' in the vernacular is one of the over-extracted fishery resources in Sagay City. It is one of the high-priced catch among fishers in coastal communities in the Philippines. High buying prices compared with other fish catch motivated small-scale fishers to target abalones and caused its overfishing.

**Keywords:** co-management approaches, fishery resources, Philippines, social preparation

### Introduction

Stock enhancement and restocking are potential measures that could either reduce the time needed to rebuild certain capture fisheries to a more productive level, or increase the productivity of some 'healthy' fisheries (Bell *et al.*, 2006). Stock enhancement primarily involves the release of cultured juveniles into wild population to augment the natural supply of juveniles from wild spawners as well as mature stocks derived from releases of hatchery-reared juveniles and optimize harvests by overcoming recruitment limitation (Bell *et al.*, 2008). Restocking, on one hand, is the process of restoring the spawning biomass of severely depleted populations of fish and shellfish to a level where they can once again provide regular, substantial yields (Bell *et al.*, 2005). Purcell (2004 as cited by Bell *et al.*, 2006) noted that restocking could also be achieved by aggregating and relocating adults of some species. Sea ranching is also an alternative way to increase productivity from fisheries habitats, wherein, animals are released for harvest at a larger size. Both stock enhancement and restocking are likely to be effective for some coastal invertebrate fisheries because the shallow inshore distribution and sedentary behavior of the species involved can create self-replenishing populations on a relatively small spatial scale (Bell *et al.*, 2005).

Stock enhancement programs were initiated as a fisheries management option in developed countries where governments prioritize and allocated funds for research and its actual implementation. The small-scale enhancement trials being done in the Philippines cannot compare with the magnitude of the releases in developed countries. These enhancement initiatives in developed countries could provide fundamental principles, lessons and motivations for adopting a fishery resource enhancement strategy for a developing country like the Philippines. For example, Japan stocked scallops *Patinopecten yessoensis* (Uki, 2006) and flounder *Paralichthys olivaceus* (Kitada and Kishino, 2006; Tomiyama *et al.*, 2008); China on shrimp *Penaeus chinensis* (Wang *et al.*, 2006); United States on red drum *Sciaenops ocellatus* (Leber, 2004); Australia on barramundi *Lates calcarifer* (Loneragan *et al.*, 2013) and Norway on lobster *Homarus gammarus* (Tveite and Grimsen, 1995 cited in Bell *et al.*, 2005).

Community-based strategies were applied in some of the abovementioned large-scale stock enhancement programs. In the stocking of one million hatchery-reared Japanese flounder juveniles annually since 1996 in Fukushima Prefecture in Japan (Tomiyama *et al.*, 2008), fishers pay 5% of their landings to fund the community-based enhancement program. Fishers also consented not to catch flounders <30 cm total length to sustain the economic gains from investments in stock enhancement. However, the economic benefits from the program declined due to reduced recapture rates and lower market prices of fish. In spite of negative net economic benefits, the stock enhancement program cannot be easily terminated because it has effectively demonstrated the importance of fishery management to fishers. Thus, it is a concern on how fishery stocking programs can be made flexible and effective in order to optimize either economic or social benefits. More recently, the approach to marine stock enhancement further emphasized the need for an integrated view of the role of enhancement within fisheries management systems, noting the stakeholder participatory and scientific approach (Lorenzen *et al.*, 2010).

In the stocking of scallops in Hokkaido, Japan, the success of stocking was attributed to the presence of cooperatives tasked to plan and implement effective use of fishery resources, aside from the fulfillment of biophysical factors such as ideal habitat, effective methods and sedentary attribute of scallops. Some of the activities developed by the fisheries cooperatives are: a) fixed levy-rate on the value of scallops harvested by each fisherman to generate funds to support future fishery management efforts; b) monitoring the attributes of the scallop population (e.g. density and survival of juveniles, and growth-rate of sub-adults in the rotational fishing area); c) production of a specified number of juveniles for release by the members of the cooperative; and d) training courses for new members on the production and harvesting of scallops must be completed before new members start fishing (Masuda and Tsukamoto *et al.*, 1998; Drummond, 2004, as cited by Bell *et al.*, 2006).

The stocking of shrimp *Penaeus chinensis* in China in 1991 yielded cost-benefit ratios up to 1:9 (Wang *et al.*, 2006). These economic benefits were largely due to substantial support from the central government and facilitated by an easy access to the existing aquaculture infrastructure. Relevant local governments also required the beneficiaries of release programs to contribute to the costs of releasing hatchery-reared juveniles. However, the implementation of the user-pay systems have been difficult in some regions such as in Bohai Sea because of the large area covered and the complexity of multi-provincial and multi-level administrations. In other smaller regions such as in Haiyangdao and Qinghai, the identification of the beneficiaries and the collection of appropriate fees to cover the cost of stock enhancement were easier. Hence, the study concluded that rebuilding the wild shrimp fishery by implementing conventional management measures supplemented by stock enhancement may present a more cost-effective approach to increasing prawn production than aquaculture because the yield from farming of shrimp is achieved with a huge consumption of resources and at a great cost.

In the Philippines, there were initial efforts in 1996 to 2001 to re-establish the spawning populations of sea urchins in Bolinao in Pangasinan by exploring complimentary outcomes of private investments in the grow-out culture of sea urchins *Tripneustes gratilla* in sea cages and restocking of hatchery-produced juveniles in unprotected seagrass areas (Juinio-Meñez *et al.*, 2008). As a follow-up activity in 2004 to 2006, higher population densities, higher incidence of recruits and steady increase in the catch per unit effort of gatherers showed that the grow-out culture in cages combined with sustained efforts of the fishing community to protect and implement harvest regulation to ensure steady recovery of the wild population proved to be a cost-effective strategy for re-establishing effective spawning populations. The cage culture operators obtained income from sale of harvested sea urchins, while those without cages benefitted from gleaning sea urchins from spill-overs.

In early 2000, stock enhancement of mollusks was identified to be one of the priority research areas at SEAFDEC/AQD to complement emerging coastal resources management initiatives that will secure food and livelihoods in fishing communities. In

2005, SEAFDEC/AQD, with funds from the Government of Japan-Trust Fund (GOJ-TF), started to collaborate with the Protected Area Management Board-Sagay Marine Reserve (PAMB-SMR) of the City of Sagay in Negros Occidental to implement a research program on *Resource enhancement of internationally threatened and over-exploited species in Southeast Asia through stock release*. These species include giant clam *Tridacna gigas*, abalone *Haliotis asinina*, sandfish *Holuthuria scabra*, and sea horse *Hippocampus* spp. The program included a study on the socioeconomic analysis of stock enhancement of threatened marine species in SMR, initially with a focus on abalone because seed production and nursery techniques are already developed at SEAFDEC/AQD (Capinpin *et al.*, 1999), studies on release strategies have commenced in SMR (Gallardo *et al.*, 2003; Lebata-Ramos *et al.*, 2013), and economic benefits for fisherfolks can be attained from trade of these species.

This paper describes the social preparations executed from 2007 to 2011 in order to orient a fishing community such as the remote island barangay of Molocaboc towards a successful stock enhancement of abalones in areas within the SMR. A community-based approach to stock enhancement is proposed to be executed and tested in this project considering the social milieu of the stakeholders in the stock enhancement of abalone, i.e. stakeholders have limited financial resources to contribute to funds for seed release programs, reluctance of local governments to prioritize and allocated public funds for stock enhancement, and the need to improve income and access to food among fisherfolk faced with depleted resources.

***Methods***

The Sagay Marine Reserve was selected to host the study on stock enhancement of threatened and over-exploited species since there is abalone fishery in the area, its catch is dwindling, and the recovery of enhanced stocks would benefit from and perform better in protected environment (Maliao *et al.*, 2004). The key activities under this study are: 1) reconnaissance and baseline socioeconomic survey of the SMR and its fishing communities in cooperation with the SMR staff; 2) capacity-building for fisherfolk-stakeholders through information, education and communication (IEC); 3) strengthening of fisherfolk organization through formation of Barangay Fisheries and Aquatic Resources Management Council (BFARMC); and 4) establishment of a community-based stock enhancement demonstration site.

To provide basis for detailed project planning, the baseline survey of 60 fishers was implemented in selected six (6) coastal barangays and two (2) island barangays in February 2007. The respondents were chosen through stratified proportionate random sampling based on number and distribution of fishers in coastal barangays in Sagay City. The outcomes of the survey led to the selection of and focus on Barangay Molocaboc for the implementation of subsequent action-oriented activities intended to identify strategies for managing released stocks. The IEC activities were conducted in Barangay Molocaboc and in other relevant areas in Sagay City mostly in 2008. Networking with and identifying the roles of various stakeholders in stock enhancement such as fishers, traders, LGU officers at barangay and city levels, local academe and other relevant people's organization were consensually determined through a series of meetings in 2009. Fisherfolks were organized into BFARMC in November 2010. Capacity building relevant to stock enhancement were implemented by training ten (10) fishermen-members of the BFARMC on abalone and sandfish seed production and nursery techniques at SEAFDEC/AQD in Tigbauan, Iloilo in 2011. Finally, the community-based stock enhancement demonstration site for abalone and sandfish was established in the intertidal flats of Molocaboc Dacu upon consensus among fisherfolks represented by BFARMC, Barangay Molocaboc LGU, PAMB-SMR and SEAFDEC/AQD.

## *Results and Discussions*

Social preparations to introduce, inform and condition stakeholders towards active participation in community-based stock enhancement of abalone and sandfish as a fishery resource management strategy were conducted in Barangay Molocaboc from 2007 to 2011. The social preparation activities conducted in the study site are categorized as: 1) reconnaissance and baseline socioeconomic survey of fisherfolks; 2) conduct of information, education and communication activities; 3) formation and strengthening of fisherfolk organization; and 4) establishment of the community-based stock enhancement demonstration site.

### *1. Reconnaissance and baseline socioeconomics survey*

The Sagay Marine Reserve 10.90°N, 123.42°E covers 32,000ha of coastal waters north of Sagay City mainland. It encompasses protected reef areas not habited by human population, namely; Carbin, Panal and Maca Reef. It also includes the island Barangay of Molocaboc with a population of 4,189 residing in three component islands called Molocaboc Dacu, Diut and Matabas (Figure 1). Of the 25 barangays of Sagay City, a baseline survey of 80 fishers was conducted in February 2007 in selected coastal barangays, namely Bulanon, Himoga-an Baybay, Old Sagay, Taba-ao, Vito and Poblacion; and in the island barangay of Molocaboc. The survey respondents reported about the declining catch of all species, i.e. 1 to 20kg in 1995, to the present catch at less than 5kg per 1 to 6-hour fishing trip. More so, there is increasing fishing pressure due to the worsening economic situation among the growing number of population in fishing communities within the SMR.

The survey respondents noted that fishery resources used to be abundant and that most fishers noted to have been part of the irresponsible exploitation of these resources. They recalled the declaration of the SMR in 1995 which have changed their fishing activities and areas because of the imposition of rules and regulations to protect the vast area of Sagay's coastal waters. There have been changes in target fishing grounds reported by fishers during the survey and prior to the declaration of the SMR. In spite of the protection, fishers reported continuing decline in catch due to a combination of fishing pressure. Only 17% of the survey respondents in 2007 were aware of stock enhancement as a fisheries management option. The fishers welcomed the concept when explained during the survey but were oblivious of the mechanisms on how and who will implement stock enhancement in Molocaboc. The survey showed the need to inform the stakeholders on the principles, importance, stocking strategies, present trade-offs and future benefits from stock

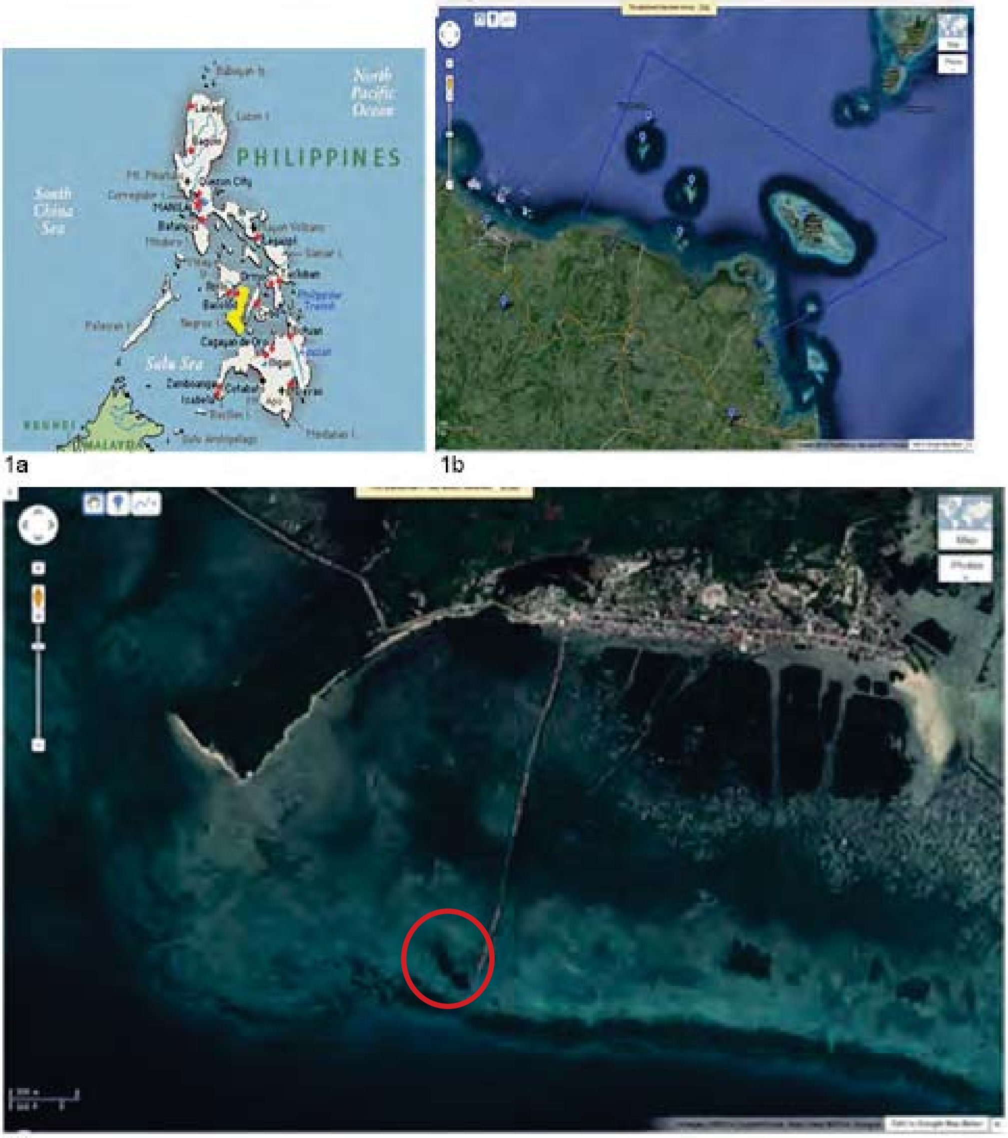


Figure 1a. Map of the Philippines showing the location of Negros Occidental province (marked yellow) in central Philippines; 1b. Aerial view of Sagay Marine Reserve demarcated by blue line in northern part of Sagay City in Negros Occidental; and 1c. Aerial view of Barangay Molocaboc showing the area of the community-based stock enhancement demo-site on the coral patch on left side at end of the 2-km footwalk.

enhancement in order to obtain the cooperation and participation of fishers and other stakeholders towards a successful stock enhancement initiative.

A multi-stakeholder consultation activity called *Participatory Action Plan Development (PAPD)* was conducted on 13 May 2008 to complement the baseline survey in February 2007. The PAPD enabled 41 stakeholder-participants categorized as fishers, local government or barangay officers, women gleaners, stockers/traders, and fishers to come-up with a community resource map, priority list of problems and corresponding solutions relevant to Sagay fisheries and the proposed collaboration on community-based stock enhancement. The PAPD outcomes enabled stakeholders to analyze their problems that potentially relate to stock enhancement initiatives in SMR. The PAPD process identified problems that were categorized as environmental, economic and socio-political. The most recognized environmental problems are: 1) flood and rising sea level experienced by the island dwellers during calamities, and 2) destruction and declining catch in intertidal flats due to mining of shells for handicrafts. Economic problems are: 1) low income from fishing due to declining catch, and 2) lack of market outlet and capital among handicraft makers. Meanwhile, the socio-political problems are about the limitations associated poor implementation of existing fishing rules and regulations that prohibit the use of illegal fishing gears such as compressor, fine mesh nets, triple nets and cyanide fishing. The PAPD results showed the fisherfolks in Molocaboc as confronted by declining catch and deteriorating economic incentives for fishing. However, fishers lack skills and opportunities to shift jobs away from fishing; and stock enhancement was perceived to offer solutions to their problems. Moreover, the local socio-political support to implement stock enhancement is limited and benefits are often attained after substantial periodic lags. Therefore, fisherfolks requested for other livelihoods to be developed to generate income for fishers affected by regulations to support stock enhancement.

In view of the potential of ecotourism as source of alternative livelihood in fishing communities, the fisherfolks were introduced to a seminar on ecotourism initiatives and opportunities in Sagay on 13 May 2008 in coordination with the Sagay City Tourism Desk. The seminar introduced ecotourism concepts and presented the Sagay City webpage to create awareness among 47 fisherfolk-stakeholders on non-resource extractive service-oriented livelihood options that may benefit local fishers. A tourist survey was also conducted in 2007 to understand the profile of visitors that will provide basis for designing and promoting ecotourism programs involving fishers. The survey showed that the top sites to visit are Cabin Reef and local resorts with preferred recreation activities such as swimming (27% of respondents), strolling in the beach (26%) and eating seafood (15%). The awareness levels of the tourist-respondents on abalone (13%) and giant clam (42%) are, however, low in comparison with corals (91%), star fish (73%), sea horse (61%) and sea cucumber (36%). The presence of these species in the waters of Sagay elicited interest among tourist, either as species to see in diving or snorkeling sites or as food in local restaurants for non-prohibited marketable species.

### *2. Information, education and communication (IEC)*

In response to the outcomes of the baseline survey which showed low levels of awareness and understanding of stock enhancement of abalone among stakeholders, IEC activities were continuously conducted in Barangay Molocaboc and other relevant areas in Sagay City. Information seminars with resource persons from SEAFDEC/AQD were held in March 2008 on the biology of abalone and giant clams, which were prioritized for stock enhancement. The seminar informed the fisherfolks and SMR personnel on abalone seed production and grow-out technology, and on the progress and significance of the giant clam stock enhancement experiments of SEAFDEC/AQD. In particular, the seminar informed the fishers on the life cycle and growth stages of these species with emphasis on the various implications of some fishing practices on recruitment and growth of fish stocks. The fishers were made aware of the spawning and larval development of donkey's ear abalone based on SEAFDEC/AQD research results (Capinpin *et al.*, 1998). Following several stakeholder consultations, an ordinance that regulates the harvesting, consumption and trade of abalones was proposed and promulgated on June 2010 by the Barangay Council of Molocaboc. The project stakeholders adhere that the ordinances, in particular the abalone catch size regulated to be >6cm shell length, will allow replenishment and promote sustainable fisheries. It is also expected to compliment the forthcoming project on abalone stock release. IEC activities also included the preparation of posters about coastal fisheries management, compliance to fishing regulations and the importance on stock enhancement in SMR. These posters were displayed and explained in local fairs and exhibits such as the Sinigayan Festival every March.

The seminar on the biology of sea cucumbers and the potential of sandfish and sea horse for stock enhancement in SMR was conducted on 25 November 2010. To develop understanding and skills for stock enhancement and prepare them for the community-based demo-site, ten fisherfolks were sent to SEAFDEC/AQD in Tigbauan, Iloilo for an introductory hands-on training in hatchery and nursery techniques. Five fisherfolks were trained on abalone and another five on sandfish for five (5) days for each species.

### *3. Strengthening of Fisherfolk Organization*

The role of fishermen's organization is critical in community-based stock enhancement projects. However, at the start of the project, there is no active fishermen's organization in Molocaboc. The organization of the Fisheries and Aquatic Resources Management Council (FARMC) at various levels from the barangay, municipality or city, provincial and national levels is promulgated through Fisheries Administrative Order (FAO) 196 - Series of 2000 of the Bureau of Fisheries and Aquatic Resources (BFAR). This Order promulgates the rules and regulations for the fisherfolk empowerment program of the government and to achieve sustainable development of fisheries and aquatic resources, attain food security and eradicate poverty among the coastal and inland fishing communities. The provisions of Section 68-79 and other related provisions of Republic Act 8550 otherwise known as the Philippine Fisheries Code of 1998 provided the basis for the formation and functioning of the Barangay Fisheries and Aquatic Resources Management Council of Barangay Molocaboc (Molocaboc BFARMC) which constitute the association of fisherfolks and similarly interested parties in the locality.

This stock enhancement project has been instrumental in forming the Molocaboc BFARMC on 24 November 2010. The organizational structure and relationships between BFARMC and other entities, including SEAFDEC/AQD and this project have been discussed with the fishers and other stakeholders. Thus, six groups of stakeholders agreed to collaborate to establish, maintain and sustain the *semilyahan* (Table 1).

Table 1. Roles and responsibilities agreed by stakeholders to maintain and secure the stock enhancement demonstration site in Barangay Molocaboc, Sagay Marine Reserve, 2010.

| SMR/ Municipal LGU | Barangay Molocaboc LGU/BFARMC/ Youth Organization | SEAFDEC/AQD | Academe/ Schools (e.g. NONESCOST) | Community (preferably through peoples' organization) | Traders, stockers |
|---|---|---|---|---|---|
| Assist partners: SEAFDEC, community, barangay LGU<br><br>Community organizing<br><br>Law enforcement (security of demo farms)<br><br>Resources management planning<br><br>Project monitoring<br><br>Conduct/ support IEC<br><br>Benchmarking of resources (abalone and sea cucumber population, natural food, predators) in demo site | Assure & provide manpower to secure demo site<br><br>Enforcement of barangay ordinance<br><br>Follow-up implementation of IEC activities<br><br>Assist in monitoring<br><br>Coordinate with municipal LGU, SMR, BFARMC<br><br>Enjoin participation of youth and "purok" leaders<br><br>Provide logistics when needed (e.g. food for manpower securing demo-site)<br><br>Provide permit to SEAFDEC to collect broodstocks | Research, technical assistance & training in biophysical & socioeconomics aspects of fisheries resource enhancement<br><br>Initial supply of seeds in demo site<br><br>Provide scientific information in drafting fishery ordinances, resource management and aquaculture livelihood | Assist in data gathering<br><br>Assist and complement in IEC activities | Revitalize POs-MAMFA with assistance from LGU/SMR<br><br>Actively cooperate in community projects<br><br>Disseminate and comply with ordinance<br><br>Provide labor (*bayanihan* style) | Support and encourage compliance to ordinance on catch size regulation<br><br>Practice fair pricing<br><br>Provide market information to SMR and SEAFDEC as requested (price, volume, quality, etc.)<br><br>Contribute in purchase or acquisition of materials for pen construction |

## *4. Establishment of Community-based Stock Enhancement Demonstration Site*

In response to the outcomes of the socioeconomic baseline assessment survey in February 2007 and a follow-up survey in 2009 which showed the need to improve awareness about stock enhancement as a fisheries management option among fisherfolks, the community-based abalone and sandfish stock enhancement demonstration site was established in January 2011 in the coralline intertidal flats, approximately 4,000sq m and located less than two (2) km from the shoreline of Molocaboc Dacu (Figure 2). The location of the demo-site, locally called by fishers as *semilyahan* or spawning area, was nominated and finally selected by the local fisherfolk members of the BFARMC on the basis of the following criteria defined by the stakeholders: 1) biophysical suitability for stock enhancement of abalone (i.e. presence of at least few wild abalone and sufficient branching corals with encrusting algae as described in Lebata-Ramos *et al.*, 2013); 2) visibility and accessibility to serve its purpose as stock enhancement demo-site; 3) safety for BFARMC members who volunteered to be on overnight duty to guard the demo-site against poachers; and 4) compliant to the multiple use zoning policy of the PAMB-SMR and Barangay Molocaboc (i.e. located in prescribed mariculture area not in navigational area).

An assessment of the nominated site started in February 2011 by visual inspection of the corals and search for wild abalone samples every month from February to June 2011 during neap tide. Abalone catch ranged from 0 to 2 individuals per month with fishing effort comprised of 3 divers simultaneously searching the nominated demo-site for one hour. The stakeholders therefore decided to proceed with the establishment of the demo-site. Basic preparations include the installation of floating signage marked with "*Semilyahan, Bawal Manginhas*", meaning "spawning area, gleaning is prohibited" to inform about the purpose of the demo-site. The demo-site was also demarcated by buoys and ropes installed by BFARMC.

On June 2011, the first trial release of 514 pieces of tagged hatchery-bred abalone juveniles in the community-based stock enhancement demo-site was participated by stakeholders such as the BFARMC, local government officials, SMR staff and some interested men and women residents in Barangay Molocaboc. The local stakeholders proposed and agreed to secure the demo-site from poachers. Every month or two weeks depending on the number of volunteers, BFARMC members sign-in in pairs to be on overnight duty from 6pm to 6am. The volunteers established their guarding protocol which include signing of guarding log-book, bringing of flashlight, whistle and food provisions and logging of incident report in record book kept by the President of the BFARMC. The volunteers were informed that they have no police power, hence, poaching incidents should only be deterred and reported to authorities such as the local government official of Barangay Molocaboc who will in turn process the incident as appropriate.

## *Conclusions and Recommendations*

Stock enhancement programs are often constrained by prohibitive implementation cost and reluctance of local governments to prioritize and allocate public funds for such purposes. This study demonstrated that small-scale enhancements can be initiated through community-based strategies. A network of strategically contiguous small community-based stock enhancement sites which can be afforded in the Philippine setting, may therefore serve some purposes similar to well-funded large-scale enhancements in developed countries. These release sites, called as *semilyahan* by fisherfolks in Barangay Molocaboc in Sagay Marine Reserve in Negros Occidental are designed to generate spill-overs that will provide future benefits in terms of "catch-for-subsistence" for many marginalized gleaners and fishers in small fishing communities.

The study showed that the decision and implementation of stock enhancement and the definition of its objectives and relevance involves the strong engagement with stakeholders. The determination of social preparation activities requires and involves continuous consultation with stakeholders through a variety of methods such as survey interviews, IECs, formation and strengthening of fisherfolk organization, and the actual establishment of stock enhancement demo-site accessible to the public. The activities tested in this study showed that the formulation of social preparation activities for stock enhancement should be founded on the following principles: 1) community-specific strategies, 2) responsive to local social settings and needs, and 3) consultative and participatory across various stakeholders.

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# Community-based Shrimp Stock Enhancement for Coastal Socio-ecological Restoration in the Philippines

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## *Abstract*

The reality of declining quality of coastal areas has been evident for many developing countries, especially in Southeast Asia. In the Philippines, rural coastal zones and estuaries are now being characterized by declining wild fisheries resources and degrading environment. This paper presents, as an example, the typical rural coastal towns of New Washington and Batan in Aklan province, Philippines and showcases how the concept of shrimp stock enhancement can provide incentives to restore the environment and provide sustainable fishing livelihood in the area.

The New Washington-Batan Estuary in northeast Panay Island, Philippines was a productive fishing ground that has been in a state of degenerating brackishwater fisheries and estuarine environment. Average daily catch composed of various species decreased from 24 kg in 1970s to 0.7 kg at present. Shrimp fisheries, the most important livelihood, declined in quality and quantity. The highly-priced and once very abundant tiger shrimp *Penaeus monodon* was replaced with smaller-sized and lower-priced species like the *Metapenaeus ensis*. These can be attributed to the conversion of 76% of mangroves to culture ponds in the past 50 years and more than 400% increase in fishing gears since the 1990s. The need to reduce fishing structures and rehabilitate mangroves is evident. However, these drastic changes directly affect fishers' livelihood. This paper explores the prospects of *P. monodon* stock enhancement as "positive reinforcement" for the estuary's rehabilitation. Number of gears per fisher may have to be reduced but shrimp catches will be relatively high-priced. Simulations with additional tiger shrimp caught due to stock enhancement show that fishers can increase income by more than 4 times from their current PhP 34 $gear^{-1}$ $day^{-1}$. Campaigns on the importance of mangrove especially as shrimp habitat can encourage local communities to reforest the estuary especially in abandoned ponds. With effective management, law enforcement, and sustained support from different sectors, shrimp stock enhancement can be a positive strategy in estuarine rehabilitation and livelihood sustainability in the New Washington-Batan Estuary.

**Keywords:** stock enhancement, *Penaeus monodon*, rehabilitation, estuary, mangroves, livelihoods

## Introduction

Fish stock enhancement programs have been done since the late 1800s and continues until now for many countries. However, crustacean stock enhancement initiatives, especially for shrimps, are limited. Only seven programs were reported in literature involving varied purposes. In the USA, Kuwait, Sri Lanka, and Taiwan, the primarily aim is to increase available shrimps in the wild for commercial capture fisheries (Bell *et al.*, 2005). On the other hand, the loss of natural coastal habitats and nurseries caused by industrialization in Japan since the 1960s has affected supply of natural shrimp seeds, hence the need for artificial stocking of shrimp juveniles (Hamasaki and Kitada, 2006). In China, excess shrimp seeds originally produced for aquaculture were instead used for release in the wild (Wang *et al.*, 2006). Shrimp stock enhancement projects in Australia were done to verify new scientific protocols and release technology (Loneragan *et al.*, 2006). However, in developing countries like the Philippines where poverty is prevalent especially in rural coastal communities, improving the lives of artisanal fishers through better harvest is the major consideration.

In the Philippines, being an archipelago of 7,107 islands, coastal zones are considered to be the most important areas for residence and livelihood, especially for more than half of the country's 100 million people living in the rural areas. This paper focuses on the New Washington-Batan Estuary as an example, located in the province of Aklan, northern Panay Island, central Philippines (Figure 1).

## Methods

Field surveys were conducted to update data and monitor the actual daily catch for the modified fish corral or stake net, locally known as *tigbakol*, the most dominant stationary fishing gear in the area (Figure 2). Twenty *tigbakol* were monitored twice monthly from January to December 2013 to establish a trend in annual harvest, catch composition and catch-per-unit-effort or CPUE. Parallel socioeconomic survey which includes questions related to the present stock enhancement project were also conducted among 200 respondents. Review of secondary data and literature were also done site-specifically, especially on the environmental and fisheries status of New Washington and Batan.

Shrimp fishery in the estuary is considered as the most important livelihood in the area (Ingles *et al.*, 1992). So, more focus was given on shrimps as a commodity, particularly the tiger shrimp *Penaeus monodon* or *sugpo* which commands the highest market value. Stock enhancement impact simulations were also based on *P. monodon*, using the following assumptions: (1) 500,000 shrimps released, (2) 330 fixed fishing gears to potentially capture the shrimps around the area, (3) conservative recapture rate of 2% after 2 months of release, and (4) even lower recapture rate of 0.5% after 3 months of release.

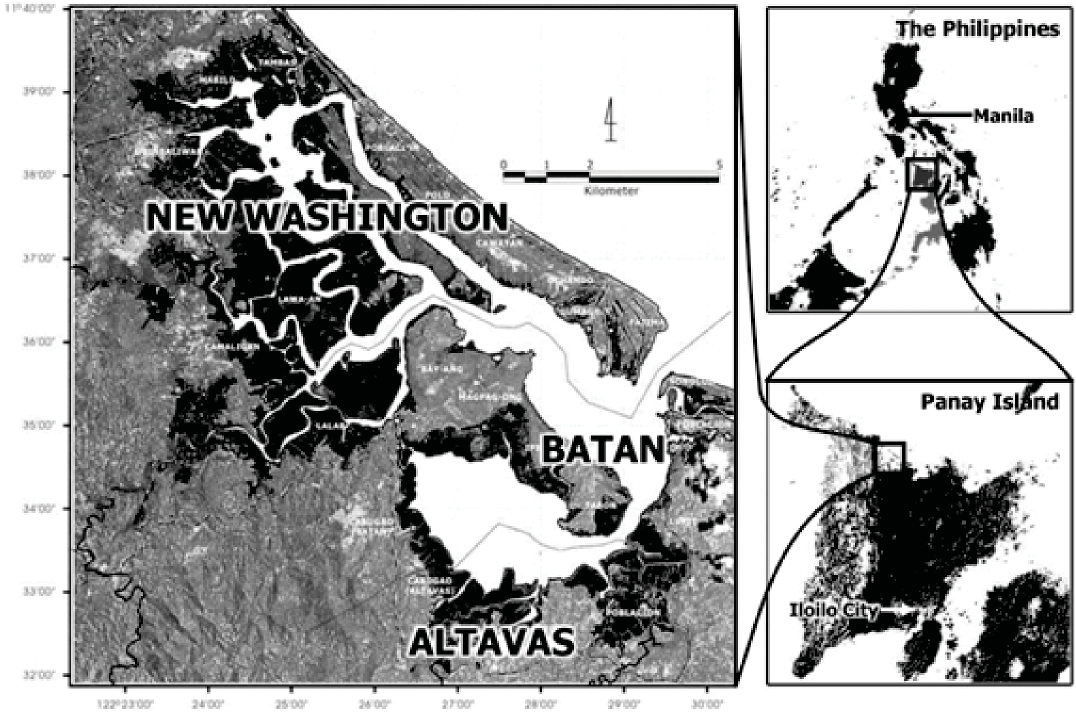


Figure 1. The study site showing the location of New Washington that composes most of the semi-enclosed estuary; Batan in the middle, and Altavas sharing a small portion in the south.

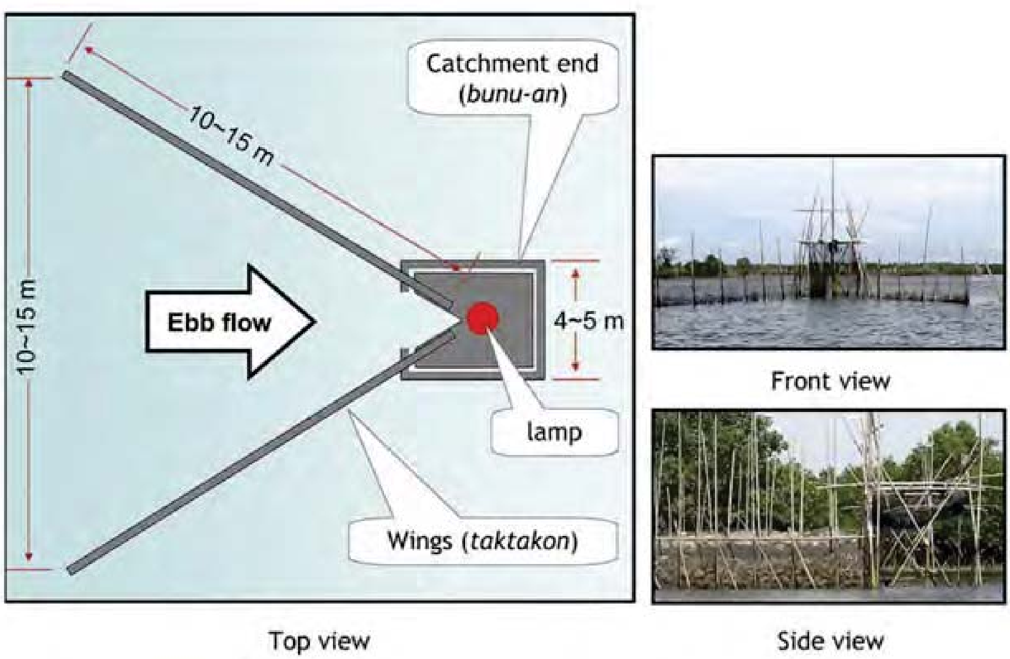


Figure 2. Photos and schematic diagram of the fixed gear *tigbakol* or fish corral.

## *Results*

### *Changes in the New Washington-Batan Estuary*

The New Washington-Batan Estuary was once considered as a very productive fishing ground with lush mangrove forests and abundant aquatic resources but now suffer from degrading environment and brackishwater fisheries (Altamirano, 2007). Mangrove forests were reduced by 76% in less than half a century, from 4,923 in 1950s to only 406 ha of thin fringing mangroves in 2008, which is viewed to have been caused by rapid development of culture ponds from 513 ha 1988 to 3,747 ha by 2008 (Altamirano *et al.*, 2010). Nevertheless, some evidences of conversion of the remaining mangroves or reclaiming riverbanks to build more ponds can still be found.

Decline in estuarine fisheries were similarly striking. Representative CPUE ($gear^{-1}\ d^{-1}$) in terms of total daily catch of various species per *tigbakol* was about 24 kg in 1970s, decreasing by about half every decade until 1.65 kg in 2006 (Altamirano and Kurokura, 2010). The most recent survey in 2013 showed an average daily catch per gear of *tigbakol* to be 0.7 kg only. Shrimp fisheries declined in quality and quantity where the highly-priced and once abundant tiger shrimp *P. monodon* was replaced in composition with smaller-sized and lower-priced shrimp species, like *Metapenaeus ensis*. In a 1978 data, *P. monodon* composed some 60% of daily shrimp catch from the estuary (Ingles *et al.*, 1992). However, catch survey in 2006 showed that tiger shrimps only composed <10% of the catch, while more than 80% are of the cheaper *M. ensis*. Most of these catch (about 70%) belongs to the smaller size classes of juveniles of less than 20 mm carapace length (Figure 3). On the average, with this size composition of shrimps, a fisher can only earn PhP 34 per day from sale of catch from one *tigbakol*. Local fishers who recalled catching about 6 kg of tiger shrimps per day in the 70s, were disappointed to catch literally nothing of the species at present. From January to December 2013, the monitoring of 20 *tigbakol* gears twice in a month (a combined total of 480 hauls) only caught a total of 19 pcs of *P. monodon*. This is equivalent to only one tiger shrimp caught for every 25 hauls of the gear.

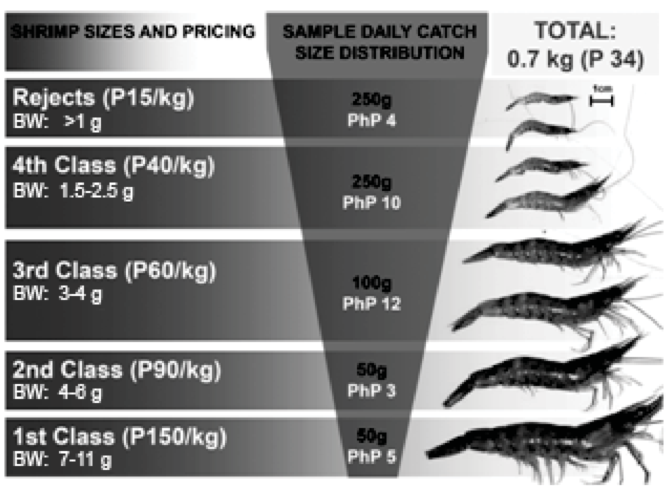


Figure 3. Average daily shrimp catch composition (mainly of *M. ensis*) from *tigbakol* (Altamirano, 2007).

In every decade since 1940s, human population in the area has been increasing by 15-25%, but slowed to only 10% in 2000. Desperate to increase income to feed an average family of 8, fishers intensified fishing effort by multiplying their fishing gears, reaching 400% more stationary gears than in 1990 (Altamirano and Kurokura, 2010). In 2006, surveys showed that fixed gears (fish corral, filter nets, lift nets) in the area already reached more than 2,300 structures. This does not include active gears like gill nets, push nets, traps and others. Surely, the estuary and rivers in New Washington is overcrowded of fishing gears where local navigation is even hampered in some areas because of bamboo structures spanning the whole width of rivers (personal observation).

Anecdotal reports showed that a number of fisheries and resource management programs have already been implemented in the estuary. However, most of these programs were grants and aids that usually only had short term effects and no observable long-term sustainability. The "top-down" nature of these programs mostly fails to reach down into the base of problems, which are the local communities. When funds have been exhausted and activities slow down, people still tend to return to what they were used to do. Driven by poverty and desperation to survive, some fishers engage in illegal fishing practices like the use of small meshed nets, and theft of other people's fishing gears and catch.

## *Main concerns in the estuary*

Interview surveys revealed that fishers and communities were well aware of the threats they are facing in the estuary. They outlined many problems that were generalized into (1) small income caused by poor catch and overcrowded fishing gears; (2) degraded environment with hardly any mangroves, shallow water with heavy siltation; and (3) poor law enforcement. Table 1 further summarizes the main problems in the New Washington-Batan estuary, as well as the direct solutions to these problems. It is with these ideas that shrimp stock enhancement is viewed to play a crucial role.

Table 1. Main problems in the New Washington-Batan Estuary and their direct solutions.

| Problems in the estuary | Direct solutions |
|---|---|
| 1) poverty situation among local fishers is worsening due to low quality and quantity of catch, especially of shrimps | 1) increase fishers' income |
| 2) intense overfishing in the area is evident with overcrowded fishing gears, and the use of illegal fishing methods | 2) reduce number of fishing gears used |
| 3) natural environment is extremely degraded where 76% of mangroves were lost, mostly to aquaculture ponds | 3) rehabilitate mangroves |

## *Can shrimp stock enhancement increase income?*

Fishers catch about 700 g of shrimps for one gear (*tigbakol*) daily, equivalent to PhP 34 (Philippine Peso), that were sold at prices respective of size class (Figure 3). Assuming that one fisher has 5 gears, the combined daily total catch for shrimps will be 3.5 kg or about PhP 170. This is still below the PhP 235 law-mandated minimum daily wage in this region on 2008 (National Wages and Productivity Commission of the Philippines, 2008). Interestingly, during heavy rains, some dikes of ponds collapse, releasing cultured shrimps out to the rivers. Consequently, fishers noted unusual catch of tiger shrimps at these times, thereby boosting their profits temporarily. This unintentional release of pond stocks exemplifies the prospects of a programmed shrimp stock enhancement as a viable technique, especially for increasing income of fishers.

Assuming that a successful release of 500,000 tiger shrimps was accomplished in the estuary, possible hypothetical scenarios on impacts on catch are presented in Table 2. With effective fishery rules and enforcement, Scenario 1 shows that two months after release and with a conservative recovery rate of only 4%, each of the 330 *tigbakol* can possibly catch about 60 pcs (900 g total) of *P. monodon* (2-3 cm carapace length, CL; 5-15 g body weight, BW), which can eventually provide PhP 135 in one day. While in Scenario 2, when shrimps are allowed to grow until 3 months after release and even with a much lower hypothetical recovery rate of 1%, each gear can potentially catch some 15 pcs (475 g total) but of larger shrimps (3-4 cm CL, 15-35 g BW). This in turn can also earn the same amount (PhP 135). Whether within scenario 1 or 2, a single gear can sell PhP 135 of tiger shrimps, on top of the PhP 34 pesos of other shrimp species, plus earnings from fish and crabs. This is a huge 400% increase in shrimp sales per fishing gear. The recovery estimates of 4% after 2 months and 1% after 3 months are very conservative, which means that potential higher recovery rates can further increase income of fishers. In comparison, common recovery rates for shrimp stock enhancement activities is around 20%; the lowest recovery rates for shrimp releases was about 5% in Japan (Bell *et al.*, 2005; Hamasaki and Kitada, 2006).

### *Can shrimp stock enhancement reduce number of fishing gears?*

The hypothetical figures in Table 2 show that a single *tigbakol* can potentially provide a conservative estimate sale of PhP 135 daily for tiger shrimps. This means that with only two *tigbakol*, a fisher can expect PhP 270 sales daily which is a little more than the PhP 235 daily minimum wage in 2008. Earnings from other species caught by *tigbakol* along with shrimps like fish and crabs will add to the PhP 270 obtained from *P. monodon* catch. Basically, this means that the number of gears can be reduced to allow only 2 gears per household. Assuming that on the average, one fisher currently owns 5 gears of *tigbakol*, a reduction to only two can mean a huge 60% decrease in fixed gears distribution in the area, if implemented well, but with improved shrimp catch quality and prices received.

Table 2. Hypothetical daily catch of *P. monodon* per *tigbakol* after a simulated stock enhancement release.

| Shrimp Size (CL, BW) | Price ($PhP \cdot kg^{-1}$) | Scenario 1: 2 months after release (pieces) weight, price | Scenario 2: 3 months after release (pieces) weight, price |
|---|---|---|---|
| 3-4cm, 15-35g | 300 | | (15) 450g, PhP 135 |
| 2-3cm, 5-15g | 150 | (60) 900g, PhP 135 | |
| 1-2cm, 1-5g | 60 | | |
| <1cm, <1g | 20 | | |
| TOTAL: | | 900g, PhP **135** | 450g, PhP **135** |

Note: US$1 = PhP42.50 (2013 annual average)

### *Can shrimp stock enhancement promote mangrove rehabilitation?*

It has been pointed out in the interviews and separate studies that majority of mangrove forests in the Batan Estuary have been cleared, mostly for aquaculture. The importance of mangroves as habitat especially for shrimps is already established (Sasekumar *et al.*, 1992; Chong *et al.*, 1996; Primavera, 1998). This suggests a very practical need for mangrove rehabilitation to allow natural revival of shrimp populations. Therefore, information, education and communication (EIC) campaigns on the importance of mangroves as shrimp habitat can encourage locals to reforest the estuary, most especially in abandoned ponds. This is a long term target which means that it is crucial that properly-guided mangrove rehabilitation be done soonest.

### *The role of the community in a stock enhancement program*

The effect of stock enhancement on the people is clear. This has also been exemplified in the kuruma prawn *Penaeus japonicas* stock enhancement in Hamana Lake, Shizuoka, Japan (Fushimi, 1999). The bottom-up approach focusing mainly on local fishers themselves has enriched their awareness and encouraged active participation in the rearing and release phases of the program. In the case of New Washington-Batan Estuary, the fisherfolk community is the most direct beneficiary and stakeholder in stock enhancement. Hence, their awareness, participation and cooperation in the enhancement activities are critical determinants of success.

The socioeconomic survey in New Washington in 2012 revealed that 82% of the respondents preferred *P. monodon* over other local species for stock enhancement project. They considered *P. monodon* a high-valued species that could give them higher income compared with grouper, snapper, crabs and other shrimp species. They claimed that *P. monodon* used to be abundant in the past, but are now seldom caught in the estuary. Although 24% of them were not aware of the stock enhancement project in New Washington, still almost all of the respondents (97%) were willing to participate in the project. However, direct voluntary participation of fisherfolks in the rearing phase of the shrimp stocks was dampened by the limitations that characterize their organization.

There is also an apparent weak fisheries governance even when the national fisheries policy gave mandate to the local government to manage its own fishery resources. The local government have not acted to effectively manage and regulate fishing activities to accord with and support the stock enhancement activities. In particular, active fine-meshed gears that easily entrap released shrimps persist to operate in the estuary. On the other hand, majority of the fishers understand the problems better than the managers and officials but they lack the power to prevent unsustainable fishing operations. Therefore, it is better to implement a “bottom-up” approach in the area where local fishers are to be given main considerations. They should also be active “participants” in the planning and implementation of the stock enhancement activities, with strong supervision from technical authorities. The clear-cut incentive of increasing income through higher sales from *P. monodon* catch is a strong motivation for the fishers to join the stock enhancement activity.

### Concluding Remarks

The usual environmental and fisheries problems are still evident in New Washington and Batan in spite of the various fishery ordinances and laws to guide sustainable fisheries. More so, there were several developmental projects implemented to prevent further degradation of the local fishery. Weak law enforcement and political will reported by fishers, together with the ineffective cooperation among leaders and local communities, further complicate the situation. The observations and results presented in this paper indicate that the New Washington-Batan Estuary urgently needs effective measures for rehabilitation. One alternative fisheries and environmental management option is through stock enhancement of the tiger shrimp *P. monodon*. Theoretically, by restoring wild populations of this highly-priced shrimp species, fishers can directly increase income. With this incentive, reduction of fishing gears is possible and mangrove rehabilitation can be promoted. The prospects of tiger shrimp stock enhancement in the area are high and the benefits are clear. However, it is important that support of sectors like the government, local universities, people's organizations, stakeholders, and local fishers must be solicited to create unbiased management plans.

