

*Improving lives through sustainable aquaculture*



# 2013 SEAFDEC/AQD Highlights



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**COMMODITIES** under R&D at SEAFDEC/AQD in the past 40 years. Artist renditions and photos are from: nautic-seafood.com [SHRIMP]; fishbase.sinica.edu.tw [MILKFISH]; daff.qld.gov.au [SEABASS]; harvestselect.com [OYSTER]; daff.qld.gov.au [POMPANO]; wasanbeta.net [CATFISH]; mitofish.iori.u-tokyo.ac.jp [SNAPPER]; changshengyuye.com [PRAWN]; J Zarate [ABALONE]; lurebook.com [RABBITFISH]; reef.crc.or.g.au [MUDCRAB]; fishbase.us [GROUPER]; sustainablesushi.net [MUSSEL]; JM dela Cruz [SEAWEEDES]; bioweb.uwlaw.edu [CARP]; worldfishingnetwork.com [TILAPIA]

# AQD's 40 years of aquaculture research and development



**Felix G. Ayson, D.Sci.**  
Chief, SEAFDEC/AQD

From its humble beginnings in 1973, SEAFDEC/AQD is now 40 years old. Here we report in brief the significant accomplishments of AQD since its establishment. People who are aware of AQD's activities say that we in AQD are humble in telling the world of our accomplishments. Since 2013 is the ruby anniversary of AQD, allow me to describe to you in brief AQD's contribution to the development of world aquaculture.

## Significant accomplishments

AQD made its first impact when its scientists completed the life cycle of tiger shrimp (*Penaeus monodon* in captivity) in 1975, and developed the induced breeding and hatchery fry production in 1977. Trainees from around the world came to AQD then to learn these techniques. An offshoot of these developments was the proliferation of small-scale or "backyard" shrimp hatcheries all over the country and in the region. Subsequent developments in culture technologies and shrimp nutrition that led to the development of formulated feeds ushered the rapid development of the shrimp aquaculture industry. It was so lucrative that some sugar cane lands in the country were converted into shrimp ponds.

Shortly thereafter, another scientific breakthrough by AQD scientists came in 1980 when successful spontaneous spawning of milkfish ('sabalo'), *Chanos chanos*, was achieved. Attempts, however, for induced spawning and larval rearing of milkfish were underway in 1976 and 1978, respectively. The first training course in AQD on milkfish hatchery fry production was done in 1984 in support of the government's National Bangus Breeding Program. Whereas in the past, the fishpond operators got their milkfish fry supply from the coastal waters, now the fry supply comes mostly from milkfish hatcheries. While refinements in milkfish fry production and farming systems technologies were being done, AQD's team of fish nutritionists determined the nutrient requirements of milkfish – their findings served as basis for the formulation of feeds that are now commercially available. With the availability of milkfish

fry and feeds, farmers now grow milkfish in pens in freshwater bodies and in cages in the coastal waters, in addition to the traditional milkfish culture in brackishwater ponds.

AQD is likewise largely responsible for the development of aquaculture technologies and hence the promotion of aquaculture of other commercially important aquaculture species. Induced spawning of rabbitfish (*Siganus guttatus*) was achieved in 1983 and year-round spawning and mass fry production in 1985. Successful induced spawning and larval rearing of Asian sea bass (*Lates calcarifer*), groupers (*Epinephelus* spp. and mangrove red snapper (*Lutjanus argentimaculatus*) were achieved in 1983, 1990 and 1992, respectively. From then on, these species became mainstays in AQD's training course on marine fish hatchery operations and management.

After the successful mass production of abalone (*Haliotis asinina*) in 1994 and the completion of the life cycle of mud crab (*Scylla serrata*) in 1997, AQD has been conducting training courses on the fry production and culture of abalone and mud crab for nearly two decades now. For this year, mud crab is the hottest commodity with numerous requests for training on the various phases of culture as well as for technical assistance from the private sector both here and abroad.

For freshwater aquaculture, AQD has contributed significantly to the full development of the culture technologies for the tilapias, catfishes including our native catfish (*Clarias macrocephalus*), carps and the giant freshwater prawn. AQD's contributions to these species are on the breeding technologies, fry production, nursery and grow-out culture technologies, and support technologies on feed development and health management. AQD's mark is also very much visible in the development of the seaweeds, oysters, mussel and kapis shell industries.

## Technology extension

These technologies will contribute to economic development only when they are adopted by the end users. Even with our limited manpower and financial resources, AQD will continue to strengthen its

technology information dissemination programs and extension activities. Scientific findings are first published in international peer-reviewed journals so that results can be verified by the experts. AQD has so far published 1,414 scientific papers in various international scientific journals. The technical information from these scientific publications are then transformed into "laymanized", easy-to-understand extension manuals for the farmers. To this day, 55 manuals have already been published.

The best way to transfer the technologies to the farmers is through the conduct of actual hands-on training courses which we do in all our stations as well as on-site. We now have more than 10,000 training alumni from 64 countries who attended the 413 training courses we conducted to date. The United Nations (UN) listed 192 member countries; we have trainees from roughly 30% of the UN member states, clearly indicating AQD's contribution to global aquaculture development. We also train the future scientists, aquaculturists, and fish farmers. In our On-the-Job Training and Internship Programs, we have so far trained 2,219 students from 78 universities and colleges, and 12 secondary schools in the country.

To accelerate the technology transfer process, we collaborated with agencies that are on the ground and have extensive reach. In the Philippines, we worked closely with DA-BFAR, local government units, the academic community and people's organizations. The Department of Fisheries from SEAFDEC member countries are our partners as well.

## Collaborative R & D

To augment our resources to implement various programs and initiatives, aggressive fund sourcing activities are done. Currently, we have on-going collaborative projects funded by the Australian Center for International Agricultural Research (ACIAR), Food and Agriculture Organization of the UN (FAO), and various Japanese organizations like the Japan International Research Center for Agricultural Sciences (JIRCAS), Research Institute for Humanity and Nature (RIHN), Ministry of Education, and the Fisheries Research

Agency (FRA) that fund the regional programs of AQD thru the Thrust Fund Projects. Projects funded by USAID and the United Soybean Board of the US have just ended but another USDA-funded development project will be implemented by AQD in the next three years. In collaboration with the state universities in the country and with funding from the Philippines' DOST-PCAARRD, AQD is implementing national science & technology programs on tiger shrimp, milkfish, mud crab, abalone, blue swimming crab and oysters. In all these projects, we make sure that intended project outputs are realized and the impact felt by the people.

### Future challenges and directions

Food security and economic prosperity of the people are among the top priorities of the governments of all SEAFDEC-member countries. Considering the push to increase fish production to feed our fast-growing population, intensification of aquaculture operations is inevitable. The challenge for us now and for AQD in particular, is how to make intensification of aquaculture sustainable and without harmful effects to the environment. Issues that need to be addressed in relation to this general challenge, among others, are:

- (1) bringing down the costs of production in aquaculture operations
- (2) finding appropriate alternatives to fish meal and fish oil in fish feeds to bring down the costs of feeds without affecting the performance of the stocks
- (3) ensuring high feed quality

- (4) pro-active and appropriate health management protocols, especially for the emerging disease problems
- (5) maintaining and re-emerging the over-all health of the aquatic environment where aquaculture activities are being conducted
- (6) ensuring that aquaculture to continue given the changing world climate

These concerns are all addressed in the five thematic areas that describe AQD's program thrusts as approved by ASEAN-SEAFDEC in 2011. Our programs now include:

- (1) quality seed for sustainable aquaculture (where we consider all approaches to produce good quality fry)
- (2) healthy and wholesome aquaculture (that includes aspects of aquatic animal health and nutrition)
- (3) maintaining environmental integrity through responsible aquaculture (where we consider the possible impacts of aquaculture to the environment and ways to mitigate these, to this effect, AQD has been espousing the environment-friendly way of aquaculture, and we have been pushing for the implementation of CCRF/CCRA or code of conduct for responsible fisheries and aquaculture in the region)
- (4) meeting the social and economic challenges in aquaculture (where we consider the social and economic impacts of aquaculture)
- (5) adapting to climate change (where we explore alternatives to prepare the aquaculture sector for possible impacts of changing world climate).

The scientific information that will be generated from our present and future activities will be very critical in this regard.

Considering all the above challenges, we will continue to implement aggressive but strategic fund-sourcing activities to implement the various programs. We will continue activities that accelerate the dissemination of viable AQD technologies. We will continue to work closely with concerned agencies of SEAFDEC member countries, and in the host country with DA-BFAR, DA-BAR, DOST, LGUs, SUCs, NGOs and POs. We will forge new collaborations and strengthen our existing collaborations with all our partners in the country and the region.

Simply, AQD will continue to pursue its mandates which are to:

- (1) promote and undertake research on aquaculture relevant and appropriate to the region
- (2) develop human resource capability in aquaculture through training and extension
- (3) disseminate and exchange these information to all stakeholders

With the support from our national government, the SEAFDEC organization as a whole, our various partners and collaborators, and the whole AQD family, I am confident that AQD will continue to fulfill its role as a significant source and provider of scientific information for further development of aquaculture in the region.

### Snapshots: ruby jubilee 2013





AQD signs 24 new agreements with stakeholders such as [L-R] the Intellectual Property Office of the Philippines, University of the Philippines Visayas, and the local government of Tagum City in southern Philippines

## 2013 agreements with stakeholders

### ACADEME

- ( 1) **Queensland University of Technology - Science & Engineering Faculty** (Brisbane, Queensland, Australia): Research collaboration and exchange of experts (2 February 2013 – 1 February 2018)
- ( 2) **University of Tokyo – Laboratory of Global Fisheries Science and Tokai University – School of Marine Science & Technology** (Japan); **University of the Philippines Visayas** (Iloilo, Philippines); and **Aklan State University** (Aklan, Phil.): Research collaboration on coastal area capability enhancement in Southeast Asia (12 July 2013 – 31 March 2017)
- ( 3) **Sophia University – Faculty of Science & Technology** (Tokyo, Japan): Conduct of a short term experiment on the reproductive physiology of seahorse at AQD (25 June 2013 – 5 July 2013)
- ( 4) **University of the Philippines Diliman** (Quezon City): Research on the development of molecular markers for milkfish broodstock management (1 June 2012 – 31 December 2015)
- ( 5) **University of the Philippines Visayas** (Miagao, Iloilo): Collaboration on instruction, research, training & extension programs (28 January 2013 – 27 January 2018) and implementation of MS thesis on seaweeds (7 November 2013 – 6 November 2014)
- ( 6) **Southern Luzon State University** (Lucban, Quezon, Philippines): On-the-job training (15 April 2013 – 15 May 2013)
- ( 7) **University of Rizal System – School of Fishery** (Cardona, Rizal, Phil.): Aquaculture research, technology & development program (8 February 2013 – 7 February 2018)
- ( 8) **Cavite State University** (Indang, Cavite, Phil.): Technical assistance and training on mudcrab hatchery and nursery operations (14 October 2013 – 13 October 2014)
- ( 9) **Aklan State University** (Banga, Aklan, Philippines): Technical assistance and training on mudcrab hatchery & nursery operations (7 October 2013 – 6 October 2013)
- (10) **Zamboanga State College of Marine Science & Technology** (Zamboanga City, Phil.): Technical assistance on mudcrab hatchery & nursery operations (23 August 2013 – 22 August 2014)
- (11) **Davao del Norte State College** (Panabo City, Phil.): Technical assistance and training on mudcrab hatchery & nursery operations (30 September 2013 – 29 September 2014)

### GOVERNMENT AND NON-GOVERNMENT ORGANIZATIONS

- (12) **Intellectual Property Office of the Philippines** (Taguig City): Establishment of an innovation and technology support office at AQD (7 March 2013 – 31 December 2015)

### A move to patent, trademark and copyright some AQD technologies

“With 40 years of research-and-development under its belt, AQD has developed a lot of aquaculture technologies,” noted AQD Chief Dr. Felix Ayson. “Given its perennial funding problems, the logical next step for AQD is to determine which technologies are patentable and which have not been disseminated as prior art. We can be ambitious and perhaps foresee a future where R&D can be independently funded from AQD’s intellectual property, from patents to trademarks and copyright.”

With this in mind, AQD signed up with the Intellectual Property Office of the Philippines (IPOPHL) on 7 March 2013 to join its network of ITSOs (innovation & technology support offices). The network is a pilot project created by IPOPHL, the country’s patent-granting government entity, in partnership with the World Intellectual Property Organization. The network was organized to provide a ready pool of science and technical experts with competence to conduct patent search and patent drafting as well as to assist in patent prosecution.

IPOPHL believes that future innovators will come from universities and R&D organizations like AQD which can concretely benefit from establishing ownership of its intellectual property.