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# BFAR-CHED Philippine National Aquasilviculture Program (PNAP) in Bataan

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## *Abstract*

Under the Philippine Aquasilviculture Program, the Bataan Peninsula State University (BSPU) rehabilitated denuded mangrove resources, established aquasilviculture technology demonstration projects as a livelihood option for fisherfolks (while caring for the mangroves they had planted) and established community-based multi-species hatcheries to increase endemic fish species in the area.

The BPSU was able to (a) plant 183, 300 mangrove seedlings where 85.96% survival was noted a year after, (b) establish 16 units aquasilviculture projects for the livelihood of the beneficiaries (planting that earned the beneficiaries P1,338, 731.90); and (c) establish community-based multi-species hatcheries that already produced an estimated 1,030,502,400 eggs of various fish species, thus increasing the wild fishery resource in the area.

The program is expected to bear potential impacts on our environment and to the lives of the marginalized people of our community through the collaborative efforts of the Bureau of Fisheries and Aquatic Resources (BFAR), the Commission on Higher Education (CHED), BPSU, Local Government Units (LGUs) and the fisherfolks.

**Keywords:** aquasilviculture, mangrove propagules, community-based multi-species hatchery

## *Introduction*

The decline in mangrove resources that serve as habitat for various fishery species has reached 383, 000 hectares from 1918- 1995 (Melana *et al.*, 2000), which means that the Philippines lost 76.6% of its mangrove areas for less than a century with an estimated national deforestation rate of 4,432ha/year between 1951 and 1988. This was brought about by overexploitation by coastal dwellers, conversion to settlements, agriculture, salt beds and industry (Baconguis *et al.*, 1993: Primavera, 1995). Apart from the foregoing, conversion to aquaculture is recorded as the major cause since around half of the 279, 000 ha of mangroves lost from 1951- 1988 were developed into culture ponds. According to the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development PCAFNRRD in 1991 around 95% of the brackishwater pond in 1952- 1987 were derived from mangroves. Likewise, according to the DENR 1995 statistics, conversion to fishponds, prawn farms, salt ponds, reclamation and other forms of industrial development have reduced the mangrove area to 117,700 ha (Melana *et al.*2000).

This very alarming loss of our mangrove resources is causing the deterioration of sea grass and coral reef ecosystem. About 70% of the Philippine coral cover has been destroyed, with about 25% still in good condition and only about 5% in excellent condition, which resulted to decline in the productivity of coastal fisheries. An estimated 670 kg reduction in fish catch per hectare of mangrove forest that is clear cut had been recorded by the Coastal Resources Management Project in1998.

In line with its mandate under Republic Act No. 8550 or the Philippine Fisheries Code of 1998, BFAR aims to achieve food security, promote sustainable development of fisheries resources, and reduce poverty incidence among fisherfolk and other disadvantaged groups. The Commission on Higher Education (CHED), on the other hand, is mandated, through Section 8 of Republic Act 7722 (the Higher Education Act of 1994), to identify, support and develop potential centers of excellence in program areas needed for the development of world-class scholarship, nation building and national development and direct or redirect purposive research by institutions of higher learning to meet the needs of agro-industrialization and development.

The Government of the Republic of the Philippines, through BFAR and CHED in collaboration with the academe and local government units concerned, is implementing the Philippine National Aquasilviculture Program, (PNAP) which aims to ensure resource sustainability, to attain food security and to alleviate poverty.

A Memorandum of Agreement (MOA) has been entered into by the BFAR and the CHED to implement the said Program. On 16 December 2011, Bataan Peninsula State University (BPSU) signed a Memorandum of Agreement with BFAR3 as one of the selected State Universities and Colleges to implement the PNAP in the province of Bataan.

### *Objectives*

The main objective of this undertaking is to implement the PNAP in order to rehabilitate denuded mangrove resources and to increase survival of mangroves through the participation of fisherfolk organizations and the local government units. This project involves capacity building seminars and trainings, provision of livelihood and improvement of capture fisheries by increasing the fish population in the area for sustainable fisheries development, food security and poverty alleviation.

Specifically, this program aimed to plant 183,300 mangroves along coastal areas of Manila bay, establish 16 units aquasilviculture technology demonstration projects for the livelihood of the fisherfolks and to set up community-based multi-species hatcheries.

## *PROGRAM CONCEPT*

### *1. Resource/habitat Rehabilitation*

1.1. Site Selection/ Validation

1.1.1. Through the Project Management Office (PMO), priority areas for habitat or resource rehabilitation were pre-identified. These include:

a. Key Biodiversity Areas (KBAs) in the province as recommended by the CENRO/PENRO.
b. Abandoned, Underdeveloped and Underutilized Fishpond Lease Agreement (AUU FLA) areas as identified in accordance with the joint administrative order on the reversion of AUU FLA areas.
c. Areas identified for reforestation/afforestation or covered by the tenurial arrangements by the DENR.
d. Areas covered by co-management agreement between the DA, DENR and LGUs.

The sites identified were validated by the PMO in terms of:

a. Willingness of the community to participate;
b. Technical suitability;
c. Mangrove species thriving in the area; and
d. Accessibility

1.2. Standard Planting Design

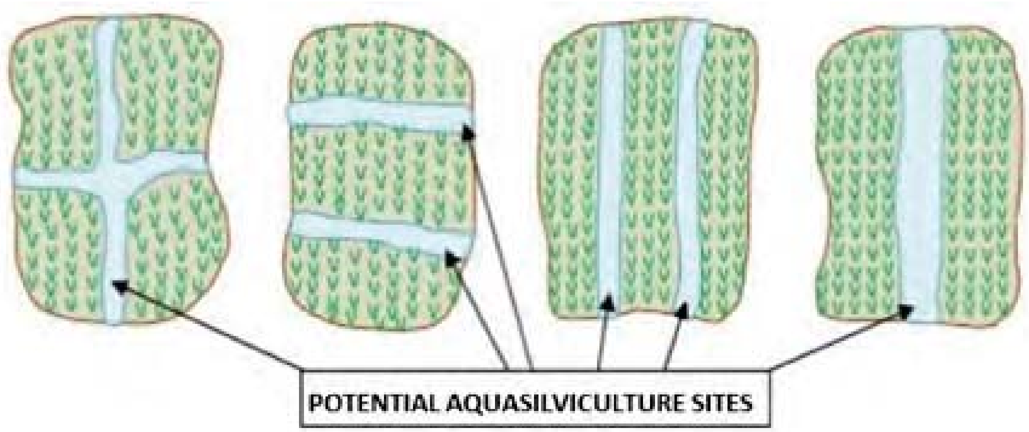
**Spacing and design**

A standard distance of 1.5 m x 2.0 m between mangrove propagules was observed, and at least 3,000 mangrove propagules needed to be planted in every hectare and that no more than 30% of the area devoted to aquasilviculture.

1.3. Budgetary Requirement and Payment Scheme

The standard cost for resource/ habitat rehabilitation were as follows:

a. P1.50 per mangrove propagule (ready for planting) gathered by the beneficiary ;
b. P2.00 per mangrove propagule planted with corresponding support stake with length of at least 2 feet and 2 inches width; and
c. P2.50 for each fully grown and live mangrove tree after one year from planting.



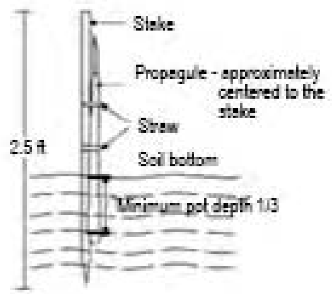




Figure 1. Planting design as prescribed in the implementing guidelines.

1.4. Selection of Beneficiaries

The PMO in consultation with the local government units and the FARMCs identified and maintained a list of qualified project beneficiaries guided by the following criteria:

a. Bonafide resident fisherfolk in the area/ project site;
b. Willingness to participate in the program and abide by the terms and conditions therein;
c. Preferably those identified and included in DSWD list of marginalized sectors

### *2. Aquasilviculture Projects*

2.1. Site Selection/ Validation

Areas identified for aquasilviculture were validated by the PMO as to its appropriateness and suitability for such purpose.

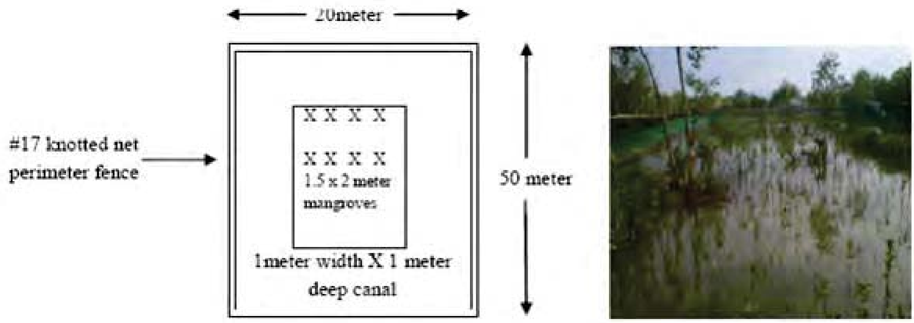



Figure 2. Layout of a 1,000 m² aquasilviculture technology demonstration project.

2.2. Standard Size and Design

No more than 30% of the area with mangrove was recommended for aquasilviculture. The suggested area was preferably 1,000 square meters per unit aquasilviculture on dead creeks, water canals and area with enough water even during low tides.

2.3. Budgetary Requirements and Payment Scheme

The total amount of P65, 000.00 was allocated as support for the establishment of the individual aquasilviculture technology demonstration project. The support will cover the following:

a. Fencing materials (nets, ropes, etc.)
b. Labor support (for excavation)
c. Portion of the farm inputs (seed stocks and feeds)

2.4. Selection of Beneficiaries

Those who participated in the resource rehabilitation activity were identified as the primary beneficiaries of the aquasilviculture projects.

2.5. The commodity species for aquasilviculture production was determined after the site suitability evaluation.

### *3. Community-based Multi-species Hatchery (CBMSH)*

The CBMSH is a strategy that was intended to:

a. Conserve, save and protect eggs of various species that are gravid when captured from the wild;
b. Take advantage of the natural productivity of the environment in the post-larval rearing and nursery of targeted species;

c. Increase the population of targeted species in the wild through stock enhancement; and

d. Utilize the abundant seed supply of targeted species for use in aquasilviculture.

3.1. Site Selection/ Validation

The PMO in collaboration with the SUC identified priority hatchery areas based on the following:

a. Existing/ operational hatcheries- were encouraged to operate utilizing the project fund to augment the operation giving priority to the multi-species hatchery project;

b. Existing/Non-operational hatcheries- were put into operation utilizing the allocated funds with counterpart funding from the SUC as per MOA including manpower complement;

c. Non-existing hatcheries- were established following the suggested design utilizing the allotted funds with the counterpart contribution from the SUC as per MOA and by considering the following criteria:

1. Required area/site was at least 1,000 square meters with at least flat/ plain terrain;
2. Availability of unpolluted marine water;
3. Availability of sea water with stable salinity not lower than 35 ppt;
4. Availability of spawners/ breeders;
5. Near aquasilviculture production areas;
6. With existing electricity;
7. Accessible to land and water transport; and
8. Availability of fresh water for domestic use

3.2. Hatchery/ Nursery Lying-in Concept

This was done through the introduction or development of a system of collecting gravid target species from fishermen, allowing them to spawn and nurse the larvae inside a designed structure under controlled conditions, until they reached the stage where they can be released into the natural habitat.

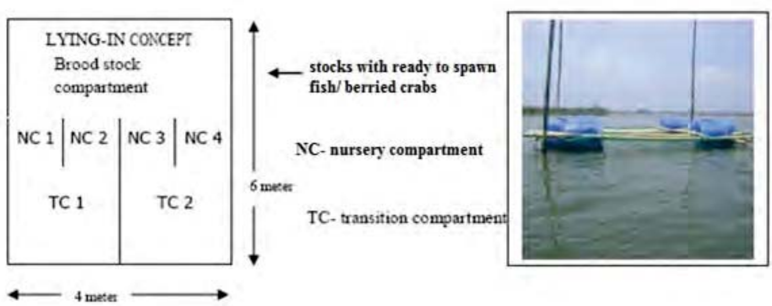



Figure 3. Recommended layout of lying-in hatchery/nursery concept.

## *PROJECT ACCOMPLISHMENTS*

Right after the orientation regarding the implementing guidelines of the program, the project management office and the BPSU team wasted no time, hence accomplished the following during the implementation of the PNAP in the province of Bataan:

### *Pre- Implementation Phase*

**Program Orientation.** Representatives from SUCs and other participating schools in Region 3 were oriented on the implementing guidelines of the PNAP at the BFAR 3 Regional Office in Maimpis, City of San Fernando, Pampanga. It was followed by a national orientation on 25 May 2012 in Sariaya, Quezon by the BFAR Director, Atty. Asis Perez.

**Coordination with PENRO and LGUs.** Right after the regional and national orientation, coordination was made with the PENRO and LGUs of Bataan through their Municipal Agriculture Offices to conduct orientation about the PNAP with their respective Municipal or City Fisheries and Aquatic Resources Management Council (M/CFARMC).

Table 1 presents the current Mangrove areas in Bataan based on the data provided by the DENR- PENRO. Out of 177 km coastline in Bataan, only 121.08 hectares of mangrove areas have remained or still exist as fish habitat in the province.

**Orientation of Fisherfolks.** There were about 637 fisherfolks coming from 28 organizations in the municipalities of Orion, Orani and City of Balanga that were oriented about the implementing guidelines of the program.

**Fisherfolks profile.** Table 3 shows the profile of fisherfolk beneficiaries taken prior to the start of the project for the implementors to have an initial data on their present status. Out of the 637 fisherfolks oriented about the PNAP, 95.77% were males and only 4.23% females. As regards to their civil status, 83.46% were married while 4.42% were separated, 7.5% were single and 4.42% were widows/widowers.

**Identification of Project Beneficiaries.** There were 16 fisherfolk organizations selected as beneficiaries of the program based on the following criteria:

a. Bonafide resident fisherfolk in the area/project site;
b. Willing to participate in the Program and abide by the terms and conditions therein;
c. Preferably, they were identified and included in the list of marginal sector of the DSWD

**Conduct of Trainings and Seminars.** Capacity building seminars that include resource protection and rehabilitation, constituency building, leadership and value formation trainings were conducted to strengthen the capabilities of the project beneficiaries.

**Signing of Memorandum of Agreement.** A formal agreement was signed between BPSU represented by the university president, Dr. Delfin O. Magpantay, and the respective chairs or leaders of the selected 16 fisherfolk organizations. Stipulated in the MOA are the roles and responsibilities of each party in the implementation of the project.

**Area Validation.** After the final identification of the mangrove areas to be

Table 1. Present mangrove areas in Bataan (DENR-PENRO, 2011).

| City/ Municipality | Barangay | Lot. No. | Area (has.) | Total per Barangay (has.) | Total per Municipality (has.) |
|---|---|---|---|---|---|
| Limay | Alangan | 1 | 0.9 | 0.9 | **0.9** |
| Orion | Daan Pare | 1 | 4.7 | 4.7 | |
| | Ormoc | 1 | 0.87 | 0.87 | |
| | Sto. Domingo | 1 | 2.51 | 2.51 | |
| | Sta. Elena | 1 | 2.52 | | |
| | | 2 | 3.19 | 5.71 | **13.79** |
| Pilar | Bantan Malake | 1 | 0.95 | 0.95 | |
| | Bantan Munti | 1 | 0.76 | 0.76 | |
| | Balut | 1 | 6.4 | 6.4 | |
| | Wawa | 1 | 1.06 | | |
| | | 2 | 0.3 | | |
| | | 3 | 0.4 | | |
| | | 4 | 0.34 | | |
| | | 5 | 0.45 | | |
| | | 6 | 1.73 | | |
| | | 7 | 1.64 | 5.92 | **14.03** |
| City of Balanga | Tuyo | 1 | 4.45 | 4.45 | |
| | Puerto Rivas | 1 | 5.53 | | |
| | | 2 | 0.62 | 6.15 | |
| | Tortugas | 1 | 13.93 | 13.93 | **24.53** |
| Abucay | Mabatang | 1 | 3.14 | | |
| | | 2 | 5.23 | 8.37 | |
| | Wawa | 1 | 1.74 | | |
| | | 2 | 2.17 | 3.91 | |
| | Capitangan | 1 | 5.1 | 5.1 | **17.38** |
| Samal | Sta. Lucia | 1 | 1.35 | | |
| | | 2 | 0.75 | | |
| | | 3 | 0.61 | 2.71 | |
| | Sapa | 1 | 2.63 | 2.63 | |
| | East Calaguiman | 1 | 2.83 | 2.83 | **8.17** |
| Orani | Kabalutan | 1 | 8.9 | 8.9 | |
| | Tapulao | 1 | 0.07 | | |
| | | 2 | 2.61 | | |

Table 2. Number of fisherfolks oriented on PNAP per locality.

| Locality/ Barangay/ Municipality/ City | Date of Orientation | Number of fisherfolks who attended |
|---|---|---|
| Pantalan Luma, Orani, Bataan | August 22, 2012 | 43 |
| Tenejero, Orani, Bataan | August 22, 2012 | 108 |
| Centro I & II, Orani, Bataan | July 31, 2012 | 46 |
| Wawa, Orani, Bataan | July 30, 2012 | 27 |
| Kaparangan, Orani, Bataan | July 26, 2012 | 51 |
| Calero, Orani, Bataan | July 25, 2012 | 20 |
| Pantalan Bago, Orani, Bataan | July 24, 2012 | 20 |
| Pantalan Luma, Orani, Bataan | June 27, 2012 | 35 |
| Pantalan Luma (Iguana), Orani, Bataan | June 20, 2012 | 74 |
| Pantalan Luma (Iguana), Orani, Bataan | June 19, 2012 | 51 |
| Pantalan Luma (Dulo), Orani, Bataan | June 18, 2012 | 39 |
| Pilapil, Orani, Bataan | June 15, 2012 | 50 |
| Pulo/Kabalutan, Orani, Bataan | June 13, 2012 | 40 |
| Balanga City FARMC | June 14, 2012 | 15 |
| Orion, Bataan | June 8, 2012 | 18 |
| **TOTAL** | | **637** |

Table 3. Profile of fisherfolks showing the average sex, civil status, highest educational attainment, average number of years in fishing, fishing equipment used and their average monthly income.

| | |
|---|---|
| Sex:<br>Male<br>Female | <br>95.77%<br>4.23% |
| Civil status:<br>Married<br>Separated<br>Single<br>Widow/widower | <br>83.46%<br>4.42%<br>7.5%<br>4.42% |
| Highest educational attainment:<br>Elementary<br>Secondary<br>Vocational<br>College | <br>56.04%<br>36.46%<br>4.17%<br>3.33% |
| Average number of years in fishing | 21-25 years |
| Kind/ Type of fishing equipment used:<br>Gill net<br>Boat<br>Lift net<br>Hook<br>Fishing rod<br>Fish trap | <br>79.63%<br>13.49%<br>4.76%<br>1.06%<br>0.79%<br>0.27% |
| Average monthly income | P2,000-P4,000 |

Table 4. Fisherfolk organizations selected as project beneficiaries.

| Name of Organization | Number of Members |
|---|---|
| 1. Samahan ng mga Mangingisdang Nagkakaisa (SAMANA), Pantalan Luma, Orani, Bataan | 42 |
| 2. Samahan ng mga Mangingisda sa Dulo (SAMADU), Pantalan Luma, Orani, Bataan | 27 |
| 3. Samahan ng mga Mangingisda sa Kaparangan (SAMAKA), Kaparangan, Orani, Bataan | 48 |
| 4. Samahan ng mga Mangingisda sa Wawa (SMW), Wawa, Orani, Bataan | 31 |
| 5. Samahang Mangingisda ng Pantalan Luma (S.M.P.L.), Pantalan Luma, Orani, Bataan | 49 |
| 6. Kapatirang Mangingisda at Makakaikasan ng Centro I & II (KAMMANCE), Centro I at Centro II, Orani, Bataan | 52 |
| 7. Makakalikasan at Mangingisdang Kinikilala sa Iguana (MMAKISIG), Pantalan Luma, Orani, Bataan | 27 |
| 8. Gabay Mangingisda ng Calero (GAMACA), Calero, Orani, Bataan | 22 |
| 9. Samahan ng Magdaragat ng Pulo (S.M.P.), Sitio Pulo, Kabalutan, Orani, Bataan | 21 |
| 10. Pulo Fisheries Development Cooperative (PuFiDeCo), Sitio Pulo, Kabalutan, Orani, Bataan | 16 |
| 11. Kaisahan sa Kaunlaran ng mga Mangingisda ng Pilapil (KAKAMPI), Palihan, Orani, Bataan | 50 |
| 12. Samahang Mangingisda ng Tenejero (SaMaTe), Tenejero, Orani, Bataan | 108 |
| 13. Samahang Mangingisda ng Pantalan Bago (SMPB), Pantalan Bago, Orani, Bataan | 15 |
| 14. Municipal Fisheries and Aquatic Resources Management Council (MFARMC), Orani, Bataan | 15 |
| 15. BPSU Aquamarine Research & Development Center, Kabalutan, Orani, Bataan | 8 |
| 16. BALANGA CITY Fisheries and Aquatic Resources Management Council (CFARMC), Puerto Rivas, City of Balanga | 10 |
| **TOTAL** | **541** |

Table 5. Mangrove areas identified, validated and qualified for resource rehabilitation.

| Location | Area Identified | Area Validated | Qualified | Remarks |
|---|---|---|---|---|
| Balanga City: | | | | |
| Puerto Rivas | 10 | 10 | 0 | Mangrove already established |
| Tortugas | 15 | 15 | 0 | Use for bird sanctuary |
| Sibacan | 30 | 30 | 20 | Abandoned fishponds |
| Orani: | | | | |
| Kaparangan | 10 | 10 | 5 | With existing mangroves |
| Pantalan Luma | 15 | 15 | 10 | Only pagatpat/palapat mangrove spp. are present |
| Kabalutan | 10 | 10 | 10 | Denuded mangrove area |
| Pulo | 30 | 30 | 20 | Denuded mangrove area |
| BPSU ARC | 3 | 3 | 2 | To use as a model site for the project |
| **TOTAL** | **123** | **123** | **67** | |

rehabilitated, the project management team together with the fisherfolk representatives and the municipal agriculturist of their respective municipalities conducted an actual site validation to evaluate each area based on the willingness of its community to participate; technical suitability; mangrove species thriving in the area; and accessibility.

Out of 123 hectares identified and validated, there were only 67 hectares that met the criteria as stipulated in the implementing guidelines of the program, forty seven from Orani, Bataan and 20 hectares in Sibacan and Puerto Rivas in Balanga City.

**Identification of Mangrove Rehabilitation Sites.** Priority areas for habitat or resource rehabilitation were pre-identified after each orientation with considerations such as: a) identified priority key biodiversity areas (KBAs) in the province to be recommended by the CENRO/PENRO, b) abandoned, undeveloped and underutilized FLA areas as identified in accordance with the Joint Administrative Order on the reversion of AUU FLA areas, c) areas identified for reforestation/aforestation or covered by the tenurial arrangements by the DENR, and d) areas covered by co-management agreement between DA, DENR, and LGUs.

### *IMPLEMENTATION PHASE*

### *Project I. Mangrove Resources Rehabilitation Project*

**A. Collection of Mangrove Propagules.** Fisherfolks including their family members collected mature mangrove propagules of "Bakawang Lalaki" or "Bakawang Babae" as specified in the guidelines.

**B. Inventory/ Counting of Collected Planting Materials.** In order to insure the validity of the actual number of collected mangrove propagules, fisherfolks were instructed to tie their propagules in bundles of 100 pc per bundle during actual counting.

**C. Payment of Mangrove Propagules.** Right after the actual counting of the mangrove propagules collected by the fisherfolks, the necessary papers were prepared for the immediate release of payment done 5-10 days after.

**D. Planting and Staking.** The suggested standard planting design and procedure indicated in the implementing guidelines were followed by the fisherfolks during planting. A bamboo stake measuring 2.5 feet by 2 inches was tied for each propagules during planting to serve as protection from water current. A planting distance of 1.5 m x 2 m was followed in open areas while patch planting was made in areas where there were existing mangroves.

**E. Resource Protection and Maintenance.** Fencing and putting up of billboards were done to ensure that the reforested areas will not be damaged by intruders. The billboards showed the provisions of Republic Act 8550 penalizing those causing damage or destruction of mangrove forest/resources.

### *Project II. Aquasilviculture Techno Demo/ Livelihood Projects*

**A. Identification and organizing of beneficiaries**

As stipulated in the guidelines, beneficiaries for the livelihood projects were from those who participated in the Mangrove Resources Rehabilitation activity. Instead of awarding the livelihood projects (Aquasilviculture) to individual fisherfolks, the Project Management Office decided to give the project to 16 fisherfolk organizations, so that even their members will benefit from the income that would be derived from their aquasilviculture techno demos.

**B. Site Identification, Validation and Establishment**

The project team together with the beneficiaries identified and validated 24 possible sites for the aquasilviculture projects that included one in Puerto Rivas, Balanga City, 22 in Orani, Bataan and one at the BPSU Aquamarine Research Center. There were sixteen (16) sites for aquasilviculture that qualified based on the criteria set forth in the implementing guidelines of the program.

**C. Training of Project Beneficiaries**

Prior to the establishment of the livelihood component of the program, which is the Aquasilviculture techno demos, the following fisherfolks attended a ten day training at the National Brackishwater Aquaculture Technology Research Center in Pagbilao, Quezon from October 15- 24, 2012:

1. Mr. Avelino V. Capuli- Chairman, MFARMC Orani
2. Mr. Reynaldo Lalican- KAKAMPI
3. Mr. Florencio Cruz- SAMATE
4. Mr. Conrado Mallari- S.M.P.L.
5. Mr. Jimmy de Jesus- SAMANA
6. Mr. Cipriano Adena- SMW
7. Mr. Hector Catahan- SMPB
8. Mr. Rodolfo Tala- SAMADU
9. Mr. Jose Sally Raymundo- PuFiDeCo
10. Mr. Rodrigo Libanan- S.M.P.
11. Mr. Rolando Benavente- GAMACA
12. Mr. Rico Alfonso- SAMAKA
13. Mr. Mario Cubacub- MMAKISIG
14. Mr. Roman Roque- KAMMANCE
15. Mr. Armando Tolentino- CFARMC Balanga City
16. Mr. Richard Deldoc- BPSU AMRC

**D. Establishment of Livelihood Projects (Aquasilviculture Techno demos)**

Each of the identified fisherfolk organization was provided with the materials needed for the establishment of their Aquasilviculture technology demonstration project to raise mud crabs and other fish species as their source of livelihood while taking care of the mangroves they planted.

A standard 20 m x 50 m design was followed to cover an area of 1,000 square meters within the rehabilitated mangrove area. Each unit was provided with 500 tilapia fingerlings, 300 crablets and 300 milkfish fingerlings for their initial stock and a corresponding amount for feeds.

***Project III. Community-based Multi-Species Hatchery (CBMSH)***

**A. Site Identification and Validation**

The project team initially identified the possible sites for the CBMSH; the in-land base was situated at BPSU Orani Campus or at the BPSU Aquamarine Research Center, the other two, which adopted the Lying- in concept was in barangay Salaman, Bagac, Bataan along the West Philippine Sea and in Orani, Bataan along Manila bay. Upon validation, the In-land based hatchery which is supposed to be situated

Table 6. Total number of mangrove propagules collected and planted, including the estimated area covered and percent that survived one year after planting.

| Location | Number collected & planted | Estimated area covered (has.) | No. survived after a year | Percent survival | Remarks |
|---|---|---|---|---|---|
| Balanga City: | | | | | |
| Sibacan | 25,950 | 6.67 | 15,570 | 60.00 | |
| Puerto Rivas | 30,000 | 11.98 | 27,000 | 90.00 | |
| Orani: | | | | | |
| Kaparangan | 31,467 | 10.49 | 29,264 | 93.00 | |
| Pantalan Luma | 20,000 | 6.67 | 18,600 | 93.00 | |
| Kabalutan | 49,300 | 16.28 | 44,000 | 89.25 | |
| Pulo | 26,583 | 8.86 | 23,127 | 87.00 | |
| **TOTAL** | **183,300** | **61.10** | **157,561** | **85.96** | |
| **Amount paid** | **P641,550.00** | | **P393,902.50** | | |

Table 7. Fisherfolk organizations identified as project beneficiaries for the livelihood (Aquasilviculture) component of the program.

| Name of Organization | Number of Members |
|---|---|
| 1. Samahan ng mga Mangingisdang Nagkakaisa (SAMANA), Pantalan Luma, Orani, Bataan | 16 |
| 2. Samahan ng mga Mangingisda sa Dulo (SAMADU), Pantalan Luma, Orani, Bataan | 10 |
| 3. Samahan ng mga Mangingisda sa Kaparangan (SAMAKA), Kaparangan, Orani, Bataan | 9 |
| 4. Samahan ng mga Mangingisda sa Wawa (SMW), Wawa, Orani, Bataan | 16 |
| 5. Samahang Mangingisda ng Pantalan Luma (S.M.P.L.), Pantalan Luma, Orani, Bataan | 11 |
| 6. Kapatirang Mangingisda at Makakaikasan ng Centro I & II (KAMMANCE), Centro I at Centro II, Orani, Bataan | 15 |
| 7. Makakalikasan at Mangingisdang Kinikilala sa Iguana (MMAKISIG), Pantalan Luma, Orani, Bataan | 15 |
| 8. Gabay Mangingisda ng Calero (GAMACA), Calero, Orani, Bataan | 16 |
| 9. Samahan ng Magdaragat ng Pulo (S.M.P.), Sitio Pulo, Kabalutan, Orani, Bataan | 15 |
| 10. Pulo Fisheries Development Cooperative (PuFiDeCo), Sitio Pulo, Kabalutan, Orani, Bataan | 13 |
| 11. Kaisahan sa Kaunlaran ng mga Mangingisda ng Pilapil (KAKAMPI), Palihan, Orani, Bataan | 16 |
| 12. Samahang Mangingisda ng Tenejero (SaMaTe), Tenejero, Orani, Bataan | 15 |
| 13. Samahang Mangingisda ng Pantalan Bago (SMPB), Pantalan Bago, Orani, Bataan | 15 |
| 14. Municipal Fisheries and Aquatic Resources Management Council (MFARMC), Orani, Bataan | 16 |
| 15. BPSU Aquamarine Research & Development Center, Kabalutan, Orani, Bataan | 8 |
| 16. BALANGA CITY Fisheries and Aquatic Resources Management Council (CFARMC), Puerto Rivas, City of Balanga | 10 |
| **TOTAL** | **216** |

at BPSU Aquamarine Research Center did not pass the criteria stipulated in the implementing guidelines of the program. Hence, the project monitoring team suggested that the CBMSH could be constructed in BPSU Orani Campus where there are already 5 units of existing concrete tanks. However, hauling of sea water for use in the hatchery was required.

**B. Project Coordination**

After the sites were validated and found suitable, the project team identified the fisherfolks in barangay Salaman, Bagac, Bataan who will be the project partners/ beneficiaries in the hatchery. Hence, coordination with the municipal mayor of Bagac, Bataan, Hon. Rommel del Rosario thru the Municipal Agriculture Officer, Mr. Baltazar T. Manducdoc was needed.

**C. Orientation of Project Partners/ Beneficiaries**

An orientation regarding the concept of the hatchery was conducted by the project team to fisherfolk beneficiaries or partners for them to know the concept of allowing gravid or mature fish species to spawn and hatch their eggs in the open, the strategy adopted in order to increase fish population in the area.

**D. Construction of Lying-in Hatchery**

The fisherfolks started the construction of lying- in hatcheries one week after the orientation. They were provided with a project plan together with the necessary supplies and materials for the project.

The project team started buying the gravid female mud crabs and blue crabs collected by fisherfolks in the area and these were allowed to spawn in the hatchery.

**E. Construction of an Inland-based Hatchery**

An inland-based multi-species hatchery was constructed in BPSU Orani Campus where the existing 5- units concrete fish tanks were situated. These were used as part of the breeding/ rearing tanks for holding different fish species for seed production. As such, stocks were produced for the aquasilviculture projects and for the enhancement of fish population in mangrove forests being established by the project beneficiaries of the PNAP. Table 9 shows the list of procured gravid/ berried mud crabs and blue crabs that spawned in the lying- in hatchery together with the estimated number of eggs produced from January 17, 2013 to December 31, 2013.

***Monitoring and Evaluation***

Regular weekly monitoring of the conditions of the newly planted mangroves as well as the whole mangrove area was done to insure higher survival.

Monthly reports of the major accomplishments of the project including disbursement of funds were regularly submitted to the regional office of the Bureau of Fisheries and Aquatic Resources in Region 3.

***Promotion and Information Dissemination of the Program***

The strategies and accomplishments of BPSU regarding the implementation of the Philippine National Aquasilviculture Program (PNAP) were promoted through the conduct of forums in the Municipal Hall of Orani, Bataan attended by Vice-Mayor Godofredo Galicia and the Municipal Council members, including the barangay chairmen of the coastal barangays of the municipalities involved. Other activities during its implementation were uploaded in the Orani MFARMC Facebook page. These were also presented in the following conferences / symposia to showcase the achievement of BPSU in the implementation of the PNAP in Bataan:

1. 1st International Organic Agriculture Conference of the International Society for Southeast Asian Agricultural Sciences (ISSAAS) in Pampanga Agricultural College
2. 3rd National Biennial Conference of the Philippine Association of Extension Program Implementers, Inc. (PAEPI) in the Lyceum University of the Philippines
3. 9th BPSU Abucay Campus R & D In-house Review at BPSU Abucay Campus on October 18, 2013-awarded as the **BEST PAPER for Development Category**
4. 24th CLARRDEC Regional R & D Symposium at Philippine Carabao Center, Science City of Munoz, Nueva Ecija - awarded as the **2nd BEST PAPER for Development Category**

The project was also visited by the following:

1. Nineteen (19) representatives from the Pamantasan ng Lungsod ng Maynila Center for University Extension Services on February 19, 2013
2. Ten (10) Chairpersons of the Municipal Fisheries and Aquatic Resources Management Council of Bataan on March 19, 2013
3. Seven (7) Media Team of Cong. Albert Garcia
4. Six (6) Master of Science in Agriculture students of BPSU on March 23, 2013
5. Forty (40) Faculty and employees of BPSU Orani and Abucay campuses
6. Seven (7) MFARC officers from Limay, Bataan
7. Twelve (12) BS Agriculture Engineering students and faculty of BPSU Abucay campus
8. Thirteen (13) Fisherfolk including two Municipal Councilors and the Municipal Agriculture Officer from Samal, Bataan for a cross-visit

## SUPPORT DEVELOPMENTAL ACTIVITY TO ENHANCE PNAP IN BATAAN

### *Training on fish processing*

A "Kaalamang Pangkabuhayan para sa mga ginang ng tahanan ng mga Mangingisda sa Bataan" was approved and funded by the BPSU Gender and Development (GAD). A training on fish processing such as boneless "tinapa" making, fish drying, sardines making, fish fillet, gourmet and the likes scheduled once a month for the selected housewife of fisherfolk beneficiaries was conducted by food processing experts from Orani campus in support of the PNAP. Right after each batch of 5 finished their training, the processing materials including a small amount of capital were awarded to them to enable them to start their business. A total of 35 beneficiaries were trained by BPSU experts and awarded P2, 500.00 each for their starting capital.

Table 8. Reported income from the beneficiaries' aquasilviculture projects during the first cycle of operation.

| Beneficiary Organization of Fisherfolks | Stocks No. | | | Harvest (pc) | Kg Harvested | | | Amount (P) |
|---|---|---|---|---|---|---|---|---|
| | Mud crab | Milk fish | Tilapia | Mud crab | Milk fish | Shrimp | Blue crab | |
| 1. KAMMANCE | 300 | 300 | 500 | 85 | 58 | 13.3 | 12.7 | 26,052.00 |
| 2. MFARMC | 300 | 300 | 500 | 178 | 40 | 17 | 13 | 11,913.00 |
| 3. SAMAKA | 300 | 300 | 500 | 296 | 41 | 11 | 22.5 | 19,670.00 |
| 4. SMT | 300 | 300 | 500 | 42 | 22.5 | 24.5 | 27 | 24,200.00 |
| 5. SMW | 300 | 300 | 500 | 258 | 12.9 | 16 | 20 | 21,250.00 |
| 6. SAMADU | 300 | 300 | 500 | 212 | 46 | 21 | 27 | 23,550.00 |
| 7. SMPL | 300 | 300 | 500 | 198 | 38 | 26.2 | 19 | 16,260.00 |
| 8. SAMANA | 300 | 300 | 500 | 324 | 54 | 19.5 | 28 | 30,050.00 |
| 9. MMAKISIG | 300 | 300 | 500 | 170 | 39 | 18 | 24 | 24,590.00 |
| 10. GAMACA | 300 | 300 | 500 | 232 | 51 | 25 | 16.5 | 27,180.00 |
| 11. SMPB | 300 | 300 | 500 | 123 | 11.2 | 9.3 | 16 | 15,047.00 |
| 12. BPSUAMRC | 300 | 300 | 500 | | | | | |
| 13. KAKAMPI | 300 | 300 | 500 | 234 | 66 | 24 | 34 | 28,180.00 |
| 14. PUFIDECO | 300 | 300 | 500 | 102 | 8.8 | 11.1 | 8.6 | 11,787.40 |
| 15. SMP | 300 | 300 | 500 | 265 | 17.5 | 4.3 | 25 | 23,550.00 |
| 16. SAMAKA** | 550 | | | | | | | |
| INCOME FROM AQUASILVICULTURE | | | | | | | | 303,279.40 |
| INCOME FROM MANGROVE RESOURCES REHABILITATION | | | | | | | | 1,623,139.00 |
| INCOME FROM GRAVID MUD CRABS & BLUE CRABS | | | | | | | | 98,195.00 |
| **TOTAL INCOME DERIVED FROM THE PROGRAM** | | | | | | | | **2,024,613.40** |

* Stocks were raised in the BPSU fishpond

**Stocks had escaped when nets were damaged by poachers

Table 9. Total number of collected and procured gravid/berried females for spawning.

| Species | Number of pieces | Cost (P) | Total number of fry/ fingerlings/ crablets produced |
|---|---|---|---|
| Mud crabs* | 124 | 12,400.00 | 248,000,000 |
| Blue crabs** | 1,557 | 38,925.00 | 778,500,000 |
| Native mud crabs | 2 | 200.00 | 4,000,000 |
| Crablets (Giant crab) | 1,100 | 4,670.00 | |
| Giant mud crabs | 10 | 1,100.00 | |
| Milkfish | 4 | 20,000.00 | |
| Sea bass | 4 | 20,000.00 | |
| Red tilapia | 25 | 500.00 | 1,800 |
| Nile tilapia | 25 | 500.00 | 600 |
| Fresh water eel | 10 | | |
| Fresh water crabs | 60 | | |
| **TOTAL** | | **98,195.00** | **1,030,502,400** |

*Estimated for an average of 2M eggs/ gravid mud crab (Pelan and Grubert, 2007)

**Estimated for an average of 0.5M eggs/ gravid blue crab (Kamrani *et al.*, 2010)

Table 10. List of trained beneficiaries for the livelihood on Fish Processing funded by BPSU GAD.

| Date of Training | No. | Trained Beneficiary | Address | Loan Granted | Remarks |
|---|---|---|---|---|---|
| 7/23/2013 | 1 | Angelina Agustin | Palihan, Orani, Bataan | P2,500.00 | |
| | 2 | Adelina Brioso | Palihan, Orani, Bataan | P2,500.00 | |
| | 3 | Josie Baltazar | Palihan, Orani, Bataan | P2,500.00 | |
| | 4 | Teresita Lacap | Palihan, Orani, Bataan | P2,500.00 | |
| | 5 | Ailina Suspene | Pulo, Orani, Bataan | P2,500.00 | |
| | 6 | Cecilia Guevarra | Pulo, Orani, Bataan | P2,500.00 | |
| | 7 | Angela Guevarra | Pulo, Orani, Bataan | P2,500.00 | |
| | 8 | Alicia Raymundo | Pulo, Orani, Bataan | P2,500.00 | |
| | 9 | Marilyn Lalican | Pulo, Orani, Bataan | P2,500.00 | |
| 8/29/2013 | 10 | Jennifer Pago | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| | 11 | Mylene Yere | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| | 12 | Joanne del Rosario | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| | 13 | Jesusa Lajara | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| | 14 | Alpay Flores | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| 10/7/2013 | 15 | Bonifacia Mendenilla | Almasen, Hermosa, Bataan | P2,500.00 | |
| | 16 | Emelita Nuqui | Almasen, Hermosa, Bataan | P2,500.00 | |
| | 17 | Caroline Salas | Almasen, Hermosa, Bataan | P2,500.00 | |
| | 18 | Leonor Mintal | Almasen, Hermosa, Bataan | P2,500.00 | |
| | 19 | Remedios Cruz | Almasen, Hermosa, Bataan | P2,500.00 | |
| | 20 | Benny Aguilar | Almasen, Hermosa, Bataan | P2,500.00 | |
| | 21 | Rosalinda Villaruel | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| | 22 | Vivian del Rosario | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| | 23 | Rosette Sanchez | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| | 24 | Rosalie del Rosario | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| | 25 | Marilyn Luega | Sitio Salaman, Pag-asa, Bagac, Bataan | P2,500.00 | |
| 11/7/2013 | 26 | Ma. Victoria Cordova | Limay, Bataan | P2,500.00 | |
| | 27 | Marissa Nuquera | Limay, Bataan | P2,500.00 | |
| | 28 | Editha Esma | Limay, Bataan | P2,500.00 | |
| | 29 | Matilde Fernando | Limay, Bataan | P2,500.00 | |
| | 30 | Rose C. Santos | Limay, Bataan | P2,500.00 | |
| | 31 | Antonia P. Bueno | Limay, Bataan | P2,500.00 | |
| | 32 | Leticia Salandanan | Limay, Bataan | P2,500.00 | |
| | 33 | Veronica Cauayan | Limay, Bataan | P2,500.00 | |
| | 34 | Erlinda Pare | Limay, Bataan | P2,500.00 | |
| | 35 | Joanne Guache | Limay, Bataan | P2,500.00 | |
| | **35** | | | **P87,500.00** | |

**_PROBLEMS AND RECOMMENDATIONS_**

## *I. Mangrove Rehabilitation Project*

1. High mortality of mangroves planted in Kaparangan, Orani, Bataan mostly due to monsoon flood. A and, according to our project beneficiaries, mangrove mortalities may have been caused by other fisherfolks .

***Solutions made: Replanting was done by the project beneficiaries and frequent visitation and patrolling were conducted.***
***Signages were also placed to inform other fisherfolks.***

## *II. Aquasilviculture Techno Demo Project*

1. Limited amount allotted for the materials for fencing of the aquasilviculture techno demo.

***Solution made: Aquasilviculture techno demos were established in cluster of twos(2), threes (3) and fours(4) within mangrove rehabilitated sites.***

2. One fisherman (a former MFARMC Officer) complained about the techno demo to the Barangay Captain of Kaparangan, Orani, Bataan; the complaints were elevated to Mayor Benjamin Serrano resulting to the temporary suspension of the establishment of the techno demo.

***Solution made: An orientation was made at the SB Hall of the Municipality of Orani, Bataan together with the Municipal Vice-mayor, councilor Rome Sicat, Barangay Captains of Palihan, Pantalan Luma, Pantalan Bago, Kabalutan and Kaparangan, MFARMC Officers, Representatives from BFAR 3 and the project team.***

3. Nets of 10 units aquasilviculture techno demo structures were intentionally destroyed by an unidentified individual causing the stocks to escape to the other unit.

***Solution made. The incident was reported to the Orani PNP and to the concerned officials of BPSU and BFAR 3. BFAR 3 replaced the stocks in the damaged aquasilviculture projects to help the fisherfolks recover their losses.***

**_Summary_**

The Philippine National Aquasilviculture Program, a BFAR-CHED collaborative project, aimed to rehabilitate our mangrove forests and at the same time improve the welfare of the marginalized sector, specifically the fisherfolks through resource rehabilitation & livelihood provisions.

Bataan Peninsula State University, after having been given the chance to implement the PNAP oriented 637 fisherfolks from 28 accredited fisherfolk organizations in Bataan. After a thorough selection of beneficiaries following the criteria set therein 16 fisherfolk organizations signed a Memorandum of Agreement with their respective Chair or Leader.

The said project beneficiaries had accomplished their targets within a period of nine (9) months only. They collected and planted a total of 183, 300 mangrove propagules covering approximately 61.10 hectares with 85.96% survival along the coastal areas of Orani and City of Balanga, Bataan where the fisherfolks earned P1,035,452.50 additional income.

They also established 16 units aquasilviculture technology demonstration projects and raised mud crabs, milkfish, blue crabs and shrimps while caring for the mangroves they planted. They reported a total of P 303,279.40 sales from their aquasilviculture projects.

An Inland Based Multi-species hatchery as one of the components of the program, has produced fingerlings of fish species local to the area. When the fingerlings reached the recommended sizes these will be released along the mangrove forest to grow. Two hatcheries were also established to allow gravid fish species being caught by the fisherfolks to lay their eggs and grow in the open, a strategy of increasing fish population and improving fish catch of the fisherfolks.

An estimated 778.5 million blue crabs and 248 million mud crab eggs were spawned through the hatchery and 4,002, 400 fingerlings produced in the Inland based hatchery, were allowed to grow in the open sea to increase their population in the area.

### *Conclusion*

The decline of mangrove resources in our country has significantly reduced the productivity of coastal fisheries due to a continuous decrease in fish capture by the fisherfolks. This is an alarming scenario that could be prevented through the collaborative efforts of the government, the academe and the most affected sectors of our society, the marginalized fisherfolks.

The strategies used in the implementation of the PNAP in Bataan were found effective due to very high survival of planted mangroves. The presence of aquasilviculture technology demonstration projects along rehabilitated mangrove areas significantly helped increase survival of mangroves since fisherfolk beneficiaries were always in their aquasilviculture projects. Fish catch according to some interviewed fisherfolks is also increasing due to continuous spawning of fish in the hatchery.

If the 61.10 hectares of mangrove areas rehabilitated during the first phase of the PNAP will reach its full potential as fish habitat, an estimated 40,937 kg of fish per year will added to the fish catch of the fisherfolks.

Even if only 1% of the 1,030,502,400 spawned or laid eggs of the mud crabs and blue crabs survived, that means around 10.3M of these species will be growing in the wild and may be harvested by the fisherfolks.

If all fisherfolks in our country will be as organized and as eager as those in Bataan, there will be no reason why this government program will not succeed. They are now very much aware that if mangrove areas will be rehabilitated and properly protected, fish capture will definitely increase.

### *Recommendation*

Various sectors of our society should do their part in improving and protecting our coastal resources. Efforts should be exerted to hasten rehabilitation of more mangrove areas to increase fish habitats coupled with the establishment of Community-based Multi-species hatcheries such as the lying-in concept hatchery in every fish farming community of the country.

Research should also be conducted to determine the rate of survival of each fish species being allowed to hatch in the open and/or in mangrove areas.

### *Acknowledgements*

Bataan Peninsula State University wishes to convey its gratitude to the Bureau of Fisheries and Aquatic Resources (BFAR 3) for the rare opportunity to be one of the implementing SUCs of the Philippine National Aquasilviculture Program (PNAP) and for the funding.

Special thanks is also due to the DENR-PENRO in Bataan for the data on the present status of mangroves in the province, to the Local Governments of Orani and City of Balanga, Bataan through their corresponding Municipal Agriculture Offices and their Municipal/City Fisheries and Aquatic Resources management Councils (MFARMCs). Likewise to the Southeast Asian Fisheries Development Center (SEAFDEC) for allowing this paper to be presented during the International Workshop on Resource Enhancement and Sustainable Aquaculture (RESA) on March 5-7, 2014, at Punta Villa Resort, Iloilo City, Philippines.

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# Marine Fish Hatchery: Developments and Future Trends

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## *Abstract*

The basic procedures for producing marine fish fry in hatcheries developed for milkfish fry production nearly 3 decades ago are the basis of fry production systems for all other marine fish species that are now reared in hatcheries in the Philippines and other Southeast Asian countries. These include large-scale microalgae production in outdoor tanks, feeding of appropriate sized rotifer grown on microalgae such as *Nannochlorum* during the first feeding phase, and shifting to larger prey such as *Artemia* towards the latter stages of production.

In recent years, the increasing demand for high-value species such as groupers, sea bass, red snapper, and pompano in both local and export markets has encouraged a number of hatcheries to produce fry to supply the requirements of fish cage farmers. Techniques are modified using information from research institutions and multi-national firms active in developing products and equipment to improve commercial production of these species. Larval feeds of appropriate sizes, forms and presentation for various larval stages incorporating essential nutrients, micronutrients, and feed stimulants are now available in the market. Diseases in marine fish hatcheries have become common occurrences such that various chemotherapeutants, vaccines, and immunostimulants are now available and increasingly being applied in fish hatcheries. Technological developments in hatchery systems, such as the use of recirculating systems, water pretreatment protocols (ozonation, mircrofiltration, UV light treatment) are also increasingly being adopted by commercial establishments.

A critical link between fry production and production of marketable fish is fingerling/juvenile production in nurseries. Fry are commonly grown in brackishwater fishponds to appropriate size for stocking in fish cages. Methods to improve growth through proper feeding and nutrition, eliminate or reduce disease occurrence and parasite infestation, reduce cannibalism in cannibalistic species such as sea bass, grouper and snappers are active areas of research. Nursery production is integrated with fry production in large commercial facilities but is also done by small-scale fish farmers who have access to fry either from the wild or hatcheries. Commercial hatcheries adopt fingerling production from well-studied species in developed countries. Small-scale farmers however still rely on zooplanktons collected from the wild such as copepods, *Moina*, mysids, and trash fish as feed. Production is dependent on availability of feed sources and susceptibility to pathogens and parasites that come with the feed. It can also be erratic since small-scale farms are vulnerable to changes in climate and weather conditions.

Further technological advancement in marine fish hatcheries will increasingly be led by commercial establishments and industries developing equipment like photobioreactor for microalgae to produce algal paste, or methods to develop intensive systems for rotifer culture. Research institutions will however need to support the needs of the small-scale farmers and

hatchery operators who may not be able to apply costly products from these companies by developing innovative simple techniques that can improve culture systems such as producing fry and fingerlings in mesocosm pond system, appropriate use of probiotics as water stabilizer, and production of zooplankton in ponds.

**Keywords:** marine fish, hatchery, larval rearing, nursery, broodstock

## *Introduction*

Fish farming has been practiced for centuries in Southeast Asia with production coming mainly from freshwater culture. Brackishwater culture of milkfish however was a major activity in the Philippines, Indonesia and Taiwan, with milkfish contributing a sizable percentage of the food fish consumed by the population. Milkfish culture has been and continues to be the main aquaculture enterprise in the Philippines with fry traditionally sourced from the sea. Milkfish is the staple food fish in the Philippines, and contributes the largest share in fish produced from aquaculture in the Philippines and Indonesia. However, since more than three decades ago, fry supply had been difficult to procure for some months of the year because of seasonal changes, adverse climatic conditions and actual decrease in volume caught by fry gatherers even during peak months. To assure continuous and reliable fry supply, milkfish breeding research was initiated at the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC AQD)in the 1970s. In collaboration with other international research institutions, the research effort led to the development of broodstock management technologies including induced spawning (Liao *et al.*, 1979), spontaneous maturation and spawning in floating cages (Marte and Lacanilao, 1986) and tanks (Emata and Marte, 1994), and larval rearing technologies (Juario *et al.*, 1984; Gapasin and Marte, 1990). Through the years, these technologies were continuously improved and refined with research on nutrition, physiology, behaviour, disease prevention and management. The broodstock and hatchery technology developed for milkfish was subsequently modified and applied in developing breeding andlarval rearing methods for other marine fish that have high commercial value such as sea bass, grouper, snapper, pompano and rabbitfish (Marte, 2003).

Fry production is the first stage in the fish farming cycle that ends in the production of marketable fish. A necessary and crucial stage however is the production of fingerlings to supply the requirements of fishponds and marine and freshwater cages. While pond culture of fingerlings for stocking in grow-out farms is traditionally practiced by milkfish farmers as part of the farming cycle, recent innovations in nursery rearing has improved production. The nursery subsector of the milkfish industry is now emerging as a lucrative business enterprise.

The basic techniques in larval rearing developed for milkfish, modifications adopted for carnivorous species and those with long larval gestation phases, technologies developed by multinational companies and the private sector to improve production in the hatchery, and recent innovations in nursery production are described in the following sections.

### *Broodstock Development and Management*

Aquaculture had been dependent on wild-caught fry and juveniles for stocking in fishponds or cages. The practice was unsustainable particularly for species such as groupers that are often caught using destructive methods such as the use of cyanide. Even for species such as milkfish whose fry is traditionally caught along the shoreline using fine-meshed nets, the numerous other fry species caught together with milkfish that are discarded contribute to the depletion of important species that are part of the marine food chain making the capture method ecologically unsound. The development of marine fish broodstock and establishment of commercial hatcheries has long been recognized as a primary means of reducing pressure on wild juvenile stocks and supply the demand for seedstock of fish farmers.

#### *Source of breeders: farmed or wild*

Fish broodstock may be caught as adults from the wild and brought to the broodstock/hatchery facility for spawning if these are reproductively ripe. Spawning techniques developed in research facilities such as injection of human chorionic gonadotropin (HCG) or luteinizing hormone releasing hormone (LHRHa) is applied at the appropriate dose and the fish are either strip-spawned or allowed to spawn naturally. Young adults are reared for several years and acclimated to captive conditions of the facility until they show signs of reproductive readiness. As with ripe adults, breeders are checked for spawning readiness and induced to spawn with hormones or allowed to spawn naturally. Facilities for rearing and maintaining marine fish broodstock are either cages located in clean, safe environments such as marine coves, or in land-based canvas or concrete tanks. For practical and economic considerations, young adults are first reared in cages or ponds to reduce maintenance cost and later transferred to land-based facilities when the fish are ready for spawning. Milkfish farmers often leave juveniles and young adults in brackish or marine ponds for 2-4 years before these are transferred to either cages or tanks.

For many marine fish, most of the nutritional requirements of the broodstock have been determined or are currently being refined by nutritionists in research institutions. Commercial feed companies or broodstock operators use the information in formulating appropriate broodstock feeds. Nutritionists determine basic protein, lipid and energy requirements of broodstock and focus on some of the essential nutrients such as highly unsaturated fatty acids (HUFAs) and vitamins that directly affect egg production and quality.

Marine fish broodstock spawn during their natural breeding season although they may be induced to spawn at other times of the year using hormonal and/or environmental triggers. Sea bass for instance may spawn outside their natural breeding season when maintained at 30-35 ppt, 29-30°C and day light regime of 13 hours. Temperature is reduced to 23-24°C for 8-10 weeks a year to simulate cold months and to allow gamete development (Fielder, pers. comm.). Changes in climate patterns appear to have an effect on spawning and egg production as observed recently for milkfish that have been spawning almost year round.

***Marine fish Larval Rearing- then and now***

The specifications and requirements for a small-scale marine fish hatchery are detailed in Sim, et. al (2005). Figure 1 illustrates the basic design of a hatchery suitable for rearing various marine fish such as milkfish, sea bass, grouper, rabbitfish, pompano and others.  Various modifications are made by hatchery operators, depending on their projected production targets, availability of construction materials, financing, market, etc. Site requirements and availability of support services will be the same for small-scale and large commercial hatcheries.

Hatchery production technologies for marine fish in the Philippines started with the development of breeding methods for milkfish in the 1980s. With the successful hatchery production of milkfish fry, research efforts to develop technologies for other marine fish such as sea bass, rabbitfish (Ayson *et al.*, 2014), snapper (Duray *et al.*, 1996), grouper (Sugama *et al.*, 2012) and pompano (Reyes *et al.*, 2014) were done  resulting in the production of fry in commercial hatcheries.

The basic milkfish larval rearing scheme is shown in Figure 2.  Newly hatched larvae are stocked at 10-20 larvae per liter in concrete or canvas tanks filled with seawater that has been seeded with the microalgae *Nannochlorum*.  Rotifers are added on the second or third day, initially at 2-3 rotifers per ml, and then gradually increased to 10-20 individuals per ml as the larvae grow. Water management involves replacing 10-20% of the rearing water with fresh seawater during the first week of larval rearing and increasing the volume  to about 50% towards the later phase of rearing. Microalgae density is maintained at 1-3 x $10^5$ cells per ml during the entire rearing period. With information on nutritional requirements of larvae, microparticulate diets have been developed and these are given at 0.5-2g/ton/day as supplemental feed for larvae as early as the $8^{th}$ day of rearing until harvest.  Milkfish larvae have a short gestation period and fry are harvested on the $18^{th}$ to $20^{th}$ day (Figure 2).

Modifications based on the larval rearing scheme developed for milkfish were adopted for the rearing of seabass, rabbitfish, grouper, snapper and pompano larvae. Larval rearing of these species takes from 50 to 60 days that may be divided into two phases: 1) an early rearing phase lasting 20 days and following the procedure used for milkfish larval rearing; and 2) an extended second phase lasting until the $60^{th}$ day of rearing where larger plankton prey such as *Artemia nauplii*, on-grown *Artemia*, copepods, or mysids are added as live food for the increasingly cannibalistic larvae (Toledo *et al.*, 1999). Artificial diets are also provided, at increasing amounts of up to 3-5g/ton/day. Similar water management methods, such as siphoning of tank bottom to remove debris and excess feeds from the $5^{th}$ day onwards and water change from 20-30% until the $20^{th}$ day, increasing to 50-70% until the 35-$40^{th}$ day, are employed. Continuous flow-through water exchange is done from the $40^{th}$ day until harvest (Figure 3).

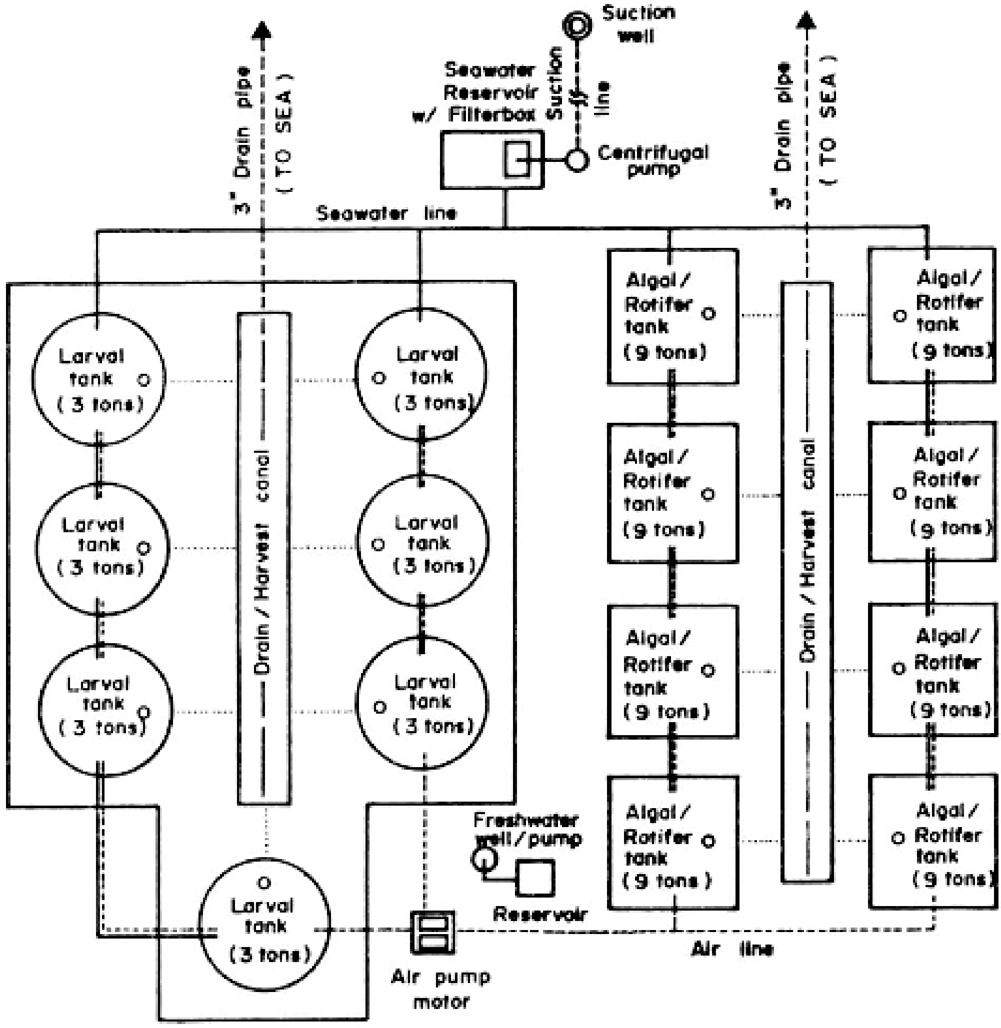


Figure 1. Layout of a typical small-scale milkfish hatchery (Gapasin and Marte, 1990).

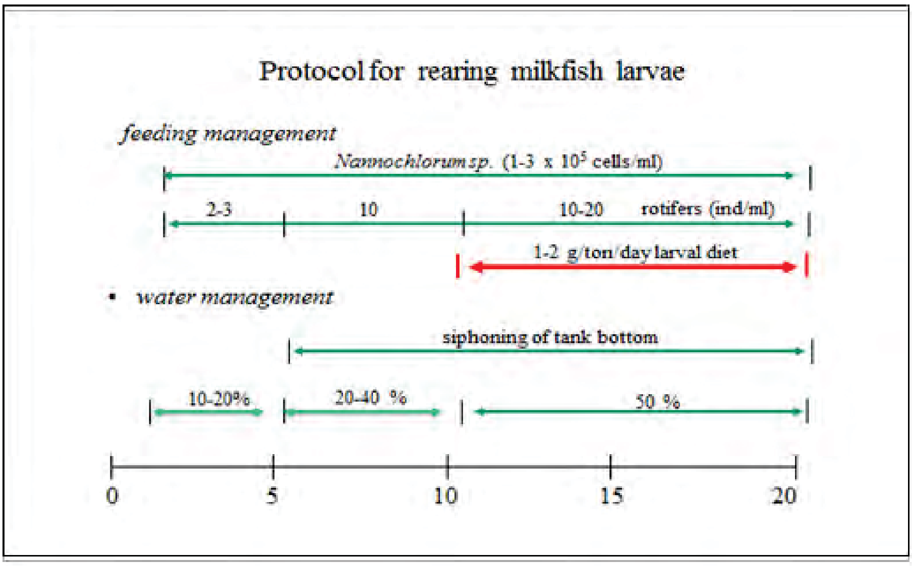


Figure 2. Larval rearing scheme for milkfish.

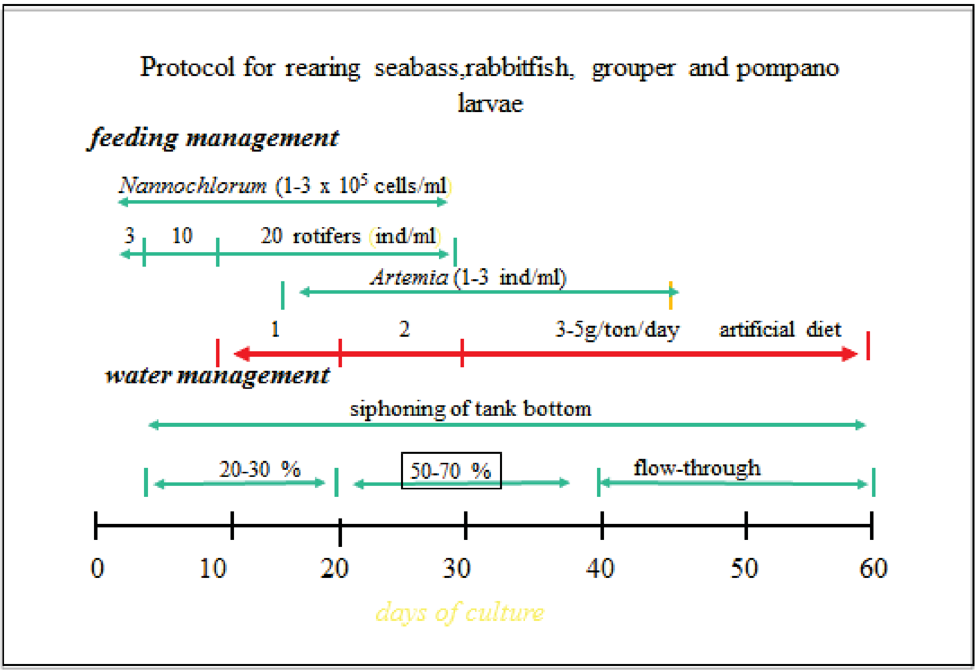


Figure 3. Larval rearing scheme for high value fish (sea bass, grouper, rabbitfish, snapper and pompano).

## *Recent Developments in Fish Hatchery and Nursery Technologies*

Microalgae and rotifers are essential first food for marine fish larvae and culture techniques continue to be improved. Since microalgae and rotifer production requires more than half of the tank facilities of a hatchery, ways to increase cell densities, and methods to produce and to preserve concentrated microalgal paste or slurry are active areas of research although some products from commercial companies are already available to big hatchery operators. Aside from the conventional method of rotifer culture in outdoor tanks using microalgae as sole food, rotifers may now be grown using a combination of Baker's yeast, commercially available *Nannochlorum* or *Chlorella* paste, and live microalgae. These innovations led to the development of super intensive rotifer system with a production efficiency of more than 50 times the conventional system. Because intensive rotifer systems may reach a density as high as 10,000 ind/ml, a system is used to remove solid wastes, neutralize ammonia levels and maintain dissolved oxygen levels higher than 4ppm (for review, see Dhert *et al.*, 2001). A high-density continuous recirculating system using sodium hydroxymethanesulfonate to neutralize ammonia was recently reported to produce large quantities of rotifers on a daily basis without the use of a biofilter and with a lower production cost than a batch culture system (Bentley *et al.*, 2008).

Rotifers and *Artemia* are deficient in highly unsaturated fatty acids (HUFA) that are essential for normal growth and survival of marine fish fry (Ogata *et al.*, 2006). Methods to enrich rotifers and *Artemia* have been developed and products for boosting fatty acid levels are now available. Various formulations containing docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) arachidonic acid (ARA) and Vitamin C can be used to enrich rotifers and *Artemia*. Enriched rotifers and *Artemia* fed to milkfish and other marine fish larvae result in improved fry survival rates and reduced morphological deformities (Gapasin and Duray, 2001). Microorganisms that produce high levels of HUFA such as *Thraustocrytrids* have been shown to improve survival rate of milkfish fry and was comparable to commercial products when used to supplement larval food (Estudillo-del Castillo *et al.,* 2009). To date, there are a number of commercial enrichment products available but these are costly.

Growing microalgae and rotifers to feed to marine fish larvae is labor-intensive. Natural food production is also unpredictable and affected by changing weather patterns especially for small-scale hatcheries that have little or no effective protection against unfavorable weather. With the development of larval diets based on the known nutritional requirements of larval and juvenile stages, microbound, and microencapsulated feeds are fed to the larvae midway during the rotifer feeding period and in most cases may completely replace live food during the latter phases of rearing. Artificial diets should be of appropriate size for the stage of the larvae, attractive to the larvae, digestible and contain nutrients needed by the larvae. In addition, the physical properties of the larval diet is critical in ensuring efficient utilization of the nutrients it contains.

Hatchery facilities range from low-cost canvas tanks of backyard hatcheries to large industrial type integrated broodstock and hatcheries. Support facilities for backyard hatcheries consist mainly of at least two seawater pumps, an aeration system, and a power generator as a back-up. Integrated broodstock and hatchery support facilities may, in addition, include systems to filter incoming water, UV facilities, or other water sterilization equipment such as ozonators to disinfect seawater. These additional equipment are usually included in recirculating systems to control entry of predators, and pathogens.

***Mesocosm Systems***

These are culture systems for fish larvae with water volume ranging from 1 to 10,000 $m^3$ where a pelagic ecosystem is developed consisting of multi-species, natural food chain of phytoplankton and zooplankton for fish larvae. Most common systems used are the pond and tank mesocosm. Cement 50-100 $m^3$ tanks or 300-1000 $m^2$ earthen ponds are cleaned and sun dried for 3-4 days and filled with filtered seawater rich in phyto- and zooplankton. The tanks are then fertilized with commercial sources of nitrogen and phosphorus. Fish larvae, just before complete yolk absorption, are introduced into the system when the abundance of the plankton is enough to support the population. It is important to have proper timing of the availability of larvae for stocking and the available quantity and quality of zooplankton population. Stocking densities vary from 0.1 to 1.0 larva per liter. In a pond mesocosm system in Taiwan, a 500 $m^2$ pond is stocked with 500,000 larvae of the giant grouper *Epinephelus lanceolatus*. The pond is provided with moderate aeration during the first 2 weeks using a single propulsion-type aerator from the 3$^{rd}$ week until harvest. Harvest is done by seine between days 30-35 when total length (TL) is about 1.8-2.5 cm. Additional zooplankton (rotifer, copepod, or mysids) are supplied when needed. Separate ponds may also be prepared to culture these food organisms. Formulated feeds are given in increasing amounts from third week after stocking. Probiotics are widely used to maintain the desired water quality. About 50,000 giant grouper fry may be harvested from this system depending on the quality of the larvae stocked, abundance of natural food and weather conditions (Toledo, personal observation).

***Nursery Phase***

Larval rearing ends after the larvae achieve full metamorphosis. Fry harvested from larval tanks or mesocosm system are often not large nor strong enough for stocking directly in grow-out farms. Milkfish fry are usually stocked in nursery ponds until they reach a size of about 2-3cm. Nursery ponds are prepared by complete drying to eliminate predators and application of appropriate fertilization to promote growth of natural food. Once the natural food are depleted, the "hatirin" are harvested and transferred to a prepared pond to grow further to 10-15 cm for stocking in grow-out farms. Formulated feed is introduced when the natural food in the pond is almost consumed.

Other high value marine species achieve complete metamorphosis at various age and size. Seabass and snub-nose pompano metamorphose between days 21-25 at a size of 1.6 to 2.2 cm TL. Mangrove snapper and tiger and green groupers metamorphose into juveniles from 2.0-2.5 cm TL at days 35-45. Newly-metamorphosed groupers and pompano are usually reared in cement tanks of about 2-5 tons in a flow-through system. Net cages in brackishwater ponds or coastal waters may alternately be used for nursery of metamorphosed snapper and seabass fry. The fry at this stage are weaned to formulated feeds from live food such as copepods, mysids or on-grown *Artemia*. There is an increasing trend in using probiotics in nursery tanks to improve the water quality and reduce water consumption for flow-through system. Size grading is done at least once a week to control cannibalism and to check for parasite and bacterial infection.

A major disease problem encountered in marine fish hatcheries and nurseries is infestations from the dinoflagellate *Amyloodinium* that occur during certain months. Small-scale hatchery operators are mainly affected by the infestation because of lack of filtration facilities, improperly located hatchery (close to rivers and other polluting establishments) and perhaps poor water management. If uncontrolled, this can cause large mortalities in larvae and considerable loss in harvestable fry (Cruz-Lacierda *et al.*, 2004). *Amyloodinium* affects milkfish, seabass, grouper and pompano larvae as early as a week after hatching and may cause massive mortalities if not controlled. Overnight bath at the larval stage in 0.50 ppm copper sulphate may eliminate the free swimming dinospore stage of the parasite but may not eradicate the trophonts attached to larvae nor the reproductive cysts (Toledo, personal observation). The parasite can be controlled at the fry stage by application of low concentration of formalin or hydrogen peroxide and freshwater bath.

Fish with long gestation periods such as seabass, grouper, rabbitfish, pompano and snapper are susceptible to Viral Nervous Necrosis (IVNN), a viral disease that is transmitted from broodstock, plankton, or infected food organisms. The disease was first reported in 2002 in 35 day-old orange spotted grouper (Maeno *et al.*, 2002) and in 14 day-old seabass larvae (Maeno *et al.*, 2004). VNN is now a major disease problem occurring in most fish reared in the hatchery, nursery and grow-out culture. Ways to prevent and control the disease has been a continuing research effort. Since VNN is transmitted from broodstock to eggs and larvae, steps to prevent VNN infection in the hatchery starts with screening broodstock using molecular tools (RT-PCR) and selection of VNN negative fish as spawners. Further screening of eggs, feeding of broodstock with artificial diets, and periodic sampling of larvae for the presence of the virus, need to be done to ensure prevention of the disease (de la Peña, 2010). Husbandry procedures such as thorough cleaning and disinfection of tanks and hatchery paraphernalia, discarding dead fish and reducing stressors to broodstock and larvae also need to be followed. A promising method to prevent viral infection in hatcheries is by enhancing the immune response of broodstock resulting in virus-resistant breeders that produce VNN-free eggs (Pakingking *et al.*, 2010). An annual vaccination regimen using a formalin inactivated virus applied to seabass enhanced neutralizing antibody titers against VNN in broodstock and antibodies transmitted to spawned eggs (Pakingking *et al.*, 2012). Development and maintenance of VNN-free broodstock as source of spawned eggs will be an essential step to ensure disease free larvae in the hatchery. Commercial vaccines currently being developed and tested by a number of pharmaceutical companies (e.g. AquaVac) are expensive and only large-scale hatchery and farm operators may be able to afford these once these become available in the market.

### *Future directions*

Farming of marine fish will increasingly rely on hatchery-produced seeds with new species added to the roster of available species that are being cultured. Research institutions and commercial establishments will actively pursue various avenues to improve production of marine fish fry. These will include improvement of the design of hatchery facilities by developing more cost-efficient filtration and sterilization facilities, improved biosecurity measures, and nutritionally superior broodstock, larval and nursery feeds. For species that are currently being produced in hatcheries, future directions will include undertaking breeding programs to improve growth rates, improve feed quality for breeders to increase egg production and adopt broodstock management procedures that will promote extension of the spawning season of seasonal breeders. Many carnivorous fish are still fed fish by-catch, hence, good quality artificial diets that are attractive to mature breeders still need to be developed. Studies to enhance resistance to diseases, identify new disease agents, and prevent vertical transmission of disease agents from breeders to fry are active research areas. However, cost-effective vaccines and vaccination procedures especially for fry and fingerlings still need to be developed to make these available to small-scale hatchery operators. There are numerous feed supplements currently available but these are costly and may not be affordable to small-scale operators. Natural food candidates that are nutritionally superior to the currently available food items, and natural sources of feed additives can reduce production cost. Efforts to identify, isolate, and develop culture techniques for these organisms need to be pursued. There are already available concentrated preserved microalgal products that are mainly used for the production of biofuels or food supplements for animals but there are very few microalgal species used in marine fish larval rearing that are amenable to preservation. Methods to concentrate, preserve and extend the shelf life of these preserved microalgae for hatcheries will need to be developed. These technologies will considerably reduce requirement for tank facilities, increase fry production potential by utilizing tanks intended for microalgae production but these need to be cost-effective and made available to commercial and small-scale operators.

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# Hatchery Management Techniques for Tiger-tail Seahorse (*Hippocampus comes*)

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## *Abstract*

Seahorse culture has been practiced throughout the world to meet the demand for global trade and reduce the pressure on wild stocks through overexploitation. Development of culture techniques for seed production of seahorses is one of the most effective measures to avoid such anthropological repercussions on the wild stocks, and is currently being conducted at SEAFDEC/AQD with the aim to produce seed for stock release to protect these internationally threatened and overexploited species in Southeast Asia. This paper describes the breakthroughs in seahorse breeding and nursery rearing. So far, we have developed water and feeding management schemes that resulted in improved reproductive performance of broodstock and higher survival and growth rates in newborn and juvenile seahorses.

We highlight the concern of providing desirable food organisms and maintenance of suitable water quality in order to maintain maximum efficiency in the management of the seahorse hatchery. Newborn seahorses fed with formalin-treated food organisms and reared in UV-treated seawater had significantly higher survival and daily growth rate based on stretched height and body weight than those fed with untreated food organisms and reared in both chlorinated and sand-filtered seawater. Broodstocks fed with mysid shrimps showed higher brood size and shorter parturition interval. Thus, improved reproductive performance as well as survival and growth of newborn seahorses were largely influenced by refinement of hatchery management techniques.

**Keywords:** *Hippocampus comes*, seahorse, breeding, nursery, hatchery management

## *Introduction*

All seahorses are listed on Appendix II of the Convention on International Trade of Endangered Species Flora and Fauna (CITES) as of May 2004 with 160 signatory nations, where ‘sustainable’ trade was allowed, meaning, trade must be controlled in order to ensure that their use is compatible with their survival. An estimated 20 million seahorses were consumed every year for Traditional Chinese Medicine (TCM). In the Philippines, the Philippine Fisheries Code of 1998 or Republic Act (RA No. 8550) Section 97 completely bans seahorse trade. However, unmanaged fishing of seahorses still persists. Interest in seahorse aquaculture reflects concern over exploitation in the wild with consequent declines in populations, and in recognition that seahorses command high prices and thus may be highly marketable (Vincent, 1996; Lourie *et al.,* 1999).

Stock enhancement is the stocking of cultured organisms to replenish or increase abundance of wild stocks. Simply said, stock enhancement involves developing successful enhancement by producing and releasing hatchery animals that survive (Leber *et al.,* 2004). Two components of a hatchery-release program are the availability of the cultured organism and the release of these organisms to the natural environment. Breeding and seed production techniques must be developed in the hatchery, while release strategies such as selection of release sites, assessment of the release micro habitat, collection of baseline data on wild populations, magnitude of stocking and development of tagging techniques essential to evaluate survival and efficiency of stocking strategies must also be investigated.

Currently, culture techniques in the seed production of seahorses is being developed at SEAFDEC/AQD with the aim to produce seed for stock release to protect internationally threatened and overexploited species in Southeast Asia. The present study addresses the main breakthroughs in seahorse breeding and nursery rearing. So far, we have tested water and feeding management schemes (Figure 1) that resulted in improved reproductive performance and higher survival and growth rates in new-born and juvenile seahorse.

***Broodstock management***

The selection of pairs of seahorses for broodstock is done after a few days observation of adult seahorses (average size of 85±2 mm stretched height (SH), 2.3±0.3 g body weight (BW)) that exhibit some distinct swimming behavior and intertwining of tails. Sexual maturity in males can be recognized by the presence of brood pouch. Female seahorses transfer eggs to the pouch of males via the ovipositor tube. Eggs are fertilized in the male's pouch. Pregnancy lasts for two weeks, after which the males give birth to live seahorses.

The broodstock seahorses are maintained in 250 L circular fiberglass tanks (Figure 2) at a sex ratio of 1 female:1 male and a stocking density of 1 seahorse 5 L-1 seawater. Temperature of the rearing water is kept at 27-28°C, salinity at 30-33 ppt, and dissolved oxygen at 5.0-7.5 ppm under a LD12:12 photoperiod. Excess feeds and feces are siphoned out from the tank bottom and 30-50% of the rearing water is replaced daily at 0800 h. Sand-filtered seawater and mild aeration are provided in the rearing tanks. Nylon twines tied to lead sinkers serve as holdfasts where the seahorses can coil their tail around.

At the present time, formulated artificial diet for seahorses are not commercially available, thus, seahorses are solely fed with live or frozen food. Seahorses are ambush predators that feed on a variety of mobile preys consisting mostly of planktonic crustaceans such as mysid shrimps, amphipods, copepods, or any tiny larvae that fits into their elongated snouts (Woods, 2002; Kendrick and Hyndes, 2005; Kitsos *et al.,* 2008). One factor to consider in prey selectivity would be the simple digestive physiology of seahorses, which enable them to prefer mysid to *Artemia*. When feeding within the water column, seahorses wait until preys come close to the mouth, whereupon, the preys are drawn up into the long snout with a rapid intake of water (Foster and Vincent, 2004). Thus the snout opening would limit the size of the prey that the seahorse can

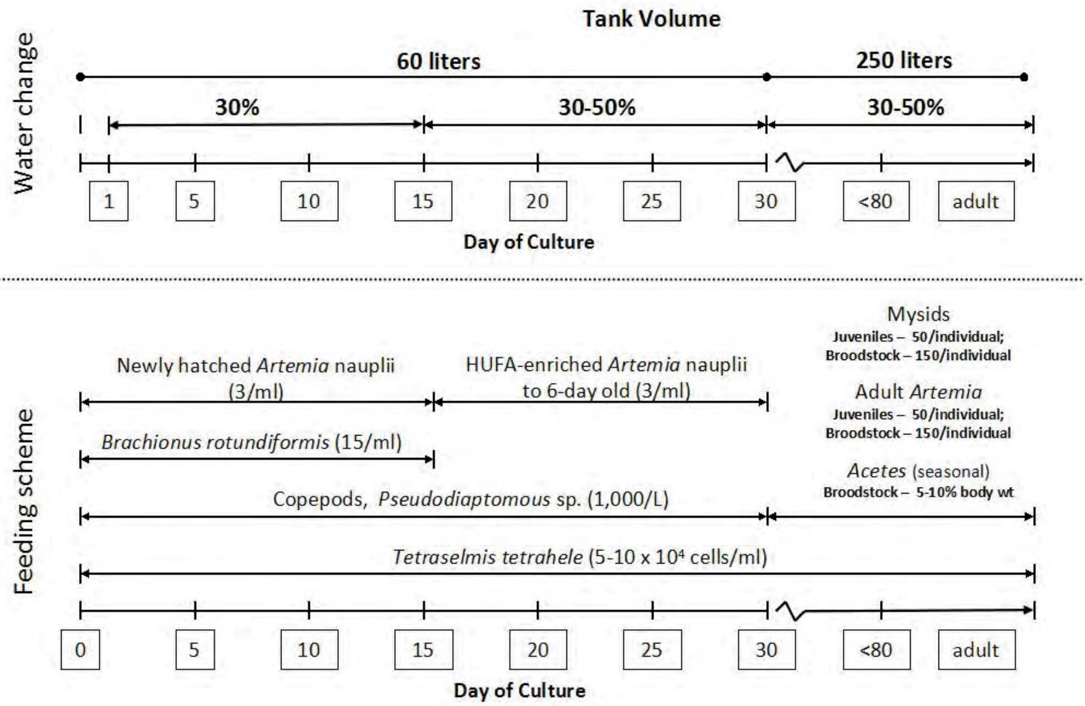


Figure 1. Feeding and water management scheme for seahorse.

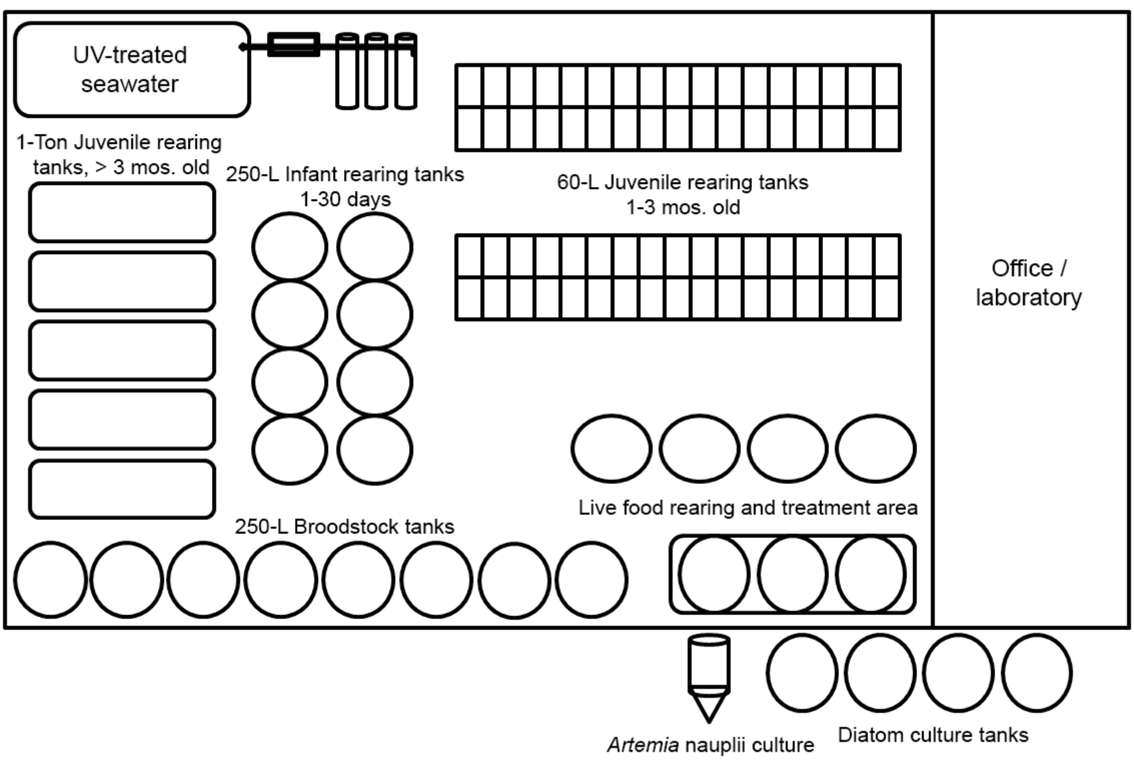


Figure 2. Layout of seahorse hatchery at SEAFDEC/AQD Tigbauan, Iloilo, Philippines.

ingest. Mysid shrimps (18-20 mm) may be longer in length compared to *Artemia* (7.8 mm), but the whole body is slender and the pleopods and pereiopods are found only on the ventral part, while the swimming appendages of *Artemia* are spread on both sides of the body.

The reproductive performance markedly improved when seahorses were fed with mysid shrimp alone or in combination with *Artemia* and *Acetes* (Buen-Ursua *et al.,* 2015). A food preference study where adult seahorses were offered a combination of mysid, *Artemia* and *Acetes* twice daily (0800h and 1400h) showed that mysid was the preferred food as a single diet or combined with *Artemia* and *Acetes*. Significantly higher brood sizes (223-292 newborn seahorses) were obtained from seahorses fed with mysid shrimps as a single diet or combined with the other natural food than diet treatments comprised of *Artemia* only, *Acetes* only and *Artemia+Acetes* which resulted in 107-152 broods. Longer parturition interval was observed in seahorses fed with single diet of *Artemia* (60 days) than those fed solely with mysid, or mysid in combination with other natural food (13-26 days). Parturition occurrence was highest when seahorses were fed mysid alone (13.3±1.5). Thus, better reproductive performance was obtained when seahorses were fed mysids alone or in combination with other natural food.

***Larval rearing of newborn seahorses (0-30 days)***

Parturition events or giving birth of male seahorses usually occur at night time or in the early morning. The male seahorse goes into labor, pumping and thrusting to release his brood. The young are miniature seahorses, also called infants, with average size of 9 mm SH and 0.004 g BW. The newborn seahorses immediately swim up to the water surface to gulp air to inflate their swim bladders. They are collected from the broodstock tanks using a scoop net and transferred to the larval rearing tank (Figure 2).

Newborn seahorses are reared in 250L tanks at a stocking density of 3 seahorses $L^{-1}$ seawater. Newborn seahorses are fed a mixture of newly-hatched *Artemia nauplii* at 3 $ml^{-1}$ and copepod *Pseudodiaptomous* sp. at 10 $ml^{-1}$ per (Figure 1). The copepods are collected using 40 µm plankton net from ponds in Trapiche, Oton, Iloilo, Philippines. Due to high infestation with the parasitic protozoans *Zoothamnium* sp. and *Vorticella* sp., in preliminary studies, copepods were washed several times with UV-treated seawater, subjected to 30 ppm formalin bath for 1 h and rinsed again in UV-treated seawater before feeding to seahorse. Feeding is done twice daily at 0800h and 1300h. The tank bottom water is siphoned out daily to remove uneaten feed and fecal matter. Around 30% of total water volume is replaced with fresh seawater. Mild aeration is continuously provided. On Day 15, nylon twines tied to a lead sinker are provided inside the tanks to serve as holdfasts for the seahorses.

Buen-Ursua *et al.,* (2011) showed that on Day 30, seahorses reared in UV-treated seawater had significantly higher growth in SH and BW (41.4 ± 0.5 mm and 0.23 ± 0.00 g) than those reared in both chlorinated (33.8 ± 1.4 mm, 0.16 ± 0.00 g) and sand-filtered seawater (32.8 ± 0.1 mm, 0.16 ± 0.00 g). Survival was higher in UV-treated seawater (65.6 ± 1.1%) and chlorinated seawater (62.2 ± 4.0%) than in sand-filtered seawater (41.1 ± 1.9%). Survival of seahorses fed with 30 ppm formalin-treated copepod and untreated copepod on Day 8 were 95 ± 2% and 79 ± 6%, respectively. On Day 15, survival was 79 ± 10% in seahorse fed formalin-treated copepod and none (0%) survived among those fed untreated copepod. Survival of seahorse with formalin-treated copepod was 65 ± 10% on Day 30.

### *Larval rearing of seahorses (1-6 months)*

After 30 days of feeding with copepods, the juvenile seahorses are collected from the 250L tanks and transferred to the 60L tank (Figure 2) for nursery rearing at a stocking density of 1 seahorse $L^{-1}$ seawater. A mixture of *Artemia* (up to 6 days old) at 3 $ml^{-1}$ and copepod *Pseudodiaptomous* sp. at 10 $ml^{-1}$ per day are fed to the juvenile seahorses until they are 2 months old. Methods of feeding and maintenance of seawater are the same as previously described for newborn seahorse. Nylon twines tied to a lead sinker are provided inside the tanks to serve as holdfasts for the seahorses.

Seahorse juveniles at 2 to 6 months old are maintained in sand-filtered seawater. They are fed with a mixture of mysids (50/individual), *Artemia* (50/individual) and frozen *Acetes* (5-10% BW). Juveniles that are less than 2 months old are sensitive to fluctuation in water temperature and availability of copepods. At this stage, the juveniles can be weaned to mysid. However, they are not able to feed on frozen *Acetes*. Survival of 2-6 months old juveniles is more stable mainly due to their ability to feed on mysid shrimps and *Acetes*. It was observed that mortalities of juvenile seahorses occur when seawater temperature decreases to 24 or 25°C.

### *Conclusions*

UV sterilization of water and formalin treatment of natural feed resulted to higher survival of the newborn seahorses, which is crucial for stable mass production of seahorse juveniles. Timely and sufficient supply of the necessary food organisms is another key factor to ensure success of seahorse seed production. The development of techniques for the mass production of mysids and copepods as natural food to support seahorse seed production needs to be further pursued to ensure available supply for seahorse hatchery maintenance. Furthermore, an efficient and reliable water supply system is important in maintaining maximum efficiency in the management of the seahorse hatchery.

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# Updates on the Seed Production of Mud Crab

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## Abstract

Widespread interest in mud crab species is increasing because these are highly prized both in domestic and export markets. Among the three mud crab species commonly found in the Philippines, *Scylla serrata, S. olivacea,* and *S. tranquebarica, S. serrata* is preferred by farmers because it is larger and less aggressive than the other species. Likewise, *S. serrata* is the most widely distributed species in the Indo-west Pacific region.

Hatchery-produced seedstock are presently used by some crab farmers in their grow-out operations. In the hatchery phase, feeding mud crab larvae with shrimp formulated diets and natural food was found to reduce the occurrence of molt death syndrome, one of the major problems in seed production. Larvae given 25% formulated diet (FD) + 75% natural food (NF; rotifers and *Artemia*) and 50% FD + 50% NF showed better performance than those larvae fed 100% FD, 100% NF and 75% FD + 25% NF indicating that usage of natural food, especially the expensive *Artemia*, can be reduced. Since the early crab instar (C) produced in the hatchery need to be grown further before stocking in grow-out ponds, two phases of nursery culture have been developed. $C_{1-2}$ are grown to 1.5-2.0 cm carapace width (CW) size in the first phase and further grown to 3.0-4.0 cm CW in the second phase. Nursery rearing is done in net cages installed in ponds for easy retrieval. A combination of mussel or trash fish and formulated diet is used as feed.

Domestication of the mud crab *S. serrata* as a prerequisite to selective breeding has been done at SEAFDEC/AQD. Likewise, defining criteria for the determination of quality of newly hatched zoeae for stocking in the hatchery was initiated. Newly hatched zoeae were subjected to starvation and stress test using formalin. Starvation failed to elicit responses that were significantly different between the good and poor quality larvae hence it is not suitable for larval quality evaluation. Based on three-year data, the formalin stress test gave mean cumulative mortalities of 2.38±0.32, 8.24±0.88, 20±1.58 in good quality larvae, and 43.74±2.39 while 22.93±4.19, 63.68±7.17, 84.29±3.88 and 97.65±1.06 for poor quality larvae at 0 (control), 20, 30 and 40 ppm formalin, respectively. As formalin level increased, cumulative larval mortality also increased regardless of the quality of the larvae. Formalin stress test proved to be a reliable method to determine whether a batch of newly hatched zoeae was of good or poor quality.

**Keywords:** *Scylla* spp., mud crab, quality seed stocks, domestication

## Introduction

Mud crab is highly prized in both domestic and export markets, thus widespread interest in its culture is increasing. Among the three mud crab species commonly found in the Philippines, *Scylla serrata, S. olivacea,* and *S. tranquebarica, S. serrata* is the most widely distributed in the western and central Indo-Pacific regions. This species is preferred for farming because it is larger and less aggressive than *S. olivacea,* and *S. tranquebarica*.

Total mud crab production in the Philippines was 14,438 t in 2010 (valued at US$86,521,000) and increased to 16,360 t in 2012 (valued at US$114,236,000) (FAO ISS, 2014). At present, the Philippines is one of the leading producers of market size mud crab from aquaculture. The major source of seedstock for farming in many countries is from the wild. The overexploitation of mud crabs and habitat losses have resulted in both reduced landings and mean capture size. The depletion of wild stocks highlights the need to develop alternative sources of seedstock like hatcheries. In Viet Nam and the Philippines, crablets are also sourced from the hatchery. In the Philippines, to stem the wild harvest, the provincial and municipal government along with the Bureau of Fisheries and Aquatic Resources have introduced ordinances that prohibit the gathering and selling of crablets (≤ 3 cm) outside the municipality of origin. This resulted to increased acceptability of hatchery-reared crabs by crab growers.

## Domestication

The life cycle of the three mud crab species, *S serrata, S. tranquebarica* and *S. olivacea* has been completed but the focus on the seed production is on *S. serrata*. Domestication of the *S. serrata* as a prerequisite to selective breeding has been initially done at SEAFDEC/AQD (Quinitio *et al.*, 2011). The criteria for the evaluation of the quality of various stages were first established for selective breeding. Stress and challenge tests were used to evaluate the quality of the zoeae and juveniles, respectively. The stress test for the zoeae is now being employed in the larval quality assessment in the hatchery (see section on **Hatchery phase**, this paper).

The bacteria *Vibrio harveyi* was used for the challenge tests to evaluate the disease resistance of each batch belonging to the base population ($P_o$) and first generation ($F_1$). Juvenile crabs from various families were injected with *V. harveyi* at $10^6$, $10^7$ and $10^8$ cfu/ml, and saline solution as control. Juveniles from batch Sam2A ($F_1$). (3 x $10^{5.6}$ cfu/crab) had the highest resistance to *V. harveyi* followed by batch Sam2C ($P_o$) (2 x $10^{5.5}$ cfu/crab), Sam2D ($P_o$) (3 x 105.4 cfu/crab) and batch CamB ($F_1$) (3 x105.5). Juveniles from batch CamA ($P_o$ ) (2 x $10^{4.4}$ cfu/crab) and Sam2B ($P^o$) (2 x $10^{4.5}$ cfu/crab) had high mortality even at low levels of *V. harveyi*. The same pattern was observed in terms of duration to 100% cumulative mortality. The median lethal dose for *V. harveyi* was estimated as $10^{5.696}$. The stress tests in hatchery-reared juveniles, using the white spot syndrome virus, are currently being done by another colleague.

The batches that passed as good quality larvae and juveniles were further reared to broodstock size in ponds and subjected to another evaluation (e.g. growth and reproductive performance), including screening for viruses, prior to selection.

***Hatchery phase***

The inconsistent survival from later zoea to megalopa stage due to molt death syndrome (MDS) remains the major problem in mud crab hatchery. It has been suggested that poor nutrition, low water temperature and prophylaxis application in the zoeal stage are some of the causes of MDS.