AQD sent five of its staff who comprised its ITSO to IPOPHL’s lectures and practicals that were conducted in various venues from March to November for a total of 13 days. An examination administered by IPOPHL in 2014 will qualify AQD staff as registered patent agents.

AQD staff in training as patent agents



- (13) **Philippine Council for Agriculture, Aquatic & Natural Resources Research & Development – Department of Science and Technology** (Los Baños, Laguna): Implementation of national R&D programs for blue swimming crabs (hatchery & nursery component) and donkey's ear abalone (2 November 2013 – 1 November 2016)
- (14) **National Research Council of the Philippines** (Taguig City, Metro Manila): A study on bacterial microbiota of tilapia as a tool for investigating emerging and re-emerging diseases (16 March 2013 – 15 March 2015)
- (15) **Bureau of Fisheries and Aquatic Resources – Central Office** (Quezon City, Phil.): Training course on fish health management in aquaculture (2 October 2013 – 15 December 2013); and with BFAR Regional Office No. IX (Zamboanga City) on the use of BFAR's ponds to test the feasibility of aquasilviculture of tiger shrimp (8 February 2013 – 7 February 2014)
- (16) **Local government of Tagum City** (Phil.): Technical assistance for mud crab hatchery & nursery operations (beginning 23 September 2013)
- (17) **Municipality of Concepcion and Brgy. Polopiña of Concepcion, Iloilo; Municipal Fisheries and Aquatic Resources Management Council; Sitio Lo-ok Small Fisherfolk Association** (Iloilo, Phil.):

Community –based sea ranching of sandfish (5 November 2013 – 4 November 2017)

- (18) **Zoological Society of London – Community Based Mangrove Rehabilitation Project in the Philippines** (Iloilo City): Organize and conduct training courses on mangrove & coastal resources management (16 June 2013 – 21 July 2013); and mangrove conservation, management & rehabilitation (7 November 2013 – 21 December 2013)

#### PRIVATE SECTOR

- (19) **San Jose Agro Marine Corp.** (Mr. Mario Santos Jr. of Lucena City, Phil.): Technical assistance on mudcrab hatchery and nursery operations (12 March 2013 – 11 March 2014)
- (20) **Samance Food Products** (Engr. Deo Grajo of Iloilo City, Phil.): Rental of cages at Igang Marine Station (14 May 2013 – 13 May 2014)
- (21) **Lutao Prawn Hatchery** (Mr. Dioscoro dela Pena of Panaon, Misamis Occidental, Phil.): Technical assistance on mudcrab hatchery and nursery operations (31 August 2013 – 12 August 2014)
- (22) **CDO Foodsphere Inc.** (Mr. Jose Ong of Zambales, Phil.): Mudcrab hatchery and nursery operations (11 November 2013 – 10 November 2014)
- (23) **Mr. Carmelo Celis** (Dingle, Iloilo, Phil.): Use of impounding facility located in Lake Vito in Brgy. Camambugan for tilapia / seabass culture (01 January 2013 – 31 June 2013)
- (24) **Mr. Juan Nepomuceno III** (Pasig City, Phil.): ABOT AquaNegosyo program on milkfish / shrimp culture (5 February 2013 – 4 February 2014)



## Honor to AQD!

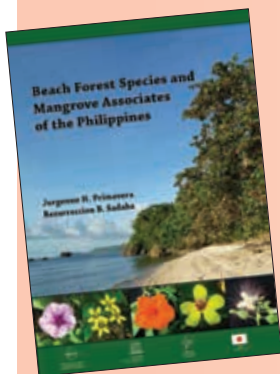
### INDIA

Scientist and AQD's head of technology verification & demonstration Dr. Emilia Quintio [LEFT, HOLDING PLAQUE] was given a citation by the Rajiv Gandhi Centre for Aquaculture (RGCA) for her contribution to the mud crab industry in India. The Tamil Nadu-based RGCA is the R&D arm of the Marine Products Export Development Authority. Dr. Quintio has been providing technical assistance to RGCA since 2004. She disseminated AQD's technologies in mud crab hatchery, nursery and grow-out by conducting training and managing field work. She helped in designing RGCA's first mud crab hatchery which was inaugurated during the 2013 ISMAF (*International seminar-workshop on mud crab aquaculture and fisheries management*) held 10-12 April 2013.

### PHILIPPINES

Scientist emerita Dr. Jurgenne Primavera bagged the *Outstanding book award* given by the National Academy of Science & Technology for the book *Beach forests and mangrove associates in the Philippines* that she co-authored with UP Visayas professor Dr. Resurreccion Sadaba. The award was given on 11 July 2013 in Manila. The book was published by AQD in 2012 with funding from UNESCO Jakarta through its Man & the Biosphere Programme and the Japanese Trust Fund.

AQD librarian Mr. Stephen Alayon [BELOW, CENTER] was given the 2013 ASLP (*Association of Special Libraries of the Philippines*) *Citation Award for Research Excellence* for helping make information and publications readily and publicly available through OPAC (online public access catalog) and SAIR (SEAFDEC/AQD institutional repository). Stakeholders can also access AQD's library collection through Web 2.0, a software allowing users to interact in a social media dialogue.



# Research and development programs in 2013

Study title	Proponent/s	Collaborating partner/s*	
<b>THEMATIC PROGRAMS</b>			
<b>Producing quality seed for sustainable aquaculture (QS)</b>			
1	Selective breeding of the Indian white shrimp <i>P. indicus</i> : I. Disease resistance in juveniles produced by captive spawner	FDP Estepa	
2	Genetics for sustainable milkfish aquaculture. I. Development and application of molecular markers in broodstock management	MR Eguia	DOST-PCAARRD <sup>1</sup> / UP <sup>2</sup> Institute of Biology
3	<i>National mud crab science &amp; technology program</i> Refinement of mudcrab hatchery technology: Improvement of larval rearing protocol	ET Qunitio	DOST-PCAARRD
4	Refinement of sandfish hatchery and nursery techniques	JP Altamirano	ACIAR <sup>3</sup>
5	Development of techniques for sustainable production of good quality captive <i>Penaeus monodon</i> broodstock and spawners and high-health fry	FDP Estepa	DOST-PCAARRD / UPV <sup>4</sup>
6	Development of molecular genetic markers for Philippine red seaweeds	MMD Peñaranda	
7	Refinement of abalone culture techniques to enhance growth, meat quality and maturation: I. Experimental hybridization of Philippine native species; <i>Haliotis asinina</i> , <i>H. glabra</i> , <i>H. ovina</i> , <i>H. varia</i> , <i>H. planata</i> II. Triploid induction in <i>Haliotis asinina</i>	MR de la Peña	
8	Improved larval rearing protocol for silver therapon ( <i>Leiopotherapon plumbeus</i> ) in outdoor tanks	FA Aya	
9	Feeding biology of the early life stages of indigenous freshwater fishes in Luzon	LMaB Garcia	UP NSRI <sup>5</sup>
10	Use of tryptophan supplementation to reduce cannibalism in fish larvae	JM Zarate	
11	Application of strategies to reduce cannibalism in the mud crab nursery	FDP Estepa	DOST-PCAARRD
12	Use of thraustochytrid <i>Schizochytrium</i> sp. LEY7 as live food enrichment for fish larvae and component in abalone formulated diets: I. Mass production of thraustochytrid in optimum culture conditions yielding highest lipid and fatty acid contents II. Utilization of thraustochytrid as live food enrichment for larviculture of tiger grouper and as component in formulated diets for aquaculture species	MR de la Peña / G Ludevese	
13	Refinement of hatchery techniques for the donkey's ear abalone <i>Haliotis asinina</i> : I. Improvement of fecundity and seed quality of breeders recently acquired from the wild II. Evaluation of genetic stocks for selective breeding	MR de la Peña	
14	Use of <i>Cocconeis</i> sp. <i>Nitzschia</i> sp. and <i>Diploneis</i> sp. as alternative benthic diatom feed species in the hatchery rearing of abalone <i>Haliotis asinina</i>	MR de la Peña	
15	Refinement of seed production techniques for high value marine fish species such as grouper, red snapper, seabass, rabbit fish and pompano: Metamorphosis and survival of high-value marine fish larvae fed sodium iodide-enriched rotifer and <i>Artemia</i>	OS Reyes	
16	Development of maturation diet for grouper ( <i>Epinephelus fuscoguttatus</i> ) broodstock: Use of paprika as natural pigment in grouper maturation diet and its effect on quality of eggs and larvae	OS Reyes	
17	Development and evaluation of maturation diet for tropical donkey's ear abalone, <i>Haliotis asinina</i> Linne	MB Teruel	DOST-PCAARRD
18	Development of techniques for sustainable production of marine annelids as feed for mud crab broodstock	VR Alava	DOST-PCAARRD
19	Refinement of the nursery cage culture technology for the sea bass <i>Lates calcarifer</i> in brackishwater pond: Determination of the optimum stocking density, feeding frequency and the effect of in-pond sorters and shelters on juvenile production	JM Ladja	
20	Refinement of mass production techniques for copepods <i>Acartia tsuensis</i> and <i>Pseudodiaptomus annandalei</i> for intensive marine fish larviculture	HS Marcial	

	Study title	Proponent	Collaborating partner/s*
21	Larval rearing of grouper ( <i>Epinephelus fuscoguttatus</i> ) using Kiko technology	OS Reyes	
22	Effect of tryptophan-supplemented diets, stocking density and feeding management on survival and growth of mud crab <i>Scylla serrata</i> in the nursery phase	VR Alava	DOST-PCAARRD
23	Microparticulate diet as alternative feed in abalone hatchery production: Comparative evaluation in small- and large-scale tank systems	MB Teruel	
24	Verification of feeding and water management techniques for mudcrab <i>Scylla serrata</i> seed production	ET Quinitio	
25	Nursery rearing techniques for seed production of <i>Kappaphycus</i> "seedlings"	MRJ Luhan	
26	Development of ice-ice resistant strains of <i>Kappaphycus</i> and technology for reduction of epiphytes	MRJ Luhan	ACIAR
27	Mass production of sex-reversed and mixed Nile tilapia ( <i>Oreochromis niloticus</i> ), and hybrid red tilapia fingerlings	DM Reyes Jr.	
28	Large-scale production of donkey's ear abalone, <i>Haliotis asinina</i> juveniles	NC Bayona	
29	Production of abalone <i>Haliotis asinina</i> juveniles at the demo hatchery	NC Bayona / R Tantiado	
30	Small-scale fish hatchery production at the Integrated Marine Fish Demo Hatchery	JM Ladja / LD dela Peña	
<b>Promoting healthy and wholesome aquaculture (HWA)</b>			
31	Optimization of milkfish seed production through feeding fortified broodstock diets: Effects on eggs and fry quality	OS Reyes / EG Ayson / FG Ayson	
32	Improvement of feed formulation for milkfish ( <i>Chanos chanos</i> ) culture in ponds and cages	RM Coloso	
33	Distillers' dried grains with solubles (DDGS) evaluation as protein ingredient for milkfish, <i>Chanos chanos</i> diets	RE Mamauag	
34	Evaluation of milkfish by-product hydrolysate as ingredient in juvenile grouper, <i>Epinephelus coioides</i> diets	RE Mamauag	
35	Improvement of feeds and management practices for mud crab grow-out culture: Pilot-scale production of pellets suitable for mud crab	RM Coloso	DOST-PCAARRD
36	Production characteristics of the giant freshwater prawn <i>Macrobrachium rosenbergii</i> cultured in cages using different grow-out management strategies	MLC Aralar	
37	Feed development for the golden pompano, <i>Trachinotus blochii</i>	MR Catacutan	
38	Evaluation of existing feed formulations for mud crab grow-out	MR Catacutan	DOST-PCAARRD
39	Use of soybean meal and soy protein concentrate as alternatives to fish meal in practical feeds for milkfish, <i>Chanos chanos</i>	RM Coloso	USB <sup>6</sup>
40	Improvement of the nutritional value of locally available feed resources for practical aquatic feeds by submerged fermentation and solid substrate fermentation using milkfish gut bacteria and/or selected fungi	RM Coloso	
41	Quantitative and qualitative analyses of the bacterial microbiota of tilapia ( <i>Oreochromis niloticus</i> ) cultured in earthen ponds as tool for investigating emerging and re-emerging diseases of tilapia in the Philippines	RV Pakingking Jr	DOST-PCAARRD / NRCP <sup>7</sup>
42	Surveillance of emerging diseases in wild and farmed mud crab: Application of conventional and molecular approaches	EA Tendencia	DOST-PCAARRD
43	Refinement of hatchery techniques for the donkey's ear abalone <i>Haliotis asinina</i> : Bacterial diversity and algal community structure in biofilms of settlement plates for abalone <i>Haliotis asinina</i> larvae	LD dela Peña	
44	The application and mode of action of probiotic <i>Bacillus</i> species in the larviculture of <i>Penaeus</i> : Identification, isolation and characterization of AHL-degrading <i>Bacillus</i> species for use as probiotics in <i>Penaeus monodon</i> culture ( <i>PhD dissertation</i> )	JLQ Laranja Jr	IFS <sup>8</sup>
45	Application and mode of action of polyhydroxybutyric acid (PHB) in the larviculture of <i>Penaeus</i> spp. ( <i>PhD dissertation</i> )	GL Ludevese-Pascual	VLIR <sup>9</sup>
46	Host responses and defense against <i>Amyloodinium ocellatum</i> infestation in marine fish species and development of control methods	EC Amar	