Rotifer and *Artemia* are the most commonly utilized natural food for crab larvae. Later megalopa stage and crab instar are fed with minced fish and mussel. At present, there are several commercially available shrimp formulated diets with various levels of HUFA and other essential nutrients that can also be fed to mud crab larvae hence, reducing the use of rotifers and *Artemia*. Feeding of mud crab larvae with shrimp formulated diets and natural food was found to lessen the occurrence of MDS. Mud crab larvae given 25% shrimp formulated diet (FD) + 75% natural food (NF, rotifers and *Artemia*) and 50% FD + 50% NF showed better performance than those larvae fed 100% FD, 100% NF and 75% FD + 25% NF indicating that use of natural food, especially the expensive *Artemia*, can be reduced (Burlas, 2014) (Figure 1).

Likewise, defining criteria for the determination of quality of newly hatched zoeae for stocking in the hatchery was initiated. Newly hatched zoeae were subjected to starvation and stress test using formalin. Starvation failed to elicit responses that were significantly different between the good and poor quality larvae hence it is not suitable for larval quality evaluation. Based on three-year data, the formalin stress test gave mean cumulative mortalities of 2.38±0.32, 8.24±0.88, 20±1.58 in good quality larvae, and 43.74±2.39 while 22.93±4.19, 63.68±7.17, 84.29±3.88 and 97.65±1.06 for poor quality larvae at 0 (control), 20, 30 and 40 ppm formalin, respectively. As formalin level increased, cumulative larval mortality also increased regardless of the quality of the larvae. Formalin stress test proved to be a reliable method to determine whether a batch of newly hatched zoeae was of good or poor quality.

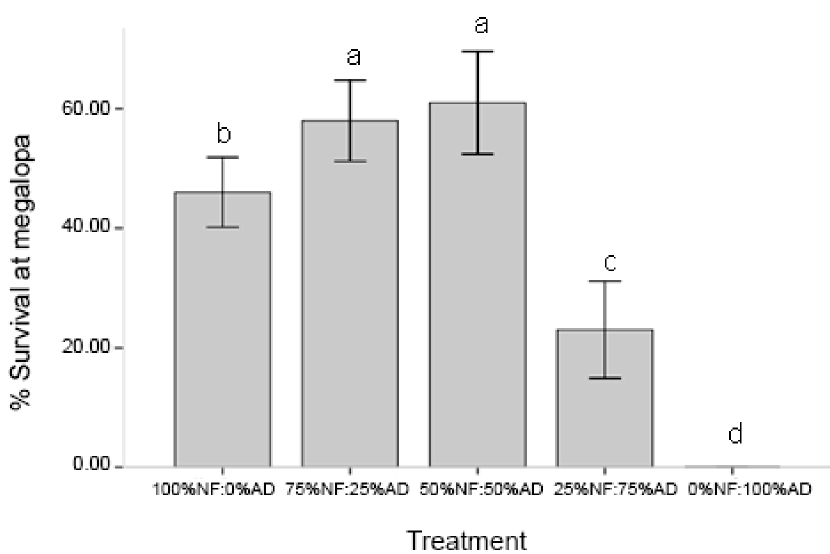


Figure 1. Survival of mud crab larvae fed various combinations of natural food and formulated diet.

To date, the success of mud crab and majority of shrimp hatcheries is still dependent on the use of antibiotics for the treatment against *Vibrio* spp., which is one of the major causes of diseases in the crustacean larvae. The use of antibiotics as treatment for *Vibrio* spp. has proven to improve larval survival (Baticados *et al.*, 1990; Diggles *et al.*, 2000). However, misuse of antibiotics may cause mortalities, incomplete molting (Baticados and Paclibare, 1992), morphological deformities (Baticados and Paclibare, 1992; Pakingking *et al.*, 2002; Lye *et al.*, 2005), and slow growth (Ferreira *et al.*, 2007) in animals. It has been observed that frequency of antibiotic application can be reduced to every 5 days in good quality mud crab larvae. Upon reaching the benthic stage of megalopae, any prophylactic treatment is stopped.

***Nursery phase***

Nursery is the intermediate phase between hatchery and grow-out. Since the megalopa or early crab instar (C) produced in the hatchery need to be grown further before stocking in the grow-out ponds, two phases of the nursery culture were developed. $C_{1-2}$ are grown to 1.5 – 2.0 cm carapace width size in the first phase and further grown to 3.0-4.0 cm CW in the second phase (SEAFDEC *et al.*, 2010). The second nursery phase was developed to address the growout farmers' preference for bigger-sized juveniles (Rodriguez *et al.*, 2007b).

Nursery rearing is done in net cages installed in ponds (Figure 2) for easy retrieval of stocks. For Phase 1, this previously involved megalopae for stocking (Rodriguez *et al.*, 2007a). However, $C_1$ is now used for stocking (Quinitio *et al.*, 2009; SEAFDEC/AQD *et al.*, 2010) in the nursery and this may be due to ease in transport at this stage. Phase 1 and 2 may be done one after the other separately or continuously using the same pond compartment. The culture period is 3-4 weeks in each phase depending on the desired size at harvest.

Figure 2. Net cages installed in ponds used for nursery rearing of *Scylla serrata*.

Phase 2 of the nursery may also be done in net lined ponds or in pens inside mangroves (SEAFDEC/AQD, 2010). The net enclosures, as in cages in ponds, prevent the escape of cultured stocks and the entry of other crab species. Stocking directly in ponds was tried but resulted in lower yields than those stocked in net cages (Rodriguez *et al.*, 2007b).

Several strategies have been tested to reduce cannibalism, one of the major problems in the nursery, and improve yield. Various shelter materials and designs are being tested in actual nursery culture. Crab instars use these shelters for hiding when they molt as they are most vulnerable to cannibalism immediately after molting.

Trimming of claws has been shown to be an effective means to reduce cannibalism (Quinitio and Estepa, 2011) and improve percentage survival of intermolt juveniles. This strategy was applied in the second phase of nursery rearing. Initial results revealed that, although trimming could be done easily with the use of nail clippers, this method did not significantly increase survival and was labor intensive.

Feed for the nursery usually consists of trashfish, mussel, or boiled chicken lungs (SEAFDEC/AQD *et al.*, 2010; Quinitio and Parado-Estepa, 2008) or commercially available formulated diets for shrimps (Shelley and Lovatelli, 2011). Several tests have recently shown that feeding a combination of minced mussel or low value fish and pelleted diet formulated for mud crab gives better survival in the nursery. Tryptophan has been shown to lessen the aggressive behavior in mud crab juveniles in the laboratory (Laranja *et al.*, 2010). Incorporation of tryptophan in the formulated diet is currently being tested in the nursery cage culture to determine if this will significantly augment profitability of nursery culture through increased survival or growth.

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# Marker-aided Genetic Stock Management: Prospects in Philippine Aquatic Biodiversity Conservation and Aquaculture

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## *Abstract*

With the advent of DNA marker-based technologies and applications, genetic stock assessment incorporating molecular marker information has become an important tool in managing resources both for aquaculture and stock enhancement. Local initiatives toward this end have been undertaken by several research and academic agencies particularly those with access to advanced molecular genetic laboratory facilities both in the Philippines and in collaborating foreign institutions. Funds coming from the Philippine Department of Science and Technology and/or international research grants have supported work on commercially valuable species such as tilapia, shrimp, mud crabs, abalone, milkfish and some high value marine fishes with a view of utilizing and in the process, demonstrating the significance of more scientific micro-level assessment of stocks. Information drawn from marker-aided genetic stock evaluation can contribute to a better understanding of the impact of how proper stock management can be more effectively achieved and how this method can gradually translate to improved yields both from culture and fisheries. This paper covers a review of the status of this technology as applied to on-going fish conservation and aquaculture production efforts in the Philippines.

**Keywords:** DNA markers, genetic stock assessment

## *Introduction*

Being an archipelagic country, the Philippines has vast water resources found inland as well as along an expansive 36,389 km. coastline. Marine areas cover territorial waters, shelf areas, coral reef areas to coastal waters while inland waters consist of swamplands, fishponds, lakes, rivers and reservoirs (Table 1). Hence, aquatic organisms abound which are for the most part, directly extracted and/or produced for human consumption. Current fish production estimates can attest to the richness of such resources. Production from capture fisheries is very diverse, ranging from aquatic plants or seaweeds at 458 metric tons (MT), and fishes at 2,363,221 MT which consist of tuna species (frigate, yellowfin, Eastern little tuna, skipjack), big-eye scad, roundscad, mackerel, anchovies, sardines, squid and slipmouth etc. Meanwhile, farmed species is estimated at 1,840,833 MT for aquatic plants or seaweeds and 767,287 MT for tilapias, carps, prawns, mud crabs, abalone, grouper, seabass, siganids, pompano, oysters, mussels, penaeid shrimps, sea cucumbers, the native catfishes and indigenous species such as the giant trevally, climbing perch, silver therapon, etc.

In 2011, the Philippines ranked seventh among the major fish producers in the world, contributing 2.79% of fish, crustaceans, mollusks and aquatic plants, to global fisheries production (Table 2). Increasing fish production holds a lot of potential in aquaculture since fish breeding and farming technologies are now well established, if not, advanced. As for capture fisheries, the challenge is in protecting the habitats (which serve as breeding grounds), from degradation brought about by anthropogenic activities apart from them being exposed to climatic changes resulting from global warming. Aquatic habitat destruction cause irreversible damage to natural biodiversity of which the Philippines is known for.

Table 1. Aquatic Resources in the Philippines (BFAR, 2012).

| MARINE RESOURCES | Area |
|---|---|
| 1. Total territorial water area (including EEZ) | 2,200,000 sq km |
| a. Coastal | 266,000 sq km |
| b. Oceanic | 1,934,000 sq km |
| 2. Shelf area (depth 200m) | 184,600 sq km |
| 3. Coral reef area | 27,000 sq km (within the 10-20 fathoms where reef fisheries occur) |
| 4. Coastline (length) | 36,289 km |
| INLAND **RESOURCES** | |
| 1. Swamplands | 246,063 ha |
| a. Freshwater | 106,328 ha |
| b. Brackishwater | 139,735 ha |
| 2. Existing fishpond | 253,854 ha |
| a. Freshwater | 14,531 ha |
| b. Brackishwater | 239,323 ha |
| 3. Other Inland Resources | 250,000 ha |
| a. Lakes | 200,000 ha |
| b. Rivers | 31,000 ha |
| c. Reservoirs | 19,000 ha |

### *Philippine Aquatic Biodiversity*

The Philippines is one of several countries in Southeast Asia that has an abundance of diverse terrestrial and aquatic biological organisms. Reference to the Philippines being the center of the center of marine biodiversity is an understatement to say the least. There are aquatic organisms that are successfully bred and farmed in captivity apart from those that thrive in natural waters. Due to overexploitation, illegal extraction and simply an inexcusable disregard for the aquatic environment and its fauna, some species are now considered vulnerable and/or threatened while many are ironically, yet to be discovered and named. Some of the known threatened/ vulnerable species are the seahorses (*Hippocampus* spp), sea turtles, abalone, Napoleon wrasse (*Cheilinus undulatus*), sea cucumber (*Holothuria* spp), clams, among others. High value marine species that are often illegally extracted from the wild include the orange-spotted grouper, Napoleon wrasse (which incidentally is also vulnerable), sharks, corals, etc. On the other hand, the Philippines has indigenous species that are known to have commercial aquaculture potential, these are the giant trevally (*Caranx ignobilis*), silver therapon (*Leiopotherapon plumbeus*), climbing perch (*Anabas testudinaeus*), freshwater sardines (*Sardinella tawilis*), to name a few.

Apart from the commercially caught and/or farmed species, several expeditions conducted by foreign scientists in collaboration with local researchers have enabled the collection and subsequent identification of new species. Attempts to document aquatic biodiversity in the Philippines began as early as 1907 when a research vessel, the USS Albatross did a two and a half year survey of aquatic resources in the Philippines. The survey encompassed rocky shores, coral reefs, mangroves, estuaries, deep ocean basins as well as freshwater lakes and rivers (Smithsonian National Museum of Natural History, http://vertebrates.si.edu/ fishes/ albatross/ philippines_exp.html).

Another expedition, under the Panglao Marine Biodiversity Project (PANGLAO 2004), was conducted from May to July 2004 in selected coastal areas in Bohol, Central Philippines to measure aquatic species richness. This involved 70 participants (from 16 countries) who worked on the molluscs and crustaceans collected in 150 km$^2$ of municipal waters covering Panglao, Dauis, Cortes, Tagbilaran and Baclayon. The samples were taken through different methods (intertidal collection, SCUBA collection, traps, tangle netting, dredging and trawling). An estimated 1200 species of decapod crustaceans and 5000-6000 mollusc species were obtained by the investigators (Bouchet *et al.*, 2009).

The most recent expedition conducted in 2011 by researchers from the California Academy of Sciences (CAS) resulted to about 300 new species being identified and several more yet to be taxonomically described and named. It is said to be the largest CAS expedition that was tasked to implement a 1 1/2 month comprehensive survey of terrestrial and marine diversity found in shallow water reefs, deep sea and terrestrial freshwater areas in the Philippines, mainly to look for new species (http://www.calacademy.org./science/ hearst/).

***Biodiversity conservation***

Food production especially from fisheries and aquaculture, is generally confronted with numerous challenges, from climate change, habitat destruction, overexploitation, inappropriate fishing practices, indiscriminate stock movement

Table 2. 2011 World Fisheries Production of fish, crustaceans, molluscs and aquatic plants (including seaweeds), by the top ten producers (BFAR, 2012).

| Major countries | Total | | Fish, Crustaceans, and Molluscs | | | Aquatic Plants (includes seaweeds) | | |
|---|---|---|---|---|---|---|---|---|
| | MT | % share | Capture | Aquaculture | Total | Capture | Aquaculture | Total |
| 1. China | 66,216,938 | 37.15 | 15,772,054 | 38,621,269 | 54,393,323 | 274,060 | 11,549,555 | 11,823,615 |
| 2. Indonesia | 13,601,785 | 7.63 | 5,707,684 | 2,718,421 | 8,426,105 | 5,479 | 5,170,201 | 5,175,680 |
| 3. India | 8,879,499 | 4.98 | 4,301,534 | 4,573,465 | 8,874,999 | - | 4,500 | 4,500 |
| 4. Peru | 8,346,483 | 4.68 | 8,248,482 | 92,200 | 8,340,682 | 5,801 | | 5,801 |
| 5. USA | 5,559,907 | 3.12 | 5,153,452 | 396,841 | 5,550,293 | 9,614 | | 9,614 |
| 6. Vietnam | 5,555,000 | 3.12 | 2,502,500 | 2,845,600 | 5,348,100 | - | 206,900 | 206,900 |
| 7. Philippines | 4,971,799 | 2.79 | 2,363,221 | 767,287 | 3,130,508 | 458 | 1,840,833 | 1,841,291 |
| 8. Japan | 4,755,453 | 2.67 | 3,761,176 | 556,761 | 4,317,937 | 87,779 | 349,737 | 437,516 |
| 9. Chile | 4,436,484 | 2.49 | 3,063,449 | 954,845 | 4,018,294 | 403,496 | 14,694 | 418,190 |
| 10. Russian Fed | 4,391,154 | 2.46 | 4,254,864 | 128,830 | 4,383,694 | 6,639 | 821 | 7,460 |

and poor management, alien species introductions, diseases, pollution, etc. (Table 3). Establishing schemes in maintaining and/or conserving biodiversity is a means of securing these resources in the light of such pressing concerns. Hence proper management of aquatic stocks should be a major consideration.

### *Managing stocks for biodiversity conservation and aquaculture*

Aquatic stock management is a method of dealing with aquatic organisms that are propagated, maintained and utilized for food or other purposes.  Any scheme that is adopted to properly manage stocks is done to minimize their depletion in natural waters and in captivity. Management of aquatic stocks may also be done with an understanding and consideration of the genetic structure of individuals, stocks and/or populations. In this context, proper management is done to reduce not only the depletion of these stocks in terms of numbers but also to minimize their genetic deterioration.

Table 3. Current challenges in fish production.

| Fisheries | Aquaculture |
|---|---|
| Depletion of fishery resources due to overexploitation; illegal fishing practices | Declining production due to poor quality seed stock |
| Genetic contamination of threatened stocks if stock enhancement is not done properly | Low yield due to diseases, improper nutrition caused by poor management practices |
| Poor catches due to displacement/predation by exotic species | Poor harvests due to displacement/predation by exotic species |
| Habitat degradation due to anthropogenic causes | Environmental degradation due to anthropogenic causes |
| Climate change, vulnerability to disasters | Climate change, vulnerability to disasters |
| Reduction of value of catches due to improper post harvest protocols | Reduction of value of yield due to improper post harvest protocols |
| Food safety issues | Food safety issues |

Stock management can be through conventional means and/or genetic (DNA marker based) methods. Traditional stock management can be done through (a) restocking and monitoring of tagged organisms, (b) regulating fishing intensity/practices through the use of appropriate fishing gears, declaring seasonal fishing ban, etc. and (c) adoption of proper breeding/farming schemes. On the other hand, genetic stock management is implemented with the use of DNA marker methods as supportive tools in planning and managing breeding and farming operations in aquaculture and/or in stock enhancement.

### *DNA markers as tools for stock management*

In managing stocks used in aquaculture and biodiversity conservation, individual tagging/marking is done to enable ease in determining stock characteristics and monitoring stock quality, movement etc. There are several tags/markers, the most common types that describe or define individual aquatic organisms in a stock or in a population are the following: (a) phenotypic description, and (b) physical tags. A third type, which requires knowledge in molecular genetics methods, is referred to as genetic markers. Phenotypic description of individual aquatic organisms can be useful for describing animals at any age. However, as such, these are not permanent for the observable traits (e.g. size, color, metric parameters etc.) are unstable for these descriptions change in response to environmental changes. Physical tags on the other hand, are physical identifiers that can help monitor/trace individual organisms. These can be anywhere from coded microwire tags, numbered physical implant tags, diet tags, fin clips, etc. The main drawback is that these may be invasive and the retention rate is not 100%. Meanwhile, genetic markers or biochemical traits that are detectable as protein variants are considered useful at any age, less invasive (except for protein analyses using allozyme markers), stable and heritable hence may provide the best alternative to the other tags, given the resource, skill and knowledge of molecular marker analysis procedures, and marker variation assessment. Table 4 shows a summary of the characteristics of all three tag/marker types.

Genetic markers such as DNA-based markers allow us to know the genetic make-up of individuals and genetic structure as well as phylogenetic relationships of stocks/populations. In aquaculture, examining stock performance would be relevant in planning how farmed fish stocks are to be managed to increase yield or production for improved fishfood sufficiency. Stock performance is assessed through economically important traits (phenotypes) that are essentially the physical expression of genes (genotypes) possessed by individual aquatic organisms. It is observed that the higher the genetic variability or the more genetically diverse stocks are, the more fit and better these are in terms of production or performance traits (growth, disease resistance, survival, etc.).

DNA markers can either be simple protein markers known as allozymes or either of two other types, namely: (a) mitochondrial DNA (mtDNA) markers (e.g mtDNA sequence data, mtDNA restriction fragment length polymorphism or mtDNA-RFLP) that are maternally inherited or, (b) nuclear DNA markers (randomly amplified DNA or RAPD, amplified fragment length polymorphism or AFLP, and microsatellite DNA markers or msDNA) which are biparentally inherited. MtDNA and nuclear DNA markers have often been used recently in view of the numerous advances in DNA marker analysis using polymerase chain reaction (PCR), automated sequencing equipment and web-based analysis software (Romana-Eguia, 2006).

Table 4. Characteristics of the different tag/marker types.

| **Phenotype/observable traits** (size, color, etc) | **Physical tags** (diet tags, coded microwire tags, fin clips) | **Genetic markers** (biochemical traits detectable as protein or DNA variants) |
|---|---|---|
| Useful for identifying/ describing animals at any age | Tags could not be used for younger animals | Useful at any age/size |
| Plastic/ unstable | May be lost as the animal grows (tag retention 85%-90%) | Intrinsic, stable |
| Heritable but expression of the traits are influenced by external factors | -- | Heritable |
| -- | Invasive for some tags | Less invasive especially for PCR-based DNA markers; small tissue can be used |

Several studies on marker-assisted selective breeding and/or stock enhancement have utilized mtDNA sequencing data and microsatellite DNA marker information as tools in stock management programs. Both methods require knowledge and skills in DNA extraction; primer development; PCR amplification of target gene marker regions; purification of DNA samples; automated sequencing; sequence data analysis and/or fragment analysis, to process microsatellite marker data; and should next generation sequencing (NGS) is done, bioinformatics or genetic data management (this is mainly for primer development and genomics work).

***DNA markers: Uses and Applications***

DNA-based markers are used to:

1. ***Discover genes, study their structure and function.*** This is referred to as genomics or genome technology research where gene maps and linkage maps are developed. Such maps are used as reference in:
   a. Marker-assisted selection for genetic improvement based on quantitative trait loci;
   b. Development of effective fish vaccines and delivery technologies;
   c. Monitoring antibiotic resistance;
   d. Diagnosis of aquatic animal diseases;
   e. Understanding the mechanisms and the genes involved in viral disease development and management e.g. determining genes involved in viral infection in shrimps (Alenton *et al.*, Tare *et al.*, this proceedings);
   f. Evaluating success in the development of transgenic fish; and
   g. Discovery of genes useful for biotechnology and pharmaceutical purposes.

2. ***Confirm and/or validate the taxonomic identity of individual organisms.*** Examples of such researches are current efforts on barcoding Philippine lake fauna (Aquilino *et al.*, 2011, Aquino *et al.*, 2011) as well as to determine traceability and mislabeling in commercial fishery products (Maralit *et al.*, 2013)

3. ***Elucidate/reveal cryptic biodiversity in marine and freshwater areas in support to the description of historical studies for marine and freshwater biodiversity.*** This is of utmost importance in countries like the Philippines which is known as to be the center of aquatic (especially) marine biodiversity.

4. ***Identify and discriminate populations, stocks.*** This is a common application of genetic markers particularly if marker-based genetic variation between and within stocks is notably high and significant. There have been several studies conducted for this purpose, mainly to generate genetic databases for aquaculture purposes. Locally, genetic differences in the population structure of farmed tilapias (Macaranas *et al.*, 1986, Macaranas *et al.*, 1995, Romana-Eguia *et al.*, 2004, 2005), wild milkfish stocks (Ravago *et al.*, 2002; Ravago-Gotangco and Juinio-Menez, 2004), farmed and natural tiger shrimp populations (Xu *et al.*, 2001),

among others, have been studied by Philippine scientists under internationally-funded projects. At present, there are several recently completed and on-going DNA marker-based stock assessment/ discrimination studies in the Philippines. Among these are on newly developed tilapia strains (Quilang and Basiao, pers. comm.), tiger shrimp wild stocks which have not been previously characterized, mud crab *Scylla* sp, abalone *Haliotis asinina* stocks and milkfish, albeit using microsatellite markers and mtDNA sequence data information. Unless otherwise stated, these on-going studies are spearheaded by SEAFDEC/AQD, funded by DOST PCAARRD under their National R&D Programs, in collaboration with the Tohoku University. Apart from applications in aquaculture, genetic markers can also be used to discriminate species produced from capture fisheries. An example is a study on distinguishing between juvenile yellowfin and big-eye tunas, both being commercially important fishery products (Pedrosa-Gerasmio *et al.*, 2012).

5. ***Monitor changes within and between stocks and determine inbreeding.*** Changes in individual organisms comprising stocks which are either simply domesticated or bred selectively to promote genetic improvement, can be detected not only phenotypically but also at the molecular level using genetic markers. A study on the use of microsatellite markers in determining genetic variability changes in selected and unselected tilapia stocks, have demonstrated this application (Romana-Eguia *et al.*, 2005). In the aforementioned study, the difference in the specific growth rate of a mass-selected and a control line of tilapia showed a reduction from 0.034% /day to 0.016% /day after four generations. Meanwhile, a higher increase in the inbreeding coefficient was noted in the mass-selected line (108%) as compared to the increase in the inbreeding coefficient of the control line (64.2%) based on genetic variability in four generations of the stocks using five microsatellite marker loci.

6. ***Compare between wild and hatchery stocks.*** An example of this is an on-going study on several wild and hatchery stocks of milkfish collected from local sources as well as from countries (Indonesia and hopefully Taiwan) where most of the commercially imported milkfish seed stock are obtained. This current undertaking has to date identified nine working microsatellite markers that will be used for genetic characterization. The ultimate aim is to enable the identification of sources of good quality milkfish broodstock and genetically improve the Philippine milkfish using marker-aided methods.

7. ***Identify specific markers or quantitative trait loci (QTLs) correlated with fitness and/or quantitative traits for use in marker-assisted selective breeding.*** This is possible if linkage map information is available to allow the determination of QTLs. Marker-assisted selection has been

Table 5. QTL research conducted in different farmed species (Liu, 2007).

| Common name | Species Name | Traits | References |
|---|---|---|---|
| Salmonids | | | |
| Atlantic salmon | *Salmo salar* | Body weight, condition factor, disease resistance, sex | Reid *et al.*, 2005; Moen *et al.*, 2004; 2004c; Grimholt *et al.*, 2003; Artieri *et al.*,, 2006 |
| Rainbow trout | *Oncorhynchus mykiss* | Albinism, condition factor, disease resistance, growth rate, killer cell-like activity, meristic traits, pyloric caecae number, precocious maturation, spawning date, upper thermal tolerance | Danzmann *et al.*, 1999; Palti *et al.*, 1999; 2001; Ozaki *et al.*, 2001; Perry *et al.*, 2001; 2005; Robison *et al.*, 2001; Martyniuk *et al.*, 2003; Nichols et al., 2003a; O'Malley *et al.*, 2003; Somorjai *et al.*,, 2003; Khoo *et al.*, 2004; Nichols *et al.*, 2004; Zimmerman *et al.*, 2004; 2005; Moen *et al.*, 2004b; Reid *et al.*, 2005; Rodriguez *et al.*, 2005 |
| Coho salmon | *Oncorhynchus kisutch* | Flesh color | Arenada *et al.*, 2005 |
| Artic char | *Salvelinus alpinus* | Temperature tolerance, growth rate, condition factor | Somoraj *et al.*, 2003; Tao and Boulding 2003; Reid *et al.*, 2005 |
| Tilapia | | | |
| Tilapias | *Oreochromis spp.* | Body and peritoneum coloration, cold tolerance, disease resistance, growth rate, immune response prolactin expression level, survival, sex determination, sex ratio, stress response | Shirak *et al.*, 2000; 2002; 2006; Streelman and Kocher 2002; Palti 2002; Cnaani *et al.*, 2003; 2004a, 2004b, 2004c; Lee *et al.*, 2003; 2004; 2005; Moen *et al.*, 2004a |
| Carp | | | |
| Common carp | *Cyprinus carpio* | Cold tolerance | Sun and Liang 2004 |
| Molluscs | | | |
| Eastern oyster | *Crassostrea virginica* | Disease resistance | Yu *et al.*, 2006 |
| Shrimp | | | |
| Kuruma prawn | *Penaeus japonicas* | Body weight, total length and carapace length | Li *et al.*, 2006 |
| Others | | | |
| Zebrafish | *Danio rerio* | Behavioral and morphological differentiation | Wright *et al.*, 2006 |

done on several commercial aquaculture species (refer to Table 5). It has yet to be done on local species.

8. ***Assess success of genetic manipulation methods such as polyploidy induction, gynogenesis and transgenesis.*** DNA markers can be used to distinguish the genetic make-up of manipulated stocks against normal, non-manipulated or genetically unmodified stocks. There are no local initiatives towards this end for development of genetically modified aquatic organisms is not encouraged in the Philippines.

9. ***Monitor the fate of stocks after deliberate or accidental release in the wild.*** This is important in conservation and stock management research. DNA markers can be utilized to trace the impact of stock introductions/ enhancements in sites where natural populations are noted as depleted. Recently, a study co-funded by the Japan Society for the Promotion of Science with SEAFDEC/AQD on the genetic impact of reseeding on natural abalone stocks in the Sagay Marine Reserve, Philippines using molecular marker profiles, was completed in collaboration with the Tohoku University.

## *Conclusion*

Several research initiatives that show the application of DNA marker technology in the Philippines was presented. In most instances, micro-level stock analysis through DNA marker applications were undertaken to provide an effective means of monitoring and managing stocks both for conservation and production purposes. Local scientific undertakings are now possible with trained human resources using laboratory facilities established in national and international research and academic institutions, through research linkages.

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# Feed Formulation for Sustainable Aquaculture

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***Abstract***

As aquaculture production of tropical fish and crustacean species becomes more intensified, practical diets need to be formulated to be cost effective and environment-friendly. Ingredients should be included to satisfy the nutrient requirements of the animal, promote optimal fish growth, and boost the income of small-scale farmers and commercial producers with minimal impacts to the surrounding environment. Feed formulation for sustainable aquaculture should aim at increasing aquaculture system performance and profitability, enhancing the animals' disease resistance, increasing attractability, palatability, and digestibility of practical diets, and maintaining environmental quality through sound feeding management and good aquaculture practices. More vigorous research and development efforts need to be supported to generate feed technologies that will ensure a steady and reliable supply of safe and high quality aquaculture products to the public while preserving the environment.

**Keywords:** practical diets, feed formulation, sustainable aquaculture

## *Healthy and wholesome aquaculture*

One of the most important thematic programs of SEAFDEC/AQD is Healthy and Wholesome Aquaculture which is an important strategy towards increasing the productivity of aquaculture systems. Ultimately, by improving performance of the aquaculture system and by maintaining environmental quality, aquaculture will be sustainable for generations to come.

Over the last few decades, increases in aquaculture production have been based on the application of aquaculture feed. Considering that about 60-70% of investment in aquaculture is due to feeds and feeding management and with an annual growth rate of roughly 5% and 75% of production being due to feed taking species, the feed demand exceeds 20 M metric tons annually. At the average percentage of crude protein for the aquafeed of about 30%, the protein requirement exceeds 6M metric tons per year. At this rate, the aquafeed industry cannot continue its dependence on fish meal and will have to find alternative sources of protein.

Feed formulation is the process of combining different feed ingredients in proportions necessary to provide the fish with proper amounts of nutrients needed at a particular stage of production at a reasonable cost. It requires knowledge about nutrients and feedstuffs, nutrient requirements, palatability, acceptability, digestibility, toxicity, as well as costs.

Proper and correct feed formulation as well as good aquaculture practices and feeding management will ensure sustainable aquaculture, aquaculture that is (a) environmentally acceptable to all stakeholders; (b) economically viable depending on the level of economic development of the particular locale or country where the operations take place; and (c) socially equitable, a concept that varies according to the differences in social parameters of a particular society.

To deliver proper nutrition and feeds to aquaculture species, several aspects need to be considered such as nutrient requirements of aquaculture species, sustainability of aquafeed ingredients (sources of raw materials that are environmentally acceptable), improved feed management by improving feed quality, farming of low-trophic level species and integration with other agricultural farming activities.

***Feeds for sustainable aquaculture***

The aims in formulating feeds for sustainable aquaculture are improved production, higher disease resistance, better attractability and palatability of aquafeeds, and stronger environmental protection.

*Improved production and profit*

Improved feed composition and better feed conversion efficiency increase fish production, lower feed cost, and minimize the production of wastes from fish farms. A balanced diet for fish is important in ensuring fast growing, healthy, and disease-free fish and shrimps. Giving food that supplies all the components of good nutrition is essential in good aquaculture practices. Nutrients provide energy sources, build tissues, and are able to regulate metabolism in fish and shrimps. These nutrients are carbohydrates, fats or lipids and fatty acids, proteins and amino acids, vitamins, minerals. Nutrient standards for complete feeds have been recommended in the recently published Philippine National Standards for aquaculture feeds. This is the output of the technical working group composed of resource persons from the academe, research and development institutions and the Philippine feed industry upon consultation with major stakeholders in the aquaculture industry in the Philippines (BAFS/PNS 84 - Aquaculture Feeds, 2010).

Carbohydrates include simple sugars, starches, celluloses, gums and related substances that are inexpensive sources of energy giving 4 kilocalories of energy per gram of carbohydrate. Thus, as much carbohydrate as the fish or shrimp can use is usually included in aquaculture diets. They are also used as feed binders (for example, bread flour, carrageenan, agar, and alginates) to make the feed stable in water. Table sugar (sucrose), glucose, lactose, bread flour, wheat flour, corn starch and cassava starch are good carbohydrates and bread flour, wheat flour, and starches are used as carbohydrate sources in fish or shrimp diets.

The ability of fish or shrimps to make use of carbohydrates in their diet varies considerably. Most carnivorous species have limited ability to use carbohydrates compared with omnivorous or herbivorous species. The carbohydrate levels in grow-out diets for various tropical aquaculture species are 25-30% of the diet, 29%, 45%, and 55% for grouper, Tiger shrimp, milkfish, and tilapia, respectively.

Fats or lipids are organic compounds that are important components of biomembranes of animals, plants, and microbes. They are nutrients that are not soluble in water, but are soluble in organic solvents like ether and alcohol. Lipid in the diet of aquatic animals has two main functions – as a source of energy and as a source of essential fatty acids that cannot be made in the body of the animal. In addition, lipids are also important sources of fat-soluble vitamins. Lipids provide a secondary storage of heat and energy in that one gram of fat or lipid gives 9 kilocalories of energy. Fish and shrimps require w3 and w6 -fatty acids in their diets because they cannot make them. The polyunsaturated fatty acids (PUFA) namely: linoleic acid (18:2w6) and linolenic acid (18:3w3), and the highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA, 20:5w3), docosahexaenoic acid (DHA, 22:6w3) and arachidonic acid (ARA, 20:4w6), are needed by fish and shrimps. Failure to provide these essential fatty acids in the diet can slow growth and consequently, a prolonged lack of these fatty acids in the diet can lead to death. Animal sources of fats or lipids in fish diets are cod liver oil (CLO), squid liver oil, and beef tallow while the plant sources are soy bean oil (SBO), corn oil, coconut oil, and sunflower seed oil. CLO is rich in w3 HUFAs and SBO is rich in w6 and also w3 PUFAs.

The crude fat levels in grow-out diets for tropical aquaculture species can vary considerably. Diets for marine fish like grouper and milkfish can contain higher levels (11-12%) of fat compared with those of shrimps or freshwater fish like tilapia (4-9%). A 1:1 combination of CLO: SBO is usually used to provide a source for both w3 and w6 fatty acids.

The essential fatty acid requirements of tropical aquaculture species also differ for warmwater tropical fishes or shrimps. Marine carnivores like grouper requires 1% of w3 HUFA from fish oil while a marine planktivore such as milkfish requires 1-1.5% w3 PUFA from fish oil and/or SBO. In contrast, a freshwater herbivore like tilapia needs w6 PUFA from SBO. Tiger shrimp needs 0.5% w3 PUFA and less than 0.5% w6 PUFA from fish oil and/or SBO. The differences are due to the varying adaptations to the combination of the predominant PUFA in the marine environment and the carnivorous preference of marine species. The unsaturated fatty acids in the marine food web are dominated by w3 HUFA originating from marine algae and carnivores consume smaller fish that depend on phytoplankton and zooplankton. The essential fatty acid requirements of freshwater fish can generally be met by w3 PUFA because freshwater microalgae generally have w3 PUFA rather than the w3 HUFA. In addition, w6 PUFA, and not w3 PUFA, can be abundant in freshwater microalgae.

Lastly, increased fish oil in finisher diets leads to increased levels of w3HUFAs in the tissues of fish. This observation has important implications for fish husbandry in ensuring the health, growth and survival of farmed fish as well as in maintaining the quality of fish products because of the benefit of consumption of fish and its content of w3 fatty acids to human health.

Proteins are needed by fish and shrimps for making new tissues (growth and reproduction), and replacing worn-out tissues (maintenance and repair). Proteins function in two ways: They provide the ten essential amino acids (histidine, methionine, arginine, threonine, tryptophan, isoleucine, leucine, lysine, valine, and phenylalanine) which cannot be made in the body of the animal and thus must be obtained from the diet. Proteins are also a tertiary source of energy in that one gram of protein can give 4 kilocalories of energy. Inadequate dietary protein will slow growth and severe lack of protein in the diet can eventually lead to death. Common protein sources in fish or shrimp diets are classified into two – animal sources such as fish meal, shrimp meal, squid meal, and meat and bone meal; and plant sources such as soy bean meal, pea seed meal, cowpea meal, and various leaf meals.

The nutritive value of dietary protein depends on the ability of the protein source to fulfill the essential amino acid requirements of fish or shrimps. The closer the profile of amino acids in the protein source to the requirement level, the higher is the nutritive value of the protein. Thus, the essential amino acids coming from the diet must satisfy the amino acid requirements of the animal to be of any nutritive value.

Complete protein sources are those that contain all the essential amino acids needed by fish or shrimps. Animal protein sources are usually complete proteins. Some protein sources especially plant protein sources lack certain essential amino acids. For example, soy beans lack methionine and are said to be limiting in methionine. Corn lacks lysine and tryptophan and is said to be limiting in these amino acids. It is therefore ideal to have a mixture of protein sources to provide a good amino acid balance needed by the animal.

The essential amino acid requirements for tropical aquaculture species such as milkfish, tiger shrimp and Asian sea bass have been determined (Borlongan and Coloso, 1993; Millamena *et al.*, 1996-1999; Coloso *et al.*, 1999, Murillo-Gurrea *et al.*, 2001, Coloso *et al.*, 2004). Aquaculture diets for these species should be formulated with amino acid levels that conform to the requirement levels for these amino acids for optimum protein efficiency.

The crude protein levels in fish or shrimp diets also vary. Grow-out diets for carnivorous species like grouper contain more protein (44% or higher) and tiger shrimp contain 42% while grow-out diets for omnivorous or herbivorous species such as milkfish or tilapia can contain much less protein (28-32%).

In addition, the crude protein levels in diets for other life stages of tropical aquaculture species are not static and can also vary. Diets for the larval stages contain higher levels of protein (38-50%) to support rapid growth as well as for broodstock stages (44-48%) to support ovarian maturation and production of good quality eggs and larvae.

Vitamins are organic substances that are present in small amounts and are vital for the health and well-being of fish and shrimps. There are two classes of vitamins depending on their solubility characteristics. The water-soluble vitamins are vitamin C, vitamin B complex, folic acid, inositol, choline, and pantothenic acid. The fat-soluble vitamins are vitamins A, D, E, and K. Fish meal, organ meats, leaf meals, yeast and other microorganisms are good sources of vitamins. The vitamin requirements for tropical species have been determined in some species but not in others (Halver, 2002). It is difficult to determine the vitamin requirements of fishes and shrimps because these are in minute amounts, the basal diet must contain purified ingredients free of vitamins, and the water medium must also be vitamin-free. Minerals help to build and maintain the tissues of fish and shrimps and regulate metabolism. There are four major classes: macrominerals – sodium, potassium, calcium, phosphorus, magnesium, sulfur, carbon, hydrogen, oxygen, nitrogen and chloride; microminerals or trace elements –iron, zinc, iodine, manganese, fluoride, copper, selenium, molybdenum, chromium, and cobalt; ultratrace elements- silicon, vanadium, nickel, tin, aluminum, and boron; and the toxic elements- cadmium, arsenic, mercury and lead. Good sources of minerals are fish meal, soybean meal, various leaf meals, seed meals, flour, and rice bran. Aquaculture diets can contain up to 2-4% each of vitamin and mineral premixes. The stable form of vitamin C, magnesium ascorbyl phosphate is used because ascorbic acid is rapidly destroyed upon contact with water. Oftentimes, additional vitamin A and E are also added especially for broodstock feeds.

The two most important factors to consider in formulating a feed for any aquaculture species for improved production are nutrient requirements and feeding behavior of the fish or shrimp. Adequate nutrients must be given to fish or shrimps for faster growth and survival and feeds that are suited to the feeding behavior of animals should be offered. The culturist must know what feed to give to a fish that swallow food whole or shrimp that nibble slowly on their food. Some fish feed on the surface, some on water column, and still others are bottom feeders. Nutrient requirements also vary with various life stages. The culturist must know the nutrient requirements of these life stages to provide good nutrition and ensure rapid growth of the cultured species and minimize the production of waste from the farming activity. Balanced nutrition leads to good growth, low feed conversion ratios, lower production costs, higher profit, and more sustainable production.

### *Higher disease resistance*

All of the essential nutrients discussed in the preceding section should be provided in the diet in adequate amounts to ensure the health of aquatic animals. Several of these nutrients and other components of the diet as well as feeding practices influence the susceptibility of fish and shrimps to various infectious and noninfectious diseases. Diseases of fish can be caused by bacteria, fungi, viruses, parasites as well as environmental and genetic factors. Fish exhibit different tolerances to different environmental conditions and disease agents.

In terms of the dietary components, nutrient deficiencies can adversely affect fish health making them more susceptible to various disease agents. However, dietary supplementation of certain nutrients at levels above the minimum requirements has shown enhancement of some immune responses and resistance to various disease agents. Of the various vitamins that are essential for fish and shrimps, vitamin C and vitamin E have several distinct metabolic effects but they both have antioxidant functions. Higher vitamin C and E levels leads to increased disease resistance perhaps because high tissue levels of these vitamins provide a readily available reservoir for use by the animal to defend itself from disease causing agents. The high levels of these vitamins have shown lower mortalities of fish challenged by bacteria or a stimulation of the nonspecific immune response (Gatlin, 2002).

Nonnutritive dietary components such as the immunostimulant b glucan may also potentially aid in disease control of fish or shrimps. These compounds are polysaccharide derivatives from yeast and fungi and act to stimulate the specific or nonspecific immune response of fish and increase survival (Gatlin, 2002). Other dietary components such as nucleotides have also been shown to enhance antibody production, increase the nonspecific immune response, increase disease resistance and survival. Nucleotides function as building blocks for DNA/RNA synthesis and cell growth, enhance stress tolerance, and enhance disease resistance.

### *Better attractability and palatability*

The overall profitability of aquaculture depends on preventing mortality during the early life stages and maximizing feed utilization during the entire duration of culture. This depends on the physical and chemical properties of the feed to ensure maximum utilization of the nutrients in the feed. It important that fish can efficiently utilize the feed and also that the nutrient components must be stable during manufacture, storage, transport, and feeding. Feeds must have the proper density, odor, size, taste, appearance and must have good binders to make it stable in the water so that nutrients do not leach out in the water before the fish can eat the feed (Pigott and Tucker, 2002). Factors that make diets attractive to fish and palatable include high quality of ingredients, use of fresh oils, fish meals with low biogenic amine content, absence of mold and low tannin content. Use of chemical attractants in the feed for fish and crustaceans is desirable. Attractants include marine based extracts and/or meals, fish meal, fish solubles, squid meal, squid liver meal, squid oil, shrimp meal, shrimp head meal, short necked clams; free amino acids- glutamate, glycine, taurine, arginine, lysine; and others such as glucose, starch, gelatin, casein, pheromones, betaine, putrescine, and cadaverine.

### *Stronger environmental protection*

Better feeding management is required for environment protection and sustainability of aquaculture systems. Excess, uneaten feeds and feces sink to the bottom sediment. Dissolved nutrients and decomposing bottom sediment increase nutrient loading, change the algal composition and production, and changes the nature of the benthic community. These conditions deplete the dissolved oxygen, and increase the production of harmful gases such as H2S by anaerobic conditions having deleterious effects on aquatic organisms. If unabated, the poor environment will cause the gradual deterioration of the ecosystem leading to fish kills and can eventually lead to the eutrophication of inland bodies of water. Eutrophication is the unnatural enrichment of the water with two plant nutrients, nitrogen and phosphorus. The phosphorus (P) requirements (0.29-0.85%) of several fish species are known. In rainbow trout, low dietary P and high vitamin D levels, decrease the soluble and fecal P levels. Low dietary P levels increase P deposition (as % of P intake) in the tissues (Coloso *et al.*, 2001, 2003). However, the use of low P diets are costly thus needs to be regulated. In rainbow trout fed 1.0 or 1.4% dietary P, growth is similar in both diets, but the low dietary P, decrease soluble and fecal P excretions. However, cost of production is higher using low P diets, thus needs regulatory measure (Sugiura *et al.*, 2005). Increased P availability from the diet can also be achieved with supplementation of phytase, an enzyme that liberates the phosphate group from phytic acid content of plant sources making it more available to the animal. Thus, to protect the environment we need to include enough non-protein energy to decrease nitrogen excretion to the environment, reduce feed conversion ratio to minimize impact to the environment, use low P diets to reduce the amount of P excreted to the environment, and use phytase to increase P availability.

***Summary and conclusion***

The concept of healthy and wholesome aquaculture is an integral component in improving and sustaining aquaculture production to provide fish protein and other beneficial nutrients. Our understanding of the principles of aquaculture nutrition and feed formulation for tropical aquaculture species has vastly improved over the years resulting in better feed formulations and better feeding practices. However, more vigorous R & D efforts in nutrition and fish health management involving various sectors of the aquaculture industry need to continue to ensure a steady, sustainable, and reliable supply of safe and quality fish beneficial to public health while preserving the environment.

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# Potential of Cowpea (*Vigna unguiculata* L.) Meal as an Alternative Protein Source in Diets for Giant Freshwater Prawn (*Macrobrachium rosenbergii*, de Man 1879)

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## *Abstract*

Growth trials were conducted to evaluate cowpea *Vigna unguiculata* (L.) meal as a potential protein source in diets for giant freshwater prawn, *Macrobrachium rosenbergii* (de Man 1879), reared in tank and lake-based cages. Five isonitrogenous (approximately 37% crude protein) and isocaloric diets were formulated where fish meal (FM) protein was replaced with 0%, 15%, 30%, 45% and 60% cowpea meal protein (or CP0, CP15, CP30, CP45, and CP60, respectively). Results of an 8-week tank trial showed that the final body weight (FBW), percent weight gain, specific growth rate (SGR) and survival of prawns were not significantly influenced by dietary treatments ($P > 0.05$), although the highest values, except for survival, were observed with CP45. In a lake-based cage trial that lasted for 16 weeks, prawns fed CP30 and CP45 had significantly higher FBW (13.1 and 14.4 g, respectively) compared to other treatment groups ($P < 0.05$). SGR (4.52–5.00%/day), survival rates (53-77%), yield (98.5-116.5 g m-2) and feed conversion ratio (FCR; 2.0-2.7) were not affected by increasing levels of cowpea meal in the diets. Based on these results, cowpea meal can be considered as an alternative protein source in diets for *M. rosenbergii*.

**Keywords:** *Vigna unguiculata*, giant freshwater prawn, growth, Laguna de Bay

## *Introduction*

The giant freshwater prawn (*Macrobrachium rosenbergii* de Man) is an economically important species for aquaculture in Asian countries such as China, India, Thailand and Malaysia. It is a promising alternative to black tiger shrimp (*Penaeus monodon*) due to its high market value and relatively low susceptibility to diseases. Presently, in the Philippines, farming of *M. rosenbergii* in natural inland water bodies such as lakes and reservoirs could be a sustainable option for the growth of aquaculture in lake-shore fish farming communities (Cuvin-Aralar *et al.*, 2007), similar to other well-known species such as bighead carp *Aristichthys nobilis*, milkfish *Chanos chanos* and Nile tilapia *Oreochromis niloticus*. Farming of this species requires a nutritionally-balanced diet for optimal growth and survival. However, the rising cost of feed hinders profitability of production due to the use of expensive protein sources such as fishmeal (McCoy, 1990; Tacon and Metian, 2015). Feed constitutes 40-60% of the operational costs for *M. rosenbergii* culture (Mitra *et al.*, 2005). Therefore, assessment of locally available sources such as plant proteins for use in feed formulations needs to be tapped and explored.

Among the alternative protein sources for fishmeal, cowpea (*Vigna unguiculata* (L.)) meal has been used to replace fishmeal in crustacean diets because of its high nutritional value and digestibility (Eusebio, 1991; Eusebio and Coloso, 1998; Rivas-Vega *et al.*, 2006). An important legume crop in the Philippines and in other Southeast Asian countries, cowpea seeds are known for their crude protein content of 23–26%, high levels of essential amino acids such as lysine and tryptophan and digestible energy. Likewise, the successful use of *V. unguiculata* has been reported for tilapia feeding (Keembiyehetty and de Silva, 1993; Olvera-Novoa *et al.*, 1997). The present study evaluated the response of *M. rosenbergii* to diets containing cowpea meal (*Vigna unguiculata*).