Study title		Proponent	Collaborating partner/s*
47	Screening of antimicrobial activities of crude extracts from Philippine seaweed	MMD Peñaranda	
48	Nursery culture of pompano ( <i>Trachinotus blochii</i> ) in floating net cages using formulated feeds	EDJ Ayson	
49	Grow-out culture of pompano ( <i>Trachinotus blochii</i> ) in floating net cages using formulated feeds	EDJ Ayson	
50	Culture of <i>Gracilariopsis heteroclada</i> Zhang & Xia in pond and in intertidal area	SS Avanceña	
51	Production of rabbitfish ( <i>Siganus guttatus</i> ) in semi-intensive grow-out culture using formulated diet in brackishwater pond	EB Coniza	
52	Demonstration of semi-intensive grow-out culture of grouper ( <i>Epinephelus fuscoguttatus</i> / <i>coioides</i> ) fed SEAFDEC/AQD phased-diet in brackishwater pond	MR Catacutan	
53	Demonstration of semi-intensive grow-out culture of pompano ( <i>Trachinotus blochii</i> ) using commercial diet in brackishwater pond	MR Catacutan	
54	Economics of producing mud crab <i>Scylla serrata</i> juveniles for soft shell crab farming	RPCB Ragus / ET Quintio	DOST-PCAARRD
55	Small-scale grow-out production in netcages of freshwater prawn, <i>Macrobrachium rosenbergii</i> , and Nile tilapia, <i>Oreochromis niloticus</i> in freshwater dam/reservoir for rural aquaculture development	DD Baliao	
56	Epidemiology study and elucidation of spread route of shrimp viral diseases in southeast Asian countries	LD de la Peña	JIRCAS <sup>10</sup>
57	Novel strategies to reduce disease incidence in mudcrab hatchery and grow-out	EC Amar	
58	Development of protocol for the production of hatchery-reared mudcrab <i>Scylla serrata</i> juveniles for soft shell crab farming	ET Quintio	DOST-PCAARRD
<b>Maintaining environmental integrity through responsible aquaculture (MEITRA)</b>			
59	Biodiversity in the milkfish and shrimp fry ( <i>semilya</i> ) fishery in the surf zones in southern and western Panay	TU Bagarinao	
60	Marine biodiversity around the SEAFDEC Aquaculture Department in Tigbauan, Iloilo	TU Bagarinao	
61	Impact of aquaculture in a freshwater environment: Biodiversity of aquatic fauna at the east and west cove of the Binangonan Freshwater Station	MLC Aralar	
62	Application of molecular markers in the conservation and management of marine genetic resources in Asia	MRR Eguia	JSPS <sup>11</sup>
63	Polyculture of sea cucumber with selected marine fish species	J Zarate	ACIAR
64	Hydrographic profiling of Igang Marine Station	JP Altamirano	
65	Determination of optimal conditions for sandfish <i>Holothuria scabra</i> culture	JP Altamirano	ACIAR
66	Carrying capacity, decision support tools for freshwater systems in Australia and the Philippines	MLC Aralar	ACIAR
67	<i>Anodontia philippiana</i> and <i>Holothuria scabra</i> as bioremediators in an intensive cage culture system	MJHL Ramos	
68	Polyculture of seabass <i>Lates calcarifer</i> and seaweed <i>Gracilariopsis heteroclada</i> in brackishwater pond	HS Marcial	
69	Development and extension of integrated multi-trophic aquaculture techniques for improvement of livelihood	Satoshi Watanabe	JIRCAS
<b>Adapting to climate change (CC)</b>			
70	Climate change and aquaculture: Effect of increasing rearing water temperature and acidity on the reproductive performance of some important tropical aquaculture fish species (rabbitfish)	FG Ayson	
71	Biological responses of plankton commonly used in aquaculture to climate change conditions	H Marcial	
72	Effect of elevated temperature and acidity on growth, early development and reproduction of abalone ( <i>Haliotis asinina</i> )	FL Pedroso	
73	Climate change and aquaculture: Effect of increasing seawater temperature and acidity on embryonic development, larval survival and subsequent performance in the hatchery of important tropical marine fish species (grouper and pompano)	FG Ayson	
74	Photosynthetic responses of epiphyte-infected <i>Kappaphycus alvarezii</i> to irradiance, pH and salinity variations (Thesis Research)	MRJ Luhan	

Study title	Proponent	Collaborating partner/s*	
<b>Meeting social and economic challenges in aquaculture (MSECAP)</b>			
75	Demonstration and adoption of cage culture of giant freshwater prawn (GFP) <i>Macrobrachium rosenbergii</i> among smallholder fish farmers in Laguna de Bay	EV Aralar	
76	Coastal area capability development through community-based stock enhancement in New Washington, Aklan, Philippines	JP Altamirano	RIHN <sup>13</sup>
<b>GOJ Trust Fund Program – Promotion of Sustainable Aquaculture and Resource Enhancement in Southeast Asia (ASEAN-SEAFDEC Fisheries Consultative Group Mechanism)</b>			
<b>Promotion of sustainable and region-oriented aquaculture practices</b>			
77	Selective breeding of mudcrab <i>Scylla serrata</i>	ET Quinitio	GOJ-TF <sup>12</sup>
78	Selective breeding of the tiger shrimp <i>Penaeus monodon</i>	FDP Estepa	GOJ-TF
79	Genetic improvement in the giant freshwater prawn, <i>Macrobrachium rosenbergii</i> (II) Assessment of effective broodstock management schemes for improved growth and reproductive performance	MRR Eguia	GOJ-TF
80	Development of hatchery techniques of emerging species	FL Pedroso	GOJ-TF
81	Development of efficient and low pollution feeds for grow-out and broodstock (freshwater prawn, milkfish, grouper, mud crab, and black tiger shrimp) I. Partial replacement of fishmeal with cowpea meal in practical diets for giant freshwater prawn <i>Macrobrachium rosenbergii</i>	FA Aya	GOJ-TF
82	Establishment of management technology for disease tolerance and sustainable aquaculture environment	EA Tendencia	GOJ-TF / RESCOPAR <sup>14</sup> (Wageningen University)
<b>Food safety of aquaculture products in Southeast Asia</b>			
83	Surveillance of chemical contaminants in aquaculture products and feeds: Micro-organism method for the detection of oxolinic acid (OXA) and oxytetracycline (OTC) in fish and shrimp muscles	EA Tendencia	GOJ-TF
84	Withdrawal period of antibiotics in fish species cultured in the tropics: Orange-spotted grouper, <i>Epinephelus coioides</i>	MT Arnaiz	GOJ-TF
85	Status and needs of primary aquatic animal health care in small scale aquaculture	EC Amar / CL Pitogo	GOJ-TF
86	Investigation of the situation of antibiotics/chemicals usage and regulations in aquaculture	RM Coloso	GOJ-TF
<b>Accelerating awareness and capacity-building in fish health management in Southeast Asia</b>			
87	Establishment of novel prophylactic and therapeutic methods for the prevention of viral infections in commercially important maricultured fish	RV Pakingking Jr.	GOJ-TF
88	Establishment of immunization regimen for the prevention of viral nervous necrosis (VNN) in high value marine broodfish	RV Pakingking Jr.	GOJ-TF
89	Evaluation of carriers for practical delivery of vaccines to shrimp, <i>Penaeus monodon</i> and other crustaceans	EC Amar	GOJ-TF
90	Parasitic and shell diseases of abalone ( <i>Haliotis asinina</i> ) in the Philippines	GE Pagador	GOJ-TF
91	Surveillance of parasite fauna of economically important freshwater fish in some Southeast Asian countries	GE Pagador	GOJ-TF
92	Molecular diagnosis and prevention of viruses in economically important fish and shrimp: Susceptibility of different shrimp species to white spot syndrome virus (WSSV)	LD de la Peña	GOJ-TF

Study title	Proponent	Collaborating partner/s*
<b>Resource enhancement of internationally threatened and over-exploited species in Southeast Asia through stock release</b>		
93 Stock enhancement of Napoleon wrasse <i>Cheilinus undulatus</i> with special reference to reproductive biology and seed production	FL Pedroso	GOJ-TF
94 Stock enhancement of seahorses <i>Hippocampus barbouri</i> and <i>H. comes</i>	SMB Ursua	GOJ-TF
95 Diagnosis of coral reef healthiness and establishment of coral replenishment technology	T Azuma / J Zarate / J Altamirano	GOJ-TF
96 Stock enhancement of mud crabs <i>Scylla</i> spp.	MJHL Ramos	GOJ-TF
97 Community managed sandfish ( <i>H. scabra</i> ) sea ranching and stock release	MFJ Nievaes	GOJ-TF
98 Socioeconomic analysis and identification of strategies for managing released stocks of abalone and sea cucumber in Sagay Marine Reserve in Negros Occidental in the Philippines	ND Salayo	GOJ-TF

\* The Philippine government funded a total of US\$ 748,743 for AQD's thematic programs on quality seed (42%), healthy & wholesome aquaculture (39%), maintaining environment integrity (11%), climate change (7%) and social & economic challenges in aquaculture (1%)

**AQD's COLLABORATING PARTNERS FOR 2013**

<sup>1</sup>**DOST-PCAARRD**, Department of Science & Technology - Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development

<sup>2</sup>**UP**, University of the Philippines

<sup>3</sup>**ACIAR**, Australian Centre for International Agricultural Research

<sup>4</sup>**UPV**, University of the Philippines Visayas

<sup>5</sup>**NSRI**, National Science Research Institute

<sup>6</sup>**USB**, United Soybean Board

<sup>7</sup>**DOST/NRCP**, National Research Council of the Philippines

<sup>8</sup>**IFS**, International Foundation for Science

<sup>9</sup>**VLIR**, Flemish Interuniversity Council

<sup>10</sup>**JIRCAS**, Japan International Research Center for Agricultural Sciences

<sup>11</sup>**JSPS**, Japan Society for the Promotion of Science

<sup>12</sup>**GOJ-TF**, Government of Japan - Trust Fund

<sup>13</sup>**RIHN**, Research Institute for Humanity and Nature

<sup>14</sup>**GOJ-TF / RESCOPAR**, Rebuilding Resilience of Coastal Populations and Aquatic Resources



## THEMATIC PROGRAMS

# Producing quality seed for sustainable aquaculture (QS)

Southeast Asia is a major contributor to world aquaculture production. It is here where refined seed production and farming techniques of major, commercially important aquaculture species have been developed and the awareness of the merits of using quality seeds has been promoted. Good quality means fit, 'clean', ideally uniformly-sized fry, fingerlings, or juveniles (and for seaweeds, plantlets) that subsequently express good performance attributes during culture. The attributes include desirable color, shape, good growth, health, efficient feed conversion, better reproduction, tolerance to and survival in poor and/or extremely adverse environmental conditions.

Success in the sustainable production of aquatic species for human consumption depends primarily on the availability of good quality seedstock and the adoption of optimal husbandry techniques. With the intensification of aquaculture systems and the environmental challenges such as those resulting from climate change, both factors – genetic quality and culture management – should be considered as equally important in ensuring a steady yield of good quality seeds and later, marketable products from aquaculture. Hence, through SEAFDEC/AQD's program on *Producing quality seeds for sustainable aquaculture (QS)*, activities that determine optimal conditions and methods in producing sufficient, quality seedstock are being pursued. Research involves the use of conventional means of stock improvement such as domestication, broodstock management, strain evaluation and selective breeding or genetic improvement of economically important freshwater and marine species. These technologies shall be verified and once mature, shall be packaged as viable or cost-effective methods for broodstock and seed production.