## Materials and Methods

### Experimental Diets

The chemical composition of *Vigna unguiculata* is shown in Table 1. Five experimental diets were formulated by replacing 0%, 15%, 30%, 45% and 60% of the FM protein with cowpea meal (CP0, CP15, CP30, CP45 and CP60). All diets were formulated to be isonitrogenous (approximately 37% dietary protein) and isocaloric. The experimental diets were tested in both tank and lake-based feeding trials.

### Feeding trials

#### Tank trial

Fifteen day-old postlarvae (0.029 ± 0.008 g mean weight) were stocked in 60-l polyethylene tanks at 15 prawns per tank and acclimatized for one week prior to actual feeding trial. Tanks were half-filled with freshwater which was maintained throughout the experiment. Tanks were provided with nets as substrates where PL

Table 1. Proximate composition (% dry matter) of cowpea meal *Vigna unguiculata.*

| | Cowpea meal |
|---|---|
| Moisture | |
| Crude protein | 23.03 |
| Crude fat | 0.28 |
| Crude fiber | 4.38 |
| NFE* | 68.65 |
| Ash | 3.66 |

*Nitrogen Free Extract

adhered after feeding. Experimental diets were fed at 20–30% of estimated biomass three times daily at 0800, 1300, and 1600 h for an eight-week period. Each diet treatment was replicated thrice. Water temperature, dissolved oxygen (DO) and pH ranged from 26.3–28.4°C, 5.62–8.88 mg $L^{-1}$ and 8.6–9.4, respectively during the rearing period.

### Lake-based cage trial

Postlarvae (PL20) were stocked in hapa net cages (L × W × H: 2 × 2 × 1.5 m) in Laguna de Bay with 15 shrimps $m^{-2}$ (0.04 ± 0.01 g body weight) and three replicate cages per treatment. Each cage was provided with two used A-nets (mesh size: 2 $mm^2$; dimension: 0.5 × 2.0 m) as shelters and suspended horizontally inside each cage. The prawns were fed experimental diets once daily (0900h) at 10, 8, 6 and 4% of estimated biomass for the 1st, 2nd, 3rd and 4th month of culture (Millamena and Triño, 1997). Total length, individual weight, weight gain and survival were monitored monthly. Production parameters such as final weight, percent weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and survival were used to evaluate the acceptability of cowpea meal in diets for *M. rosenbergii*.

Water quality was monitored inside the cages between 0800 and 0900 hours for the duration of the experiment. DO levels ranged from 3.47–6.95 mg $L^{-1}$ and temperature was noted between 25.8–28.4°C. pH readings varied from 7.5–8.4 during the trial period.

### *Data analysis*

The results for growth, feed conversion ratio (FCR) and survival were analyzed using one-way ANOVA followed by Tukey's post hoc test when significant differences were detected. Survival data were arcsine transformed prior to statistical analysis. All statistical tests were performed using the Number Cruncher Statistical System (NCSS 07.1.4 version) 2007 Software (Hintze, 2007).

## *Results*

### *Tank trial*

The results for survival and growth after an eight-week tank trial are shown in Table 2. The experimental diet CP45 gave the highest mean weight, percent weight gain and SGR, but there were no differences among treatments ($P > 0.05$). However, a gradual decrease in growth performance was observed at CP60. Survival rates ranged from 83 (CP45) to 93% (CP15) and no significant difference was detected among treatments.

### *Lake-based Trial*

FBW ranged from 10.1 to 14.4 g with significantly higher FBW at CP30 and CP45 compared to other treatments ($P < 0.05$). SGR (4.52–5.00%/day) and survival rates (53.4–77.2%) did not differ significantly among treatments. The experimental diet CP60 gave the best survival rates but the poorest FBW and SGR among the experimental diets. Production ranged from 98.5 g $m^{-2}$ (CP0) to 116.2 g $m^{-2}$ (CP60) and feed conversion ratio (FCR) between 2.00 (CP30) and 2.72 (CP60). No significant differences were found among treatment means for yield and FCR (Table 3).

Table 2. Growth and survival parameters monitored in *Macrobrachium rosenbergii* postlarvae fed diets with varying levels of cowpea *Vigna unguiculata* meal for 8 weeks during the tank trial.

| Diets | Parameters | | | |
|---|---|---|---|---|
| | FBW (g) | Weight gain (%) | SGR (% d$^{-1}$) | Survival (%) |
| CP0 | 0.230 ± 0.045[a] | 693 ± 155[a] | 3.43 ± 0.35[a] | 88.3 ± 7.6[a] |
| CP15 | 0.249 ± 0.038[a] | 759 ± 129[a] | 3.57 ± 0.24[a] | 93.3 ± 5.8[a] |
| CP30 | 0.271 ± 0.064[a] | 833 ± 221[a] | 3.69 ± 0.40[a] | 91.7 ± 7.6[a] |
| CP45 | 0.288 ± 0.037[a] | 892 ± 128[a] | 3.81 ± 0.21[a] | 83.3 ± 11.5[a] |
| CP60 | 0.208 ± 0.029[a] | 617 ± 99[a] | 3.27 ± 0.24[a] | 90.0 ± 5.0[a] |

FBW = final body weight, SGR = specific growth rate
Initial prawn weight, 0.029 ± 0.008 g; [1]SGR = $(\ln wt_{final} - \ln wt_{initial})$/days of culture × 100
Survival = actual count at harvest/initial stock × 100
Column means followed by different letter superscripts are significantly different at $P < 0.05$

Table 3. Production parameters for *Macrobrachium rosenbergii* fed diets with varying levels of cowpea *Vigna uinguiculata* meal for 16 weeks during the lake-based cage trial.

| Diets | Parameters | | | | |
|---|---|---|---|---|---|
| | FBW (g) | SGR (% d$^{-1}$) | FCR | Survival (%) | Yield (g m$^{-2}$) |
| CP0 | 10.6 ± 1.03[a] | 4.55 ± 0.13[a] | 2.36 ± 0.12[a] | 62.2 ± 8.39[a] | 98.5 ± 4.04[a] |
| CP15 | 11.1 ± 1.18[a] | 5.00 ± 0.57[a] | 2.13 ± 0.26[a] | 62.8 ± 0.96[a] | 104.0 ± 9.56[a] |
| CP30 | 13.1 ± 0.42[b] | 4.97 ± 0.24[a] | 2.00 ± 0.20[a] | 55.0 ± 7.64[a] | 108.4 ± 15.4[a] |
| CP45 | 14.4 ± 2.92[b] | 4.97 ± 0.31[a] | 2.18 ± 0.20[a] | 53.4 ± 4.24[a] | 109.3 ± 32.5[a] |
| CP60 | 10.1 ± 0.76[a] | 4.52 ± 0.22[a] | 2.72 ± 0.04[a] | 77.2 ± 8.22[a] | 116.2 ± 9.03[a] |

Column means followed by different letter superscripts are significantly different at $P < 0.05$

## *Discussion*

The present study was conducted to evaluate the potential use of *Vigna unguiculata* as an alternative protein source in diets for *M. rosenbergii*. Based on chemical composition, cowpea meal has a high nutritional value (23% crude protein). Likewise, the nitrogen free-extract (NFE) or the carbohydrate content of *V. unguiculata* showed that it can be an excellent source of energy in crustacean diets.

In terms of biological performance, results of the tank study indicated that the growth performance of *M. rosenbergii* PL fed the control diet was inferior to prawn fed cowpea meal-based diets. SGR, in particular, was comparable to or even higher than the findings of Du and Niu (2003) who achieved an SGR of 2.5% day$^{-1}$ when soybean meal was used to replace FM in diets for the same species. Growth performance improved with increasing levels of cowpea meal protein, but the best results were obtained at 30 to 45% substitution. However, the inclusion above 45% resulted in diminished performance in terms of mean FBW, suggesting that mixing of cowpea meal with low levels of FM protein may have contributed to the slower growth of *M. rosenbergii* PL. Cowpea meal contains several inherent anti-nutritional factors such as trypsin inhibitor which may interfere with feed utilization. This however, may not be the case as the trypsin inhibitor activity (TIA) in the cowpea meal ranged from 23.7–31.6 TIU/mg protein as reported by Ologhobo and Fetuga (1984) and Rivas-Vega *et al.,* (2006) and even lower than those reported for soybean meal (106 TIU/mg of sample) (Kakade *et al.,* 1974), suggesting minimal impacts of any trypsin inhibition. On the other hand, while analysis of essential amino acids (EAAs) of experimental diets have not been determined, high inclusion level of cowpea meal at 60% is likely to be limiting in EAAs such as methionine and has resulted in poor growth . Survival rates (83–93%) of *M. rosenbergii* PL in the present tank study were comparable to or slightly lower than those reported by Roy *et al.,* (2009).

Similar trends in growth were also observed in the lake-based feeding trial with cowpea meal-based diets performing better than the control diet (CP0), which has FM and shrimp meal (*Acetes* sp.) as protein sources. *M. rosenbergii* are omnivore species which can efficiently digest both plants and animal protein sources (Ashmore *et al.,* 1985). This explains the best growth performance at CP45 experimental diet in this study. However, the mean weights of the prawns fed the best performing diet (CP45; 14.4 g) after 120 days are considerably lower than those obtained by Cuvin-Aralar *et al.,* (2007) for similar stocking density (15 prawns $m^{-2}$; the mean weight after 150 days is 26.3 g), but comparable with the mean sizes at higher stocking density (90 prawns $m^{-2}$; the mean weight at harvest is 14.3 g). Differences in the final size or weight at harvest were attributed to the initial size of PLs used at the start of the experiment (0.04 g, this study vs. 0.40 g in Cuvin-Aralar *et al.,* (2007). Nevertheless, SGRs obtained in the present study (4.52–5.00% $d^{-1}$) were considerably higher than those obtained by Cuvin-Aralar *et al.,* (2007) (2.68–3.02% $d^{-1}$) and Ghosh *et al.,* (2010) (3.55–3.75% $d^{-1}$).

The better survival of prawns in cages at CP60 (77.2%) followed by CP15 (62.8%) and CP0 (62.2%) has resulted to smaller size of prawns at harvest (10.1, 10.65 and 11.1 g for CP60, CP0 and CP15, respectively). Conversely, the relatively lower survival at CP30 (55.0%) and CP45 (53.4%) produced larger prawns (13.1–14.4 g), which were comparable to or even higher than those obtained by Cuvin-Aralar *et al.,* (2007), who reported survival rates ranging from 36.9–55.3%. Lower survival rates achieved in these diets maybe attributed to heterogeneous individual growth (HIG), possible entry of predators and competitors inside the experimental cages, and cannibalism (Ranjeet and Kurup, 2002; FAO, 2002; Cuvin-Aralar *et al.,* 2007). Nonetheless, FCRs obtained in the present study were comparable to those reported in other studies (FAO, 2002; Cuvin-Aralar *et al.,* 2007). Shrimp production varied from 98.5 to 116.2 g $m^{-2}$, which is generally higher than those reported in pond culture in Asian countries such as India (12–45 g $m^{-2}$) (Ghosh *et al.,* 2010).

In summary, the present study shows the potential of cowpea (*Vigna unguiculata*) meal as an alternative protein source in diets for *M. rosenbergii*. Cowpea meal can replace FM at 30–45% inclusion level with no adverse effects on growth and production of this species reared under laboratory and lake-based conditions.

***Acknowledgements***

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# Application of the United States Soybean Export Council Program's Soy-optimized Floating Feeds and Low Volume, High Density Cage Aquaculture Technologies

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## *Abstract*

The United States Soybean Export Council's (USSEC) Soy-In-Aquaculture (SIA) project in the Philippines introduced the Low Volume High Density (LVHD) cage culture production methodology in 2003. The aim of this technology is to maximize farmers profit, improve productivity, reduce feed conversion ratios (FCR) and limit environmental degradation. The Philippine fish farmers were very conservative and hesitant about adopting the USSEC SIA Low Volume High Density (LVHD) cage culture technology, particularly the new feeding techniques using extruded floating feeds. This conservative attitude was highlighted with different projects using Nile tilapia (*Oreochromis niloticus*), milkfish (*Chanos chanos*) and snubnose pompano (*Trachinotus blochii*) in USSEC SIA LVHD cage feeding demonstrations conducted in different commercial farms in the Philippines.

**Keywords:** low volume high density, extruded floating feeds, target biomass, cage positioning, feeding management

## *Introduction*

The United States Soybean Export Council (USSEC) program under the Soy-In-Aquaculture (SIA) Project conducted different feeding demonstrations using Nile tilapia (*Oreochromis niloticus*), milkfish (*Chanos chanos*) and snubnose pompano (*Trachinotus blochii*) at different commercial cage farms in the Philippines. The objective of the USSEC Low Volume High Density cage production technology demonstration was to show the correct application of the following: (a) USSEC-developed satiation feeding technique, (b) least-cost formulated soy-optimized aquafeeds, (c) feed enclosures in the cages to prevent floating feeds from exiting the cages, and (d) cage positioning for better water exchange in the cages, which is critical for good results in a high density system.

### Materials and Methods

For the Nile tilapia feeding demonstrations, five units of 3 x 3 x 2.5 m or 22.5 $m^3$ bamboo floating cages were used in a commercial farm in Taal Lake, Batangas, Philippines. Tilapia fingerlings of about 7.5 g were stocked in the 22.5 $m^3$ cages at a density of 8,700 fish per cage. Fish in all five cages were of uniform size and age at stocking. Tilapia production targets were 300 g per fish and 2,250 kg per cage, or 100 kg $m^{-3}$ of cage volume. Tilapia were fed thrice daily with USSEC 36/7 soy-optimized extruded floating feed for tilapia weighing 7.5 g to 50 g, and fed twice daily with USSEC 32/6 extruded floating feed for tilapia 50 g to harvest. These feeds were formulated by USSEC and produced domestically in the Philippines. The five cages were treated as replicates of a single feed treatment, with fish in all cages fed to satiation twice to thrice daily every seven days, using the USSEC satiation feeding technique.

For the milkfish feeding demonstrations, four units of 5 x 5 x 4 m or 100 $m^3$ bamboo floating cages were used in Panabo City, Davao del Norte, Philippines. Milkfish fingerlings of about 32.0 g were stocked in the 100 $m^3$ cages at a density of 7,800 fish per cage. Fish in all four cages were of uniform size and age at stocking. Milkfish production targets were 500g per fish and 3,750 kg per cage, or 37.5 kg $m^{-3}$ of cage volume. Milkfish were fed five times daily with USSEC 34.7/9.8 soy-optimized extruded floating feed for milkfish 25 g to harvest. These feeds were formulated by USSEC and produced domestically in the Philippines. The four cages were treated as replicates of a single feed treatment, with fish in all cages fed to satiation five times daily every seven days, using the USSEC satiation feeding technique.

For the pompano feeding demonstration, three units of 3x3x3 m or 27 $m^3$ floating steel cages were used in Cebu, Philippines. Pompano fingerlings of about 19.0 g were stocked in the 27 $m^3$ cages at a density of 2,150 fish per cage. Fish in all four cages were of uniform size and age at stocking. Pompano production targets were 500 g per fish and 1,075 kg per cage, or 39.8 kg $m^{-3}$ of cage volume. Pompano were fed twice daily with USSEC 43/12 soy-optimized extruded floating feed for fish from 19 g to harvest. These feeds were formulated by USSEC and produced domestically in the Philippines. The three cages were treated as replicates of a single feed treatment, with fish in all cages fed to satiation twice daily every ten days, using the USSEC satiation feeding technique.

In all the demonstration cages, the nets were made from a rectangular nylon mesh cage net which were suspended and weighted down to maintain the cage shape against water currents. As the fish grew, the mesh sizes of cage nets were increased to maximize water exchange. Each cage was equipped with an internal feed enclosure and a light blocking cover as specified in the ASA-IM LVHD Manual “Principles and Practices of High Density Fish Culture in Low Volume Cages”.

Cage management was based on the USSEC cage production model. Fish in all cages were sampled once per month on about the same date each month. At the conclusion of the project, all cages were completely harvested and all fish weighed. All of the harvested fish were enumerated when weighed to obtain an average fish size and fish survival. Results were used to determine fish survival, average fish weight, gross fish production and feed conversion ratio (FCR).

### *Results*

Tilapia was fed for 132 days between 29 July and 08 December 2011. Tilapia grew from an average of 7.5 g to 300 g in this period and yielded an average production of 1,572 kg cage$^{-1}$ or 70 kg m$^{-3}$. Mean survival rate was 60% and average FCR was 1.47:1.

Milkfish were fed for 133 days between 31 May 2012 and 11 October 2012. Milkfish fed with 34.7/9.8 grew from an average of 32 g to 379 g, with a mean FCR of 1.92:1. Average production was 2,776 kg cage$^{-1}$ or 27.8 kg m$^{-3}$ with a mean survival rate of 99%.

On the other hand, pompano were cultured for a total of 142 days between 29 July and 17 December 2008. Pompano fed USSEC 43/12 diet grew from an average of 19 g to 338 g, with a mean FCR of 2.38:1. Average production was 763 kg /cage or 28.2 kg m$^{-3}$ with a mean survival rate of 99.1%.

### *Discussion*

This is the first time for most of the cooperators to try the USSEC feed-based LVHD cage culture technologies. The feed manager and technicians required some time to learn the satiation feeding technique during the first months of culture. This unfamiliarity with the USSEC feeding management technique could have been one factor why the target weight of some fish was not attained.

Despite the problems encountered in implementing these new technologies, at the end of each project, the fish farmers were able to appreciate the positive aspects of the USSEC LVHD cage culture technology. The farms that have adopted the use of maximum cage volumes, target biomass densities, proper cage positioning, use of high quality extruded floating feeds and USSEC satiation feed management have recognized its benefits compared to their traditional commercial culture methods and have improved their production.

### *Acknowledgements*

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# Utilization of Sensors and SMS Technology to Remotely Maintain the Level of Dissolved Oxygen, Salinity and Temperature of Fishponds

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## *Abstract*

Due to the occurrence of fish kills in various fish producing areas in our country, millions of pesos and opportunities for the Filipino people had been put into waste. Bataan Peninsula State University (BPSU) collaborated with the Central Luzon Association of Small-scale Aquaculture to devise strategies to address the said problem and prevent further losses.

More often than not, a fish kill can be attributed to the low level of dissolved oxygen (DO) in the water, decrease or increase in salinity and sudden increase in temperature, which usually occur after heavy rainfall, flooding or high tide, or high levels of ammonia due to decomposing organic matter and high temperature during summer.

For these reasons, BPSU researchers tested the use of radio frequencies and installed sensors in different areas of the fishpond at various depths to remotely monitor the levels of DO, salinity and temperature of the water. Once these reach critical levels, the installed system which comes with a specific program, will send an alarm through radio frequencies via Short Messaging Services (SMS) technology on the cellular/mobile phone of the caretaker or the fishpond operator. Upon receiving the alarm, caretakers were able to adjust the levels of dissolved oxygen, salinity and temperature of the water by remotely switching on the air compressor or the electric water pump using their cellular/ mobile phone, thus preventing losses due to fish kills.

**Keywords:** fish kill, dissolved-oxygen (DO), salinity, radio frequency, sensors

## *Introduction*

The increasing activity of people affects the environment. Changes in the environment can be seen as part of climate change, from massive rainfall, floods, high tide, high temperature, etc. and these have brought about unstable production of food and food scarcity. The traditional way of maintaining the environmental conditions suitable for food production has not provided solutions to these present-day concerns which otherwise can be solved through precise and quick responses.

In 27 May 2011, the World Mind Network and the Batangas Fish kill Research Group reported a massive fish die-off in Taal Lake, Batangas. Over 800 metric tons of bangus and tilapia died in the areas of Laurel, Talisay, Tanauan City, and San Nicolas. In mid-June, more fish died, this time near Cuenca and Lipa City. More fish kills occurred until the month of July. In Taal Lake alone, the Department of Environmental and Natural Resources (DENR) reported that from 27 May until 8 June, 2,056 metric tons of bangus were counted as losses from 239 fish cages in 9 municipalities, and this amounted to P144 million. Fish kill losses in Taal Lake and Pangasinan have reached P190 million.

There were several theories to explain the die-off. The predominant one is that the beginning of the rainy season resulted in low oxygen levels in the lake. The problem with this is that the rainy season happens at almost about the same time every year, and yet fish kills like this one are very rare. A major factor is that there are still 14,000 fish cages in the Lake, even though the government in 2009 has put forth a regulation to reduce this to a maximum of only 6,000 cages.

Some believe that overfeeding of fish in commercial cages caused the die-off. Others hypothesized that increased emissions of hydrogen sulfide and sulfur from Taal volcano may be a factor. These were noticed during earlier fish kills.

Advanced technologies such as sensors and mobile phones lessen the need for people to be physically present in some activities so that they can spend more time in doing more productive things, and yet be assured that his/her job, company or business will still be managed efficiently through special monitoring and controlling facilities.

### *Objectives of the Study*

The study aimed to develop an automated monitoring system with controlling facility using sensors and a database management system to remotely monitor the dissolved oxygen (DO), salinity and temperature of water in fish culture enclosures, particularly in ponds, thus preventing fish kills.

Specifically, it aimed to: 1) use wireless technology in maintaining the required level of DO, temperature and salinity in the fish production system; 2) prevent or at least lessen damages brought about by fish kills for sustainable food production; 3) create a program in the microprocessor that would give a preset environment condition in an automated aquaculture system as a solution to problems with regards to DO, salinity and temperature; 4) remotely monitor and maintain the DO, salinity and temperature for the species being raised in the fish ponds; and 5) assess its contribution to the aquaculture industry and fish pond operators.

### *Materials and Methods*

The following were the factors considered in designing the project:

*Installation* – the server, the heart of the system, must be located in a safe and suitable facility near the location of the fish pond.

*Flexibility* – the system must be able to read, store, send, alter, manipulate and process the data from the program through the database.

*Storage capacity* – the server must have a high powered memory capacity.

*Availability* – the programming language used must be accessible in order to build a better database system.

*Usability* – it must be easy to use, understand, access and control.

*Competitiveness* – it must be able to help the various sectors in the fishery industry specifically, aquaculture by remotely monitoring and maintaining favorable environmental conditions.

A schematic diagram of the operational design for the automated monitoring system is presented in Figure 1. Meanwhile, the actual experimental setup layout is shown in Figure 2.

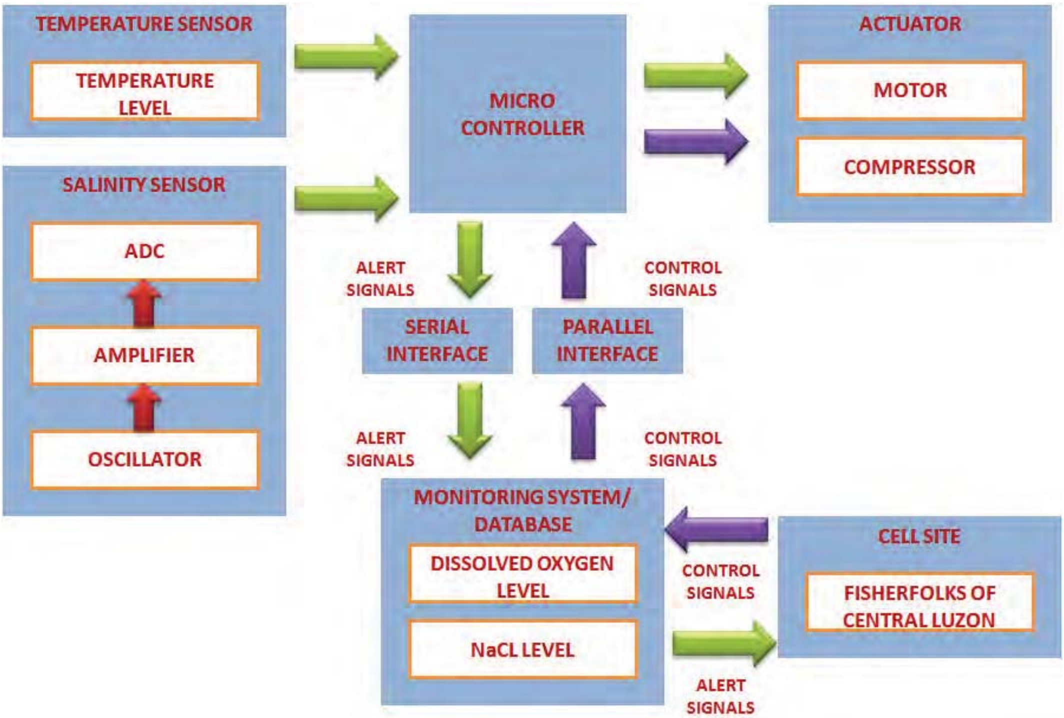


Figure 1. The operational design of the water monitoring and alarm system and how each component interfaces with each other.

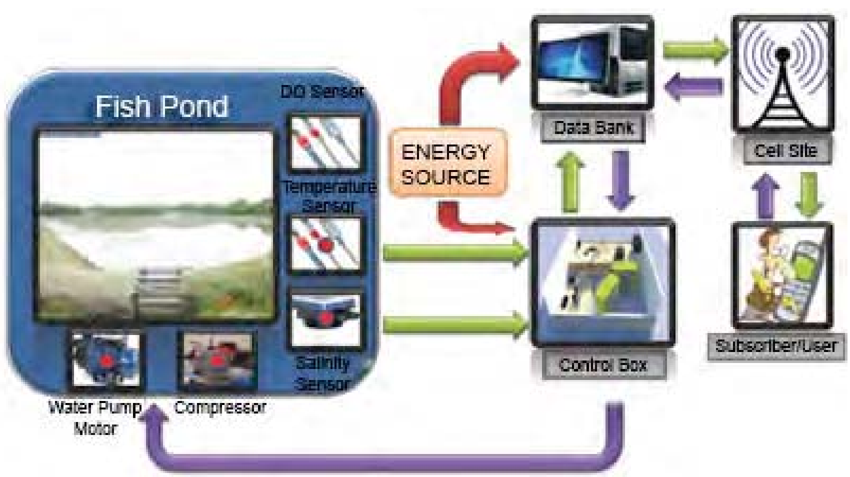


Figure 2. The layout of the experiment.

## Results

Data on the levels of dissolved oxygen, salinity and temperature of the water in the experimental pond were successfully collected through the use of sensors and recorded in the micro controller unit (MCU) for analysis and interpretation, and transmitted via SMS to the mobile phone of the owner whenever a critical level had been reached. The compressor and electric water pump were also successfully switched on and off through the mobile phone of the owner even if he was not near his pond to adjust the DO, salinity and temperature of the water to desired levels. This means that the system could be a tool to remotely monitor and maintain a suitable environmental condition for the fish species being raised in the fishpond.

For temperature vs salinity, the data collected show that an increase in temperature will also cause an increase in salinity level making the relationship of the two parameters linear (please refer to Figure 3). The temperature versus DO, graph shows that DO content decreases as temperature increases (Figure 4). Finally, Figure 5 shows that when the value of salinity increases by 0.5, the dissolved oxygen decreases by approximately 0.02.

## Discussion

The study proved that sensors and wireless communication systems can be used to remotely monitor the dissolved oxygen, salinity and temperature levels of water in the fishpond. It also proved that through the use of these advanced technologies we could also remotely manipulate and control devices and equipment such as air compressors and electric water pumps used in the fishpond, thus preventing fish kill.

## Conclusion

Fish kill could be prevented if the levels of dissolved oxygen, temperature and salinity will not be beyond critical levels. This could be remotely monitored and manipulated through the use of sensors and wireless communication systems.

Table 1. Oxygen saturation at different temperature and salinity levels.

| Oxygen Saturation Based on Temperature and Salinity | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Temperature (dec C) | Salinity (ppt) | | | | | | | | | |
| | 0 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 |
| 0 | 13.11 | 13.06 | 13.02 | 12.97 | 12.93 | 12.88 | 12.84 | 12.79 | 12.75 | 12.70 |
| 5 | 11.49. | 11.45 | 11.42 | 11.38 | 11.34 | 11.30 | 11.26 | 11.23 | 11.19 | 11.15 |
| 10 | 10.19 | 10.16 | 10.13 | 10.10 | 10.07 | 10.03 | 10.00 | 9.97 | 9.94 | 9.91 |
| 15 | 9.14 | 9.11 | 9.08 | 9.05 | 9.03 | 9.00 | 8.97 | 8.94 | 8.92 | 8.89 |
| 20 | 8.27 | 8.24 | 8.22 | 8.19 | 8.17 | 8.15 | 8.12 | 8.10 | 8.07 | 8.05 |
| 25 | 7.54 | 7.52 | 7.49 | 7.47 | 7.45 | 7.43 | 7.41 | 7.39 | 7.37 | 7.35 |
| 30 | 6.92 | 6.90 | 6.88 | 6.86 | 6.84 | 6.82 | 6.80 | 6.78 | 6.76 | 6.75 |
| 35 | 6.38 | 6.36 | 6.34 | 6.33 | 6.31 | 6.29 | 6.28 | 6.26 | 6.24 | 6.23 |
| 40 | 5.90 | 5.89 | 5.87 | 5.85 | 5.84 | 5.82 | 5.81 | 5.79 | 5.78 | 5.76 |
| 45 | 5.47 | 5.46 | 5.45 | 5.43 | 5.42 | 5.40 | 5.39 | 5.38 | 5.36 | 5.35 |
| 50 | 5.08 | 5.07 | 5.06 | 5.04 | 5.03 | 5.02 | 5.01 | 5.00 | 4.98 | 4.97 |

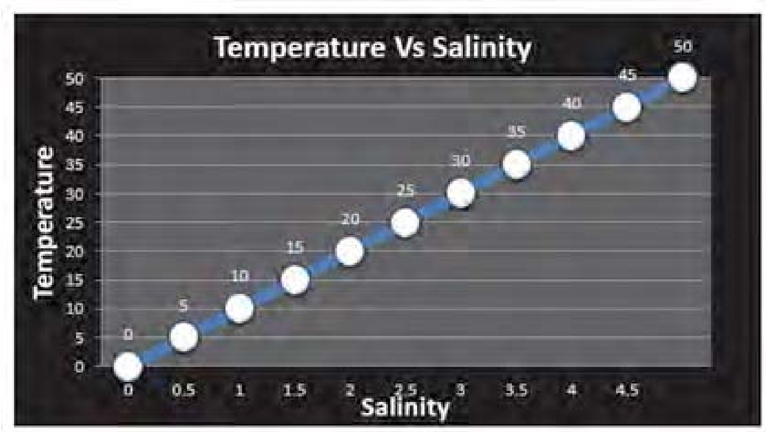


Figure 3. Changes in temperature and corresponding salinity levels in the experimental pond.

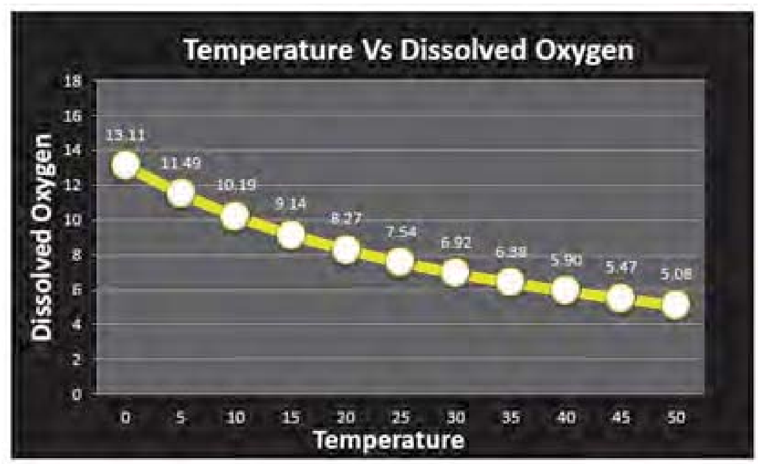


Figure 4. Dissolved oxygen and corresponding temperature levels in the experimental pond.

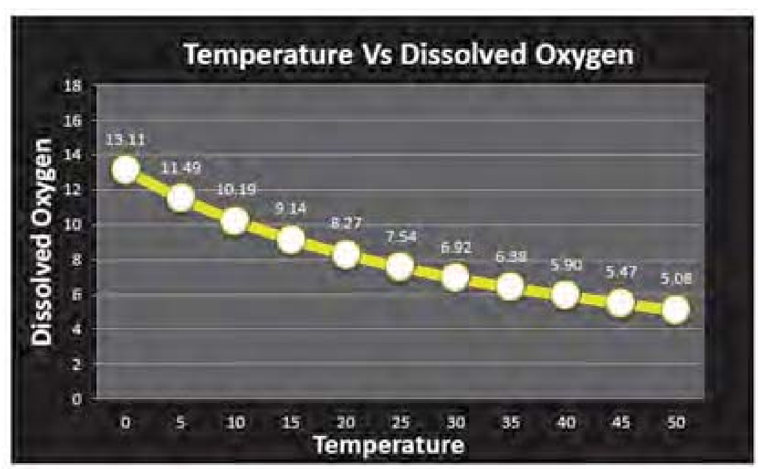


Figure 5. Dissolved oxygen at different salinity levels in the pond.

## Recommendation

Fishpond operators could adopt this system to remotely monitor and control the condition of water in their fishponds, thus preventing losses due to fish kill. Similar studies could be made with other fish production systems such as those in Laguna and Taal Lakes, Pangasinan and other parts of the country affected by massive fish kills.

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# Reaching the Poor Through Aquaculture: The Case of Technology Adoption in Rural Communities at West Central Philippines

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## *Abstract*

Aquaculture is promoted for food security and poverty alleviation in developing countries. This study examines the socio-economic impact of aquaculture technologies extended to calamity-stricken rural communities in Nueva Valencia, Guimaras, representing the marine water fishery and in Dumarao, Capiz, representing the inland freshwater fishery at west central Philippines. The adoption pathway employed in both sites was community-based and participatory. The survey was conducted among cooperators and non-cooperators, randomly selected in equal numbers in two sites with 60 respondents each per site using a pre-tested interview schedule.

Results showed that aquaculture is an acceptable technology both for cooperators and non-cooperators. The venture is a profitable business either done individually or collectively through an association, if managed properly. Milkfish cage culture, however, needs big capital that technology adoption among local fisherfolk (Guimaras) is limited. In contrast, tilapia cage culture enables small farmers/fishers in Dumarao to venture on their own. Dumarao growers were able to innovate using local materials like bamboo poles to make their cages afloat instead of drums or plastic containers as buoys. There were, however, environmental, technological and institutional issues deterring technology adoption in both sites. Climate change and institutional issues were the more prevalent concerns of Dumarao growers. The technological issues like fluctuating market price, cost of feeds, and fry supply were more enunciated in Guimaras.

**Keywords:** aquaculture, technology adoption, rural communities, marine waters, freshwater

## *Introduction*

The human population dependent on fish as their primary source of animal protein is expected to grow by 2 billion to 8 billion in the next 25 years (van der Zijpp *et al.*, 2007). Meanwhile, world production of capture fisheries has leveled out (FAO, 2007). Reliance on aquaculture for food supply has become even greater with production from 31 to 59 million metric tons since 1995, accounting for almost half (45%) of the world's food fish (Subasinghe *et al.*, 2009; FAO, 2006). Aquaculture does not only bridge the supply and demand gap of aquatic food, but also generates employment, and alleviates poverty (Irz *et al.*, 2007; Subasinghe *et al.*, 2009; Srinivasan *et al.*, 2010; Soto-Zarazúa *et al.*, 2011).

In the Philippines, the aquaculture sector showed increasing production trend and has posted the highest growth (18%) compared with municipal (2.4%) and commercial (1.7%) sectors in 2005 (BFAR, 2006). NSCB (2012) reported in 2009 that among the nine basic sectors in the country, those engage in fishing had the highest (41.4%) poverty incidence, while those in farming come in second (36.7%). Poverty in fishing communities is further exacerbated by the declining catches of municipal fisheries for over the past 20 years (Irz *et al.,* 2007).

Through the Institutional Capacity Development for Sustainable Aquaculture (ICDSA)[1] project of the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC/AQD) aquaculture technologies are taught to rural communities as supplemental livelihood. ICDSA uses multidisciplinary, community-based and participatory approaches in the transfer of technology (Agbayani and Toledo, 2008). The introduction and adoption of technologies, however, affect the different spheres of society- be it social, economic, political, cultural or ethical – in different modes and paces (Daňo, 2007). Conversely, there are also constraints that hinder or retard the uptake of the technology in rural communities. There is a need to examine the socioeconomic impact of the aquaculture interventions in ICDSA sites, particularly in marine and freshwater fishery. Positive outcomes of technology adoption may pave the way not only for livelihood improvement and poverty alleviation in rural communities, but it will also become an essential component of integrated rural development.

This study aims to analyze the socio-economic impact of the transfer and adoption of aquaculture technology among coastal dwellers and farmers in rural communities. Specifically, it aims 1) to document changes over time, resource use and socioeconomic conditions in study sites with the adoption of aquaculture technology; 2) to examine the factors that contribute or impede the acceptability and adoption of technology; and 3) to determine whether there are differences in knowledge of and attitudes among community members (growers & non-growers) and between locations (marine vs. freshwater) towards aquaculture technology adoption.

## *Material and Methods*

### *Study Sites*

The study was conducted in four villages (barangays) in Western Visayas, central Philippines where aquaculture was introduced to calamity stricken rural communities with differing culture environments under the ICDSA project. The sites were in Nueva Valencia, Guimaras, representing a marine water area and in Dumarao, Capiz, representing a freshwater area (Figure 1). Each study site is composed of two villages (Sto. Domingo and Magamay in Guimaras; Codingle and Tamulalod in Dumarao). The villages in Guimaras were selected based on its location (adjacent villages) and size of the community.

[1] ICDSA protocol is discussed extensively in Agbayani, RF and Toledo, JD. 2008. Institutional capacity development for sustainable aquaculture and fisheries: strategic partnership with local institutions. In K. Tsukamoto, T. Kawamura, T. Takeuchi, T. D. Beard, Jr. and M. J. Kaiser, eds. *Fisheries for Global Welfare and Environment, 5th World Fisheries Congress 2008, pp. 435–448.*

Nueva Valencia is a third class coastal municipality of Guimaras province. It is considered a tourism capital and major fishing ground of the province. It has the highest number of fishers among the five municipalities of Guimaras. In 2006, an oil spill[2] stretching over 15 miles and reaching 50-75 in width affected the rich fishing ground, the mangroves, and other marine life of Nueva Valencia. As a consequence, about 5594 families were directly or indirectly affected and the total losses of the municipality were estimated at PhP 237 million (Provincial Government of Guimaras and PEMSEA, 2012).

Dumarao is an inland 2nd class municipality of Capiz province. It is the 4th leading rice-producing municipality of the province. About 23.7 ha of agricultural lands spanning four villages were submerged with water due to unfinished dam construction in 2006. The river expanded approximately from 2-4 m to 50-100 m in width and became deeper from 2 m to 5-8 m in depth. The submerged land cost PhP 526,844 and most (72%) of the 36 affected farmers were from Barangay Tamulalod[3]. One of the affected villages was excluded from the assessment and validation for concerned parties did not file claims on time.

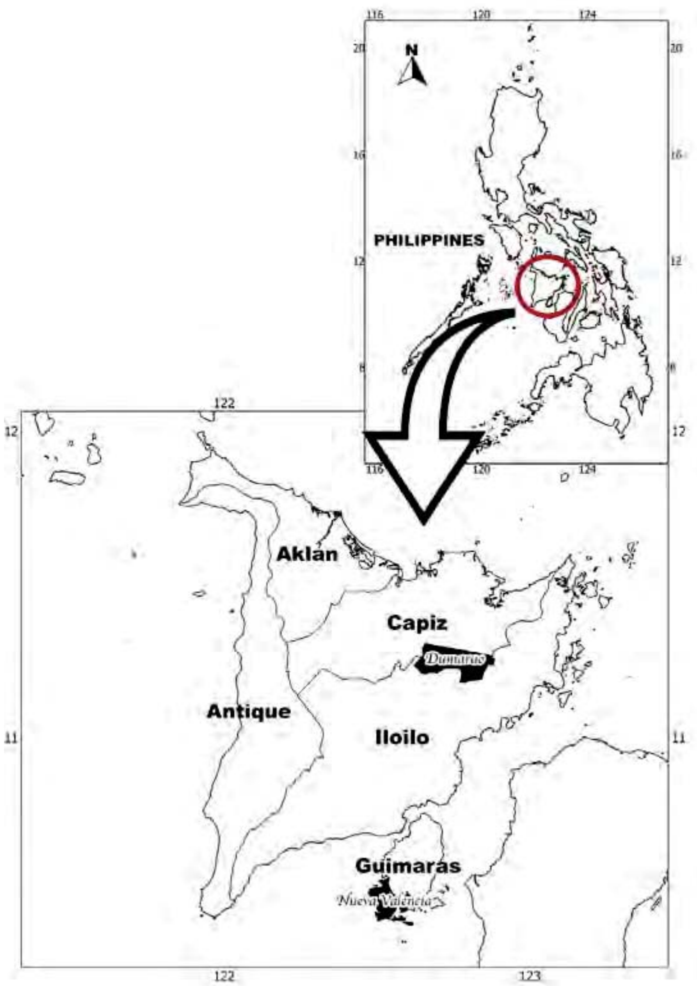


Figure 1. Study sites.

[2] MT Solar 1 tanker carrying 2.7 million liters of bunker fuel sunk in marine waters a few km from Nueva Valencia.
[3] Source: Result of Survey and Validation of CIP claimants - Oct 2-13, 2006.

## *Technology Transfer*

The adoption pathway used in both sites was community-based and participatory although the aquaculture interventions vary in each site. The community is represented by an organized group or Peoples Organizations that chose among themselves the members that would undergo the training. Table 1 shows the profile of the peoples' organizations (POs) whose members were the major participants and beneficiaries of the project.

Thirty trainees or six trainees each from five small-scale fishers' association[4] of Nueva Valencia's four villages were trained at SEAFDEC's Mariculture Park at Igang Marine Station. The trainees cultured milkfish in three 10 m x 10 m x 6 m net cage with a stocking density of 24,000 fry per cage for six months. After the successful runs, each association operated their own milkfish cage culture in their respective villages. Harvest and post-harvest handling were part of the training including value-adding activities, e.g. deboning of milkfish, for members. Petron[5] funded the project as part of its support to rehabilitation and ecological recovery program of Guimaras. Included in the fund support was the social preparation of POs to which a non-government organization with expertise in entrepreneurial development and institutional capability building was hired for the purpose.

Table 1. Profile of peoples organizations involved in ICDSA[1] projects in study sites.

| People Organization | Location | Number of members | | | Existing livelihood projects | Year registered |
|---|---|---|---|---|---|---|
| | | Male | Female | Total | | |
| Sto. Domingo Fisherfolk Association | Sto. Domingo, Guimaras | 27 | 19 | 46 | • Milkfish cage culture<br>• Gasoline trading | 2005; DOLE[2] |
| Magamay Small Fisherfolk Association | Magamay, Guimaras | 79 | 50 | 129 | • Milkfish cage culture | 2009; DOLE |
| Dumarao Fishfarmers Multi-purpose Cooperative[3] | Dumarao, Capiz | 24 | 6 | 30 | • Tilapia cage culture | 2008; CDA[4] |

[1]Institutional capacity development for sustainable aquaculture; [2]Department of Labor and Employment; [3]Not all members are residents of Barangay Tamulalod and Codingle; [4]Cooperative Development Agency

[4] Only Sto. Domingo and Magamay associations were included in this study.
[5] Petron Corporation chartered the tanker.

The Provincial Government of Capiz funded the ICDSA project in Dumarao. About 25 participants from different villages attended the season-long training in 2007. Eight net cages (4 m × 4 m × 1.5 m) were installed and stocked with tilapia, catfish and freshwater prawn (ulang) in a demonstration farm in Badbaran River. The Acting Officer of the Municipal Agriculture Office formed the participating community-members into Dumarao Fish Farmers Multi-Purpose Cooperative (DFFMPC). The demonstration farm was turned over to DFFMPC and the proceeds of their harvest were re-invested and rolled-over for operations. The members opted to focus on tilapia for they found it more viable in their area compared to other species. It was noted that technical support from the local government office was minimal in the absence of trained staff on fisheries and aquaculture.

### *Methods*

Field surveys were conducted in 2010-2012 among 30 non-cooperators and 30 co-operators/adopters each per site using a pre-tested interview schedule. Cooperators/adopters refer to members of an organized group or individuals who benefited or adopted the technology after the season-long training. The non-cooperators are those who compete with the use of water resources in the area. The respondents were randomly chosen from a list of fishing households provided by local officials for non-cooperators and from the associations, in the case of cooperators. Secondary data such as ICDSA reports on Dumarao and Nueva Valencia season-long training and results of ICDSA mini-workshop, among others, were gathered and reviewed. Key informants were the Village Heads and officials, Association/Cooperative Heads, Municipal Agriculture Office staff, technicians, and a family of fishers.

Production data were gathered among adopters of technology in project sites. Cost-benefit analysis was used to determine the viability of the enterprise. Mann-Whitney U Test was used to determine the differences between Guimaras and Dumarao co-operators on the constraints and benefits gained from technology adoption. Focus group discussion was used to validate gathered data.

### ***Results***

Most (27%) respondents were in the age bracket of 48-58; the youngest, 18 and the oldest, 73. All had formal education and 77% of them were married. Most (40%) Dumarao co-operators reached college level while majority (57%) of Guimaras co-ooperators only attained elementary level. Likewise, majority (43%) of Dumarao co-operators were farmers, mostly (60%) from Barangay Codingle. In Guimaras, the co-operators were mostly (50%) fishers and the majority (67%) were from Barangay Magamay.

### *Adopters and Dispersal of Technology*

Tilapia cage culture in Dumarao was small-scale. Most growers owned one cage with size ranging from 4 x4 m to 4 x 10 m (Table 2). All had positive income since they had pre-agreed price and harvest arrangements to avoid competition. Harvest was sold locally, along the road. Adopters claimed that the technology provided opportunity for them to use their submerged farmlands for aquaculture venture. They ranked tilapia cage culture second (33%) to farming (55%) as the most important household occupational activity that contributes to their household income, particularly those in Brgy. Tamulalod. They maintained that farm goods can be used as collateral for loan, but not fish harvest. However, 44% of them conceded that fish farming is not laborious compared to farming. They also ranked fish farming second (34%) to fishing (50%) as a source of food.

It was noted that initial adopters of tilapia cage culture were Dumarao's better off residents. These were not sustained when operations were relegated to hired labor. Similarly, the PO's aquaculture venture was not sustained. The water depth in culture area became shallow during 2010 long dry spell. The PO also had organizational problems leading to its demise as a group. Nonetheless, the adopters, who were also members of the cooperative, increased to fifteen (5 in Tamulalod; 10 in Codingle), but the number reversed in the latter part of the survey. Some adopters innovate using excess surface water for backyard pond. Two cooperators became hatchery operators (one for commercial scale and the other, for personal use). Informants claimed that the technology spread to six other villages with some adopters serving as resource persons. Two fertilizer dealers of the municipality addressed the growers' feed needs. Others developed interest on other species that an on-site demonstration on induced spawning of catfish was facilitated. Most (83%) of Dumarao respondents claimed that the tilapia production volume was not enough to meet community fish requirements.

Milkfish cage culture in Guimaras showed varied results (Table 3). Only Sto. Domingo fisherfolk association gained profits in its two production runs. The harvest was sold in Iloilo fishing port where it competed with other fish species for higher price. The Sto. Domingo PO retained small portion of their harvest for retail to members. Some members deboned the milkfish, gaining higher profit. The PO's share of the production income was 20% while the 80% went to the four technicians (caretakers) who divided it equally among themselves Compared with Dumarao, most (56%) of them claimed that aquaculture is better than farming. Only two private investors aside from the PO's own venture were adopters of the technology in the area. Private investors hired trained PO members as technicians. Nonetheless, Village Heads (Brgy. Magamay and Sto. Domingo) were not inclined to grant permit for new entrants on cage culture claiming that water bodies within their control were small. Culture operations of POs were on hold for lack of funds.

There was a heightened interest on aquaculture as a source of added income among members of Sto. Domingo PO. They were awaiting for the approval of their proposed sea cucumber grow-out culture which they submitted to a non-government organization for funding. Some of its members also showed interest for the seeding of their coastal waters with sea cucumber to enhance its productivity. They claimed that it is easy for them to monitor the growth of sea cucumber and oversee the area for their coastal area is just small.

### *Factors Affecting Adoption*

The aquaculture issues in two sites are generally classified into: 1) environmental issues, 2) technical issues, and 3) institutional issues. The freshwater culture operation in Dumarao was most affected by climate change. The growers experienced high water temperature; low water level, and profuse growth of giant water lettuce during long dry spell in 2010. But during rainy season, they had to live through flooding, and the siltation, thereafter. Dumarao growers were also in quandary whom to deal with regarding their problems on the unfinished dam construction for it is affecting their culture operations. The local government unit could not address their problems claiming the project was not turned-over to them. Both sites had similar technical issues such as cost of feeds, market, and fry supply, among others, but these were significantly ($p<0.001$) pronounced in Guimaras than in Dumarao (Table 4). In terms of benefits, the technology as a source of cash income was highly significant ($p<0.01$) among Dumarao growers. Resource utilization on the other hand is significant in Guimaras co-operators.

### *Attitude Towards the Technology*

The respondents showed positive attitude towards aquaculture. Majority of them claimed that existing culture operations did not affect their own activities (59%) or the water quality (75%) of their marine/freshwater resource base. Most of them (81%) believed that their resource base is an open access, but only 41% respondents liked to limit the number of cages to avoid congestion and pollution. This sentiment, however, was not shared by 72% Dumarao respondents.

Table 2. Cost-benefit analyses of tilapia cage culture of sample respondents in Dumarao, Capiz.

| Technical Assumption | A | B | C | D | E |
|---|---|---|---|---|---|
| Size of cage | 4x4x4m | 4x5x4m | 4x10x4m | 4x5x4m | 4x6x4m |
| No. of cages | 1 | 1 | 2 | 8 | 2 |
| Total stock | 1,000 | 2,000 | 2,000 | 7,000 | 2,000 |
| Production/m$^2$ (kg) | 4.68 | 5.5 | 1.0 | 1.66 | 2.29 |
| Net income | 3,382 | 3,649 | 5,302 | 3,143 | 3,372 |
| Total operating cost | 4,118 | 7,531 | 4,298 | 28,657 | 9,798 |
| Total investment cost | 757 | 1,160 | 2,000 | 11,950 | 3,372 |
| ROI (%-depreciation excluded) | 447 | 315 | 265 | 26 | 64.85 |

Table 3. Cost-benefit analyses of milkfish cage culture of fisherfolk associations in Nueva Valencia, Guimaras.

| Technical assumption | Brgy Sto. Domingo PO | | Brgy. Magamay PO | |
|---|---|---|---|---|
| | 1st run | 2nd run | 1st run | 2nd run |
| Size of cage | 10x10x6m | 10x10x6m | 10x10x6m | 10x10x6m |
| No. of cages | 1 | 1 | 1 | 1 |
| Total stock | 20 | 20 | 20 | 20 |
| Production/m$^2$ (kg) | 42.05 | 44.25 | 27.97 | 33.67 |
| Net income | 31,945 | 20,736 | -84,527 | -105,165 |
| Total operating cost | 417,803 | 458,584 | 381,502 | 418,308 |
| Total investment cost | 134,509 | 134,509 | 134,509 | 134,509 |
| ROI (%-depreciation excluded) | 47.50 | 30.83 | -125.38 | -78.18 |

Table 4. Cooperators' difficulties and benefits in adopting the technology by location (n=60).

| Variables | Guimaras | Dumarao | Mann-Whitney U | Z |
|---|---|---|---|---|
| 1.Difficulties | | | | |
| Supply of fry | 33.71 | 25.57 | 302* | -1.976 |
| Credit | 41.92 | 17.67 | 77.5*** | -5.606 |
| Feeds | 38.69 | 21.60 | 183*** | -3.996 |
| Harvesting | 37.24 | 23.00 | 225*** | -4.301 |
| Market | 37.83 | 22.43 | 208*** | -4.123 |
| 2. Benefits | | | | |
| Source of cash income | 24.22 | 35.58 | 267.5** | -2.759 |
| Utilization of resource | 34.81 | 21.65 | 184.5* | -3.159 |

***= p<0.001; **=p<0.01; *= p<0.05

## *Discussion and Conclusion*

The findings showed that adoption of aquaculture technology is acceptable to both farmers and fishers and even for non-growers as long as it does not impinge on their own activities in the same resource base. This implies that zoning and corresponding enforcement are two important factors that will avert future conflicts on resource use and help ensure sustainability of the venture. This also necessitates pro-active local legislation on resource allocation especially in Dumarao where fishery management is still wanting, more so in aquaculture.

The venture showed positive income either operated as an individual or cooperative undertaking. In the latter, the organization must be stable and well-managed. The size of membership may have affected members' participation in culture operation. Group size proved to be unwieldy, and the lack of social preparation affected PO's success (Baticados *et al.*, 1998). Unless income from culture operation is substantial, fishers will remain fishing. The milkfish cage operation in Guimaras is a cooperative venture, thus, the sharing of benefits is spread to all members. Failure on their cage operation might cause disintegration of PO membership. Thus, there is a need for their organization to diversify operation that requires less capital, e.g. sandfish culture or value-adding activities. This is to cushion the impact of the venture's poor performance on members. Interchangeably, other modes of partnership must be explored that would pay for opportunity loss of the adopters.

The spread of technology is faster among fishers/farmers requiring low capital investment. Thus, it should be given as an option to rural folks if viable in the area.

The effect of climate change is more felt in freshwater culture operation than in marine culture operation affirming ADB's disclosure (ADB, 2005). The involvement of LGUs is important both in legislation and in facilitating solutions to articulated concerns of adopters.

Aquaculture indeed provides food security and income to rural communities. However, the transfer of technology requires an adoption pathway that is easily and effectively understood by the beneficiaries. In the transfer of technology, the experts must be able to anticipate, identify, and make a follow-through on the transferred technology. If feasible, he/she should facilitate in addressing issues or concerns of adopters. Because of climate change, small-scale growers should be informed of an array of aquaculture livelihood options feasible to the locality to enable them to make wise decision on the technology appropriate for their skills, interest, and affordability.

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### *Suggested Readings*

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# Marine Biodiversity at the SEAFDEC/AQD Research Stations in Iloilo and Guimaras, Philippines

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## *Abstract*

Species inventories were recently made in and around the research stations of the SEAFDEC Aquaculture Department to facilitate subsequent monitoring. AQD's Tigbauan Main Station (TMS, since 1973) faces the deep open waters of the Panay Gulf and Sulu Sea and is flanked by densely populated fishing villages operating nearshore fish corrals, gillnets, longlines, and beach seines. In 2013–2014, sampling at the sand-gravel intertidal and monitoring of the catch of the various gears showed at least 579 species from 213 families, including 252 species of fishes, 228 mollusks, 48 crustaceans, 12 cnidarians, 9 echinoderms, 16 seaweeds, sea turtles, and sea snakes inhabiting the nearshore areas off TMS. Any adverse effect of the TMS hatcheries and laboratories is difficult to discern on top of the continuous intense fishing and habitat disturbance. AQD's Igang Marine Station (IMS, since 1980) is in a cove under the rocky cliffs of southern Guimaras, behind several islands facing the Panay Gulf and Sulu Sea. IMS includes 40 ha of seagrass beds and sandflats around five rocky islets and two 6–12 m deep basins where broodstock and grow-out cages are moored. IMS is flanked by many fish corrals operated by fishers who live in villages in nearby coves. Fishers on outrigger boats also use gillnets and spears, and others glean for mollusks and echinoderms inside IMS. In 2011–2012, some 786 species in 261 families were collected or photographed at IMS, including 74 species of fishes, 40 crustaceans, 391 mollusks, 44 echinoderms, 87 cnidarians, 47 poriferans, 24 ascidians, and 12 bryozoans, and sea snakes living among 48 seaweeds and 4 seagrasses. Biodiversity at IMS seems high despite 35 years of operation of the fish cages and the continuous fishing, gleaning, and boating by the locals. Several species of filter-feeding invertebrates grew on the cage nets and platforms but were not found in the natural habitats. The cages provide additional attachment surfaces for many species; these biofoulants presumably reduce water flow into the cages but they also remove nutrients and particulate wastes and help maintain good water quality. Nevertheless, siltation is evident under the cliffs inside the cove, and the sandflats may be expanding over the seagrass beds. AQD's 16-ha Dumangas Brackishwater Station (DBS, since 1998) is flanked by freshwater Talaugis River, by hundreds of hectares of mangrove-derived fish ponds, and by Pulao Creek and an extensive mudflat with fringing mangroves at the northeastern end of Iloilo Strait. In 2009–2010, 16 ponds with water areas from 0.5 to 0.9 ha were sampled during harvest of the experimental crops. At least 90 species of non-crop fishes lived in the DBS ponds, along with 35 crustaceans, 60 mollusks, three echinoderms, two cnidarians, and a water snake. The snails *Cerithideopsilla* spp., *Cerithium coralium*, and *Batillaria* spp. were very abundant in the ponds. Almost all the same species in the ponds, plus many others, were found in the adjoining fringing mangroves with ~10 species of trees. The ponds serve as proxy for mangrove lagoons that harbor the young of migratory fishes as well as all life stages of resident species. Several non-crop species inside the IMS cages and the DBS ponds are harvested by the pond workers and contribute to nutrition and income. Aquaculture farms should be managed for high biodiversity to ensure sustainability. Ways are suggested for SEAFDEC/AQD to do so at its aquaculture research stations.

**Keywords:** species inventories, biodiversity, sustainability

## *Introduction*

Marine resources and ecosystems must be adequately studied and known to be sustainably used. Such knowledge has been generally taken for granted or overlooked by aquaculture practitioners and even research institutions. Partly as a result of such historical oversight, aquaculture has been chastised both in the scientific literature and by the media for a wide range of environmental and social impacts, including (i) pollution (uneaten feeds, excreta, silt, pathogens, debris, nitrogen and phosphorus); (ii) the fish meal trap; (iii) loss of biodiversity; and (iv) poor people becoming poorer (Primavera, 1993; Phillips, 1995; Naylor *et al.,* 1998, 2000; Holmer *et al.,* 2002).

Loss of biodiversity due to aquaculture has been widely assumed but not much documented in the Philippines, except the reduction of the mangrove area from 418,000 ha to 117,000 ha as pond area increased from 73,000 ha to 261,000 ha between 1950 and 1995 (Bagarinao, 1998, 1999). Indeed, that species have been and are lost due to aquaculture? For many aquaculture areas, the baseline species composition in nearby natural aquatic habitats has not been studied, and the later (current) species composition has yet to be studied. To address such neglect even belatedly, species inventories were conducted at the three research stations of the SEAFDEC Aquaculture Department in Tigbauan and Dumangas, Iloilo, and in Igang, Guimaras.

## *Materials and Methods*

### *Species inventory at Tigbauan Main Station*

SEAFDEC/AQD's Tigbauan Main Station (TMS, since 1973) in Buyuan, Tigbauan, Iloilo, faces the deep open waters of the Panay Gulf and further to the west and south, the Sulu Sea (Figure 1) and is flanked by densely populated fishing villages. The TMS beach front is ~540 m long, with black sand and gravel, the high tide debris line ~5–10 m from the water line at lowest low tide, the beach slope ~30–40° (Figure 2). In 2013, the sand-gravel intertidal fronting TMS and eastward to Buyuan Creek was surveyed several times during daytime negative low tides and all attached species (e.g. seaweeds, sea anemones) and stranded species (e.g., seaweeds, opisthobranchs, jellyfish) were photographed and recorded, and the unfamiliar specimens preserved in formalin. Buried species were not included (not dug out). Empty but intact mollusk shells and echinoderm testa found at the beach were included and considered as those of Tigbauan resident species.


Figure 1. Google Earth view of the three aquaculture research stations of the SEAFDEC Aquaculture Department in Panay and Guimaras Islands in Central Philippines: TMS, Tigbauan Main Station; DBS, Dumangas Brackishwater Station; IMS, Igang Marine Station. Panay Gulf opens into the Sulu Sea to the west and south.

Figure 2. SEAFDEC/AQD's Tigbauan Main Station. A. Aerial view circa 1996, showing the seawall jetties traversing the beach and the effluent pipes emptying onto the beach as creeks and puddles; B. Some of the hatcheries and two seawater reservoirs, circa 1996; C. View of the TMS beach and nearshore area in 2014. Visible in all three photos are the nearshore fish corrals, the current versions of which were sampled in 2013-2014.

In March–April 2013 and February–March 2014, the catch of two fish corrals (locally known as 'punot' and 'tangkop'), 3–4 gill nets ('pukot'), 2–3 beach seines ('sahid'), 1–2 longlines ('labay'), and a big fish basket ('bubo') operated nearshore off Buyuan village were monitored in the early morning and sometimes in the late afternoon when the catch was landed. All species landed were examined and photographed and specimens of the unwanted species were preserved in formalin. The species caught by fishing gears were wide-ranging but entered Tigbauan and TMS waters presumably in the course of foraging and migration.

### *Species inventory at Igang Marine Station*

SEAFDEC/AQD's Igang Marine Station (IMS, since 1980) is in a cove surrounded by the rocky cliffs of southern Guimaras, behind several islands facing the Panay Gulf and the northern Sulu Sea (Figure 1). Cages for milkfish were set up at a marine cove in Igang about 1980 and a 50 ha marine cove with islets was reserved for SEAFDEC about 1986. IMS includes 40 ha of seagrass beds and sandflats around five rocky islets and two 6–12 m deep basins where broodstock and grow-out cages are moored (Figure 3). In 2003, the Igang Mariculture Park (IMP) was established to anchor commercial marine cages of private operators. IMS is flanked by many fish corrals operated by fishers who live in villages in nearby coves. Fishers on outrigger boats also use gill nets and spears inside IMS, and gleaners walk around the seagrass beds exposed during negative low tides. Motorized outrigger boats carrying IMS personnel and goods, and now also tourists, traverse the IMS habitats every day.

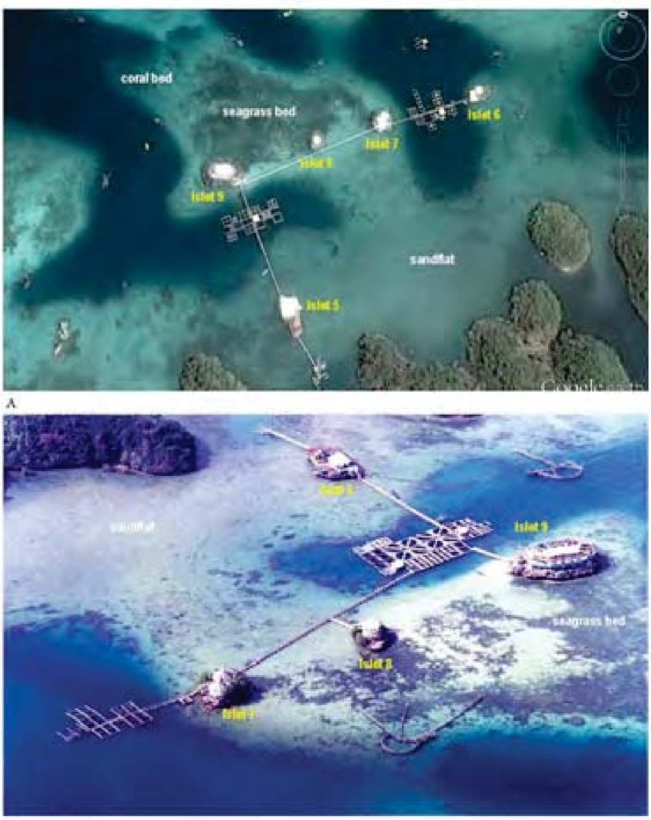


Figure.3. SEAFDEC/AQD's Igang Marine Station. A. Google Earth view taken in February 2015, showing the five rocky islets, the seagrass bed, the sandflat, coral bed, and the 6–12 m deep basins for broodstock cages near Islet 9, and growout cages near Islet 6; B. Aerial view looking south, circa 2000, showing the different habitats and the cage basins looking much the same as today. Both photos show some of the 50 or so fish corrals that local fishers operate within 200 m of IMS.

Several visits were made to IMS during the northeast monsoon months between September and June in 2010–2012 when the calm weather and the daytime negative low tides allowed field work around the station, particularly the intertidal around the five rocky islets and the connecting seagrass beds, sand flats, and silty coves. All attached marine plants and invertebrates and all species living among them were examined closely and photographed. Buried species were not dug out. All empty but intact mollusk shells and echinoderm testa found inside IMS were considered as those of resident species. The aquaculture platforms and nets were examined for attached species, and the fishes inside and outside the fish cages were recorded and photographed during harvest of farmed milkfish and seabass under AQD-approved projects. Whenever gillnetters, spear fishers, and gleaners were found operating inside IMS, their catch was also recorded and photographed.

### *Species inventory at Dumangas Brackishwater Station*

SEAFDEC/AQD's 16-ha Dumangas Brackishwater Station (DBS, since 1998) is flanked west by the freshwater Talaugis River, north and south by hundreds of hectares of mangrove-derived fish ponds, and east by Pulao Creek and an extensive mudflat with fringing mangroves at the northern end of Iloilo Strait (Figure 1, Figure 4). The 16 DBS ponds (with water areas from 0.5 to 0.9 ha) are used in technology verification experiments and production runs (Baliao *et al.,* 1998; Coniza *et al.,* 2010; Jamerlan and Coloso, 2010; Madrones-Ladja *et al.,* 2012; Jamerlan *et al.,* 2014). In 2009–2010, all ponds were sampled during harvest of the experimental crops when the ponds were totally drained. Bycatch species were collected, identified, and enumerated fully.


Figure 4. SEAFDEC/AQD's Dumangas Brackishwater Station, Google Earth views taken 2014. A. DBS (in the white rectangle) lies in the midst of large tracts of fishponds derived from mangrove land, with Talauguis River at the west end and Pulao Creek to the northeast; B. The 16 experimental ponds (several subdivided for replicates), with the mangrove greenbelt at the east end.

DBS has a mangrove greenbelt (30 m wide x 180 m long) fronting, but separated by a high concrete dike from Pulao Creek, and subdivided into one large and six small compartments by concrete fences for a past experiment. The mangroves and mollusks in the DBS greenbelt were documented in October 2009. In addition, a local fisher was hired to set a tidal enclosure net ('pahubas') outside the DBS greenbelt in October 2010, and all the fishes and crustaceans that were caught were photographed and identified.

### *Identification of species*

For identification of the commercially important fishes, crustaceans, and mollusks, the main reference was the six-volume FAO Species Identification Guide for Fishery Purposes, West Central Pacific (Carpenter and Niem, 1998a, 1998b; 1999a, 1999b; 2001a, 2001b). Other taxonomic references include Masuda *et al.* (1984), Kuiter (1992), Rainboth (1996), Kimura and Matsuura (2003), Matsuura and Kimura (2005), Yoshida *et al.* (2013) for fishes; Springsteen and Leobrera (1986), Okutani (2004), and Poppe (2008a, 2008b; 2010, 2011) for mollusks; Schoppe (2000) for echinoderms; Colin and Arneson (1995), Richmond (1997) for invertebrates; Trono (1997), Calumpong and Menez (1997) for seaweeds; and Primavera *et al.* (2004) for mangroves. Also useful were pictorial accounts of marine biodiversity in the Philippines and the South China Sea (Chou and Alino, 1996; Allen, 1998, 2000; White, 2001). Many species of sponges, bryozoans, and tunicates could not be identified to scientific names. Marine botanist Lawrence Liao identified the unfamiliar seaweeds, carcinologist Jose Christopher Mendoza identified the unfamiliar crabs, and ichthyologist Helen Larson provided advice with the unfamiliar gobies.

## *Results and Discussion*

### *Biodiversity nearshore off Tigbauan Main Station*

The TMS shore and the adjoining Buyuan shore (about 1 km long) is depauperate in intertidal flora and fauna, compared to Igang Marine Station and Dumangas Brackishwater Station. No seagrasses, no corals, no attached invertebrates, but seasonal seaweeds, and seasonal strandings of sea hares, jellyfishes, salps, and other pelagic invertebrates. This is mainly because the seabed off TMS is unconsolidated gravel and sand and provides limited and unstable habitat surfaces and crevices for flora and fauna. Also, the rough weather during the southwest monsoon overturns the seabed and disrupts life cycles. However, the intensive fishing during the northeast monsoon brings to shore so many species of fishes, cephalopods, and crustaceans. In 2013–2014, some 579 species from 213 families in major marine taxa were collected, photographed, and inventoried, including 252 species of fishes, 228 species of mollusks, and 48 species of crustaceans (Table 1). More species could be expected with continued sampling at other times of the year; if the infauna were included; if the microscopic species were sampled; and if the subtidal was surveyed underwater.

This study is the first documentation of the marine biodiversity off Tigbauan, Iloilo in southern Panay, Philippines. This southern coast has had many notable megafauna visitors, many of which have been documented by SEAFDEC FishWorld since 2000: five species of sea turtles (Bagarinao *et al.*, 2010; Bagarinao, 2011), the sunfish *Mola mola*, the whale shark

Table 1. Biodiversity in the nearshore areas fronting the Tigbauan Main Station, Iloilo, Philippines, 2013–2014.

| Phylum | Class/Order | Families | Species | Representative species |
|---|---|---|---|---|
| Chordata | Selachei | 3 | 3 | *Rhincodon typus, Odontaspis ferox* |
| | Batoidei | 2 | 3 | *Mobula kuhli, Pteroplatytrygon violacea* |
| | Osteichthyes | 80 | 246 | *Carangoides spp., Lutjanus spp., Nemipterus* spp., *Upeneus spp., Arothron* spp., *Mola mola* |
| | Reptilia | 2 | 7 | *Chelonia mydas, Eretmochelys imbricata, Lepidochelys olivacea, Hydrophis* spp. |
| | Urochordata | 2 | 3 | *Thalia, Doliolum, Tethys* |
| | Ascidiacea | 1 | 1 | *Pyrosoma* |
| Crustaceans | Penaeidea | 2 | 7 | *Acetes* spp., *Penaeus* spp. |
| | Brachyura | 13 | 28 | *Portunus* spp., *Calappa* spp. |
| | Anomura | 3 | 3 | *Coenobita violascens* |
| | Palinura | 1 | 3 | *Panulirus versicolor, P. ornatus* |
| | Stomatopoda | 1 | 1 | *Harpiosquilla harpax* |
| | Thalassinidea | 1 | 1 | *Thalassina anomala* |
| | Cirripedia | 4 | 4 | *Balanus amphitrite, Tetraclita squamosal* |
| | Isopoda | 1 | 1 | *Ligia exotica* |
| Mollusks | Gastropoda | 38 | 122 | *Harpa major, Aplysia* spp., *Cypraea* spp. |
| | Bivalvia | 23 | 96 | *Placuna placenta, Alectryonella plicatula* |
| | Cephalopoda | 6 | 10 | *Octopus* spp., *Sepia* spp., *Photololigo* spp. |
| Cnidarians | Anthozoa | 4 | 6 | *Stichodactyla haddoni, Aiptasia diaphana* |
| | Scyphozoa | 5 | 5 | *Aurelia aurita, Cassiopea medusa* |
| | Hydrozoa | 1 | 1 | *Plumaria* sp. |
| Annelids | Polychaeta | 2 | 2 | *Marphysa* sp., *Eunice* sp. |
| Sipuncula | | 1 | 1 | *Chloeosiphon aspergillus* |
| Echinoderms | Asteroidea | 3 | 3 | *Astropecten monacanthus* |
| | Echinoidea | 3 | 3 | *Astropyga radiate* |
| | Holothuroidea | 1 | 1 | *Opheodesoma serpentine* |
| | Ophiuroidea | 2 | 2 | *Ophionereis* sp., *Ophiactis* sp. |
| Plantae | Chlorophyceae | 3 | 10 | *Ulva=Enteromorpha* spp., *Acetabularia* sp. |
| | Phaeophyceae | 4 | 5 | *Dictyota ceylanica, Rosenvingea intricate* |
| | Rhodophyceae | 1 | 1 | *Hypnea spinella* |
| | **All** | **213** | **579** | |

*Rhincodon typus*, the tiger shark *Galeocerdo cuvier*, and the dwarf sperm whale *Kogia sima* (Bagarinao, unpublished data). The fishing gears also brought in small deep-sea fishes: the lanternfish *Benthosema pterotum*, the barracudina *Lestidium*, and the snaggletooth *Astronesthes lucifer*. The lionfishes *Pterois* spp., the puffers *Arothron* spp., and the sea anemone *Stichodactyla haddoni* and its commensal *Amphiprion polymnus* were quite common nearshore off TMS.

Above all, the commercial fishes were very diverse in species, sizes, and value—sharks, rays, eels, sardines, mullets, needlefishes, groupers, snappers, threadfins, slipmouths, jacks, round scads, goatfishes, barracudas, mackerels, and the occasional sailfish *Istiophorus platypterus* and milkfish *Chanos chanos*. Cephalopods, crabs, and large shrimps made up a small part of the catch, but included many species. Moreover, the TMS and Buyuan beaches had a high complement of gastropod and bivalve shells, many of them intact and indicative of live animals nearshore.

The TMS tanks discharge large volumes of seawater laden with uneaten plankton, wasted feeds, as well as feces and other metabolites of the hatchery species (and rarely, bacteria and viruses from diseased stocks). This polluted sea water goes through a maze of drain pipes and canals onto the TMS beach. The AQD laboratories, restrooms, and housing complex also discharge large volumes of fresh water laden with various chemicals and sewage into drain canals that mostly open onto the beach as well. Seasonal blooms of the green seaweeds *Ulva=Enteromorpha* and *Chaetomorpha* occur at the TMS and Buyuan beaches during the calm water months, but these have been rapidly consumed by corresponding swarms of sea hares (*Aplysia* spp., *Bursatella leachii*, etc., collectively called by the local term 'kalamputay') that leave behind a huge volume of egg masses. Biological pollution by TMS is undeniable, but the current level of enrichment seems to be within carrying capacity, and adds to the food supply without diminishing the oxygen supply. It is fortunate that TMS is located on an open coast with relatively steep slope and strong tidal currents from the Panay Gulf and the Sulu Sea. The pollutants from TMS apparently are quickly diluted and broken down.

TMS broodstock tanks, plankton tanks, and hatcheries have increased over the years and have multiplied in seawater requirements. All this sea water passes through sand filters and is stripped of particulates including the larvae and juveniles of countless marine species. The sand filters are effective (except during the stormy months) and very few marine species (sea anemone *Aiptasia diaphana*, green seaweeds *Ulva* spp.) can be found in the drain canals inside TMS. Many species (barnacles, sponges, crabs, hydrozoans, even the black coral *Antipathes* sp., etc.) grow on the screens of the seawater intake pipes and have to be regularly removed.

Any adverse effect of the TMS hatcheries and laboratories is difficult to discern on top of the continuous intense fishing and habitat disturbance. So many nearshore species are harvested every day from the water column and from the bottom by various and numerous fishing gears. It is estimated that the resident fishers in Buyuan harvest from the 1 km coast an average of ~500 kg of fishery products every day during the northeast monsoon period (October–May), but a lot less during the southwest monsoon when the fish corrals can not operate. Such high level of extraction of fishes, shrimps, and cephalopods is detrimental to the marine ecosystem. Nearshore fishing gears use fine-mesh nets to catch the small sergested shrimps *Acetes* spp. ('hipon' or 'alamang') and anchovy *Stolephorus* spp. larvae ('lobolobo'), but they also catch large amounts of other small animals of no commercial value but of great ecological importance (e.g., as prey for complex food webs). Several species of small crabs as well as swarming sea hares were not eaten but thrown out of the water to prevent entanglement in fishing nets and interference in seining. Yet, the fisheries sector has not been sufficiently criticized or regulated for this wanton waste of biodiversity.

### *Biodiversity in the seagrass beds, rocky islets, and sandflat at Igang Marine Station*

In 2011–2012, some 786 species in 261 families were collected or photographed at IMS, including 74 species of fishes, 40 crustaceans, 391 mollusks, 44 echinoderms, 87 cnidarians, 47 poriferans, and 24 ascidians (Table 2). Different species combinations were found in the varied habitats — seagrass beds, rocky bases of the islets, sand flats, silty-muddy inner cove, cage netting, and cage platforms (plastic drums floats, bamboo frames). Most invertebrates and seaweeds growing on the net cages and platforms were also found in the natural habitats, but some were not. IMS has a very different species composition than TMS although some species occurred in both stations. More benthic species occurred at IMS because of the protected cove environment, varied habitat types, and the stable substrates. Among the attached seaweeds, seagrasses, corals, sponges, sea squirts, and oysters lived a variety of mobile echinoderms, snails, jellyfish, small fishes, and sea snakes—in a colorful albeit often turbid aquatic forest only a few meters deep. Larger fishes come in with the high tides, and the sunfish *Mola mola*, the sea turtles *Chelonia mydas* and *Eretmochelys imbricata*, and the dugong *Dugong dugon* occasionally strayed into the IMS cove. Artificially reseeded giant clams *Tridacna gigas* have grown large in the IMS sandflat.

Table 2 includes only those that were readily seen when walking around the station during daytime low tides, and none of the microscopic species, nor the infauna, nor the subtidal coral terrace. Presumably missing in the inventory were the species that came into the station only during high tides, or at night, or during the southwest monsoon months between June and September. Certainly more species could be found at IMS if sampling is continued. The IMS species inventory adds information on the marine biodiversity in Guimaras, which has been studied in part by the University of the Philippines-Visayas. IMS has many of the same species photographed in the wild by Kuiter (1992), Colin and Arneson (1995), Allen (1998, 2000), and White (2001), but the IMS specimens did not look as clean and healthy.

Biological pollution by IMS —from fish excreta, uneaten feeds, and occasional diseases— is undeniable. The fish cages also probably impede water flow around the seagrass beds and sandflat and into the inner cove from Islet 5. The seagrass beds are heavily silted and turbid, the sandflat may have expanded, and the inner cove and cliff sides are deep in mud. Still, the IMS species count (Table 2) seems high despite 35 years of operation of the aquaculture

Table 2. Biodiversity in the seagrass beds, sandflats, and rocky islets at Igang Marine Station, Guimaras, Philippines, 2011–2012.

| Phylum | Class | Families | Species | Representative species |
|---|---|---|---|---|
| Chordata | Osteichthyes | 37 | 74 | *Pterois* spp., *Canthigaster* spp., *Siganus* spp., *Apogon* spp., *Aeoliscus strigatus, Mola mola* |
| | Reptilia | 2 | 3 | *Chelonibia mydas, Eretmochelys imbricata, Hydrophis cyanocinctus* |
| | Mammalia | 1 | 1 | *Dugong dugon* |
| | Ascidiacea | 7 | 24 | *Oxycorynia fascicularis, Didemnum* spp. |
| Porifera | Demospongiae | 25 | 47 | *Spheciospongia vagabundus, Xestospongia exigua, Adocia viola, Theonella, Haliclona* |
| Cnidarians | Anthozoa | 27 | 75 | *Dendronephthya* spp., *Sarcophyton* spp., *Cerianthus* spp., *Heteractis crispa, Acropora* spp., *Goniopora* spp., *Fungia* spp. |
| | Scyphozoa | 4 | 5 | *Versuriga anadyomene; Cassiopea andromeda* |
| | Hydrozoa | 5 | 7 | *Millepora* spp., *Plumularia* sp. |
| Annelida | Polychaeta | 6 | 7 | *Sabella* spp., *Reteterrebella* sp. |
| Platyhelminthes | Turbellaria | 1 | 2 | *Pseudoceros* sp., *Pseudobiceros* sp. |
| Crustaceans | Penaeidea | 1 | 2 | *Penaeus semisulcatus* |
| | Stenopodidea | 1 | 1 | *Stenopus hispidus* |
| | Caridea | 2 | 6 | *Lysmata amboinensis, Alphaeus* spp. |
| | Brachyura | 9 | 16 | *Pilumnus vespertilio, Lissocarcinus orbicularis* |
| | Anomura | 1 | 8 | *Diogenes megistos, Calcinus laevimanus* |
| | Palinura | 1 | 1 | *Panulirus ornatus* |
| | Stomatopoda | 1 | 1 | *Nanosquilla* sp. |
| | Cirripedia | 5 | 5 | *Balanus amphitrite, Tetraclita squamosa* |
| Mollusca | Bivalvia | 27 | 140 | *Tridacna gigas, Malleus malleus, Atrina vexillum, Trachycardium rugosum* |
| | Gastropoda | 50 | 244 | *Conus* spp., *Cypraea* spp., *Cymatium* spp., *Nassarius* spp., *Cerithium* spp., *Morula* spp. |
| | Cephalopoda | 2 | 5 | *Octopus* spp., *Sepioteuthis lessoniana* |
| | Polyplacophora | 1 | 2 | *Acanthopleura spinosa, A. gemmata* |
| Echinodermata | Asteroidea | 5 | 10 | *Protoreaster nodosus, Culcita novaeguineae* |
| | Echinoidea | 7 | 16 | *Toxopneustes pileolus, Diadema setosum* |
| | Holothuroidea | 2 | 10 | *Pearsonothuria graeffei* |
| | Crinoidea | 1 | 6 | *Comanthus alternans* |
| | Ophiuroidea | 1 | 2 | *Ophiocoma scolopendrina* |
| Bryozoa | | 8 | 12 | *Schizoporella serialis, Stylopoma, Zoobotryon* |
| Hemichordata | Enteropneusta | 1 | 1 | *Balanoglossus* sp. |
| Plantae | Chlorophyceae | 8 | 23 | *Caulerpa* spp., *Halimeda* spp., *Codium* spp., *Ulva=Enteromorpha* spp., *Neomeris vanbossae* |
| | Phaeophyceae | 3 | 9 | *Padina* spp., *Dictyota* spp., *Turbinaria* spp., *Sargassum* spp., *Colpomenia sinuosa* |
| | Rhodophyceae | 6 | 16 | *Gracilaria* spp., *Halymenia* spp., *Acanthophora* spp., *Amphiroa* spp., *Sporolithon* spp. |
| | Angiospermae | 3 | 5 | *Thalassia hemprichii, Enhalus acoroides, Halophila ovalis, Rhizophora mucronata* |
| | **All** | **261** | **786** | |

cages, and despite continuous fishing and gleaning by the local villagers since long before IMS. But most species occurred in low densities, and many were found singly or just once. Some species occasionally or seasonally became abundant—e.g., the horned sea star *Protoreaster nodosus*, the black sea urchin *Diadema setosum*, and the edible sea urchin *Tripneustes gratilla* (which was avidly harvested). Such population explosions may have been responses to seagrass and seaweed blooms due to nutrient enrichment from the cages, or just natural fluctuations.

Several species of sponges, barnacles, bryozoans, ascidians, and oysters not found in the natural habitats were found growing on the net cages and platforms as biofoulants. Cage structures evidently provided additional surfaces for settlement of seaweed spores and planktonic larvae of many invertebrates that otherwise could not find space or food in the adjoining natural habitats. Cage aquaculture adds structural substrate, food items, and refuge for a variety of species, and can enhance biodiversity in the marine habitats it occupies. Eggs and larvae of all sorts of organisms are always in the water ready to get into the cages and attach to the nets and supporting structures. Over time, these extraneous organisms grow, go through community succession, and interact with the farmed species in various ways. The seaweeds that grow on the cages absorb nitrogen and phosphorus from the fish feeds and wastes, and provide food for grazing snails and crabs. The biofoulant filter-feeding invertebrates remove the particulates (feeds, feces, plankton) from the cage. The older the cages, greater fouling is noted. The greater the biodiversity there is and the more effective the biological recycling, the less outward pollution is observed. The biofoulants impede water flow into the cages but they also help maintain good water quality. Before the nets are fouled, small fishes continually go in and out of the cages and partake of the feeds given to the crop species. Some of these fish stay and grow in the cages with the farm crop.

Cages that are kept in the water long enough often yield a wide variety of extraneous species, most of them small, many of them edible, many others ornamental, and all of them contributors to farm sustainability and ecosystem balance. Some of the extraneous fishes are harvested with the farmed fishes, and are in effect cage bycatch. Some bycatch are eaten by the cage workers, but many are too small or toxic and just left to die when nets are removed from the water. In addition, all attached species die when cages, floats, and associated structures are taken out of the water as part of farm management. This practice can be changed. For cage farms to contribute to biodiversity, extraneous species (if not big enough for eating) should not be left high and dry to die. Instead they can be thrown alive overboard or scraped off fresh and deposited onto denuded sandflats, seagrass beds, rocky shores, or mudflats, where they may reattach and survive.

On top of the biological pollution and siltation, gross carelessness by fishers, gleaners, and boatmen has seriously damaged the IMS habitats. Local fishers including IMS personnel walk on the seagrass beds during negative low tide to harvest edible bivalves, gastropods, and sea urchins. Seagrasses and seaweeds are trampled, corals and sponges are broken, sediment is dug up, rocks are turned over, and the resident flora and fauna displaced and driven to a marginal existence in turbid water. Every day, the IMS service boat and several tourist boats traverse the IMS seagrass beds, sandflat, and coral beds, causing further damage. Thirty-five years on, it is time for the IMS habitats to be rehabilitated or protected in some way.

### *Biodiversity in the ponds and mangroves at Dumangas Brackishwater Station*

At least 312 species in 117 families were found at DBS, 210 species in the ponds plus 102 more in the mangrove greenbelt (Table 3). Some 90 species of fishes and 35 species of crustaceans lived in the ponds along with 60 mollusks, only 10 of these crop species and the others naturally seeded by the tides. Sixteen species of mangrove gobies and sleepers resided in the ponds. Three gobies often became very abundant: *Acentrogobius viganensis, Pseudogobius javanicus,* and *Mugilogobius cavifrons*, collectively called in the local dialect as 'dalodalo'. Two others were common: *Acentrogobius janthinopterus* and the almost transparent *Gobiopterus panayensis*. The 'bagtis' or *Glossogobius aureus* was not abundant but grew larger and was prized as food by the pond workers. Another mangrove resident, the larva-like priapiumfish *Neostethus amaricola* was found in some fish ponds in small schools. The pond bycatch also included juveniles of several species of commercial fishes (*Elops hawaiiensis, Eleutheronema tetradactylum*, mullets, jacks, slipmouths, mojarras), forage species (*Ambassis* spp.), and the large eels *Muraenesox cinereus* and *Pisodonophis cancrivorus*. The tilapia *Oreochromis mossambicus* and the mosquitofish *Gambusia affinis* were found in the ponds but not in the mangroves outside.

The DBS ponds also harbored 6 species of penaeid shrimps, 4 palaemonids, 6 portunid crabs, and 4 grapsids (Table 3). Only the smaller *Macrobrachium* species were found at DBS, and no *M. rosenbergii*. Some volume of *Varuna litterata* was obtained as bycatch from nearly all ponds, and a surprising crop of naturally seeded *Portunus pelagicus* was harvested from one pond. The small shrimp *Acetes erythraeus* and the mysid *Prosopodopsis orientalis* were sometimes very abundant in some ponds. Burrowing crabs like *Neosarmatium* spp. weaken earthen dikes. Many other small crabs (*Episesarma* spp., *Uca* spp., etc.) were common in the main canals and the mangrove greenbelt, but rare inside the ponds.

Mollusks in the DBS ponds included 38 species of bivalves and 22 species of gastropods (Table 3). The oysters *Saccostrea* spp. and *Crassostrea* spp. were a voluminous edible bycatch, and the snails *Cerithidea cingulata*, *Cerithium coralium*, and *Batillaria multiformis* were very abundant and considered pests. In the mangrove greenbelt were found several mollusks not found in the ponds. On both tree trunks and concrete walls clung the holed oyster *Enigmonia aenigmatica*, the coffee murex *Chicoreus capucinus*, the delicate *Cerithidea quadrata*, the black-blotched *Nerita planospira*, and the pulmonates *Cassidula mustelina*, *Onchidium* sp., and *Peronia* sp. On the leaves were glued the periwinkles *Littoraria* spp., and in the mud and on the roots crawled the orange bead snail *Sphaerassiminea minuta*.

Table 3. Biodiversity in the ponds and mangroves at Dumangas Brackishwater Station, Iloilo, Philippines, 2009–2010.

| Phylum | Class/Order | Families | Species | Representative species |
|---|---|---|---|---|
| Chordata | Osteichthyes | 43+7 | 90+34 | *Elops hawaiiensis, Acentrogobius* spp., *Gobiopterus panayensis, Neostethus amaricola* |
| | Reptilia | 1 | 1 | *Cerberus rhynchops* |
| Crustacea | Penaeidea | 2 | 7 | *Penaeus* spp., *Metapeneus* spp., *Acetes erythraeus* |
| | Caridea | 2 | 6 | *Macrobrachium* spp., *Nematopalaemon tenuipes* |
| | Brachyura | 3+1 | 10+16 | *Thalamita crenata, Varuna litterata, Uca* spp. |
| | Anomura | 1 | 3 | *Clibanarius* spp. |
| | Stomatopoda | 1 | 3 | *Chloridopsis scorpio, Oratosquilla gravieri* |
| | Thalassinidea | 1 | 1 | *Thalassina anomala* |
| | Mysida | 1 | 1 | *Mesopodopsis orientalis* |
| | Cirripedia | 3 | 4 | *Balanus Amphitrite* |
| Mollusca | Bivalvia | 14+5 | 38+31 | *Crassostrea belcheri, Saccostrea cucculata, Enigmonia aenigmatica, Isognomon* spp. |
| | Gastropoda | 7+6 | 22+21 | *Cerithidea* spp., *Cerithium coralium, Telescopium telescopium, Littoraria* spp., *Chicoreus capucinus, Sphaerassiminea minuta, Nerita planospira* |
| Cnidaria | Scyphozoa | 2 | 2 | *Cassiopea medusa* |
| Annelidae | Polychaeta | 2 | 3 | *Capitella capitata* |
| Brachiopoda | | 1 | 1 | *Lingula ungguis* |
| Echinodermata | Holothuroidea | 1 | 1 | *Holothuria coluber* |
| | Echinoidea | 2 | 2 | *Diadema setosum* |
| Plantae | Angiospermae | 8 | 10 | *Avicennia marina, Sonneratia alba, Rhizophora* spp. |
| | Chlorophyceae | 1 | 3 | *Enteromorpha=Ulva, Chaetomorpha* |
| | Rhodophyceae | 2 | 2 | *Gracilaria* spp., *Catenella caespitosa* |
| | **All** | **97+20** | **210+102** | |

Thus, the DBS ponds and mangroves are still biodiverse systems, despite intended monoculture for many years and in contrast to the general perception that aquaculture causes loss of biodiversity. Herre and Mendoza (1929) recorded 40 species of fishes, 20 crustaceans, and several snakes and birds in milkfish ponds in the Philippines in the 1920s. Brackishwater ponds are evidently leaky, even the better ones like those at DBS, and the young of many mangrove animals find their way into ponds, survive, and grow despite net screens, liming, ammonium sulfate or teaseed treatment, and chlorination. Despite the absence of mangrove trees, and as long as tidal water flow is maintained, ponds act as proxy mangrove lagoons that harbor the young of migratory fishes as well as all life stages of resident species.

Several bycatch species were eaten or sold by the pond workers, but the small and abundant gobies were often used to feed crabs and carnivorous fishes stocked in the ponds. Indeed, ways should be developed to manage gate screens, water supply, soil preparation, and crop species to maintain a biologically diverse, balanced, healthy pond environment, produce an extra crop of bycatch species, and improve farm economics.

***Conclusion and recommendations***

As part of the implementation of the SEAFDEC-sponsored Regional Code of Conduct for Responsible Fisheries, greater conscious effort must be applied towards managing aquaculture farms for high biodiversity and low pollution. This study provides some of the biodiversity information needed to formulate strategies to keep the SEAFDEC/AQD research stations cum aquaculture farms full of life, non-destructive, and sustainable.

SEAFDEC/AQD does not know what biodiversity has been lost due to its aquaculture operations because there had been no species inventories done at its research stations before 2009. Now we have a good idea of the present biodiversity, and we have baseline species inventories as of 2008–2014 on which future monitoring can be compared. The main difficulty with biodiversity monitoring is the fact that TMS, IMS, and DBS, as well as other aquaculture farms, are sited in multi-use water bodies and the effect of aquaculture on biodiversity is difficult to discern over the effects of fisheries, boating, settlements, and other uses.

***Next steps***

1. Voucher specimens of the species from TMS, IMS, and DBS should be properly documented and deposited in the AQD Museum of Aquatic Biodiversity. If physical specimens can not be obtained, then at least good photographs.

2. A permanent exhibit of the marine biodiversity at TMS, IMS, and DBS should be set up at SEAFDEC FishWorld for the science and environment education of the Filipino (*Sambayanang Pilipino*)

3. The TMS, IMS, and DBS species inventories and photographs should be published as hardcopy books, digital books, and online databases. These books will serve three purposes:

    - For SEAFDEC/AQD to use in monitoring biodiversity at its aquaculture stations in the future;

- For farmers and government regulators (Bureau of Fisheries and Aquatic Resources) to use as species identification guides to baseline-survey and monitor other aquaculture sites in the Philippines (and Southeast Asia); and
- To add to the marine biodiversity literature for students, teachers, and researchers to use as general taxonomic guide for marine habitats in the Philippines (and Southeast Asia).

4. A training-workshop on biodiversity survey and species identification should be offered to BFAR and Department of Environment and Natural Resources (DENR) technicians assigned to environmental monitoring. The training should be done on site at TMS, IMS, and DBS.

5. Monitoring of biodiversity at TMS, IMS, and DBS should be continued as a regular program or standard operating procedure of AQD, with the Stations Heads as Head Monitors. The monitoring program should eventually include subtidal surveys, infaunal sampling, quantitative analysis, and other protocols not carried out in 2009–2014.

6. Institute strategic interventions to protect biodiversity at TMS, IMS, and DBS over the long term. Some strategic interventions are described below.

### *Protect the nearshore habitats off TMS: declare an MPA*

More than the biological pollution caused by AQD effluents, the serious threat to nearshore habitats and biodiversity off TMS comes from the intensive collective fisheries off Buyuan. SEAFDEC/AQD cannot regulate fishing, but it can protect the nearshore habitats right in front of TMS, to prevent the operation of the beach seine, and allow seaweeds and benthic animals to settle and stabilize (before and after the monsoon waves and storms). Beach seines stir up the subtidal and intertidal sediment; overturn gravel where seaweeds grow and animals hide and feed; and catch the small animals and larvae concentrated by the waves and currents at the surf zone. Many of these small animals are left to die on the beach, and even when eaten are really a waste of marine life.

Although the effect of TMS on nearshore biodiversity is not noticeably adverse, AQD now has good opportunity to protect the habitat and possibly maintain or increase biodiversity. AQD can do the following:

- Request the Tigbauan local government unit (LGU) to designate the TMS intertidal and subtidal area (~500 m long, 20 m seaward of the jetties, within 10 m deep) as a marine protected area (MPA), not because it is high in biodiversity but so that it can be so;
- Construct and install large heavy rocks or concrete multi-faceted modules in this MPA to add topographic relief and stable habitat surfaces for shore animals and plants. The species that recruit and mature inside the MPA can serve as broodstock to seed the adjacent nearshore waters; and
- Monitor the biodiversity in the MPA over the years.

### *Rest and rehabilitate the IMS habitats: Build a road and bridge and discontinue boats*

More than the biological pollution and siltation due to the IMS fish cages, gross carelessness by fishers, gleaners, and boatmen has seriously damaged the IMS habitats. In particular, every day over the last 35 years, the IMS service boat has traversed the seagrass beds, sandflat, and coral beds around the station. During low tides, the outriggers, hull, and propellers hit and break coral heads and sponges, entangle seaweeds and seagrasses, churn up the sediment, the damage obvious but undocumented. In recent years, several tourist boats a day traverse IMS to visit the fish broodstocks in cages. IMS guards have not been able to keep local boatmen, fishers, and gleaners off IMS, but AQD can otherwise manage the resources at IMS. AQD can discontinue the use of the IMS service boat and disallow the use of tourist boats in order to rehabilitate the damaged seagrass beds, sandflat, and coral terrace.