AQD's main goal for its QS program is to generate, verify and promote technologies to ensure the sustainable production of quality seed stock for aquaculture as well as for stock enhancement. More specifically, the objectives are to:

- (a) develop good quality broodstock for both traditional and emerging species through domestication, genetic and nutritional intervention, and the implementation of proper stock management protocols;
- (b) improve quality and production of seedstock through the refinement of hatchery and nursery management methods;
- (c) develop schemes for the production, management, maintenance and dissemination of genetically selected and improved stocks;
- (d) produce sufficient seedstock through the adoption of economically viable seed production systems; and
- (e) build the capability of fishfarmers and other industry stakeholders in appropriate breeding and larval rearing technologies through training, extension and information dissemination

Annual sampling of milkfish broodstock at AQD's integrated fish broodstock-hatchery complex is done before the start of the spawning season (March to November). The 30-year old broodstock have an average length of 100 cm and weight of 11 kg. These have been producing good quality eggs since the start of the broodstock-hatchery complex in 1999. In 2013, more than 40 million eggs were produced from 68 spawnings. About 96% of the eggs were fertilized, and 83% successfully hatched. From the more than 31 million larvae produced, 20 million larvae were sold to the private sector

## Development of good quality broodstock and implementation of proper stock management protocols

Good quality breeders are important both for foodfish production and for production of seeds for stock release and conservation. Stocks for both purposes must be genetically diverse to ensure fitness when farmed/released in specific environments. For stock release, the seeds produced by quality spawners will have to be genetically similar to the receiving population in the stock enhancement site to maintain the local population's genetic integrity. Supportive breeding, or the use of broodstock from the enhancement site, is often suggested to prevent further deterioration of the stocks in the release area. The following are the accomplishments for all the R&D activities that address the first objective.

### Commercial species

Shrimps (*Penaeus monodon*, 8-14g juveniles and 20-30g sub-adults) are currently grown to become potential broodstock (F<sub>2</sub>) for the sustainable production of good quality captive shrimp breeders. The growth and breeding performance of these F<sub>2</sub> generation stocks shall be compared to the growth and reproductive efficiency of the P<sub>0</sub> and F<sub>1</sub>.

Molecular markers that will identify stocks and consequently aid in determining genetic quality are currently being developed for several commercial aquaculture species. For seaweeds, molecular markers for *Kappaphycus* and *Eucheuma* are being developed. Seaweed samples or variants (n=171) were collected from major farming regions in the Philippines, namely: Bohol, Cebu, Cuyo, Cagayancillo in Palawan and mainland Palawan, Sulu, Tawi-Tawi and Zamboanga. DNA extraction and PCR amplification protocols for mtDNA coxI, cox 2-3, nuclear ITS 1-2 and chloroplast rbc target region in both species have been optimized. The mtDNA from all red seaweed variants can be amplified with the cox 1 gene primers. Among seaweed samples where the cox 1 gene and other target regions were sequenced, preliminary results showed that the cox 1 sequence of the giant green variant was similar to that of other *K. alvarezii* isolates. Similarly, the cox 1 sequences of the spinosum variant and "sacol" green variant of *E. denticulatum* are similar to published sequences of other *E. denticulatum* isolates. Another giant brown variant and *Kappaphycus* species isolate "Sur" was found to be closest to *E. denticulatum*.

Additional sequences for the rest of the samples are necessary to enable the construction of a more robust and reliable phylogenetic tree that will show the genetic relationships among the different samples using the cox1 gene. Another study, which ultimately aims to improve existing seaweed strains, deals with the isolation and culture of protoplasts from red seaweeds with potentially superior genetic traits.

With support from the Philippine DOST, collaborative studies between AQD and University of the Philippines on the development of quality milkfish broodstock through conventional stock monitoring and management protocols are underway. Local hatcheries have been surveyed and a molecular marker-based broodstock management method on the Philippine milkfish *Chanos chanos* stocks is on-going. Samples (n=50/stock) were obtained from about 20 populations of wild and/or hatchery bred broodstock (including stocks from Indonesia where some seedstock used in commercial grow-out operations are sourced). Initially genome sequence information determined through next generation sequencing was done to identify potential microsatellite markers that can be used to genetically characterize the aforementioned stocks. After optimizing DNA extraction and PCR amplification protocols, about 55 potential microsatellite markers were noted to be polymorphic, and nine of these were finally identified as utilizable in genetic stock diversity assessment based on the analysis of four stocks. Several other stocks (~ 6 to 11) have yet to be analysed. Once this work is completed, genetic differences among these stocks shall be determined and information on the best possible sources of milkfish broodstock shall be known.

Apart from genetic intervention, nutritional methods to improve egg production and quality have been done by way of the inclusion of pigments in the diet. A maturation diet for grouper (*Epinephelus fuscoguttatus*) has been formulated and feeding trials on its effect on seed quality and production is on-going.

A maturation diet has been formulated to improve reproduction in the donkey's ear abalone. Nutrient baseline data for abalone broodstock have been established and used as starting point in the production

of a maturation diet for both wild and hatchery-bred stocks. The biochemical composition of eggs from seaweed- and formulated diet-fed abalone broodstock were likewise determined and correlated with spawning success and larval quality in feeding experiments. It was noted that there is no significant difference in % protein between eggs spawned from broodstock fed either seaweed or formulated diet although eggs spawned from broodstock fed seaweeds were significantly smaller than those fed the formulated diet. Dietary formulations with varying levels of protein/energy have also been done and their effects on abalone reproductive performance were tested using wild and hatchery conditioned broodstock. The reproductive performance of wild sourced abalone improved with an increase in dietary protein/energy levels. The feeding experiments will be continued in a larger scale to demonstrate the efficacy of the maturation diet in commercial scale.

Efficient low-pollution diets for use in giant freshwater prawn *Macrobrachium rosenbergii* (GFP) broodstock are being developed and tested. Grow-out diets were prepared to be isonitrogenous (35% crude protein) and isocaloric (346-350 kcal/100 g diet). These diets were used for the tank and lake-based grow-out culture of GFP postlarvae (PL). Broodstock diets were also formulated to be isonitrogenous (38%) and isocaloric (378 kcal/100 g diet). Sardine fishmeal was replaced with cowpea meal at varying levels (diet 1 = 0, diet 2 = 15, diet 3 = 30, diet 4 = 45, and diet 5 = 60%) in grow-out and broodstock diets (diet 1 = 0, diet 2 = 10, diet 3 = 20, and diet 4 = 30%). Feeding trials in lake-based cages showed that specific growth rate (4.5–5.0%/day) and survival rate were not affected by the replacement of sardine fishmeal with cowpea meal in the test diets. GFP fed commercial prawn diet had survival rate (64%), mean body weight (13.98 g) and specific growth rate (4.73%/day) comparable to the performance of the GFP fed test diets.

Meanwhile, GFP spawners stocked in tanks at 1 M: 4 F sex ratio and fed treatment broodstock diets showed that after 72 days, GFP fed diet 1 (0% replacement) had the lowest survival (75%), which was significantly different from the GFP fed diets 2-4 (~92%). The percentage of berried females was highest in GFP fed diet 3 and 4 (92%), followed by those fed diet 1 (75%), and lowest in GFP fed diet 2 (50%). Fecundity increased with increasing levels of cowpea meal in the diet. The females produced 1,462

to 3,147 larvae per hatching event. The average number of newly-hatched larvae varied from 135 larvae g<sup>-1</sup> female in prawns fed diet 1 to 331 larvae g<sup>-1</sup> female in prawns fed diet 4. Future breeding and feeding experiments shall consider the use of newly acquired wild prawn stocks to confirm whether similar or better results can be obtained.

## Emerging species

Efforts are being undertaken to develop broodstock conditioning methods for the sandfish *Holothuria scabra* since it has been observed that old spawners decrease in body size and exhibit reduced reproductive performance. Some sandfish stocks were placed in wider broodstock holding pens in the Igang Marine Station to allow them to be reconditioned for three months. Once reconditioned, their sizes and spawning performance were recorded. Apart from conditioning techniques, sandfish reproductive performance is being improved through refinements in the spawning protocols. Cold temperature

shock, introduction of water current/movement and simulation of rising tide within tank enclosures have been tested with the view of increasing spawning frequency and production output. Thermal shock trials had comparable results regardless of temperature. Of four spawning trials, the warm method gave spawning success of 32% for males and 12% for females. Coldwater method induced 31% of the males and 13% of female breeders to spawn. Spawning runs in 2013 which totaled 19 resulted to varying production levels. The total fertilized eggs stocked ranged from 48,000 to 7.6 million while the survival rate from hatched eggs into stage 1 juveniles (1-3 mm) averaged 1%.

Meanwhile, larval rearing trials, wherein the ideal egg stocking density is being determined, showed that the highest hatching rate was observed in the stocking density of 125/L. Verification runs have yet to be conducted to confirm this result. For nursery production, stocking density experiments (150, 250, 500 and 700 individuals / 8 m<sup>2</sup> hapa) are ongoing.

Growth was noted to be satisfactory during the first month of culture but declined in succeeding months. This indicates that the natural biofilm in hapa nets can only optimally support sandfish growth in cages for a month. High stocking density was also tested (1000 and 2000 individuals per hapa). Growth rates were low, ranging 0.02-0.04g/day, with survival rates at 68-76% after a month.

A preliminary experiment to determine size-at-stocking in nursery hapas was done. Small (0.58 g), medium (1.38 g), and large (2.7 g) sandfish juveniles were used. After one month, growth rates were 0.03 g/d for small, 0.015g/d for medium, and -0.01 g/d for large group. Survival was highest with large individuals (95%), followed by medium (91%), and small (71%).

## Species for stock enhancement

The Napoleon wrasse *Cheilinus undulatus* research is being pursued at AQD primarily to understand its reproductive biology and consequently enable the production of seedstock for use in stock enhancement activities. Efforts are made to develop broodstock of Napoleon wrasse. A preliminary survey was conducted in the Igang Marine Station where sightings of small Napoleon wrasse juveniles were noted in the corals near the seagrass area. Fin clips of some of the specimens collected namely from IMS (n=3), Bohol (n=1) and Tawi-Tawi (n=10) were sent to Hokkaido University for genetic characterization. More samples are to be collected.



AQD's sandfish hatchery [BELOW] with broodstock from the Igang Marine Station



PHOTOS BY JM DELACRUZ

## Refinement of hatchery and nursery management methods to improve seedstock quality and production

### Commercial species

The refinement of mass production techniques for copepods (*Acartia tsuensis* and *Pseudodiaptomus annandalei*) is continued to support intensive marine fish larviculture. *A. tsuensis* and *P. annandalei* samples were isolated from samples collected from a river mouth in Anini-y, Antique and at AQD's Dumangas Brackishwater Station (DBS). Life history parameters including life span, age at first maturity, fecundity, and sex ratio were investigated. Individual culture experiments showed that *P. annandalei* cultured at 29±1°C, 30 ppt and fed *Tetraselmis tetrahele* produced 30-106 offspring in a life span of 12-42 days, while *A. tsuensis* raised at 29±1°C, 30 ppt and fed *Chaetoceros* sp. produced 60-124 offspring and life span ranged from 6-20 days.

Mass culture of *P. annandalei* was undertaken in a 250-li tank at initial stocking density of 200 ind/li (mixed stages). Copepods were cultured in a 30-li tank with the bottom replaced with a net to automatically sieve the nauplii, and installed inside the 250-li tank. Daily nauplii production ranged from 18 to 350/li, which peaked on the first week of culture. On the other hand, *A. tsuensis* produced eggs and nauplii ranging from 36-400/l that also peaked on the first week of culture. Both species ceased to produce offspring on the third week, although they are still alive up to one month of culture. Eggs/nauplii produced were further cultured in a separate tank, however massive mortality was observed during the first few days of culture and survival rate (up to copepodite stages) ranged from 0-16%. The copepod nauplii produced can be used in rearing fish larvae. Because of the difficulty in sustaining production of copepods in tanks, pond-based copepod culture is recommended.

In search for potential natural food as an alternative to *Artemia* in intensive production of marine fish, life history parameters and mass culture techniques of the euryhaline cladoceran *Diaphanosoma celebensis* were undertaken. The highest fecundity of *D. celebensis* (89 nauplii) was obtained in *Tetraselmis tetrahele*-fed treatment at the 10-15 ppt, but this is not significantly different from the *Chaetoceros calcitrans*-fed group.

*D. celebensis* were fed to seabass larvae. Results of the feeding experiments showed that seabass larvae fed *D. celebensis* were significantly bigger than *Artemia*-fed larvae. Nutritional (proximate and amino acid) components of *D. celebensis* fed different microalgae as well as of *Artemia* and seabass larvae will be determined.

To improve the production of marine fish larvae, nutritional intervention by way of tryptophan supplementation in the diets were used to minimize cannibalism. Preliminary trials using grouper larvae which involved weaning fish to a formulated diet were started but proved to be difficult as the larvae were very sensitive to stress associated with routine tank maintenance. Seabass larvae were then tried and the larval rearing trial proceeded well. Samples of larval seabass have been collected for serotonin level analysis and for correlation with aggressive behavior.