After 35 years, it is time to build a road and bridge to IMS from the main road going to Barangay Dolores. At the nearest road junction, the distance from the town road to Humaraon Cove is about 600 m (shorter than the feeder road going to Landasan Cove), and the bridge over Humaraon Cove to IMS would be about 500 m long. From Igang bridge, it is about 3 km to this proposed junction. Dolores is served by many public jeepneys to and from Jordan pier. Guimaras is now in a road-building mania and the provincial government might fund and build a feeder road leading to IMS. AQD can do the following:

- Lobby the Guimaras LGU to build a feeder road to Humaraon Cove (directly opposite IMS Islet 5) from the town road leading to Barangay Dolores. Make the case that this feeder road brings goods and services to the people in Humaraon; eliminates the dangers of boat travel and increases tourist visits to IMS and tourism income to Guimaras; eases the transport of harvested fish from the mariculture park to the Guimaras markets; and facilitates the transport of personnel and materials to and from IMS;
- Construct a wooden bridge from the feeder road over Humaraon Cove to the IMS house on Islet 5. This bridge can be like the one between Islets 6, 7, 8, and 9, and can use wooden planks from the mahogany trees in Tigbauan. Another bridge could be built over the sandflat from Islet 5 to Islet 9 where the lab and growout cages are located;
- Discontinue the use of the IMS service boat for routine transport (but standby for emergencies or urgencies). Arrange for a Dolores jeep to shuttle IMS personnel to and from Humaraon Cove;
- Disallow tourist boats from IMS but let tourists visit via the road and bridge. Manage the tourists and disallow food, drinks, and wastes at IMS; and
- Monitor the status of the IMS habitats over the years.

### *Green up DBS: Integrate BMP into SOP*

SEAFDEC/AQD advocates responsible aquaculture and has done several experiments to develop various best management practices (BMP) for brackishwater ponds. However, these BMPs are hardly in evidence at DBS now. There is no trace of the muchtouted aquasilviculture, and none of the recommended effluent treatment through arrays of oysters, mussels, and seaweeds. To maintain credibility, as well as get good results, DBS should integrate BMPs into SOP in the ponds, and not just do them as a project. Since brackishwater ponds serve as proxy mangrove lagoons and nursery habitats for a variety of species, BMPs should be developed to allow ponds to be' leaky', maintain a biodiverse and healthy pond environment, and produce an extra crop of bycatch species.

AQD can institutionalize BMP-SOP for a greener DBS. The BMP-SOP can include the following:

- Use hatchery-reared postlarvae or juveniles or 'fry' (better nourished, no predators and competitors);
- Rear fry to a larger size in a nursery pond or tank before stocking in large ponds with tidal water supply (so the stocks have a head start over the extraneous species);
- Reconfigure the ponds such that influent water comes through the main canal from Pulao Creek, but the effluent water leaves through another main canal into Talauguis River (to minimize self-pollution);
- Plant and grow stands of *Avicennia* mangroves (with readily available seedlings), or arrays of oysters, mussels, and seaweeds in the main canals to remove particulates and nutrients from both the incoming tidal water and the effluents;
- Manage 1–2 large ponds at the east end for aquasilviculture with a clump of *Avicennia* mangroves at the center;
- Extend the mangrove greenbelt by planting *Avicennia* outside the concrete dike facing Pulao Creek and the mudflat to remove particulates from the influent water before it enters the main gate;
- Monitor, measure, and record the pond bycatch from all experiments and production runs. Such data should be analyzed for trends and economic value;
- Collect the bycatch and feed it to carnivorous farmed species to reduce feed costs and pollution. Bycatch may also be given away to pond workers; and
- The small gobies that are not to be collected should be released alive from ponds back into the mangroves and mudflats during draining and harvest (as a simple restocking scheme).

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# Targeting Essential Gene Utilizing RNA Interference to Protect the Ailing Shrimp/Prawn Industry Against WSSV

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## *Abstract*

The white spot syndrome virus (WSSV) remains to be the most widespread and devastating infectious agent that has hit particularly the marine shrimp aquaculture industry worldwide. To date, there are no known effective strategies that can combat WSSV infection. This study aimed to elucidate host-pathogen interaction through the functional study of host - gene. Utilizing RNA Interference, the function of contig23 (c23) in the shrimp genome, identified to have high homology with WSSVORF-325, was determined. Three set-ups were prepared for treatment of c23-, GFP-dsRNA, and PBS using *Macrobrachium rosenbergii* freshwater prawns. Each treatment group was challenged with WSSV and survival rate was recorded. C23-, and GFP-dsRNA injected prawns showed a significant survival rate of 100%, in contrast to 20% of the PBS injected prawns at 10 days post-infection (dpi). Results showed that injection of c23- and GFP-dsRNA prior to challenge with WSSV, delayed and reduced mortality in contrast to PBS-treated prawns, which showed high mortality. Gene expression analysis showed silencing of both WSSV and c23 at day 3 post-WSSV challenge. This study proved that c23-dsRNA has a protective effect on WSSV-challenged prawns and highlights its involvement in the infectivity of WSSV in *M. rosenbergii*.

**Keywords:** WSSV, host-pathogen interaction, shrimp genome, gene expression analysis

## *Introduction*

The inflicting diseases on today's aquaculture have wreaked havoc on the sustainable growth of the shrimp culture industry worldwide. In the Asia-Pacific countries, the industry reported annual losses of about 4 billion US dollars yearly. Shrimp is an indispensable source of revenue, as this commodity accounts for almost 20% of aquaculture commodities in trade worldwide. A large fraction of the damage to the industry is undoubtedly caused by viruses, which already accounted to have over 20 strains that of which affected both penaeid shrimp wild stocks and commercial production.

The white spot syndrome virus (WSSV) is one of the most potent and widespread pathogen (Flegel, 2006) affecting the shrimp culture industry. This virus can spread the disease rapidly in a span of 2-10 days post-infection and can bring 100% cumulative mortality (Flegel, 2006). The insufficient modes of sanitation and the growing number of shrimp cultures, coupled with uncontrolled trade movement among countries, account for the optimum conditions for the virus to spread to almost all farming areas (de la Peña *et al.*, 2007). Although there are already some methods which displayed efficacy against the virus under experimental conditions, no effective treatments have been available to address WSSV problem in the field (Dang *et al.*, 2010).

Understanding the underlying molecular interaction between the host and pathogen is very critical in creating strategies to prevent diseases. A novel approach to understand host-pathogen interaction is the utilization of RNA interference (RNAi) technology. RNAi is a post-transcriptional gene silencing process in which double-stranded RNA (dsRNA) triggers the silencing of a cognate gene. Insurmountable evidences have pointed out the efficiency of RNAi in studying gene function and its implication in mounting antiviral responses in eukaryotes. In the shrimp system, a number of studies have demonstrated the effectiveness of RNAi in studying shrimp-pathogen interaction. Injection of WSSV gene-specific dsRNA efficiently suppressed viral replication in penaeid shrimp (Kim *et al.*, 2007; Robalino *et al.*, 2007) and suppression of yellow head virus (YHV) replication by cognate-dsRNA, significantly reduced mortality in the black tiger shrimp.

On the other hand, in the shrimp genome, several genes that can be potentially linked to anti-WSSV responses in shrimp had been identified (He *et al.*, 2005; Wang *et al.*, 2006; Zhao *et al.*, 2007). A bacterial artificial chromosome (BAC) library of the kuruma shrimp *Penaeus (Marsupenaeus japonicus)* genome revealed 27 open reading frames (ORFs) that are surprisingly homologous to the predicted proteins that code for WSSV. Two of the homologs found in kuruma shrimp (MjORF16 and MjORF18) and their WSSV homologs (WSSVORF-332 and WSSVORF-285, respectively) were utilized to demonstrate the involvement of both WSSV and shrimp homologs in the infectivity of WSSV in *Penaeus (Marsupenaeus) japonicus* (Dang *et al.*, 2010). One of the 27 ORFs identified in the genome of kuruma shrimp is contig 23 (c23), which has high homology to WSSVORF325 and codes for MjORF158 in the kuruma shrimp genome.

Here, we elucidated the function of contig 23 gene *in vivo* by utilizing RNA-interference technology.

## Materials and Methods

### Laboratory Set-up and Shrimp Acclimatization

Freshwater prawn *Macrobrachium rosenbergii* weighing 3-5 grams were purchased from Southeast Asian Fisheries Development Center (SEAFDEC) Binangonan, Rizal, Philippines. One hundred (100) juveniles were reared in filtered recirculating de-chlorinated tap water tanks maintained at 25-28°C and 0 ppt salinity. Feeding was *ad libitum* on a daily basis.

### Preparation of Virus Stock Inocula and Median Lethal Dosage (LD50)

WSSV stock was isolated from WSSV-infected *Penaeus monodon* obtained from SEAFDEC, Iloilo, Philippines. WSSV infection was confirmed through Polymerase Chain Reaction (PCR) using WSSV-specific primers (Table 1). Viral isolation was done following the procedure in the study of Rout *et al.,* (2007). One hundred (100) µL of the viral isolate was injected to 5 healthy *M. rosenbergii* juveniles, from which a new viral stock was isolated and used for the challenge test, following the same procedure mentioned above. The WSSV viral stock was then stored at -80°C until the commencement of the challenge test. The virus concentration used in the challenge test was determined based on the median lethal dose ($LD_{50}$).

### *dsRNA synthesis*

For the production of double-stranded RNA (dsRNA), optimization of the conditions was based on the methods developed by Maningas (2008). dsRNAs were generated *in vitro* using the T7 RiboMAX Express Scale RNA Production System (Promega, USA) following the protocols provided by the manufacturer. Briefly, T7 promoter sequence was incorporated to gene specific primers for c23 (Table 1) to produce sense and antisense strands separately. Two separate PCR reactions with a single T7 promoter were set up for each dsRNA to generate two separate single promoter PCR templates for *in vitro* transcription. The primers used to amplify the region are shown in Table 1. The resulting PCR products (with T7 promoter) were quantified through the use of Perkin Elmer Lambda 40 UV-VIS Spectrophotometer and normalized to attain similar concentration for the transcription experiment.

Transcription was performed by utilizing Promega Transcription T7 polymerase kit to yield single-stranded RNAs (ssRNAs). Two µL of PCR products were utilized for transcription and the reaction yielded 20 µL of ssRNA. Equal amounts of ssRNAs were mixed together to anneal the RNA strands and were incubated at 70°C for 10 minutes and allowed to cool at room temperature for about 20 minutes. After cooling to room temperature, the resulting dsRNAs were further purified following the protocol provided by T7 Ribomax Express Large Scale RNA Production System and were quantified again using the same spectrophotometer. The synthesized dsRNAs were quantified to make sure all samples were of equal concentration for the *in vivo* RNAi experiment (1 mg/mldsRNA).

### *Amplification, Sequencing and Analysis of c23*

The target sequence, c23, was amplified through PCR and the primers used are in Table 1. The PCR mix contained the following: 1x buffer, 2 mMdNTPs, 0.6mM Primer (Forward and Reverse), 1 unit Taq, 5.7 ml ddH2O and 1 mg DNA template. The following thermocycler conditions were utilized: initial denaturation at 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, 72 °C for 1 min and final extension at 72°C for 10 min. The PCR products were viewed in 1% agarose gel stained with ethidium bromide to check for the presence of amplified DNA.

The following reaction was prepared for capillary sequencing: 1 mLBigDye terminator reaction mix, 3.5 mL 5x BigDye sequencing buffer, 1 mL template DNA, 1 ml primer and 13.5 mL distilled water. The thermal profile used is as follows: one cycle hold at 95°C, 25 cycles 95 °C for 10 sec, 50 °C for 5 sec, 60 °C for 4 min. and final hold at 4 °C. The resulting product was purified then sequencing was carried out in Applied Biosystem 31301 Analyzer. The DNA sequence acquired was utilized for the Basic Local Alignment Search Tool (BLAST) search in order to establish its phylogenetic

Table 1. List of primer sequences for RT-PCR.

| Primer name | Nucleotide Sequence |
|---|---|
| Contig-23 | F 5' ACCGCTACTGACGACAACG3'<br>R 5' CACTCGCTCCGTTAACAAGG3' |
| T7 Contig-23 | F 5' TAATACGACTCACTATAGGACCGCTACTGACGACAACG3'<br>R5'TAATACGACTCACTATAGGCACTCGCTCCGTTAACAAGG3' |
| GFP<br>(Maningas, et al., 2008) | F 5'ATGGTGAGCAAGGGCGAGGA3'<br>R 5'TTACTTGTACAGCTCGTCCA3' |
| T7GFP<br>(Maningas, et al., 2008) | F5'TAATACGACTCACTATAGGATGGTGAGCAAGGGCGAGGA3'<br>R 5'TAATACGACTCACTATAGGTTACTTGTACA GCTCGTCCA3' |
| WSSV<br>(Flegel, 2006) | F 5'GTACGGCAATACTGGAGGAGGT3'<br>R5'GGAGATGTGTAAGATGGACAAG3' |
| EF-1α<br>(Maningas, 2006) | F 5'ATGGTTGTCAACTTTGCCCC3'<br>R 5'TTGACCTCCTTGATCACACC3' |
| β-actin<br>(Liu, et al. , 2006) | F 5'AACTCCCATGACATGGAGAACATC3'<br>R 5'TCTTCTCACGGTTGGCCTTG3' |

relationship with other genes available in GENBANK. The evolutionary history of the query sequence was inferred by using the Maximum Likelihood method based on the Tamura-Nei model in MEGA5.

### *In vivo gene silencing of c23-dsRNA*

Three set-ups were prepared in the Wet Laboratory and each contained 12 *M. rosenbergii* as test animals. The set-ups corresponded to experimental (c23-dsRNA treated), treated control (GFP-dsRNA) and untreated control (PBS). One group was injected with 5mg of c23-dsRNA (suspended in 100 uL PBS) and another with 5 mg of GFP-dsRNA (suspended in 100 uL PBS). The negative control group was injected with 100μL of PBS. After dsRNA/PBS injection, the animals were left untouched for 24 hours to recuperate, then injection with WSSV followed. Two hours after WSSV infection corresponded to Day 0 post infection (p.i.). All the animals were injected intramuscularly at the 3$^{rd}$ abdominal segment. The tanks were maintained at 22-25°C and 0 ppt salinity. There were four sampling days for the gene expression analysis: day 0, 1, 3, and 7. Tissues (hemocytes and gills) were collected from three randomly sampled shrimps for RNA extraction. Total RNAs were reverse transcribed to cDNA using M-MLV reverse transcriptase following the protocol provided by the manufacturer (Invitrogen, USA). The inhibitory effect and specificity of the c23-dsRNA were determined by RT-PCR using equal amounts of cDNA as templates.

### *PCR and RT-PCR analysis*

PCR was carried out to confirm the WSSV-free status of experimental animals and detect the presence of WSSV particles at the persistent infection stage.

Time-course RT-PCR was utilized to evaluate the interaction of homologous shrimp, c23, with WSSV infections and to elucidate the sequence-specific gene silencing by c23-dsRNA. Gills and hemocytes were dissected out from three individual samples for each set-up and sampling day (Day 0, 1, 3, 7 p.i). Total RNAs were isolated using Trizol (Invitrogen, USA) and quantified by UV Spectrophotometer to ensure that all samples were of equal concentration when used for RT-PCR analysis. One microgram of each sample was reverse transcribed to produce single-strand cDNA with the use of SuperScript™ First-Strand Synthesis System following the protocols recommended by the manufacturer. Transcripts were visualized in 1.5% agarose gel stained with ethidium bromide.

The following thermocycler conditions were followed for all PCR and RT-PCR set-ups: initial denaturation, 95°C for 5 minutes, followed by 30 cycles of (1) denaturation at 95°C for 30 seconds, (2) annealing at 55°C for 30 seconds and (3) extension 72°C for 1 minute then final DNA extension at 72°C for 5 minutes. The transcripts were visualized and analyzed in a gel electrophoresis using 1.5% agarose. The expression of EF served as the reference gene.

### *Challenge Test*

Three set-ups, each with ten (10) *M. rosenbergii*, were prepared for the challenge test and were housed in the Wet Laboratory of TARC. A total of six plastic aquaria (72.3 cm x 52 cm x 44 cm) equipped with re-circulating water tank system were utilized for the set-up. The aquaria were maintained at about 22–25°C and 0 ppt salinity prior to the experiment. Protective efficiencies of c23-dsRNAs against WSSV infection were determined by intramuscular injection at the 3rd abdominal segment. The juveniles were injected with either 100 mL of PBS or 5µg of c23-dsRNA or GFP-dsRNA suspended in 100 mL PBS prior to challenge with the viral inoculum (100µL of $10^{-2}$ diluted WSSV stock). The injection of GFP-dsRNA served as the unrelated dsRNA control group. Cumulative mortality rate was recorded daily up to 10 days after infection.

### *Statistical Analysis*

The survival data were analyzed through the use of Kaplan-Meier survival with a chi-square test using GraphPad Prism Software and differences were considered significant at $p<0.05$.

## Results

### *Phylogenetic analysis*

The query sequence (c23) consisted of 597 base pairs and was compared with other sequences from NCBI (Figure 1). In addition to the other homologous sequences from the database, c23 sequence obtained from *Penaeus (Marsupenaeus) japonicus* was also included in the analysis. Sequencing results showed that c23 is part of the *Macrobrachium rosenbergii* genomic DNA and the sequence has 27% identity (having an E value of $1e^{-5}$) with WSSVORF-325. The sequence was aligned with other sequences obtained from BLAST and a Phylogenetic tree was constructed. Figure 1 shows related sequences with c23, and WSSVORF-325 was one of those which branched out from the c23 sequences obtained from *M. japonicus* and *M. rosenbergii*. WSSVORF-325, homologous to vp25, is an envelope protein responsible for the systemic infection of its host by the virus. In constructing the phylogenetic tree, the Maximum Likelihood (ML) method was utilized for the analysis of the sequences. In addition to this, the test of phylogeny made use of the Bootstrap method using 1000 Bootstrap replicates and gaps were not included in the analysis.

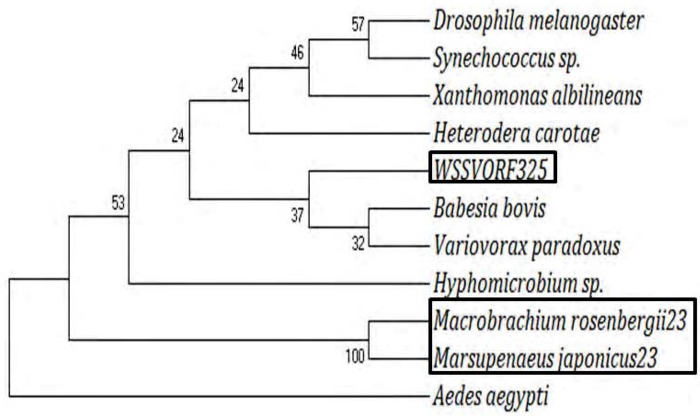


Figure 1. Molecular Phylogenetic analysis by Maximum Likelihood method of c23. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-3282.2441) is shown.

### *Expression of c23 in different tissues*

Utilizing cDNAs obtained from mRNAs of a normal/healthy *M. rosenbergii*, the expression pattern of c23 in different tissues was examined through RT-PCR analysis. As shown in Figure 2, c23 is expressed in all tissues examined and were highly expressed in the gills, heart, hepatopancreas, intestine and hemocyte. Elongation Factor (EF-1α) was used as the internal reference control and was positively amplified, with similar levels, from all tissues of normal shrimp.

### *Interaction of c23 with WSSV infection*

To elucidate the expression pattern of c23 in WSSV-infected prawns, RNA was isolated from the hemocytes and gills of three PBS-treated samples at four time intervals (Day 0, 1, 3, and 7 p.i.). cDNAs were derived from isolated RNA and were analyzed through RT-PCR. Only hemocyte and gill cDNAs were utilized as templates for the gene expression analysis because these two important tissues were essential in the progression of viral diseases and these templates showed consistent expression using normal tissues. EF-1α was used as the reference gene. WSSV specific primers from other regions with a target size of 200bp, were utilized to indicate expression of WSSV. In addition, the expression of other WSSV gene confirmed that the high mortality observed in PBS-treated shrimps was indeed due to the WSSV infection (Figure 3A).

Figure 3B shows the expression of WSSV in the GFP-dsRNA treated prawns. This further shows that the animals were still infected with WSSV despite their survival during the experiment period.

### *Sequence-specific silencing by c23*

To further explain the reason behind the high survival rate observed in c23-dsRNA treated shrimps, the expression of c23 was also determined through RT-PCR. Using cDNAs derived from gills and hemocytes as templates, the expression level of c23 and WSSV was completely silenced starting Day 3 p.i. as shown in Figure 4. This result indicates that c23-dsRNA has a specific, inhibitory effect on the expression of the target gene, c23.

### *Effect of c23 on WSSV infection*

To assess the effect of the WSSV homolog c23 in WSSV infection, the mortality of c23-dsRNA-injected *M. rosenbergii* after challenge against WSSV was monitored. In PBS-treated prawns, mortality was observed starting on day 6 p.i. although 30% of the stock survived on the last day of sampling. In those treated with c23-dsRNA, no mortality was observed through the sampling days (Day 0 till10 p.i.). High survival rate was also observed in GFP-dsRNA treated stocks. The dsRNA-treated stocks showed 100% survival while those that were PBS-treated showed 30% survival after Day 7 p.i. (Figure 5). Delayed onset of mortality was also observed in other species of shrimps *Penaeus (Marsupenaeus) japonicus* and *Penaeus*

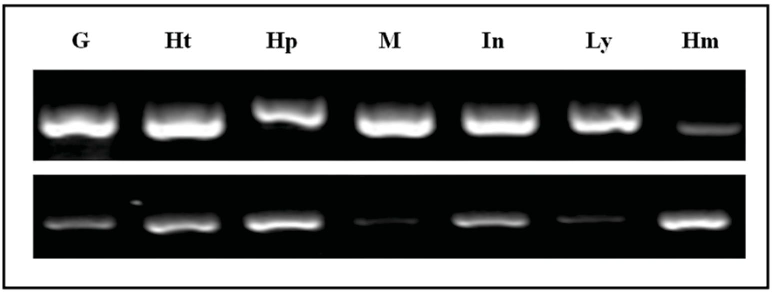


Figure 2. Expression of c23 in different tissues of healthy *Macrobrachium rosenbergii*. First lane corresponds to expression of EF-1α (reference gene) while the second lane corresponds to expression of c23. **G**: gills; **Ht**: heart; **Hp**: hepatopancreas; **M**: Muscle; **In**: intestine; **Ly**: lymphoid organ; and **Hm**: hemocyte.

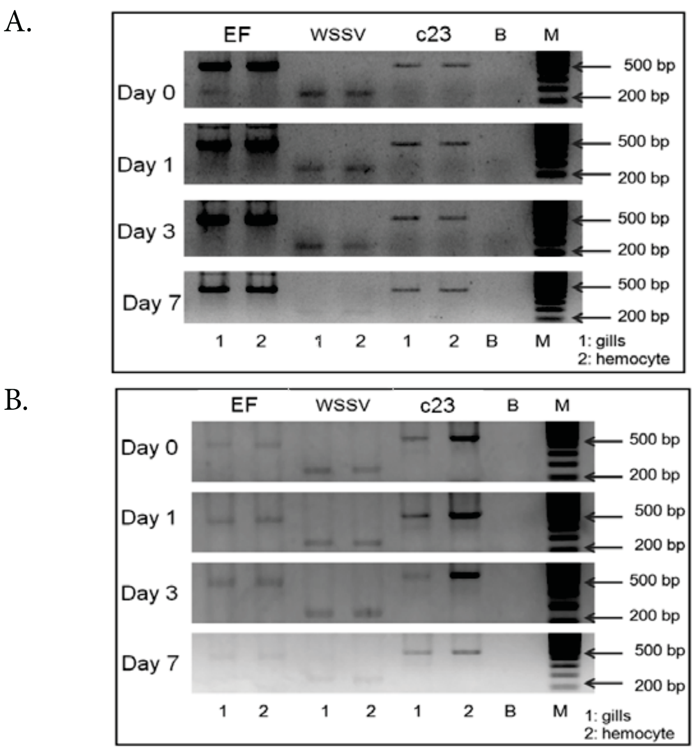


Figure 3. Expression of WSSV and c23 in gills (1) and hemocytes (2) at 0-7 dpi. A. PBS treatment; B. GFP treatment. **EF**- elongation factor was used as a reference gene. **M**-marker, **B**- blank (master mix without the DNA template).

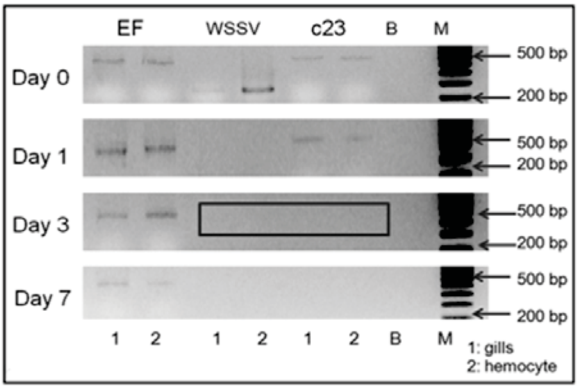


Figure 4. RT-PCR analysis. Sequence-specific gene silencing by c23-dsRNA in gills (1) and hemocytes (2) of infected *Macrobrachium rosenbergii* juveniles. **EF**- elongation factor was used as a reference gene for successful DNA amplification. **M**-marker, **B**- blank (master mix without the DNA template).

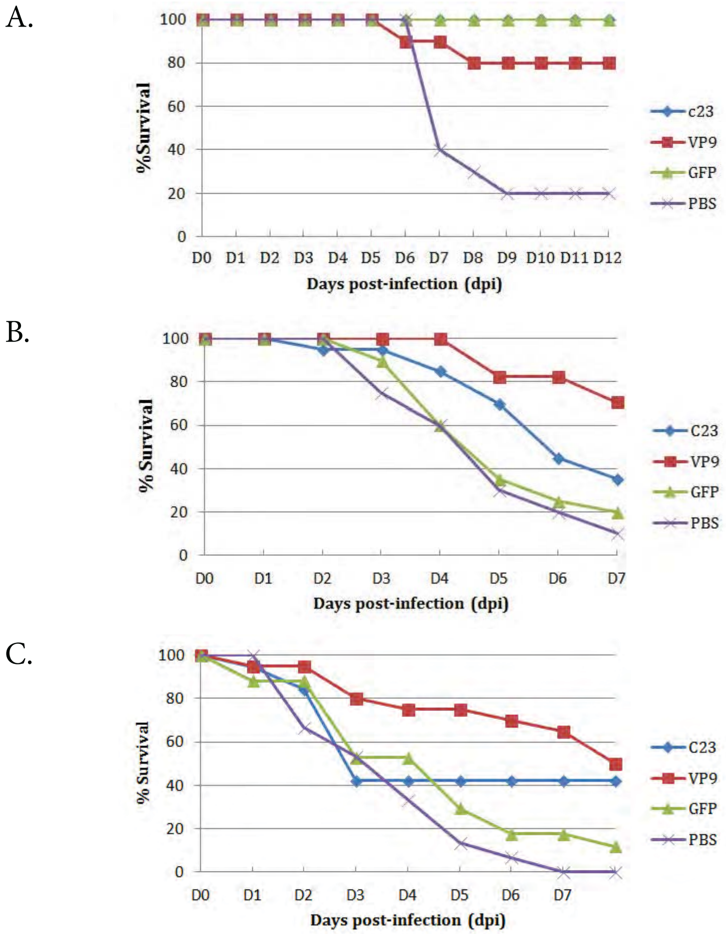


Figure 5. Survival of dsRNA-injected and WSSV-infected shrimps and prawns after WSSV challenge. A. *Macrobrachium rosenbergii* B. *Penaeus monodon*; and C. *Penaeus (Marsupenaeus) japonicus*. The animals were injected with either PBS (control) or c23-dsRNA, VP9-dsRNA, and GFP-dsRNA) and immediately challenged with WSSV (10-2 dilution from stock).The difference between the control and experimental set-ups (PBS vs GFP-dsRNA, PBS vs c23-dsRNA, PBS vs VP9-dsRNA) is statistically significant while no significant difference was observed between GFP-dsRNA vs. c23-dsRNA, based on paired t-test, ($p < 0.05$).

*monodon)* after c23-dsRNA treatment, highlighting the role of c23-dsRNA as a 'protective gene' for WSSV-infected shrimps. Silencing c23 significantly reduced and delayed mortality in all three species of crustaceans. Thus, the high survival rate observed in dsRNA-treated shrimps suggests that treatment of dsRNA be it specific (c23-dsRNA) or non-specific (GFP-dsRNA) in the WSSV-infected shrimp has a protective effect.

Results of the PCR assays, through the use of WSSV primers that had a 200 bp target size, confirmed that the mortality in all WSSV-challenged *M. rosenbergii* was due to WSSV infection.

## *Discussion*

The importance of the shrimp industry in the country mandates shrimp farmers to focus on issues of high density production as well as specific pathogen-free rearing. However, like any other organisms that are reared in great numbers and produced in high densities, these cultured shrimps are easy targets of microbial and viral pathogens. In this case, one of the effective approaches is to better understand the animal's immune system (Hirono *et al.*, 2010) in order to devise successful schemes in preventing infection. This study worked on c23, a WSSV homolog previously found in Kuruma shrimp and elucidated its possible role in the infectivity of WSSV in *M. rosenbergii*.

Phylogenetic relationship of the contig 23 with other genes from other organisms showed its high homology with WSSVORF-325. Based on the tree generated, it was shown that the contig 23 from two species of crustaceans, *Penaeus (Marsupenaeus) japonicus* and *Macrobrachium rosenbergii* clustered together showing high similarity, while WSSVORF-325 branched out from them. This result is intriguing and intensifies the speculation of "viral mimicry" in WSSV. The high homology WSSVORF-325 might be one of the virus' ways of 'adapting' to its host. Thus the supposition that the virus is mimicking some genes of its host to evade immune response, as observed in other vertebrate viruses (Koyama *et al.*, 2010), is also a possibility in WSSV.

In understanding the pathogenesis of viral diseases, it is essential to have knowledge on the complex interactions between the virus and its host (Dang *et al.*, 2010). A study conducted on *M. japonicus*, revealed the presence of multiple WSSV-like genes in its genome that strongly suggests that similar mimicking mechanisms or horizontal gene transfers can also be seen in this virus group. Such information provides a good starting point for understanding unknown WSSV-host interactions since the current database does not have information on homologous proteins (Alcami and Koszinowski, 2000). The attempt to elucidate the complexity of WSSV-shrimp interaction led to the study of mechanisms mediated by RNAi in the shrimp antiviral response. RNAi has been utilized to study specific pathogens, immune related genes and antiviral mechanism in shrimp immunity (Maningas *et al.*, 2008). It comprises of a cascade of related cellular processes wherein the introduction of dsRNA suppresses the expression of the gene based on sequence homology between the dsRNA trigger and the target gene (Robalino *et al.*, 2005).

In this study, gene expression analysis was done using hemocyte and gill cDNAs, since these two important tissues were essential in the progression of viral diseases. The gills are usually the organ that is highly affected by the virus after infection while the hemocyte is an essential organ in mediating the first line of defense and plays an integral role in the overall invertebrate immune system. In invertebrates, the most important role of the circulating hemocyte is the protection of the animal against invading microorganisms by participating in recognition, phagocytosis, melanization and cytotoxicity (Hirai *et al.*, 2004).

Here, we elucidated the role of c23 earlier found in Kuruma shrimp, as a WSSV-homolog, in the infectivity of WSSV in *Macrobrachium rosenbergii*. RNAi was induced through *in vivo* experiment by injecting synthesized dsRNAs intramuscularly in the 3rd abdominal segment of WSSV-challenged prawns. Results showed that day 3 after introduction of c23-dsRNA, expression of c23 was silenced. In previous studies, the introduction of gene-specific dsRNA can cause systemic silencing in shrimps. Extracellular dsRNAs were internalized by shrimp cells *in vivo*, as evidenced by the induction of gene silencing which is known to be an intracellular phenomenon (Robalino *et al.*, 2009). This reasoning implies the presence of cell surface receptors in shrimp that mediates the uptake of dsRNA; in a previous study, it was speculated that perhaps the dsRNA is being taken up by shrimp cells (Maningas *et al.*, 2008) either by using an endocytic pathway similar to those reported for the scavenger receptor-mediated endocytosis in *Drosophila* S2 cell (Ulvila *et al.*, 2006) or the endocytic pathway mediated cell entry of dsRNA in *Caenorhabditis elegans* (Saleh *et al.*, 2006). In *C. elegans*, the RNA interference deficient-1 (sid-1) locus involved in transmitting the silencing signal between cells has been identified. SID1 encodes a protein of 11 transmembrane domains and has a structure suggestive of an import–export channel that probably functions as a receptor. In shrimp, the presence of Sid-1 homolog *(Lv-Sid-)1* has been reported,. It was revealed that *Lv-Sid-1* plays a potential role as a channel for dsRNA. In addition, knockdown experiments on *Lv-Sid-1* gene with sequence-specific dsRNA caused mortality of up to 80% death within two days post infection (Labreuche *et al.*, 2010).

In the shrimp system, researchers were able to show the effectiveness of RNAi technology in elucidating the functions of the genes, Transglutaminase (TGase) and clotting protein (CP), in the shrimp system (Maningas *et al.*, 2008). Systemic gene silencing across different tissues tested (gills, heart, hemocyte, hepatopancreas, intestine and lymphoid organ) demonstrated the efficiency of injecting naked dsRNAs into the shrimp's system. This kind of uptake was similarly observed in previous RNAi studies on different species of shrimps (Dang *et al.*, 2010; Robalino *et al.*, 2007; Kim *et al.*, 2007).

This study showed that injection of c23-dsRNA delayed and reduced the mortality due to WSSV infection; hence, it denotes the suppression of the freshwater prawn's ORF (c23) homologous to WSSV proteins specifically vp25/vp28 which are major viral envelope proteins, such further justifies the role of c23 in the infectivity in *M. rosenbergii* (also in *M. japonicus* and in *P. monodon*) to WSSV. The high % identity (100%) posed by WSSV325 with vp25/vp28 gave some definite answers on the role of this contig in the infectivity of the virus in its host. Vp28 has a signal function for transport and also responsible for the membranous structures associated with

WSSV infection *in vivo* (Durand *et al.*, 1997). In addition to this, it is noteworthy to mention that vp28 plays a crucial role in systemic WSSV infection in shrimp in that it binds to the shrimp cells in a low-pH environment and aid viral entry into the cytoplasm (Yi *et al.*, 2004). With this information at hand, it is then safe to say that c23 might be working the same way as vp28, thus explains the findings of this study. The silencing of both WSSV and c23 observed in Figure 4, demonstrated the interaction of the WSSV-homolog with WSSV, therefore suggesting that c23 is involved in the infectivity of WSSV in this species. In the study on kuruma shrimp, it was found that injecting shrimp with MjORF16-dsRNA and MjORF18-dsRNA (both ORFs are known to be homologous to WssvORF-332 and WssvORF-285, respectively) followed by WSSV challenge test delayed and reduced mortality (Dang *et al.*, 2010). These findings suggest that suppression of host ORFs homologous with predicted WSSV proteins combat spread of the virus thereby denoting that homologous MjORFs may function in the infectivity of WSSV to its host.

While no mortality was observed in GFP-dsRNA treated prawns, silencing of the WSSV gene did not transpire. This raises the question: what causes the prawns to survive despite WSSV infection? In the moth system, injection of dsRNA representing GFP, a sequence foreign to the moth, was shown to reduce melanization induced by baculovirus infection (Angthong *et al.*, 2010). This implies that other than sequence-specific dsRNA-mediated gene silencing, injection of foreign dsRNA (GFP-dsRNA) may induce another type of antiviral response which helped the WSSV-infected prawns to survive. In a previous study performed in 2004, the findings suggested that exposure of marine shrimps to dsRNA triggered innate antiviral immunity in a sequence-independent manner (Robalino *et al.*, 2004). Nevertheless, the mechanisms underlying the said phenomenon as well as its occurrence in other invertebrate taxa remains unknown, but it is clearly shown that recognition of dsRNA by another pathway, RNAi, is widely distributed among invertebrates and likely an important component of the invertebrate antiviral response.

As stated above that the injection of dsRNA can induce innate antiviral immunity among shrimps in a sequence-independent manner, succeeding studies on shrimp antiviral immunity showed that same antiviral response was also observed among shrimps after the introduction of sequence-specific dsRNA. In another experiment conducted, the data collectively demonstrated that strong dsRNA-mediated antiviral immunity is dependent upon the sequence homology between the dsRNA trigger and the viral targets. The study thus proposed that partial protection induced by dsRNA of diverse length, sequence, and base composition can be observed from virus-infected shrimps (Maningas *et al.*, 2008).

In line with what were previously done on elucidating the effect of introduction of sequence-specific dsRNA, this study demonstrated that injection of c23-dsRNA was able to induce specific down-regulation of the expression of endogenous genes homologous to WSSV ORFs. Furthermore, it strengthened the fact that administration of dsRNA homologous to viral genes can induce a potent and virus-specific antiviral response that may result in a highly effective control of viral diseases, specifically WSSV.

The complexity of generating homologs by viruses is still a labyrinth; one hypothesis that can be derived from it is that the presence of this WSSV homolog actually helps the virus to evade its host's immune response through 'mimicking'. Since it is present in the host's genome, the entry of viral DNA will be 'masked' and will be considered as a 'self' instead of an antigen thus making the host susceptible to infection while it strengthens the virus' infectivity. The possibility of an enhanced viral infection is at a high stake in the presence of these homologs, since they are not considered as 'nonself' by the host, the immune response genes will not be readily released therefore giving the virus the bigger chance to continue replicating. In another perspective, the presence of these homologs in the genome of WSSV might be one of the mechanisms of WSSV infection. Mutation in the genome of viruses is very rampant and occurs rapidly. These homologs may be mutations that make the virus 'adaptable' to its host's environment, especially to the immune response machinery. In this way, entry of viral DNA will be a lot easier and viral replication will be at full speed because there will be no interference by the immune response genes of the host. As shown in this study, the silencing of c23after injection of c23-dsRNA to shrimps challenged with WSSV reduced and delayed mortality. This further supports the speculation that c23 is probably involved in the infectivity of WSSV and that the absence of this contig builds up a 'protective wall' in the host's immune system that resists the initiation of virus replication and triggers the release of immune response genes.

The biological interaction between viruses and their hosts is a delicate balance of actions and counteractions between host immune system and virus escape mechanisms. Having shown that viral immune evasion, possibly through viral mimicry by production of homologs, is observed in vertebrate viruses, this study is also directed towards the path of explaining the reason behind the presence of WSSV-homologs in the shrimp genome. It is more like a question of which is mimicking between the two organisms: is it the host mimicking the WSSV genes to evade infection or the WSSV duplicating these specific genes to escape the immune response of its host? The observed gene silencing of WSSV-homolog demonstrated in this study tends to answer the latter question: that the production of homologs by WSSV is one mechanism of the virus to be able to escape the antiviral response of its host and continue replicating as shown in Figure 1 where c23 is clearly a part of the shrimp genome. The expression of c23 in three different species (*Macrobrachium rosenbergii, Penaeus (Marsupenaeus) japonicus* and *Penaeus monodon*) further strengthens the hypothesis that the WSSV homologs are involved in the virus' mechanism by mimicking the host's gene to evade the immune response of its host during infection.

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# ABSTRACTS OF ORAL PRESENTATIONS

# SEAFDEC/AQD Stock Enhancement Initiatives: Release Strategies

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## *Abstract*

The Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) started its Stock Enhancement Program more than a decade ago with the first stock enhancement initiative on the mud crab *Scylla* spp. funded by the European Commission. This was followed by another stock enhancement program in 2005 supported by the Government of Japan Trust Fund. In preparation for its implementation, a Regional Technical Consultation on Stock Enhancement of Species Under International Concern was convened in Iloilo City, Philippines in July 2005 to identify species for stock enhancement. During the meeting, seahorses *Hippocampus* spp., giant clam *Tridacna gigas*, abalone *Haliotis asinina*, and sea cucumbers *Holothuria* spp. were among the priority species for stock enhancement work.

Stock enhancement, restocking and ranching are management approaches involving the release of wild or hatchery-bred organisms to enhance, conserve or restore fisheries. This paper reports SEAFDEC/AQD release activities and some of the release strategies that have been established for mud crabs, giant clams and abalone.

**Mud crab, *Scylla* spp.** – Studies on the mud crab, conducted from April 2002 to November 2005, evaluated the effectiveness of releasing wild and hatchery-reared (HR) crabs in the mangroves of Ibajay, Aklan, Philippines where preliminary studies demonstrated declining fishery yields, abundance and size of crabs (Lebata *et al.*, 2007). Comparison of survival and growth of wild-released and HR *Scylla olivacea* and HR *Scylla serrata* demonstrated the effect of nursery conditioning, size-at-release and species differences. Overall yield and catch per unit effort (CPUE) increased by 46% after stock enhancement trials. Recapture rates of released crabs were highest in wild-released *S. olivacea* and in crabs measuring 65.0–69.9mm carapace width (CW) and lowest in non-conditioned HR *S. serrata*. Growth rates were highest for conditioned HR *S. olivacea* and lowest for conditioned HR *S. serrata* (11.7 and 3.7 mm month$^{-1}$, respectively). Fishing mortality was highest for *S. olivacea*, whereas natural mortality was greater for *S. serrata*. Conditioning hatchery-bred animals before release is also important in obtaining higher survival. *S. olivacea* was the more appropriate of the two species for release in mangrove habitats inundated with low-salinity water. However, there is a need for site-specific studies to evaluate the effectiveness of releases (Lebata *et al.*, 2009). It is important to consider the following factors when releasing mud crabs: 1) hatchery-

reared mud crabs should be conditioned in ponds prior to release to increase chances of surviving in the wild; and 2) bigger crabs have better chances of survival in the wild compared with smaller ones; in this case, crabs measuring 4.5 cm CW or bigger during release had the highest recapture rates.

**Giant clam, *Tridacna gigas*** – To restore the diminishing population of the giant clams *Tridacna gigas* in Sagay Marine Reserve (SMR), Negros Occidental, central Philippines, two size classes [8- and 10-cm shell length (SL)] of hatchery-bred *T. gigas* were reared in an adjacent ocean nursery for subsequent restocking to Carbin Reef upon reaching escape size of ≥20 cm SL. Average growth rates of 0.67 cm month$^{-1}$ did not significantly differ for both sizes. However, survival after 382 days of rearing *T. gigas* was significantly higher in the 10-cm SL clams than the 8-cm SL clams (96 and 83%, respectively). For future restocking projects, the use of 8-cm SL clams is recommended because the lower survival of this size class is compensated by its cheaper price. While rearing the clams to attain grow-out size, the population of wild clams (Family Tridacnidae) in Carbin Reef was assessed using ten 50 x 2-m belt transects. Four species of tridacnid clams have been recorded: *Hippopus hippopus*, *Tridacna crocea*, *T. maxima*, and *T. squamosa*. *T. crocea* comprised 12.5–93.9% of all the clams observed in all ten transects. There was a significant difference in clam density between species (ANOVA, F = 6.94, P<0.001), with *T. crocea* having the highest density. Living *T. gigas* were absent, but presence of dead shells was indicative of its presence in the reef in the past. It can be expected that the release of hatchery-bred *T. gigas* juveniles in Carbin Reef could provide future breeders that will repopulate this reef and the adjacent reef communities (Lebata-Ramos *et al.*, 2010). For the giant clam restocking activity, among the lessons learned were to: 1) first rear giant clams in ocean nurseries until escape size of 20 cm SL because they are less vulnerable to predators when they have attained this size; and 2) rear them in shallow reefs with 0.5-1.5 m deep water during low tide because better growth was observed in giant clams reared in shallow waters with warmer temperatures (mean±SE 29.5±0.24°C, range 26-31°C).

**Abalone, *Haliotis asinina*** – The lucrative returns brought by abalone fisheries caused overexploitation and decline of the wild population. In the Philippines, SEAFDEC/AQD has successfully produced *Haliotis asinina* seeds in the hatchery. Aside from utilizing these seeds in aquaculture, they are also being considered for future stock enhancement endeavors of the department. This study aimed to evaluate post release behavior, recapture and growth rates of hatchery-reared abalone juveniles released in the Sagay Marine Reserve. From the two release trials conducted, results showed that abalone of shell length >3.0 cm had lower mortality during onsite acclimation and utilized transport modules as temporary shelter for a shorter period after release. Both wild and hatchery-reared abalone preferred dead branching corals with encrusting algae as their habitat. Recapture rates were comparable between the wild (7.97%) and hatchery-reared (HR2) abalone (6.47%). Monthly growth rates were almost the same between wild (0.25 cm, 4.0 g) and hatchery-reared (HR1: 0.27 cm, 4.6 g; HR2: 0.35 cm, 3.8 g) abalone. Moreover, hatchery-reared abalones were recaptured up to 513 days post-release, indicating viability of released stocks in the wild. Results of releases revealed that hatchery-reared abalone can grow and survive with their wild conspecifics (Lebata-Ramos *et al.*, 2013). Through this study, it was noted that: 1) abalone should be released at a minimum size of 3 cm SL; 2) they should be transported from the hatchery in PVC transport modules; 3) they should be acclimated on site prior to release to eliminate mortalities caused by transport stress; and 4) transport modules should be placed on the release site, letting the abalone move freely out of the modules into their natural habitat.

In all releases, it is important to tag the released stocks to separate them from their wild conspecifics. Numbered dymotapes were used for giant clams, diet tagging  for the abalone, and coded microwire tags in mud crabs. In stock enhancement, it is also important to consider security of the release area. Releases should be done in more secured habitats such as marine protected areas rather than in open access areas where fishing is uncontrolled.

**Keywords:** stock enhancement program, seahorse, giant clam, abalone, sea cucumber

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# Responsible Shrimp Culture Through Ecological Approach

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***Abstract***

Aquaculture is the fastest food-producing sector. It is the farming of aquatic organisms, like crustaceans, fish, molluscs and plants. Culture of aquatic organisms, particularly shrimps, is usually done in earthen ponds with some intervention in the rearing process to enhance production. Some of these processes to increase production are pond preparation, regular stocking, feeding, and the use of probiotics and other chemicals to improve soil, water quality, shrimp growth and immunity against diseases. The long range effect of the use of probiotics and other chemicals on the environment and on shrimps is unknown. Despite the various inputs, diseases continue to plague the industry, which could be due to the deteriorating environmental conditions that cause stress in shrimps thus making them susceptible to infection. Furthermore, chemicals and nutrients from aquaculture may affect biodiversity of the receiving environment.

Responsible aquaculture is a sustainable development approach that meets the needs of the present generation without compromising the ability of future generations to meet their own needs. There should be a good balance between satisfying human needs while maintaining or enhancing the quality of the environment and conserving natural resources. Human health or food safety as well as economic efficiency and/or livelihood opportunities should be taken into consideration. Responsible shrimp culture through ecological approaches to improve environmental conditions is herewith described.

Ecological approaches recognize the interactions between an aquaculture farm and the external environment, including environmental resources and local communities. Ecological approaches to improve environmental conditions identified from cross sectional, longitudinal and tank studies may be classified into culture systems and phases of pond production: pond preparation and rearing. Two culture systems are identified to improve water quality: 1) the use of the greenwater system, and 2) the presence of mangrove in the receiving environment. Among the pond preparation practices, sludge removal, crack drying of pond, and liming were identified. Toxic substances as well as organic matter, which provide nutrients necessary for the growth of microorganisms, are removed during sludge removal and crack drying of the pond sediment. Liming to pH 11 kills most harmful microorganisms including the white spot syndrome virus; it also kills unwanted species in the shrimp pond like fish and crabs. During the rearing phase, abundant supply of natural food, low stocking density, less input, addition of fermented *Avicennia alba* leaves, use of molasses and rest periods are some of the important farming practices that reduce risk of disease occurrence. Other reported practices are crop rotation, biofloc technology, aquaponics, and integrated multi trophic aquaculture.

**Keywords:** shrimp culture, responsible aquaculture, ecological approaches

# Estimation of Energy Budget of Sea Cucumber, *Holothuria scabra*, in Integrated Multi-trophic Aquaculture

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## *Abstract*

Continuous intensification of aquaculture production has brought about environmental issues associated with eutrophication worldwide. Environmental deterioration such as hypoxia and sulfide production due to water and sediment eutrophication originating from aquaculture effluents have been problematic, resulting to sporadic disease outbreaks and fish kills in the Philippines.

Integrated multi-trophic aquaculture (IMTA) is one of the promising methods for sustainable aquaculture as it also provides a supplementary source of income to the fish farmers. IMTA is a polyculture system that integrates culturing of fed species (e.g. finfish) the main commodity, organic extractive species (e.g. deposit and filter feeding benthos) and inorganic extractive species (e.g. seaweed). In this study, IMTA techniques were established for small-scale coastal fish farmers in the Philippines, with sea cucumber (*Holothuria scabra*, commonly known as sandfish), as the key species. Sandfish commands the highest price in tropical sea cucumber species.

Nitrogen (N) budget of sandfish in polyculture with milkfish (*Chanos chanos*) and Elkhorn sea moss (*Kappaphycus alvarezii*), both of which are commonly cultured in the Philippines, was estimated using a simple closed box model.

Information on stocking density, stocking size, mortality, growth, feed ration, feed assimilation, $NH_4$-N production and $NH_4$-N absorption of these species was obtained from a series of experiments and existing literature. Culture conditions were as follows: 26 g milkfish were cultured in a 5 x 5 x 4 m cage at an average stocking density of 36.7 ind/m$^3$ (i.e. usual practice in the Philippines) with an initial feeding ration of 10% of body weight which was gradually decreased to 4% over time; 10 g sandfish were cultured in a 5 x 5 x 0.3 m cage hung under the milkfish cage to trap particulate N waste (i.e. feces and leftover feed) from milkfish culture at a stocking density of 35 ind/m$^2$; the stocking weight of Elkhorn sea moss line culture was 10 kg. The culture period was 200 days.

It was estimated that milkfish culture under the above-mentioned schemes cumulatively produced 145 kg of particulate N, and milkfish and sandfish together excreted 60 kg of $NH_4$-N in 200 days of culture. Daily assimilation rate of the particulate N by sandfish ranged from 3.4 to

12.4%, and 6.4% of the particulate N was estimated to be removed by sandfish during the entire 200 days of culture. Daily absorption rate of $NH_4$-N by Elkhorn sea moss increased exponentially with time and reached 100% at 125 days of culture. Cumulative $NH_4$-N from milkfish and sandfish excretion was estimated to be depleted by 162 days of culture.

For complete utilization of particulate N by sandfish by the end of milkfish culture period (i.e. zero emission), sandfish stocking density should be 805 ind/$m^2$, which is 200 times as high as that in existing sandfish aquaculture operations in countries such as Viet Nam and New Caledonia. The purpose of sandfish culture in IMTA should be emphasized in terms of its economic advantages and not very much on environmental integrity. Cages for sandfish culture should be designed in such a way where only a small fraction of organic matter from milkfish culture (i.e. about 6% in this culture scheme) enters it to avoid sediment quality deterioration and possible death of sandfish. Elkhorn sea moss on the other hand seems very efficient in bioremediation capability.

**Keywords:** IMTA, sandfish, milkfish, nitrogen, box model, excretion, eutrophication

# Post-larval Rearing Strategies in Sandfish (*Holothuria scabra*) Culture

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## *Abstract*

Various post-larval rearing methods were compared to determine which scheme would give the most yield of newly settled (visible) juvenile stage (> 1mm body length). Five types of post-larval rearing methods were tested: T1- planktonic diatom only (*Chaetoceros calcitrans, Cc*), T2-benthic diatom *Navicula (Nsp)* as biofilm and concentrate, T3- *Navicula* as biofilm + *Cc*, T4- *Spirulina* as paste on settling plate + *Cc*, and T5- *Spirulina (Sp)* as paste on settling plates + *Nsp* concentrate. An experiment was conducted in small (3-li) aquaria using a cohort of Day 14 (post-fertilization) sandfish larvae. Simultaneously, three of the 5 post-larval rearing methods (i.e. T2, T3 and T4) were done in medium scale (30-li) aquaria to determine how a conventional method (T2) employed in a pilot sea cucumber hatchery in Central Philippines compared with method observed in Viet Nam (T3) or with a hybrid method (T4). Visible post-settled juveniles were counted weekly for the next three weeks and expressed as percentage yield. After three days of rearing, transparent but visible early settled juveniles were observed. Mean percentage (%) juvenile yield in week 1 was highest in T1 (*Cc* only)(17% + 1.3) followed by T3 (*Sp* + *Cc*) (14% + 1.6) in a 3 li scale. Yield increased and peaked in week 2 especially for rearing methods with *Nsp* while those without (e.g T1 and T2) declined dramatically by week 3. In the 30-li scale, the highest mean yield was consistent with T5 (*Nsp* + *Cc*) until Week 3 (12% + 11.2). The mean juvenile yield on the 2$^{nd}$ and 3$^{rd}$ week were better than the 2% average for this stage or the 2.5% “benchmark” based on experiences in the Philippines and Viet Nam as indicated in published references.

**Keywords:** post-larval rearing methods, sea cucumber

# Induced Breeding of Giant Trevally, *Maliputo* (*Caranx ignobilis*)

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## *Abstract*

The giant trevally, *maliputo* (*Caranx ignobilis*), a highly prized and most popular indigenous migratory fish in Taal Lake, Batangas, Philippines, was induced to spawn using various hormones (to assess hormone efficacy on spawning performance). Different feeding regimes used in the larval rearing of this species were also evaluated. Sexually mature breeders, 5 to 7 years old with at least 0.5 mm oocyte diameter and 60% of ova at GVM stage were injected intramuscularly, in two doses, with: (a) 1,000 IU/kg BW human chorionic gonadotropin (HCG); (b) 100 µg/kg BW luteinizing hormone releasing hormone analogue (LHRHa); and (c) 5 mg/kg BW carp pituitary extract (CPE), at five breeders per hormone treatment. Uninjected fish served as the control. Treated fish were released and allowed to spawn spontaneously in 40-ton (5m diameter) circular tanks.

Successful spawning was achieved during the months of March to July (28-30 ppt salinity; 27.6-29.25°C). *Maliputo* eggs are pelagic, clear and spherical, with a single oil globule and mean diameter of 0.8 mm. Ovulation period was 24-36.5 hours after 2$^{nd}$ injection in HCG-treated fish and 25-52 hours for LHRHa-injected fish. Only one of the CPE-treated fish spawned after 27 hours but eggs were not fertilized. Uninjected control fish did not spawn. Eggs were hatched in 11-13 hours in HCG treatment and 11-17 hours in LHRHa. Mean number of spawned eggs (3,500-4,000 eggs•gram$^{-1}$) was higher in HCG treatment (223,068 eggs•kg$^{-1}$ breeder at 58.27g•kg$^{-1}$ breeder) than LHRHa (176,524 eggs•kg$^{-1}$ breeder at 50.44 g•kg$^{-1}$ breeder). Fertilization and hatching rates were both higher in LHRHa (60.88% and 71.07%, respectively), than HCG treatment (30.53% and 43.06%). Mean number of produced larvae was higher in LHRHa treatment (56,040 larvae•kg$^{-1}$ breeder) compared to HCG-treated fish (41,547 larvae•kg$^{-1}$ breeder).

Hatched larvae (1.6 mm mean length) reared for 30 days in 3m x 3m concrete tanks using the standard protocol for marine finfish hatchery attained a maximum survival of 4.47%. Complete metamorphosis was observed after 26-28 days (8.1 mm mean length). Successful larval rearing was attained using greenwater (*Nannochloropsis* sp.) technology fed with live food (*Brachionus* sp. and *Artemia salina*). Critical periods were days 1-7 and days 19-22 when heavy mortalities were observed.

Being the first recorded spawning in captivity of *Caranx ignobilis* in the Philippines, the results of this study provides an important baseline data and is a major step towards the development of a hatchery technology for *maliputo* in the country as well as for seed enhancement of its natural habitat. The project has provided 400,000 *maliputo* larvae to private hatcheries for larval rearing trials while 100,000 larvae were seeded in Balayan Bay and 5,000 fingerlings released in Taal Lake.

**Keywords:** induced breeding, giant trevally, *Caranx ignobilis*, Philippines, hatchery technology

## Seed Production of the Blue Swimming Crab (*Portunus pelagicus*)

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### *Abstract*

The blue swimming crab, *Portunus pelagicus,* is becoming a commercially important species in the Philippines. The expanding export market for crabs has led to intensified collection and has threatened the wild stocks. This decline has prompted the need for proper management of the remaining resources, and interest in the establishment of hatchery facilities to produce crablets for reseeding and aquaculture.

This paper presents the production method for *P. pelagicus* seedstock at the Guiuan Marine Fisheries Development Center. Experiments to improve larval rearing were conducted as well. For seed production, larvae at the zoeal stage were taken from wild-caught berried crabs hatched in 100-L circular drums filled with sand-filtered seawater (32-34 ppt, 28-30°C). Zoeae were stocked at 100 zoea $L^{-1}$ in rectangular tanks and fed rotifers at 30 ind $ml^{-1}$ for the first 4 days. Newly-hatched *Artemia* were given at 1-2 ind $ml^{-1}$ at zoea 3, and increased to 5 ind $ml^{-1}$ at zoea 4 to the megalopa stage. *Chlorella* sp. was maintained in the rearing tanks at 50,000 cells $ml^{-1}$ as food for rotifers and for water conditioning. Water exchange was done daily at 30-50%, except for the first 5 days of rearing. The development from zoea 1 to megalopa and megalopa to the first crab instar took 10-12 and 4-6 days, respectively. Longer larval development was observed at lower temperature (<26°C). Cannibalism and deteriorating water quality were identified as major causes of mortality. Survival of zoea 1 to megalopa was higher in chlorinated seawater (7.5+2.58%) compared to UV-treated (6.5+1.73%) and sand-filtered (4.0+2.58%) seawater. Trials involving the use of different tank background colors showed that the survival of larvae was highest in black tanks (9.0+1.00%) as compared to those white tanks (1.5+0.58%).

In nursery rearing, megalopae were stocked at 1-2 ind $ml^{-1}$ in wide tanks or concrete pond. Strategies to reduce cannibalism were done by providing shelters and sand substrate (>10 cm). As soon as the megalopa molted to crab instars, they were given minced fish, shell meat, *Acetes* and formulated crab feeds twice daily, *ad libitum*. After 21 days, crablets were collected manually after partially draining the water in concrete pond. The crablets produced were released in identified fish sanctuaries and marine protected areas region-wide for resource enhancement and for aquaculture research purposes.

**Keywords:** blue swimming crab, *Portunus pelagicus*, larval rearing, nursery

# Potential Genetic Impacts of Hatchery-Based Resource Enhancement

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## *Abstract*

The global population according to the United States Census Bureau has reached 7 billion as of October 2013. The continuous growth in human population will continue to put tremendous pressure on food production. The demand for fish as source of good protein is no exception. In 2011 total capture fisheries supplied 90.4 million tons of food and total aquaculture provided 63.6 million tons. While aquaculture production has increased dramatically, more than 50% of fishery production still depends on capture fisheries. Overexploitation of wild fish stocks has become one of the biggest problems in global fisheries. Stock enhancement has become a potential viable strategy for marine fisheries in danger of collapse. With the tremendous progress made in the breeding and larval rearing techniques of marine species, hatchery-based stock enhancement is now operated in many stock enhancement programs. However, many questions are raised in the use of hatchery-reared fish in stock enhancement. This paper will discuss genetic considerations in stock enhancement in developing countries.

**Keywords:** stock enhancement, hatchery-reared fish, genetic considerations

# Good Aquaculture Practices (GAqP): Setting Directions for Harmonized Regional Standards - The Philippine Experience

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## *Abstract*

A milestone process on how Good Aquaculture Practices (GAqP) emanates in Philippine aquaculture and its integration to the ASEAN harmonized standardization efforts is discussed in the paper. The management model, value chain and draft Philippine National Standard of the GAqP code are presented and evaluated as to its impact to trade and marketing, socioeconomic considerations, food safety and technology.

**Keywords:** Good Aquaculture Practices, Philippine National Standard

# The Importance of Mangroves to Capture and Culture Fisheries

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## *Abstract /Outline*

### *A. Mangrove background*

- area: 14 to 16 M ha in tropics/subtropics
- threats: conversion to settlements, agri/aquaculture, ports, etc.

### *B. Mangrove valuation*

- various goods and services, e.g., coastal protection, fisheries, etc.
- total valuation:
- fisheries valuation: relative (to other services) vs absolute (food security)
  % protein in diet of low-income groups

### *C. Mangrove-associated fisheries*

- by taxa → permanent residents: fish, mollusks, crustaceans, other invertebrates
  → transients (nursery function): crustaceans, fish
- by fisheries → artisanal/small-scale, including gleaners (= food security of coastal poor)
  → commercial
- by food group: mainly protein (vs carbohydrates, nutrients)

### *D. Nursery function of mangroves*

- availability of food
- shelter from predation
- complex physical structure (aerial roots, canopy shade, high turbidity, fine sediments)

### *E. Global food production*

terrestrial vs aquatic → wild → marine/b'water → mangroves
→ seagrass beds
→ coral reefs
→ deep-sea
wild → freshwater
aquatic → culture (depend on wild seed, fishmeal/oil)

### *F. Brackishwater Pond Aquaculture*

- ecological footprint
- 4:1 mangrove-pond ratio
- Mangrove-Friendly Aquaculture models

### *G. Mangrove Rehabilitation*

- seafront planting vs (abandoned) pond-mangrove reversion
- tenurial status of abandoned ponds
- FLA system

**Keywords:** mangrove, mangrove-associated fisheries, rehabilitation

# Stock Enhancement? Why Bother

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***Abstract***

There are two approaches to resource enhancement of depleted wild fish stocks: through stock enhancement where aquaculture science plays a central role, or through improved management of fish stocks. This paper presents an argument that despite major advances in stock enhancement technologies (tagging, genetic mapping, numerical modeling techniques), major hurdles in policy framework, science and information gaps, risk mitigation protocols and capacity gap remain. These factors are associated with high and recurring cost that requires medium to long-term solutions that ultimately, improving management and governance to recover depleted stocks will still be the best option available.

**Keywords:** resource enhancement, policy framework, management, governance

# ABSTRACTS OF POSTER PRESENTATIONS

## Resource Assessment of Sea Cucumber in Northern Iloilo, Central Philippines

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A resource assessment of sea cucumber was conducted in six out of eight coastal towns in northern Iloilo, a fisheries rich area facing the Visayan Sea in the central Philippines. A yearlong assessment was conducted in 2012. Fishery dependent survey was done with the use of survey questionnaire translated into dialect. Six trained enumerators administered the questionnaires to 114 gatherers and 18 local traders. Fishery independent survey involving Belt Transect Method (BTM) for intertidal areas and Timed-Search Method (TSM) for subtidal areas were conducted in 21 GPS (Global Positioning System)-referenced sampling stations. Sample specimens were also collected and prepared for taxonomic identification. External morphology, internal structures (dissected samples) and spicule analysis were used in the identification.

Fishery dependent survey showed that gleaning (40%) is the most dominant extraction method used. Various methods were also employed including the dangerous compressor diving and the destructive karas, a method using a rake-like device to scrape the sea bed. In terms of volume, the most heavily exploited sea cucumber belongs to the *Stichopus* groups. The trade of sea cucumber is dominated by island-based traders. Almost half of the traders are women, signifying that trading is a woman's domain as well. Derived monthly income from sea cucumber trade ranges from PhP 2,000–3,000 for gatherers and PhP 2,000–5,000 for the traders.

Fishery independent survey resulted in the identification of six sea cucumber genera (*Bohadschia, Holothuria, Paracaudina, Pseudocholochirus* and *Stichopus*). Of the 32 species found belonging to the six genera, only 16 were identified up to the species level. Samples of unidentified specimen were sent to the University of the Philippines – Marine Science Institute (UP MSI) laboratory for molecular taxonomic identification. In terms of species count, the most dominant genera is the *Holothuria* with nine identified and seven unidentified species. *H. impatiens* is also the most dominant sea cucumber found in the area. Further, the recorded catch per unit effort (CPUE) for fishery-independent survey is 3–4 pcs/diver/hr.

The resource assessment showed that the trade of sea cucumber is dictated by economic value rather than by ecological abundance. While the scale and extent of sea cucumber fishery in northern Iloilo is small-scale and island based, the study highlights the need for trade regulation and stock enhancement of heavily exploited species as extraction affects the ecological distribution of sea cucumber stocks in the area.

**Keywords:** sea cucumber, northern Iloilo, resource assessment, trade regulation

# Optimization of Feeding and Growth Conditions for Hatchery-bred Larvae of Indigenous Philippine Silver Perch, *Leiopotherapon plumbeus* (Perciformes: Terapontidae)

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The Philippine silver perch, locally known as *ayungin*, is an endemic fish species and is considered as a potential candidate for aquaculture and for stock enhancement. However, high mortality associated with early larval stages presents a significant bottleneck to its latent commercialization. Culture experiments considered interactions among prey proportions, growth conditions and their consequences on fish growth performance and survival. Two phases of the experiment were conducted: (1) a short duration feeding trial utilizing different prey proportions of *Brachionus calyciflorus* and *Moina macrocopa* and (2) an indoor larval rearing technique that ensured optimum growth and survival of juveniles. Findings of this research will be used to propose an efficient rearing strategy addressing the aquaculture of this indigenous species.

**Keywords:** Philippine silver perch, larval stages, rearing strategy, indigenous

## Development of a Simple, Rapid, Cost-effective Diagnostic Kit for WSSV

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Shrimp aquaculture is one of the most important sources of income and livelihood in the Philippines. For the past two decades, the white spot syndrome virus (WSSV) has adversely affected the production of the Philippine shrimp industry resulting to losses in revenue. Shrimps infected by the virus experience up to 100% mortality, 3 to 10 days post-infection. One way of controlling the disease is early detection, which remains to be too complicated and inaccessible to shrimp farmers. Being a DNA virus, the first step to WSSV diagnosis is the isolation of high-quality DNA suitable for polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP). Using readily available and affordable reagents, a DNA extraction protocol has been especially developed for rapid WSSV-detection; DNA has been successfully extracted from the pleopods of shrimps and the results were comparable with that of commercially available kits from Promega and Zymoresearch. LAMP has been optimized for WSSV detection in the temperature range of 55°C to 68°C and was shown to be faster and ten times more sensitive than conventional PCR. This study together with a locally fabricated machine, offers a more convenient, practical and efficient way of detecting WSSV, with the advantage of using non-invasive means of obtaining shrimp tissue therefore not losing any shrimp meat in the process.

**Keywords:** shrimp aquaculture, WSSV diagnosis, LAMP

# Larval Rearing of Silver Therapon (*Leiopotherapon plumbeus*) in Outdoor Tanks

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Silver therapon (*Leiopotherapon plumbeus*, Kner 1864), locally known as *ayungin*, is an important freshwater food fish species found in Laguna de Bay, the largest lake in the Philippines. Its market price is twice that of other most sought after freshwater fishes such as tilapia and milkfish. However, intense fishing pressure on the species has significantly reduced the wild stock in Laguna de Bay. Studies to develop hatchery techniques for this indigenous freshwater fish species are therefore needed to produce seedstock for possible culture and wild stock rehabilitation.

This study highlights the successful larval rearing of silver therapon in outdoor concrete tanks. Larvae reared in outdoor tanks with natural food (grown two weeks beforehand) reached the juvenile stage (40 days after hatching (DAH)), suggesting the presence of some suitable live food organisms in pre-conditioned rearing water. However, larval survival rates were low (11.58 ± 6.56% at stocking density of 0.9 larvae $l^{-1}$), which is probably linked to the density of food items, particularly during the onset of exogenous feeding or due to high stocking density of larvae. To improve the availability of natural food for the larvae, fertilization of the rearing water in the outdoor tanks stocked with larvae at two densities (0.4 and 0.6 larvae $l^{-1}$) was performed. Larval growth and survival were improved at stocking density of 0.4 larvae $l^{-1}$ than at 0.6 larvae $l^{-1}$. Diet composition of first-feeding silver therapon larvae in outdoor tanks inoculated with cultured microalgae (*Chorella sorokiniana*) and zooplankton was also determined. Larvae were able to consume rotifers and some phytoplankton beginning at 2 DAH and larger preys such as cladocerans and insect larvae starting at 12 DAH.

The efficacy of raising silver therapon larvae in outdoor tanks using ambient lake water was also evaluated. Larvae reared in ambient lake water grew well but survival (48.44 ± 7.85%) was significantly improved in treatments where tropical almond or talisay *Terminalia catappa* leaves were added during the first two weeks of larval rearing.

**Keywords:** silver therapon, larval rearing, outdoor tanks

# Preliminary Trials on the Effects of Weaning and Larval Diets on Survival and Growth of Silver Therapon (*Leiopotherapon plumbeus*) Larvae

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Success in larval rearing of silver therapon can be achieved through early weaning of fish larvae from live food to artificial diet. Two experiments were carried out to investigate the effects of (a) weaning age (abrupt and gradual) and (b) larval diets (artificial and live foods) on survival and growth of silver therapon (*Leiopotherapon plumbeus*). In the first experiment, larvae were randomly stocked in round 4-l plastic basins at 15 larvae per basin to provide triplicates of four weaning age treatments (8, 14, 20 and 26 days after hatching or DAH, respectively). Larvae were fed thrice daily for 21 days with commercial feed (CF) and with copepods (COP) which served as the control. Larvae weaned at 26 DAH had the highest survival, body weight and total length among the treatment groups, which were comparable with that of the control. In the co-feeding protocol, larvae were fed *Artemia nauplii* (ART) as the control group and co-fed with either zooplankton i.e. 50% COP + 50% CF or 50% ART + 50% CF for 8 (8-15 DAH), 6 (14-19 DAH) and 4 (20-23 DAH) days, and suddenly weaned to FM until 21 days. Survival ranged from 22.2 ± 16.8 to 40.0 ± 24.0% between treatments, but was still lower than the control (88.9 ± 3.8%). Body weight and total length were significantly higher in larvae with co-feeding for 4 days (70.1 ± 2.8 mg; 18.1 ± 0.8 mm), but were still lower than that of the control (142.8 ± 7.6 mg; 22.3 ± 0.3 mm).

In the second experiment, 26-day old larvae were stocked in 20-l glass aquaria at 4 larvae $l^{-1}$. Larval diets ((I) commercial prawn feed (38% crude protein); (II) *Artemia nauplii*; (III) copepods; and (IV) free-living nematode *Panagrellus redivivus*) were given twice daily for 28 days. Survival was highest in larvae fed *Artemia nauplii* and poor in copepod fed larvae. Final total length (TL) of larvae fed prawn diet was higher than those fed copepod or nematodes. However, best growth was noted in larvae fed *Artemia nauplii* (TL= 24.30 ± 0.81 mm; BW = 156 ± 8 mg; specific growth rate or SGR = 5.33 ± 0.19%/d).

**Keywords:** silver therapon, weaning age, larval diets

# A Preliminary Study on the Diagnosis of Coral Reef Healthiness and Establishment of Coral Replenishment Technology

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Field surveys for coral reef through line-intercept-transect (LIT) and temperature profiling using data-loggers were done at three layers of 5, 10, and 15 m depths in coral reef areas, Nogas Island, Anini-y, Antique, Philippines. Preliminary data based on the LIT survey showed that both coverages of substrates by any type of organism and by Scleractinia decreased in the deeper layers. For Scleractinia, *Porites* sp. occurred predominantly in all the depth layers with the occurrence decreasing with depth. Temperature fluctuation was largest in the 5 m depth layer, where effects of tidal level were also confirmed. While the average temperature decreased with depth, this did not differ beyond 1°C between 5 and 15 m layers during November 2012 to March 2013. Fragments of the *Porites* sp. and *Acropora* sp. were sampled and transferred to aquaria at the Tigbauan Main Station of SEAFDEC/AQD. *Acropora* sp. sampled from the deepest layer alone showed bleaching and thereafter, a part of the fragments regained the color. Experimental trials to clarify the effects of ocean acidification and warming on the health of the coral using the live fragments of *Porites* sp. showed decreasing trends in both photosynthetic rates and daily growth rates in acidic condition (pH = 7.6), while decrease of zooxanthellae density was observed under warmer conditions (31°C ) for one month. A new methodology for the determination of density of zooxanthellae was established using the fragments of *Porites* sp. In this study, the need for studies on several coral communities as well as further basic research on coral biology, particularly, responses to the changing environments are discussed for diagnosis of coral reef healthiness and establishment of effective coral replenishment technology.

**Keywords:** *Porites* sp., *Acropora* sp., coral reef healthiness, coral replenishment

# Preliminary Assessment of the Abundance and Fishery of Snapping Shrimp (*Alpheus* sp.) in Calape, Bohol, Philippines

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Snapping shrimp *Alpheus* sp. is abundant in Calape, Bohol, particularly in coastal areas with a wide intertidal zone, mangroves and rich mud banks. Locally known as "takla", it is considered as one of the major seafood delicacies in the municipality. An initial assessment of the natural population and fishery of the snapping shrimp was conducted. Using quadrat sampling, mean density was noted at 7 individuals $m^{-2}$, body weight ranging from 3.87-12.86 g and total length at 4.78-7.44 cm. The largest individual was identified as male having larger claws, the size being two times larger than that found in females. Apart from actual field sampling, a total of 80 shrimp gatherers were surveyed to obtain relevant fishery information for the snapping shrimp. The snapping shrimp is sold in the local public market and traded in hotels and seafood restaurants at PhP50.00 $bundle^{-1}$ (a bundle averaging 20 pieces) and PhP180.00 a kilo. The current average catch per gatherer is at 75 pieces on a daily basis which is relatively lower compared to the average catch in the 1980s and 1990s ranging from 150-300 pieces $day^{-1}$. Destruction of mangrove swamps due to fishpond conversion, unabated mangrove cutting for commercial firewood production and unrestricted gathering of snapping shrimps were identified as possible causes for the decline. These baseline data are essential for the local government and the community to come up with appropriate protection and conservation measures. An intensive and comprehensive study on the snapping shrimps ecology and biology also need to be conducted to provide basis for sound and holistic management of this valuable resource.

**Keywords:** snapping shrimp, Bohol, initial assessment, management

# Modelling the Impact of Different Stress Agents on Holothurian Immunity

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Due to dietetic and pharmacological values of many species of sea cucumbers (Holothuroidea, Echinodermata), and depletion of their natural resources, the species of little or no commercial value attract attention as new raw material resource, and methods of their aquaculture are developed. Both monitoring and supporting the health of animals in natural and artificial conditions demand the approaches providing reliable markers. This study compared the influence of two stress agents, namely lead and bacterial toxin *Yersinia pseudotuberculosis* (TcTYp), on some of the markers of immune response of the Far Eastern holothurian *Eupentacta fraudatrix*. Phagocytes (P2 type) were isolated after 48h treatment of holothurians *E. fraudatrix* with $Pb(NO_3)_2$ (2 and 4 mg/L). In another experiment, coelomocyte were incubated with TcTYp (0.2 and 0.5 μg/g) for 18h. Apoptosis level and FITC-conjugated concanavalin A (con A) and binding of lectins from *Glycin Max* and *Dolichos biflorus* to P2 surface receptors, and activity of antioxidant enzymes were measured.

Lead induced an increase in catalase and decreases in superoxide dismutase and glutathione-S-transferase and glutathione reductase activities at dose of 2 but not 4 mg/L. 2 mg/L lead also increased apoptosis level. Noteworthy, receptors to lectins from *G. Max* and *D. biflorus* were poorly expressed in the control, and significantly expressed under lead treatment at a dose of 2 but not 4 mg/L (*D. biflorus*) or decreased at a dose of 2 but not 4 mg/L (*G. Max*). Binding con A was significant in the control and additionally increased under treatment with 2, but not 4 mg/L. Meanwhile, TcTYp also induced reversed concentration-dependent effect on apoptosis: 48h incubation with 0.5 μg/g decreased apoptosis, and 0.2 μg/g-increased it. Additionally, 0.2 μg/g TcTYp decreased binding con A and *D. biflorus* lectin. Commercially available catalase restored % lectin binding to the control level.

Data obtained indicate that lead and TcTYp differently influenced phagocyte activity, and complex definition of apoptosis level and activity of antioxidant enzymes. Finally, variations in expression of cell surface receptors may be useful for estimation of the level of stress damage to holothurians.

**Keywords:** sea cucumber, stress agents, immune response

# Growth and Survival of Nile Tilapia (*Oreochromis niloticus*) Juveniles Fed Diets with Varying Levels of Irradiated Chitosan

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Chitin is a natural biopolymer and the second most abundant after cellulose. Chitosan, a derivative of chitin which is soluble in acidic aqueous media, is used in many applications like food, cosmetics, biomedical and pharmaceutical products. It is used in agriculture for enhancing growth in crops while in aquaculture, chitosan is believed to improve the immune response of fish to stress-inducing agents, thus enhancing survival and possibly growth. This preliminary study was conducted to investigate the effects of various concentrations of irradiated chitosan on the growth performance of Nile tilapia, *O. niloticus*. Fish was fed with a control diet and three formulated diets containing increasing levels of irradiated chitosan (10g, 20g and 50g $kg^{-1}$). Juvenile *O. niloticus* was fed once daily for 21 days. The ration was based on 5% of the fish biomass. Tilapia fingerlings (n=30 per tank) of uniform size were randomly distributed in four experimental groups each with three replicates following a completely randomized design. Growth and food utilization parameters were measured. Specific growth rate (SGR), mean weight gain (MWG), mean length increment (MLI) and feed conversion ratio (FCR) were computed and analyzed using ANCOVA. Results from the feeding trials showed no significant difference (P>0.05) in the different performance parameters under the different fish feed treatments. MWG, MLG, SGR and FCR varied in the stocks fed different fish feed treatments but with no significant differences. The results also showed 45-62% survival ratio. These suggest that although there is no significant difference between treatments and control, irradiated chitosan-supplemented diets do not retard the growth of *O. niloticus*. Chitosan should be studied further to determine how it can improve the growth performance, feed utilization and immune response of Nile tilapia.

**Keywords:** irradiated chitosan, Nile tilapia, growth performance

# Perceptions on the Effects of Maritime Activities on the Philippine Aquatic Ecosystem

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Throughout history, humans create an impact on marine ecosystems. May it be positive or negative, such impact is long-term and shapes the overall image of the system. As humanity increases its number, so does the impact it creates. Humanity has relied on the oceans for food, recreation and for various economic opportunities. Overfishing and pollution affects the life in the seas. Advancements in fishing technology, such as tracking technologies and better transportation have reduced fish stocks significantly when matched with non-sustainable practices, such as dredging and trawling. Intentional dumping from sewages, industrial run-off and chemicals has brought about pollution in the seas. Though some pollution may be accidental, like oil spills, it still causes adverse effects to the sea. Excess nutrients coming from fertilizers and intensive farming practices have resulted to what is known as eutrophications. Lastly, changes in the marine environment have led to the introduction of invasive alien species and marine organisms, which are oftentimes difficult to eradicate. Such effects have made researchers rethink of various ways to maintain marine activities while sustaining its ecosystem.

Through this study, the researchers determined the effects of maritime activities on the population of aquatic creatures directly from the seafarers, and found out their perspectives on how to remedy such effects and sustain the marine ecosystem.

This research is descriptive in nature, conducted with 100 purposively selected seafarers from Manila. The participants were given a questionnaire that asked for demographics and their perceptions on the effects of maritime activities on the marine ecosystem and ways to provide solutions to minimize or avoid its negative impacts.

Findings show equal distribution on gender, with majority at the low socioeconomic level (47%) and are Tagalog in ethnicity (56%). The seafarers believe that the maritime activities cause harm on aquatic creatures (43%), limits propagation of aquatic species (36%), makes them prone to mortality (11%) and cause pollution to the atmosphere (10%). Likewise, their perception of minimizing the consequences lies within the proper conditioning of ships and running them in good condition (57%), maintaining a clean place for the marine inhabitants (28%) and creating a good waste-renewal system (15%). The results of the paper is directed towards proper handling and maintenance of the shipping industry and strict supervision for waste management.

**Keywords:** marine ecosystems, maritime activities, waste management

# Preliminary Trials on the Optimization of Hormone Dosages for Induced Breeding of Philippine Silver Perch, *Leiopotherapon plumbeus*

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The silver perch *Leiopotherapon plumbeus*, locally known as ayungin, is an endemic freshwater fish that is commercially valuable as it commands a high price in the local market. Due to excessive fishing and other potential causes such as predation by invasive alien species, the local *L. plumbeus* stocks are observed to be depleting hence there is a need for an induced breeding protocol to propagate silver perch and conserve what remains of the resource. In this study, 30 females (total length or TL: 109.4 ± 12.2 mm; total body weight or TBW: 20.3 ± 6.1 g) and 60 males (TL: 97.1 ± 11.6 mm; TBW: 13.4 ± 5.5 g) were injected once intra-muscularly with different doses of hormones. Various dosages of human chorionic gonadotropin (HCG), luteinizing hormone releasing hormone analog (LHRHa) and salmon gonadotropin releasing hormone (sGnRH) were evaluated to identify the most effective dosage and hormone that resulted to high ovulation, fertilization and hatching rate. For the hormone sGnRH, 20, 30 and 40 µg/kg body weight (BW) and 1, 2 and 3 µg/kg BW for LHRHa were the dosages used in the experiment. The dosage used for HCG is 50 IU/g BW and served as the control. The findings of the experiment determined that the use of 20 µg/kg body weight of sGnRH resulted to high ovulation, fertilization and hatching rates. The result of the experiment would provide an efficient protocol for the local fishermen so they can produce, on demand, a large supply of this high quality fish species.

**Keywords:** silver perch, induced breeding protocol

# Distribution and Abundance of Hard Clam Shells *Meretrix meretrix* Along the Coastal Areas of Panguil Bay, Lanao del Norte, Philippines

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*Meretrix meretrix* is believed to be abundant in Panguil Bay and in the absence of relevant fishery statistics, it is useful to quantify their biomass with a view to determining their fishery potential. This study aims to monitor the gonadal development, identify the associated macrofauna and determine the distribution and abundance of hard clam shells *M. meretrix* along the coastline of four municipalities of Lanao del Norte at Panguil Bay. Four sampling sites were selected and established to achieve and relate some generalities using the transect-quadrat method. Clams were counted, measured and identified. Physico-chemical parameters were also noted every sampling. *M. meretrix* was found to be most abundant in Raw-an Pt. Baroy (28-542 pcs./$m^2$) followed by Mayao, Lala (0.3-26 pcs/$m^2$). *M. meretrix* at Aloha Tubod occurred in low densities (0.1-4 pcs/$m^2$). No hard clam shell was found in Taguitic, Kapatagan. Mean length differed significantly at the three locations. The coastal area of Mayao, Lala, had the highest diversity (H' = 4.236737) in terms of shell species identified and recorded during the twelve months sampling period. Most of the shells dissected were sexually immature with male shellfishes being more predominant than females. Differences in distribution, density and length size of hard clam shells were compared in this study at four locations. Anthropogenic causes e.g. exploitation as well as environmental parameters such as salinity levels and sediment quality are suggested to be the main cause of the variation. These results will be used as baseline information to properly manage hard clam shell resources in Panguil Bay.

**Keywords:** abundance, hard clam shell, Panguil Bay, distribution

# Growth Performance of Brackishwater Enhanced Selected Tilapia (BEST) Reared in Brackishwater Ponds

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Tilapia culture contributes greatly to world aquaculture production. Several tilapia strains have been developed locally and one of these has been developed for brackishwater aquaculture. BEST or the Brackishwater Enhanced Selected Tilapia was developed by the Bureau of Fisheries and Aquatic Resources with the aim of promoting brackishwater culture of this commodity to further improve tilapia production in the Philippines. This study will determine the growth performance of BEST reared in organically fertilized brackishwater ponds at three different stocking densities.

The study was conducted in nine (9) units of 200m$^2$ ponds. Pond preparation was done following standard procedures that include drying, liming, teaseed application and fertilization. Stocking was done after twenty days when primary productivity in the ponds was noted. Three culture systems were used, namely: extensive (Treatment 1 or T1), semi-intensive (Treatment 2 or T2) and intensive (Treatment 3 or T3) systems. The study was conducted for 120 days. Results showed that fish in ponds in treatment T1 had an average body weight (ABW) of 52.1g, T2 stocks with ABW of 223.1 g and T3 stocks with an ABW of 214.5 g. Meanwhile, T1 stocks had a survival rate of 91.9% while stocks under T2 and T3 had survival rates of 80 % and 84% respectively. T1 results showed losses amounting to P382 since total revenue of P958 is smaller than total cost of P1,340. T2 harvest on the other hand registered a total revenue of P 10,368 and total cost of P 4,375 hence net return of P 5,634. T3 also had total revenue of P 21,419 with a total cost of over P 8,498 giving a net return of P12, 471. T2 showed a return on investment (ROI) of 128% and a payback period of 0.77 year. T3 had an ROI of 139% and payback period of 0.71 year. Average feed conversion ratio (FCR) in two runs for two treatments are the same at 1.1 Water parameters like DO, salinity, temperature; water level and pH were also taken. Water level in the ponds ranged from 70 to 74 cm, salinity at 6.3-6.9 ppt, DO at 1.5-1.7ppm, temperature at a constant 29°C, monthly pH ranges were from 7.1 to 8.0 and transparency of 34-47 cm.

**Keywords:** Brackishwater Enhanced Selected Tilapia, brackishwater ponds, stocking densities

# First Record of Laem-Singh Virus in Black Tiger Shrimp (*Penaeus monodon*) in the Philippines

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Laem-Singh Virus (LSNV), a single-stranded RNA virus that causes growth retardation in *Penaeus monodon*, is also known as Monodon Slow-Growth Syndrome (MSGS) virus. Black Tiger shrimps afflicted with this virus exhibit unusual dark color, a weight gain of less than 0.1 g in 1 to 2 weeks, unusual yellow markings, "bamboo-shaped" abdominal markings and brittle antennae. It was first detected in Thailand and the virus quickly spread to neighboring Asian countries such as Malaysia and Singapore. The shrimp economy of countries where infections have occurred experienced losses in the export of live shrimps and broodstocks. An earlier study in 2009 reported that LSNV was not present in the Philippines. However, since no follow-up researches were done in the succeeding years, this study was conducted to detect the presence of virus in selected sites of Luzon. Results based on biased sampling method and RT-PCR data indicated that LSNV is indeed present in the country. This is further supported by DNA sequence data, showing 100% identity with LSNV India isolate. Phylogenetic analysis showed that the Philippine isolate clustered closely with other LSNV isolates. The outcome of this study might have implications in the current practices in the Philippine shrimp aquaculture industry

**Keywords:** Laem-Singh Virus, growth retardation, tiger shrimp

# Reproductive Biology of Christian Crabs (*Charybdis feriatus*, Linnaeus, 1758) in San Miguel Bay, Philippines

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The reproductive biology of *Charybdis feriatus* was investigated from April 2012 to March 2013 to determine gonad maturity, gonado-somatic index (GSI), fecundity, breeding cycle and size at first maturity. Every month, samples of 30 specimens were randomly collected. Gravimetric and volumetric methods were used to estimate fecundity. Results showed that *Charybdis feriatus* breeds continuously with a distinct period of reproductive activity during the northeast monsoon. Spawning peaks in January where higher values of GSI, mature, and berried female crabs were observed. Female gonad (ovary) weights range from 0.5 to 18.1 grams while mean GSI of female and male were 7.35% and 6.27%, respectively. Berried females were present year round, the highest occurring in December (50%). Fecundity ranged from 1,513,660 to 6,357,133 eggs. The smallest reproductively active female was 8.3 cm. Fecundity was highly correlated with size ($r^2$=0.92).

In view of these observations, the following options are recommended for crab fishery resource management: (1) to set a closed season for crab fisheries, (2) to regulate catches by not taking egg-bearing swimming crabs, and (3) to allow egg-bearing crabs to release its eggs and the larvae in fishing grounds and/or marine protected areas.

**Keywords:** reproductive biology, *Charybdis feriatus*, resource management

# Shrimp Metabolism: The Roles of Lactate Dehydrogenase (c31), Glycogen Phosphorylase (c34) and Protein Kinase (PK) as Revealed by RNA Interference

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Energy metabolism is well-studied in vertebrate systems, providing insights on the genes and mechanisms involved in different pathways necessary for the survival of an organism. Yet, such studies are still lacking in invertebrate systems much more in shrimp. An earlier study has showed several contigs from the black tiger shrimp to be homologous to white spot syndrome virus (WSSV), a devastating pathogen in shrimp, including contig 31-WSSVORF82 (c31) and contig 34-WSSVORF21 (c34). This study aims to unveil the roles of three genes: c31, c34 and protein kinase (PK) in the shrimp system and its possible role in WSSV-infection. Rapid amplification of cDNA ends-polymerase chain reaction or RACE-PCR was used to obtain the full-length sequence of c31 and c34, followed by *in vivo* gene silencing using RNAi technology, and intramuscularly injecting dsRNA to WSSV-challenged *Macrobrachium rosenbergii* and *Penaeus (Marsupenaeus) japonicus*. Gene expression followed for healthy shrimps and dsRNA-treated shrimps.

*Mrc*31 was revealed to be the enzyme lactase dehydrogenase (LDH), commonly released during tissue damage and is a marker for disease. The most parsimonious tree pictured *Mrc*31 to be sister clades to LDH of other shrimp species, *Penaeus monodon* and *P. vannamei*, supported with 100% and 72% bootstrap values, respectively. Mrc34 was highly homologous to the glycogen phosphorylase (GP) enzymes of other organisms including that of another shrimp, *M. japonicus*, bearing a bootstrap value of 99%. For PK, phylogenetic analysis revealed that the three open reading frames (ORFs) from *P. monodon, M. rosenbergii* and *P. japonicus* have 30% homology to WSSV-PK supported by a 98% bootstrap value. Mortality data from dsRNA-treated and WSSV-infected shrimps showed that treatment with dsRNA-LDH, GP and PK had significantly higher survival rates compared to that of the controls, Phosphate Buffered Saline (PBS) and Green Fluorescent Protein (GFP). Silencing the three genes in the shrimp has rendered some protective effect against the virus. Gene expression showed that all three genes are present in immune-related organs such as the gills, hepatopancreas and hemocyte. This study is the first to report the possible identities and functions of contigs 31, 34 and PK providing valuable data on the shrimp's genome.

**Keywords:** WSSV, contig 31, contig 34, protein kinase, gene expression

# SUMMARY OF THE WORKSHOP

## SUMMARY OF THE WORKSHOP

Workshops on the two focal topics: sustainable aquaculture and resource enhancement, were conducted on the final day of the meeting. Working groups were formed to identify issues and recommend strategies to address such challenges. Tabulated are summaries of the discussion outputs from each group:

### Aquaculture

| *Issue* | *Recommendation* |
|---|---|
| *Decreased price of aquaculture commodities/ increased cost of production* | Shift to high value species<br>Value adding to low-priced aquaculture commodities (e.g. adding omega-3 fatty acid)<br>Identify and prioritize top five high value species to focus on in the next 5 years<br>Identify value chain analysis for different species<br>Enhance quality control<br>Apply competitive enhancement for aquaculture products (i.e. quality of products from ASEAN for export should be competitive with products from other regions)<br>Immediately translate/package available technologies which are ready for application and commercialization<br>Use alternative feed ingredients (i.e. alternative to fish meal and highly digestible ingredients) to reduce cost of inputs<br>Apply efficient feeding management<br>Harmonize standards in line with ASEAN integration |
| *Accumulation of inbreeding in domesticated stocks; loss of genetic ability to adapt to climate change* | Collect relevant existing data; monitor pedigree of aquaculture stocks; bring in more geneticists |
| *Low technology/lack of technology* | Identify specific technologies that need to be addressed |
| *Lack of public support/established links between academe/research institutions/ private sector* | Increase public awareness on the importance of aquaculture |
| *Need for more community-based aquaculture; lack of extension program and capacity building programs for small scale fish farmers* | Empower small-scale/small holder farmers to enable them to apply GAqP |

| Issue | Recommendation |
|---|---|
| *Poor seed quality* | Apply genetic traceability of aquaculture stocks and verifiable certification of seed stocks for aquaculture<br><br>Use molecular markers for genetic tracing and certification of seeds<br><br>Adopt a system in providing certification for genetic tracing and for stock certification<br><br>Establish a system for genetic tracing and data bank for information on various stocks<br><br>Global gene bank for valuable species (international collaboration)<br><br>Regulatory policies to address inbreeding<br><br>Provide verifiable information to allow people the option to choose which stocks to use<br><br>ISO certification to address poor seed quality |
| *Proliferation of invasive species in inland waters; introduction of exotic species in relation to disease transfer* | Come up with concrete policies/guidelines/regulations on farming of exotics; strictly enforce/implement existing policies; also strictly report escapees from farms |
| *Aquaculture competes with from other alternative uses of land and water resources; e.g. conversion of areas for aquaculture converted to other uses* | Apply zoning for marine aquaculture areas |
| *Aquaculture waste management* | Apply polyculture systems |
| *Dependence on the use of antibiotics* | Develop appropriate vaccines; develop disease resistant strains<br><br>Discontinue use of prohibited antibiotics.<br><br>Strictly implement GAqP<br><br>Use immunostimulants and natural antimicrobials; use probiotics |

## Stock Enhancement

| *Issue* | *Recommendation* |
|---|---|
| *No clear understanding of resource enhancement* | Create core group to establish a common definition/ description of resource enhancement; review the existing guidelines; establish protocols |
| *Lack or limited technical knowledge of local government units (LGUs); lack of consultations with stakeholders or academe* | Enhance information, education and communication in local government units through the League of Municipalities in the Philippines<br><br>Identify all groups doing similar work (resource enhancement) and seek assistance from them (NGOs, University, Research Institution, National Agency) |
| *No comprehensive planning and project design; project implementation is too fast* | Conduct community consultation before implementation<br><br>Provide emphasis in community/ participatory research<br><br>Careful step by step process of implementation (not skipping crucial steps) |
| *Project duration is not enough; donor-driven deadlines; unclear exit strategy of projects* | Project transition must be smooth; implementation must be continuous and evolving; Longer project needed? (e.g. Indonesia coral project with 3 phases, 15 years total) |
| *Political term-dependent projects* | Enhance coordination with established institutions (NGOs, stakeholders, academe, etc.)<br><br>Involve other government agencies (BFAR, Coast Guard, DENR, etc.)<br><br>Include project in Municipal Economic Development Plan |
| *Incomplete baseline assessment; lack of monitoring mechanisms* | At least one year of baseline information gathering before any intervention; scientific information and indigenous knowledge (experience); social preparation<br><br>More collaboration with agencies in collection of information |
| *Lack of impact assessment* | Conduct a follow-up on the same sources of information (from baseline: scientific and traditional) |
| *Data collection problem* | Empower local community to gather reliable information; wiki-type information entry; logbook entry |

| Issue | Recommendation |
|---|---|
| *Stock enhancement being done as political "stunt act" rather than "science-based" fishery management option* | Advanced information to LGU/agencies about benefits of projects before implementation |
| *Location of projects: use of MPA as release site* | Careful selection: carrying capacity evaluation: complete baseline information |
| *Alternative livelihood and difficulty of coastal fishers to adapt* | No sudden change from existing norm (i.e. type of livelihood) |
| *Lack of sustainable supply of seed* | Release bigger juveniles instead of larvae; establish seed production technology |
| *Seeds (particularly the hatchery-bred stocks and their potential genetic impacts when released in the wild)* | Use of native/endemic wild broodstocks compared with bred broodstock |
| *Seeds (as potential carrier of disease causing agents)* | Screening tests; use disease-free stocks; quarantine; vaccination; regular monitoring |

# ANNEXES

# Annex 1. WORKSHOP COMMITTEES

***Organizing Committee***

| | |
|---|---|
| Chairperson | : *Dr. Teruo Azuma, GOJ-TF Co-Manager* |
| Vice-Chairperson | : *Dr. Ma. Junemie Hazel Lebata-Ramos, RD Head* |

***Scientific Program Sub-committee***

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***Secretariat***

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***Information, Documentation, and Publication Sub-committee***

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| Chairperson | : *Dr. Eleonor Tendencia* |
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***Administration and Finance Sub-committee***

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| Vice-Chairperson | : *Ms. Jiji Rillo* |

***Food, Accommodation, and Travel Sub-committee***

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***Physical Arrangement, Security, Tour and Transport Sub-committee***

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