In the pompano *Trachinotus blochii*, optimum conditions for breeding and seed production were determined. The potential use of copepods as larval feed was tried as alternative to *Artemia*. Feeding treatments were either *Artemia* and/or copepod fed singly or in combination to 15-day old pompano larvae. Best survival (96%) was in the treatment fed with copepod while highest weight and length increment were noted in *Artemia*-fed larvae (94 mg and 12.7 mm, respectively). Early metamorphosis was observed in copepod-fed larvae compared to the other treatments. Larvae fed the *Artemia*-copepod combination showed better tolerance to hypoxic and high saline conditions.

A verification study on the brackishwater nursery pond culture of pompano that assessed the optimal stocking density, feeding frequency and effect of in-pond sorters on the yield of seabass juveniles has been completed. The use of in-pond sorters did not improve fry survival. Without in-pond sorters, survival was higher in fish stocked at low (500/m<sup>3</sup> with 87%) than in high densities (1000/m<sup>3</sup> with 73% and 1500/m<sup>3</sup> with 67%). Survival in those with in-pond sorters were lower at 52%, 42% & 16%, respectively. As for the practice of regular sorting, regular size-sorting (1-2 times per week) gave better survival after 63 days (22-31%)

than when sorting was not done (0.7-2%). Meanwhile, the optimum stocking density for 2.5 cm juveniles is 100/m<sup>3</sup>. The survival of fish stocked at 100/m<sup>3</sup> was better (45%) than when fish were stocked at 150 (28%) or 200/m<sup>3</sup> (19%).

In another experiment, seabass juveniles were stocked in enclosures with 2, 4, or 6 units of PVC shelters (1 unit = 2,500 cm<sup>3</sup> volume) or without shelter. No sorting was done during culture. Results showed that mean survival rates were low (5.6-6.8%) and did not differ among treatments. However, ATL (9.7-12.3 cm) and ABW (10.4-11.3 g) in fish reared without shelter or provided with only 2 units shelter were higher than when more shelters were installed. Similarly, frequency of feeding (3, 4, or 5 times daily) did not also improve survival, ABW or ATL.

Meanwhile, a verification study on the nursery culture of pompano in floating netcages compared the use of pompano diet with a siganid diet. Pompano fry grew better when fed pompano feeds (daily growth rate or DGR 1.29 and 1.22 vs. 0.93 g/day for big size fry and 0.79 vs. 0.68g/day for the small size fry) than the siganid diet. A confirmatory run is underway to verify the observation.

For milkfish, growth and reproductive traits of broodstock fed fortified vs. non-fortified (control) diets were compared. Fortified diets meant inclusion of phospholipids, carotenoids, vitamin C, beta carotene and arachidonic acid. It was noted that more spawning episodes were observed in young milkfish broodstock fed fortified diets compared to old stocks (17-29 year-old). On the other hand, older stocks fed fortified diets produced more eggs. Fertilization and hatching rate of eggs produced by the aging stocks was comparable to the newly acquired breeders. Finally when milkfish larvae were on-grown to test the effect of stocking density on growth and survival of milkfish fry, those stocked at high densities of 50 larvae/liter survived poorly compared to those at the lowest density of 15 larvae/liter or 9% in contrast to 56%.

To improve the seed yield of selected high value marine fish species such as grouper, red snapper, seabass, rabbitfish and pompano, sodium-iodide enriched rotifers and *Artemia* were used as feed for larvae. Metamorphosis and survival of the same high value species were noted. Improved survival rate was observed for both red snapper and pompano when fed sodium iodide-enriched rotifer and

*Artemia*. The duration of sodium iodide enrichment (short vs. long) was found to affect the survival of red snapper but not of pompano.

A strain of thraustochytrid *Schizochytrium* sp. (LEY7) was used to enrich feeds for fish larvae and abalone. Mass production is on-going as are trials for different methods of thraustochytrid processing. Either freeze-dried and frozen, concentrated and frozen or fresh concentrated thraustochytrids can be used for enriching rotifers. Feeding trials using pompano larvae revealed that larvae fed rotifer enriched with hatchery prepared emulsion and larvae fed rotifers enriched with either freeze-dried and frozen or frozen concentrated thraustochytrids had high body weight increase than the other preparations. Moist thraustochytrid concentrate was also incorporated in abalone juvenile diets. The feeding trial using abalone diet containing thraustochytrid as source of lipids (4-8%) is on-going.

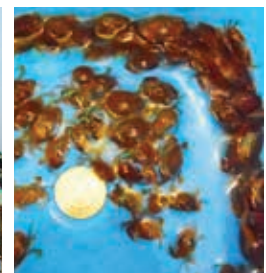
*Cocconeis* sp., *Nitzschia* sp. and *Diploneis* sp. have been used as alternative benthic diatom feed species for the hatchery rearing of abalone. Preliminary runs for food preference and digestion efficiency determination are on-going. Meanwhile in a related study, results of feeding continuously with (a) *Nitzschia* or (b) *Nitzschia* and then *Cocconeis* or (c) *Nitzschia*, *Cocconeis* then *Diploneis* /or (d) combination of *Amphora* and *Navicula* throughout or (e) the combination then *Cocconeis* showed that *Nitzschia* and *Cocconeis* are promising diets in the hatchery rearing of the abalone based

on final survival rates. Feeding with *Nitzschia* also showed high settlement rate in abalone larvae. Post-settlement survival (3% at 10d) was similar for treatments where abalone were fed *Nitzschia* sp. and *Amphora* sp. + *Navicula*. After 60d of rearing, higher survival was observed in abalone fed continuously with *Nitzschia* (0.44%) followed by those fed a combination of *Nitzschia* and *Cocconeis* (0.22%). Bigger juveniles were noted in abalone fed a combination of *Nitzschia* and *Cocconeis* (SL: 0.74 cm; BW: 0.09 g) and in abalone fed solely with *Nitzschia* (SL: 0.73 cm; BW: 0.08 g) but were not significantly different with abalone juveniles fed the other diatom diet.

Another nutritional intervention to improve abalone hatchery production is the use of microparticulate diets (MPD) as alternative feed. An agar-bound MPD has been formulated based on the nutrient profile of post larval abalone, and has been found to improve post-larval settlement and survival. Feeding trials in large scale tank systems using optimal conditions established in smaller tanks are underway.

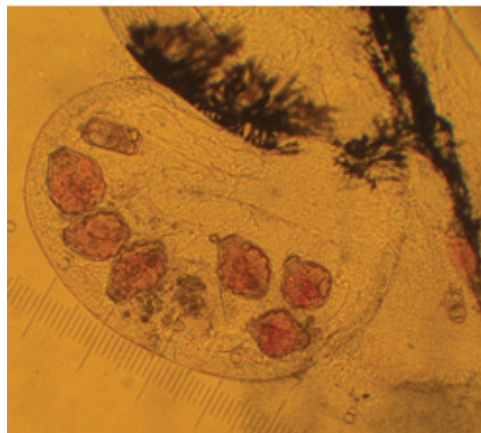
The influence of stocking density and tryptophan-supplemented diets on the survival and growth of mudcrab *Scylla serrata* in two nursery phases of rearing are being determined. For phase one based on stocking density, crabs reared for 4 weeks at 30/m<sup>2</sup> had higher survival (60%) than those stocked at 50/m<sup>2</sup> (48%). Crabs fed mussel and formulated diet with 0.5% tryptophan survived better (64%) than those fed mussel alone, mussel and basal formulated diet, or mussel and formulated diet with 0.75% tryptophan.

Crabs stocked at 5/m<sup>2</sup> and 10/m<sup>2</sup> and subjected to the same feeding regimes, survived better in diet 1 or in the mussel and basal formulated diet combination. Survival was better in a lower stocking density of 5/m<sup>2</sup> than at 10/m<sup>2</sup>. In phase two, crabs fed TRP-supplemented diets in phase one were again fed the same diets. Those that were stocked at 5/m<sup>2</sup> showed highest survival of 63% in the mussel plus basal artificial diet. As for stocking density, crabs stocked at 5/m<sup>2</sup> yielded survival of 57%, which is higher than at 10/m<sup>2</sup> (51%). In another experiment, crabs not subjected to TRP supplemented diets in phase 1 were tested for growth and survival using the same feeding treatments with TRP diets. The survival of crabs fed mussel with basal formulated diet gave the highest survival (59%). Stocking density of 5/m<sup>2</sup> promoted better survival (57%) than the 10/m<sup>2</sup> (49%). It was noted that tryptophan did little to reduce incidence of cannibalism. In this regard, modifications to further reduce the risk of competition and cannibalism are being tried and these are: (a) reduction of culture duration from four to three weeks per phase and (b) use of a more complex substrate design or more substrates to maximize surface area and interstitial spaces. The same experiments were repeated with modifications. Crabs stocked at 30/m<sup>2</sup> had higher survival (68%) than those at 50/m<sup>2</sup> (51%); natural food promoted high survival (64%). For phase two, at stocking density of 5/m<sup>2</sup>, increasing dietary TRP level decreased the survival. A similar trend was noted in crabs stocked at 10/m<sup>2</sup>. Crabs stocked at 5/m<sup>2</sup> had higher survival (89%) than in 10/m<sup>2</sup> (82%).



Harvested *Scylla serrata* crablets [ABOVE] from the nursery net cages in ponds [LEFT] at AQD's station in Dumangas, Iloilo





PHOTOS BY FAYA

Early stage *Leiotherapon plumbeus* reared in outdoor concrete tanks: larvae at the initiation of exogenous feeding two days post-hatching [UPPER LEFT] and at 4 days [BOTTOM LEFT]. Gut contents show zooplankton consumed by first-feeding larvae [RIGHT]

Refinements such as determination of the optimal NF (natural food):FD (formulated diet) ratio and feeding rate are also being conducted. In both phases, it was noted that a combination of mussel and formulated diets gave best results while no significant variation was noted when different feeding rates were used.

Other approaches to improve hatchery and larval rearing protocols also include: (a) the evaluation of commercially available shrimp formulated diets that can be used for mudcrabs and (b) use of immunostimulants, prophylactics and disinfectants. In the study comparing commercially available shrimp diets for mudcrab larval rearing, feeding the larvae with 'BP Nippai', 'Monodon' and 'Japonicus' feeds gave good results though not significantly different from each other. Incomplete molting, one of the major signs of molt death syndrome (MDS), was least observed in larvae fed formulated diets.

The amount of natural food (NF), rotifers and *Artemia*, to be replaced with formulated diet in larval rearing was determined. Preliminary results showed that larvae fed 25%FD + 75%NF and 50%FD + 50%NF had better survival than those larvae fed 100% FD, 100% NF and 75%FD + 25%NF. There are plans to compare larval performance using a SEAFDEC-formulated diet for mud crab larvae and the three shrimp formulated diets. Meanwhile, the application of antibiotics, antimicrobial nitrofurans, disinfectant, therapeutic and probiotics was done. Larvae treated with antibiotics commonly used in the hatchery survived the longest (9-10 days) compared with larvae in the other treatments (5-6 days).

### Emerging species

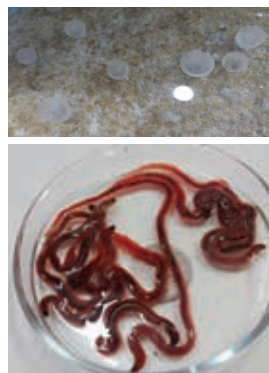
Silver perch *Leiotherapon plumbeus* is a promising species for commercial inland aquaculture because it is indigenous and commands a good market price. Studies on the type of fertilizer and the optimum

stocking density for therapon larval rearing were conducted.

The effects of different fertilizer types namely: organic fertilizer (OF, chicken manure); inorganic fertilizer (IF, 16-20-0); and no fertilizer (NF) on larval rearing were tried and results showed that larvae stocked in tanks with OF had the highest specific growth rates (7.2% d<sup>-1</sup>), compared with those in the IF (5.9% d<sup>-1</sup>) and NF (6.3% d<sup>-1</sup>) treatments. As for the stocking density, growth was found to be significantly higher at stocking density of 0.4 larvae l<sup>-1</sup> (SGR: 6.9%/d) than at 0.6 larvae l<sup>-1</sup> (SGR: 5.2%/d) at 30 days after hatching (DAH). Survival rates (0.4 larvae l<sup>-1</sup>: 35.4%; 0.6 larvae l<sup>-1</sup>: 14%) were not affected by stocking density.

In addition, two feeding trials were performed to investigate the effects of weaning age (abrupt and gradual) and larval diets (formulated and live foods) on survival and growth of silver therapon. In the abrupt weaning treatment, survival, final body weight (FBW) and total length (TL) significantly increased with weaning age. Survival of larvae weaned at 26 DAH (37%) did not significantly differ with that of the control (47%). The highest values for final body weight (FBW) and total length (TL) were observed in larvae weaned at 26 DAH (93 mg; 19 mm) which were comparable to that of the control group. However, there was no significant difference observed on specific growth rate with increasing weaning age.

In the second experiment, the effects of feeding one commercial prawn feed (38% crude protein) made into fry mash and three live foods (*Artemia* nauplii, zooplankton/copepods, free-living nematode *Panagrellus redivivus*) were investigated. Survival was best in larvae fed *Artemia* nauplii (95%) and poor in copepod-fed larvae (41%). Final TL of larvae fed prawn diet (24 mm) was higher than those fed zooplankton (19 mm) and nematodes (17 mm) and comparable to larvae fed *Artemia* nauplii (24 mm). The best growth (FBW) and SGR was also obtained in larvae fed *Artemia* nauplii (5%/d).



Harvest of marine annelid *Marphysa mossambica*: cocoons [TOP] and tank-grown bait-sized annelids [ABOVE]

PHOTOS BY VR ALAVA

## Species for stock enhancement

The seahorses, *Hippocampus barbouri* and *H. comes* are continuously being propagated at AQD for possible stock release. Meanwhile, monthly on-site assessment at Molocaboc Island, Sagay City was conducted to monitor baseline population of wild seahorses. The seahorses were collected from fringing coral reefs during night time at low tide. Most of the animals were found coiling their tails among corals, while only a few were found on the seaweed *Sargassum* sp. Local divers who are also residents of the island assisted in the collection of wild seahorses. After an hour of diving, the collected seahorses are weighed, measured and graded based on the gonad development stage. Sampling is being done continuously except for some months in the year when strong waves were noted in the sampling site.

To determine good sources of wild seahorses, DNA analysis of samples from several collection sites were done. Sequencing of mtDNA cytb and 16s rRNA genes were done on *Hippocampus comes*, *H. barbouri*, *H. kuda*, *H. spinosissimus* and a pipefish species. Wild seahorses from Molocaboc Island were all identified as *H. comes*, while two species of wild seahorses from Panal Reef (also in Sagay) were identified as *H. comes* and *H. spinosissimus*.

Phylogenetic trees were constructed to show relationship among seahorse species. The population structure of wild seahorses (Molocaboc) and hatchery-reared *H. comes* showed 3 to 4 haplotypes. Population structure analysis showed low to medium haplotype diversity ( $h = 0$  to  $0.5$ ) and very low nucleotide diversity ( $\pi = 0$  to  $0.0015$ ). Eight polymorphic microsatellite loci from related seahorse species were tested, of which only four loci showed consistent results after preliminary fragment analysis sequence in 4 seahorse individuals. However, when Molocaboc (21-24n) and hatchery-reared (21n) seahorses were screened for population structure analysis, results from only one locus was found to be interpretable and utilizable.

## Development of schemes for the production, management, maintenance and dissemination of genetically selected and improved stocks

### Commercial species

Selective breeding programs have commenced for selected crustaceans with support from the Government of Japan Trust Fund (for mudcrab, shrimps and freshwater prawns), AQD and DOST (for shrimp and the mudcrab). For the mudcrab, stress tests (particularly formalin tolerance and disease challenge response tests) were done to determine strain level differences in the effort to identify stocks with better fitness attributes. Details on the shrimp and mudcrab studies are covered in the GOJ-TF part of this report.

For the giant freshwater prawn, the approach for genetic improvement is to assess and determine the effective broodstock management scheme to enhance growth and/or reproductive performance. This year, potential broodstocks from two sources, Calumpit, Bulacan and Pampanga River were on-grown for growth and reproductive performance comparison. The growth trial in cages is on-going and will be completed in early 2014 after which the breeding performance assessment will be implemented using stocks from the growth study. Broodstock management methods (cross breeding and optimal sex ratio) effectively tried for the AQD hatchery stocks from Calumpit will be assessed using the different stocks.

Hybridization is currently being done on the local commercial abalone species (*Haliotis asinina*) by crossing this with other Philippine abalone species, *H. planata* and *H. glabra*, to enable the production of stocks/species with improved traits. A new trial was done using *H. asinina* female and male *H. planata* (from Palawan). The larvae (1,875,000 pcs) were stocked and settlement rates were 0.66% and 0.42% on day 5 and day 10, respectively. The surviving stocks are now 1.4–1.5 cm SL abalone juveniles (approximately 4,949 pcs). The juveniles will be reared until 2.5 to 3.0 cm for grow-out rearing in sea-based cages for growth and maturation studies.

Meanwhile, in an earlier growth comparison trial, in terms of body weight (BW) and shell length (SL) pure *H. asinina* attained optimum growth at a shorter period of time (BW at 180 days of

culture or DOC – 26.13g; SL: 210 DOC – 4.91cm) compared to hybrid HAFPM (or hybrids of *H. asinina* female parent and *H. planata* male parent) (BW: 210 DOC – 26.55g; SL: 240 DOC – 4.81 cm). After 300 DOC, the survival of hybrid HAFPM was higher (73%) compared to that of pure *H. asinina* (66%).

In a related study, after 180 DOC, the third hybrid HAFVM (cross of female *H. asinina* and male *H. varia*) attained optimum growth similar with pure *H. asinina*. The new hybrid (HAFVM) is bigger (SL: 4.43cm; BW 21.63g) compared to pure *H. asinina* (SL: 4.28; BW: 18.15). The survival of both strains is also similar (100%).

Efforts to produce triploids using caffeine and 6DMAP are also underway. Higher % of normal trocophore larvae was observed in caffeine treated eggs (10 min: 82%; 5 min: 85%) compared to 6DMAP treated eggs (10 and 5 min: 75%). Both samples of potential hybrid stocks and presumptive triploid abalones were sent to Hokkaido University for genetic analysis and confirmation of success in hybrid and triploid induction.

Ice-ice disease in seaweeds is a problem. Methods to develop resistant strains of the seaweed *Kappaphycus* and reduce epiphytes are being studied. Bacteria were collected from ice-ice infected *Kappaphycus* from various local farm sites. Crude bacteria did not cause ice-ice in two variants of *Kappaphycus* (plantlets). Older branches will be tested.

Meanwhile, epiphytes were collected from infested seaweeds but failed to induce settlement of spores on *Kappaphycus* plantlets. Older thalli will be tested for induction of epiphyte.

Another study involves the evaluation of the performance of haploid and diploid *Kappaphycus*. Preliminary results showed that growth rates of sporophytes and gametophytes are significantly different in the land-based nursery. Growth rates of gametophytes and sporophytes are not significantly different when grown in lines. No epiphytes and ice-ice disease was observed when tissue-cultured *Kappaphycus* were on-grown in net cages and culture lines.

Meanwhile, fertilized and unfertilized *K. alvarezii* were planted in lines randomly. Incidence of ice –ice disease was significantly lower in fertilized (9%) than in unfertilized (97%) seaweeds.

Finally, a breakthrough worth noting with regards to seaweed research at AQD is the completion of the life cycle of *K. alvarezii* *in vitro*.

### Adoption of economically viable systems to produce sufficient seedstock

Several fish/shellfish production projects are being implemented at AQD to demonstrate the viability of small-scale and/or large-scale seed production systems.

Mass production of sex-reversed and mixed-sex Nile/red tilapia fingerlings as well as large scale production of abalone juveniles, are continuously being done. In the small-scale Nile tilapia hatchery, new female spawners were used for spawning starting this year. A total of 160 females and 40 males were distributed into 4 cages (3 x 4 x 1 m) and 4 units of rectangular tanks (3 x 5 x 1 m). Approximately 300,000 swim-up fry were collected from January to June 2013. Eggs collected from spawning cages were hatched in jars, and 70,000 fry/fingerlings were produced.

For the abalone, juvenile production is ongoing from two hatchery facilities (large scale and backyard type demo-scale) at AQD. In the large scale abalone hatchery facility, a total of 97,235 abalone juveniles (SL: 5mm-8mm) were produced from January 2013 – November 2013. Survival rate from veliger larvae until 90 days of rearing ranged from 0.01% – 1.10%. In the demo hatchery facility, a total of 30,352 abalone juveniles were produced with survival rate ranged from 0.13%-0.58%.

Harvested abalone juveniles were reared further in the nursery tanks until they reached 1.1 – 1.5 cm or bigger which is the size ideal for disposal. From January to December 2013, a total of 2,800 abalone juveniles (SL: 1.1 - 3.0cm) were produced.

## Capacity-building of fishfarmers and other industry stakeholders on appropriate breeding and larval rearing technologies

Some DOST-funded studies, like those under the mudcrab program, include a component on technology dissemination. To effectively conduct such activities, the private sector is involved in on-site technology demonstration. One of the private entrepreneurs (Acantilado Aqua Resource Corporation in Samal, Davao) participating in the adoption of mud crab hatchery technology produced about 2,000 crab instar in the first trial. A technician trained at AQD was detailed in their hatchery to provide assistance and training on the culture of natural food and larval rearing. On the other hand, a collaborator in Quezon produced 19,000 crab instar. Their technician also underwent training at AQD.

There were five private hatchery operators that entered into an agreement on the hatchery operation with AQD:

- Acantilado Aqua Resource Corp., Samal, Davao
- CDO Foodsphere Inc., Zambales
- Lutao Prawn Hatchery, Misamis Occidental
- Mari-al Hatchery, Dalahican, Quezon
- Rey Acap Hatchery, Iloilo

Apart from the on-farm demonstration activities covered by externally supported studies, AQD published a manual on natural food production for marine fish species and offered several specialized training courses to local government representatives, private sector investors and fisherfolk. These were on tilapia, giant freshwater prawn, and catfish; seaweed and algae culture; marine fish, abalone, mud crab, sandfish and tiger shrimp. [See overleaf]. In addition to these customized courses, on-the job trainees were accommodated in the AQD hatcheries during the year. Moreover, technical assistance was also provided to local and international private sector clients through the *Agree build operate & transfer aquanegosyo* program. Notable among these is the technical support extended to the ACDI-VOCA project in Timor Leste.

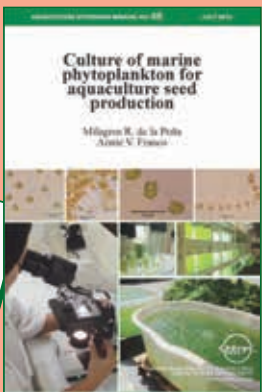
Technology dissemination on mud crab in Timor Leste: AQD scientist Dr. Emilia Quintino demonstrates how to identify mature female crab



Technology development & demonstration: mud crab nursery set-up (12 m<sup>2</sup> cages) at AQD's Dumangas Brackishwater Station

PHOTOS BY VR ALAVA

PHOTO COURTESY OF ET QUINTINO



## Extension manual

### *Culture of marine phytoplankton for aquaculture seed production* By Milagros de la Peña and Annie Franco

The 32-page extension manual describes the biology, culture techniques and maintenance and mass propagation of 12 phytoplankton species used as live feed in marine hatcheries. It is the 55th title in AQD's aquaculture extension manual series.

The lead author Ms. de la Peña is an AQD researcher working with the live food production team, making substantial contributions in the standardization and improvement of microalgal culture techniques and in maintaining and preserving AQD's algal culture collection. Co-author Ms. A. Franco is a senior technical assistant, maintaining AQD's microalgal strains and serving as practical instructor in training courses with live food as component.



Manual authors [Ms. M de la Peña, INSET] flanked by AQD officials and a stakeholder from Myanmar who received a complimentary copy during the booklaunching on 10 July at AQD's Tigbauan Main Station

Seaweeds: a trainee prepares laboratory culture materials for tissue culture



PHOTO BY RT BAUTISTA

Marine fish: a trainee from Kenya samples grouper broodstock

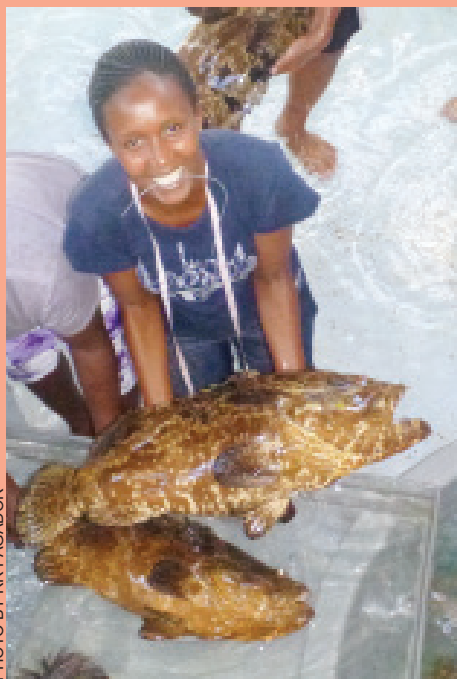


PHOTO BY RR PAGADOR

## Regular and specialized training

For 2013, AQD organized and conducted 14 sessions of 11 courses under the QS program which were attended by a total of 68 trainees from 14 countries. These courses were AQD's regular offering or organized according to the specifications of requesting stakeholders.

**Freshwater commodities @ BFS.** Training courses on seed production and culture of giant freshwater prawn (4 – 8 March), tilapia (12 – 16 March) and catfish (1 – 5 April) were conducted at AQD's Binangonan Freshwater Station (BFS) in Rizal. A total of 15 participants (five in each course) completed the three courses. They had lectures on the biology & sustainable management of farmed commodities and aquaculture economics. Also, hands-on practical sessions were done to give the trainees an actual experience in fish farming operations.

**Grouper hatchery**, held 14 – 27 January at AQD's Tigbauan Main Station (TMS) in Iloilo. This is a special course organized for a hatchery manager and a seaman from Nueva Ecija and Bohol. The participants attended lectures and practicals on biology of grouper, broodstock sampling and induced spawning, culture of live food organisms, egg sampling, larval counting & stocking, feed preparation and cage culture. They also went to AQD's Igang Marine Station to observe mariculture practices.

**Seaweeds nursery techniques**, 04 – 13 February, TMS. Four BFAR (Bureau of Fisheries and Aquatic Resources) technical staff coming from Iloilo (1), Negros Occidental (1) and Antique (2) completed the special course. AQD resource persons lectured on topics such as biology & taxonomy of red algae, *Gracilaria* culture, nursery & grow-out culture of *Kappaphycus*, common diseases of seaweeds and food safety in seaweed production & processing. In addition, the participants had practical demonstration on taxonomy & identification of seaweeds, natural food production, preparation of laboratory culture materials & media, and production of micropropagules. They also went to AQD's Igang Marine Station to observe the sea-based nursery for seaweeds.

**Algal culture**, 18 – 22 February, TMS. Two NFRDI (National Fisheries Research & Development Institute of DA-BFAR) technical staff attended lectures and practicals on the biology & culture of phytoplankton, counting & isolation techniques, monitoring & harvesting of culture and application of microalgae in aquaculture. The special course is needed for R&D projects of NFBC (National Fisheries Biological Center), one of NFRDI's research centers which is developing a phycology laboratory.

**Marine fish hatchery**, 3 – 10 May and 25 June – 31 July; TMS. The first of two sessions was tailor-made to the needs of a university lecturer from Malaysia. The second session is AQD's regular, month-long and yearly offering with fellowships from the GOJ-Trust Fund. For 2013, seven trainees coming from Kenya (4), Myanmar (1) and Philippines (2) finished the international course which included lectures on biology of marine fishes, broodstock management, larval rearing and hatchery operations as well as natural food production, feed development, fish health management and marketing strategies. The participants also had hands-on experience on rearing

PHOTO BY G GENZOLA



Mud crab: trainees ablate and tag crabs in their practicals to spawn crabs

PHOTO BY JM DELA CRUZ



Sandfish: a trainee transfers broodstock induced to spawn by thermal shock

PHOTO BY R LACIERDA; INSET BY D CATEDRAL



Shrimp: trainees identify shrimp larval stages (eg. postlarvae, inset)

marine commodities. They visited private hatcheries and fish farms where they saw how an actual commercial marine fish hatchery functions. Trainee Mr. Anthony Mutua of Kenya's Marine Fisheries Research Institute shared his thoughts: "Capture fisheries could not sustain the needs of the people anymore and we need to find alternatives. Kenya is strongly promoting aquaculture. At AQD, we were able to gather in-depth knowledge of techniques to rear marine fish species — grouper, siganid, pompano and milkfish which are also available in Kenya — and I would like to thank AQD resource persons and staff for training us."

**Abalone hatchery & grow-out**, 14 May – 04 June, TMS. This is a regular course with fellowships funded by GOJ-TF and was attended by Cambodia (1 trainee) and Philippines (2 trainees). It was aimed at providing participants with technical knowledge and skills on the broodstock management, spawning, larval rearing, and nursery of the abalone. Topics covered were broodstock management; induced spawning techniques; production of natural food organisms for abalone larvae; feeds & feeding; seaweeds culture; larval, nursery and grow-out culture techniques.

**Mud crab hatchery, nursery & grow-out operations**, 21 May – 12 June, 24 – 28 June, 23 September – 09 October, and 20 – 29 November; TMS. This is AQD's most requested course, held four times this year. The first session is a special course attended by government personnel, entrepreneurs including crab farm owners from Indonesia (1), Philippines (7) and Bangladesh (2). Lecture topics and practical exercises included broodstock management, induced spawning techniques; production of natural food organisms; larval, nursery and grow-out culture techniques.

The second session was organized for entrepreneurs from Singapore (2), Mauritius (1) and the Philippines (1).

The third session was conducted with funding from DOST (Department of Science & Technology) for eight participants from Cavite State University, Aklan State University, Davao del Norte State College and the local government (LGU) of Tagum City. "From this course we have learned that we need to update our research facilities in our respective places," said Mr. Astrolome Maningo, multi-species hatchery in-charge of LGU Tagum. The training is part of the agreement among AQD, some state universities and LGUs with laboratory, hatchery and pond facilities to hasten the promotion and dissemination of mud crab hatchery and nursery technologies in the Philippines.

The fourth session had 14 learners from the Philippines (7); Madagascar (5); Reunion Island, France (1); and Japan (1). It was also a combination of lectures and practical exercises which help the participants understand the process of starting and managing a mud crab hatchery. "I am confident that I will be able to set up a profitable and sustainable mud crab aquaculture business in my country," said Mr. Vincent Bernier, one of the trainees from Madagascar. Meanwhile, Mr. Alfonso Lim of the private sector in the Philippines, said, "I decided to come back to improve the system in my farm and luckily, this training was organized. I gained knowledge in operations, established connections with the scientists & experts, and I gained confidence to start a mud crab hatchery." As for the academe, Mr. Aldrin Mel Macale of Bicol University said that the information learned in the training will be shared to people and will be used in further research on mud crab hatchery.

**Sandfish seed production, nursery & management**, 02 – 17 October, TMS.

Six trainees successfully finished the course comprised of lectures (20% of course schedule, where the theories behind the techniques are described) and practicals (80%, done to gain the actual experience). The trainees had only good words for AQD. "What I like about the training is that you don't have to be an aquaculturist to understand the lectures because it's so basic. I am very confident now and I think I can produce sandfish," said Mr. Ali Shafeeg of the Maldives. Mr. Shafeeg also said that he will come back to train to learn about other commodities and will recommend AQD to other people in his country who are interested in aquaculture, even wishing for the Maldivian President to see what AQD has to offer.

Associate scientist Dr. Jon Altamirano, the technical lead person of the sandfish course, noted that the relationship between AQD and its trainees goes beyond the time they spent training at TMS. AQD was requested by Mr. Keith Ng, a former sandfish training participant from Century Marine Products (Kota Kinabalu, Malaysia) to assess hatchery facilities and recommend improvements in operations. Century is into sandfish farming and processing, and has been relying on natural sandfish juvenile stocks. Dr. Altamirano, who was sent in Kota Kinabalu by AQD in November 2012, said that the Tanjung Badak multi-species hatchery research complex shows high prospects for expansion of sea cucumber production.

**Shrimp hatchery operation & management**, 05 - 19 November, TMS.

Completing the course were three trainees from Cambodia (Mr. Em Thearith), Egypt (Dr. Mona Azzan Hasssan Abdat), and the Philippines (Ms. Nori Abegail Acebes). The trainees were thankful that the AQD resource persons unselfishly shared their knowledge and skills. "I found that everything in AQD is up-to-date, from knowledge to experience, and now I am sure I can start my hatchery in a scientific way," said Dr. Abdat. In addition, Mr. Thearith said that he will apply what he learned from the course in Cambodia. The course had lectures and practical sessions on larval rearing, eyestalk ablation, natural food production, feed preparation, shrimp disease monitoring, shrimp grow-out management and others. The trainees also visited the AQD stations in Dumangas, Iloilo and Igang, Guimaras which, according to Ms. Acebes, had helped her in planning how to put up her own hatchery.

## Hatchery production of marine and freshwater species at AQD

Species	Number of larvae/fry sold to fish farmers	
	Larvae	Fry
Pompano	50,000	71,470
Grouper	2,000,000	9,347
Seabass	100,000	1,188,864
Rabbitfish	-	157,350
Milkfish	20,705,000	9,062,750
Tilapia		170,364
Giant freshwater prawn		83,020

PHOTOS BY DEVCOM



Packing of rabbitfish fry for transport [LEFT] and stocking of milkfish larvae in a larval rearing tank

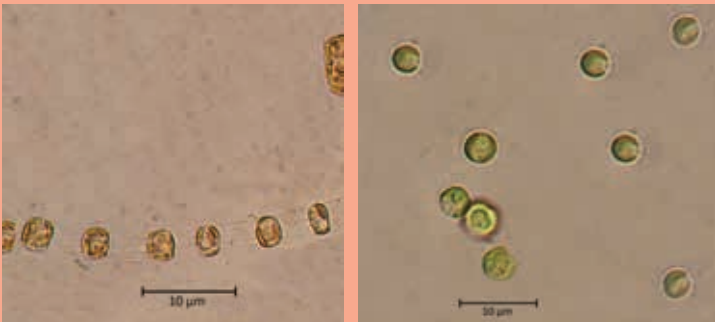
## Larval food production

To support AQD's hatcheries and research activities, the larval food laboratory served 115,760 liters of microalgal starters and 70 kg (wet weight) of *Artemia* biomass. The laboratory also sold 3,559 liters of microalgal starters/rotifers to 87 clients comprising of the private sector (local, 50%; foreign, 7%); academe (28%); and government institutions (15%).

Of the 3,559 liters of liquid starters sold, *Skeletonema tropicum* made up 41%; *Nanochlorum* sp., 34%; *Chaetoceros calcitrans*, 15%; *Brachionus rotundiformis*, 2.6%; *Tetraselmis tetrahele*, 2.3%; *Chlorella sorokiniana*, 1.5%; *Navicula ramossissima*, 1.3%; *Thalassiosira* sp., 1.3%; *Isochrysis galbana*, 0.8%; *Amphora* sp., 0.1%; *Rhodomonas* sp., 0.1%; *Chaetoceros muelleri*, 0.1%; *Chlorella ellipsoideae*, 0.1%.

Concentrated microalgal *C. calcitrans* paste was also produced. Eight test tube cultures of *C. calcitrans*; four of *Thalassiosira* sp.; one of *S. tropicum*; and two each of *I. galbana*, *N. ramossissima*, and *C. sorokiniana* were sold. Fertilizers/media sold: 112 liters TMRL, 80 li F medium and 13 li Conwy.

PHOTOS BY D CATEDRAL



*Skeletonema tropicum* [LEFT] and *Nanochlorum* sp. are best-sellers in 2013



PHOTOS BY RH LEDESMA



PHOTO BY JM DELA CRUZ

Rabbitfish harvest from the semi-intensive grow-out culture at AQD's brackishwater ponds in Dumangas, Iloilo [TOP-MIDDLE]; sacks of feeds formulated and milled at AQD

# Promoting healthy and wholesome aquaculture (HWA)

Aquaculture plays a big part in the global fish production and in meeting increasing demand for fishery products. As countries move to intensify aquaculture production for both local and international markets, problems continue to arise especially with the many challenges (eg. economic, ecological, climatic changes) that the industry is facing.

HWA is one of the thematic programs being undertaken by SEAFDEC/AQD to provide solutions to these problems and make available a more effective approach to sustainable food-fish production that will provide the protein needs of the growing human populace. This is one means by which yield from various production systems may be optimized with the least impact on the environment. The optimization and production sustainability shall be based on best management strategies and good aquaculture practices. R&D on these aspects are continuously being done.

The strategies invoked in the HWA program concentrate on nutrition and feeding to promote healthy farmed aquatic animals, disease diagnosis, control, monitoring, surveillance of aquatic animals, environmental integrity, certification, and food safety. The main goal is to improve aquaculture production through innovations in nutrition and feeding and fish health management and in preservation of the environmental integrity of aquaculture.

For the nutrition and feeding aspects, studies have been conducted to reduce the dependence on fish meal as the source of protein in formulated feeds. The focus is on the utilization of soybean meal, soy protein concentrate, mung bean meal, cow-pea meal, distillers dried grains with soluble (DDGS), and milkfish by-product hydrolyzate. The search for suitable alternative fish feed ingredients now requires an application of combined biochemical engineering and manufacturing to enhance the nutritional composition of non-traditional protein sources. There is the need to choose fish meal substitutes and other feed ingredients that will not have any bad effects on fish health and likewise will not alter meat composition of the animal. Extension and transfer of technologies when available are being implemented in order to better educate the fish farmers of their responsibilities about proper feed

management and efficient feeding practices as key components for farm profitability and sustainability as well as in the reduction of environmental pollution. Further, continuous refinements in fish feed development are likewise being carried out since feed is a very important key determinant of fish health.

For the fish health component, environment-friendly approaches including the development of probiotics comprised of indigenous microbiota isolated from wild and cultured fish and crustaceans (shrimps and crabs) and their rearing environments, and the isolation of bioactive compounds from Philippine seaweeds have been undertaken. While *in vitro* data provide some promise, their practical application *in vivo* either through direct inoculation or indirectly via feed supplementation warrant further investigation. Surveillance of viral, bacterial, and parasitic pathogens through the use of conventional and molecular approaches has also been instituted to identify emerging and re-emerging diseases affecting cultured fish and crustaceans. Convenient and economically sound methods for the detection of etiologic agents implicated in mass mortalities have been explored *in situ* (farm level), however, optimization of these methods still require thorough investigation. Finally, the usefulness of terrestrial plant extracts as antibacterial agent and as well as potent upregulator of genes involved in innate immunity of fish and crustaceans is being examined. Data that will be generated from these studies will serve as springboard in the formulation of pragmatic and environment-friendly protocols for sustainable production on cultured organisms.

## Finding fish meal substitutes and developing effective feed management schemes

Experiments on optimizing feeding management of milkfish with a SEAFDEC-USB (United Soybean Board) diet at AQD's Igang Marine Station was completed. Results showed that after 120 days of culture (DOC), daily feeding (treatment 1) had the highest mean weight gain of 1,118% and average body weight of 418 g from the initial stocking weight of 35 g. Culture period for the other treatments (treatment 2, feeding skipped every other day; treatment 3, feeding skipped every two days) was extended to try attaining the harvest weight of >400 g. However, fish were short of the target size, weighing 326 g and 352 g, respectively, for treatments 2 and 3.

Results of experiments to test the performance of cowpea and mung bean-based diets for milkfish in brackishwater ponds after 84 DOC has been completed. Milkfish fed the mung bean and the cow pea based diet had higher ABW of 448 g and 421 g, respectively, from the initial body weight of 103 g and 101g. Those fed the commercial diet attained only 368 g ABW. Feed conversion ratios (FCR) for the two diets (1.55 for mung bean and 1.35 for cow pea) were likewise better than the commercial diet (FCR=1.71).

To improve nutritional value, locally available feed resources (ipil-ipil leaf meal and cowpea meal), were processed or fermented through microbial action. For ipil-ipil leaf meal as substrate, three trials using *Bacillus cereus* isolated from the gut of milkfish as inoculum were done in 9L scale (treatment A: 10<sup>6</sup> CFU/ml; treatment B: negative control). Results showed that the crude protein and enzyme activities of treatment A (except for phytase) were significantly higher compared to B.

For cowpea meal as substrate, the first trial yielded high activities for carbohydrases and phytase and moderate activities for protease and lipase. Fermented cowpea also showed higher enzymatic activities (except for protease) compared to fermented ipil-ipil.

Proximate composition of milkfish offal and DDGS (distillers dried grain soluble) showed these unconventional feedstuff to have 38% and 30% crude protein, respectively. Feeding trials to test the performance of a hydrolysate-based diet for grouper and a DDGS-based diet for milkfish are on-going. The ingredients are eyed as potential protein sources to replace fish meal.

## Developing feeds for different growth stages

There appears to be no sparing effect of lipid on protein in diets developed for pompano fingerlings (1.7g). Lipid dietary level at 8%, 11%, and 14% made no difference in terms of growth rate in three levels of protein (38%, 46%, 54%). Final weights of fish from an initial weight of <2 g were 31 g, 43-49 g and 52-54 g for the respective protein levels regardless of dietary lipid. Pompano weighing 2 g and 70 g, both grown in tanks and cages, showed high protein requirements which did not taper off even at 54% protein level. Fish are probably after some specific amino acids that have not been provided even at the highest level of protein in this experiment.

Mud crab fed SEAFDEC-formulated diet containing the highest (48%) protein level had 100% survival and reached up to third molt in tanks compared with crab fed trash fish and commercial feed. Initially, crab fed trash fish molted ahead of crabs fed formulated diets, but this changed in the latter part of the culture. On the other hand, commercial feed was more water-stable compared to SEAFDEC diets. Experiments to find better binders are underway.

## Promoting better FCR and adequate fish nutrition

For the pilot-scale production of pellets suitable for mud crab, results of feed preference test showed that the crab preferred spherical balls and cube shapes compared to spaghetti-like or tablet shapes. The preference maybe attributed to the crab's easy grasp of balls and cubes when using their chela or walking legs. For crabs weighing 50-100 g, feed consumption is 3% of their body weight although this did not differ significantly among the shapes tested.

Experiments were done to test the effective substrate area, stocking density and shelter orientation appropriate for the production of giant freshwater prawn (*Macrobrachium rosenbergii*) in cages. Results showed no significant differences in length, weight, survival, condition factor of juvenile prawn given 0, 40, 80, or 120% substrate area. Periphyton productivity in cage culture seemed to best support the lowest stocking density (5pcs/m<sup>2</sup>), giving significantly higher mean weight, daily growth rate and specific growth rate of prawn compared with densities of 10 and 15 pcs/m<sup>2</sup>. However, no significant effects on survival and on natural food biomass were observed among the stocking densities tested. Shelter orientation also had no significant influence on growth and condition factor of prawn stocked at 10-15/m<sup>2</sup> but it mattered on the survival of prawn stocked at the lowest density.

Semi-intensive grow-out culture of pompano *Trachinotus blochii* using commercial diet in brackishwater ponds is feasible. Two batches of pompano juveniles (initial ABW of 68 g and 54 g) fed commercial diet grew to ABW of 402 g and 413 g after 165 days of culture (DOC). Survival is 100% in both batches.

Sensory test of the pompano revealed fresh odor, sweet flavor, and soft texture. Product net income is P140,161 with 226% ROI, 0.43 year payback period, P161 production cost/kg and 1,192 kg break-even volume.

Semi-intensive grow-out culture of rabbitfish *Siganus guttatus* using formulated diet in brackishwater ponds is economically feasible. At 120 DOC, ABW of 278 g was attained with 99% survival and FCR of 2.3-2.5.

Small-scale grow-out production in net cages of *Oreochromis niloticus* and freshwater prawn *Macrobrachium rosenbergii* in a freshwater dam using SEAFDEC formulated feed has been shown to be technically and economically feasible.

Crablets were produced from production runs and nursery studies conducted at AQD's Dumangas Brackishwater Station, and stocked in two stocking densities. After 53 days, juveniles reared at 0.5 ind/m<sup>2</sup> attained 47g BW and 27% survival rate while those stocked at 1 ind/m<sup>2</sup> had 41g BW and almost the same survival. All the crabs were transferred to plastic cages to grow them as 60-90 g soft-shelled crabs. Culture is ongoing.

For the seaweed *Gracilariopsis heteroclada*, it was noted that average growth rate was higher when cultured using the fixed bottom line method (4.33% day<sup>-1</sup>) compared to the broadcast method (3.81% day<sup>-1</sup>). The highest growth was attained in the month of May and August and lowest in the month of July. Economic analysis of pond production showed a high ROI of 1,055% for fixed bottom line method and 863% for the broadcast method. Although results already indicated the profitability of the fixed bottom line method, growth performance can still be improved by optimization of the fertilization scheme.



Harvest from the semi-intensive grow-out culture of pompano at AQD's brackishwater ponds in Dumangas, Iloilo: a seine is used to gather fish to the side in the half-drained pond, after which the stock is temporarily held in a net cage before being iced and sold

PHOTOS BY JM DELA CRUZ



## Investigating the efficacy of probiotics and ensuring biosecurity on-farm

As part of the effort to develop *Bacillus* as a probiotic for shrimp larviculture, the degradation capacity of different enriched AHL-degrading mixed bacterial cultures was determined using the plate diffusion method. [AHL is acyl homoserine lactone.] The bacterial strain *Chromobacterium violaceum* CV026 was used as a bioreporter for detecting AHL. *Pseudomonas* strains P3/PME 6000 and P3/PME 6863 were used as negative and positive controls, respectively. Results showed that the enriched mixed bacterial cultures from sea sediments produced the highest degradation rate ( $1.63 \text{ mg l}^{-1} \text{ h}^{-1}$ ) while the lowest degradation rate was observed in P3/PME 6863 ( $0.13 \text{ mg l}^{-1} \text{ h}^{-1}$ ).

The effects of PHB (polyhydroxybutyric acid) supplementation in the proximate composition, total lipids and fatty acid methyl ester (FAME) profile of shrimp *Penaeus monodon* postlarvae were examined. Experimental diets with 0 (control diet), 2 and 5% PHB supplementation in SEAFDEC-formulated shrimp diets were fed twice daily at 9 and 16 h. Feeding trial lasted until test animals reached 10x its initial wet weight. Total length and weight gain of shrimp fed the dietary treatments significantly differed, with the highest increase recorded in shrimp fed 2% PHB. Survival on the other hand was highest for shrimps fed 5% PHB, but survival after 24 h exposure to ammonia concentration ( $94 \text{ mg L}^{-1}$ ) did not significantly differ among the shrimp groups.

To study bacterial diversity and algal community structure in biofilms of settlement plates for abalone *Haliotis asinina* larvae, microbes were allowed to grow by inducing unsuccessful larval rearing run with sub-optimal feeding and 75% reduction in water exchange. After 3 months of culture, 3,535 abalone juveniles were harvested (0.24% survival rate of the initial 1,500,000 veliger larvae stocked). A total of 67 bacterial strains were isolated from the biofilm of abalone rearing plates.

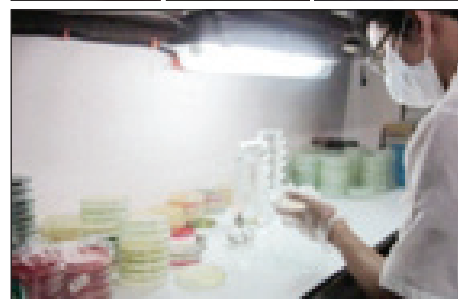
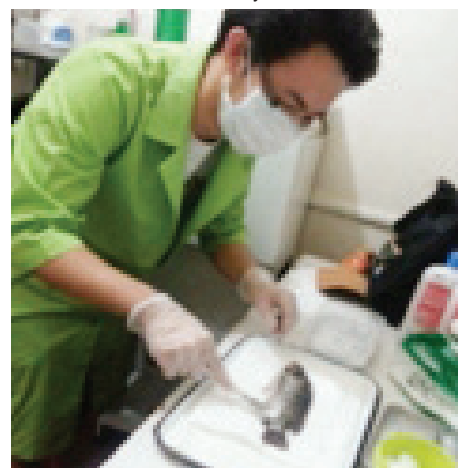
Quantitative and qualitative analyses of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in

earthen ponds are used as tools for investigating emerging and re-emerging diseases of tilapia. Fortnightly samplings from May to August 2013 were done on pond water and sediment, gills and intestine of tilapia in six grow-out ponds in Balaring (n=4) and Lantad (n=2), Silay City. The total heterotrophic aerobic bacterial count (THABC) in the water and sediment ranged from  $1.4 \times 10^3$  to  $8.9 \times 10^5$  CFU/ml and  $4.2 \times 10^3$  to  $1.3 \times 10^6$  CFU/g, respectively. Presumptive *Vibrio* count (PVC) in the pond water ranged from  $4.0 \times 10^1$  to  $7.2 \times 10^3$  CFU/ml whereas in the sediment, it ranged from 0 to  $2.2 \times 10^4$  CFU/g. In addition, the presumptive *Pseudomonas* and *Aeromonas* count (PPAC) ranged from  $3 \times 10^1$  to  $5.2 \times 10^3$  CFU/ml and  $2 \times 10^1$  to  $3.4 \times 10^3$  CFU/g, respectively, for pond water and sediment. The THABC in the gills and intestine of tilapia (5 fish/sampling) ranged from  $4.8 \times 10^5$  to  $5.6 \times 10^7$  CFU/g and  $1.6 \times 10^4$  to  $6.3 \times 10^7$  CFU/g, respectively. On the other hand, PVC ranging from  $1.4 \times 10^3$  to  $3.2 \times 10^6$  CFU/g and  $4 \times 10^1$  to  $1.3 \times 10^6$  CFU/g were respectively noted in the gills and intestine of tilapia examined. Additionally, the PPAC in the gills of fish examined ranged from  $1.3 \times 10^4$  to  $6.9 \times 10^6$  CFU/g while in the intestine, it ranged from  $2.8 \times 10^2$  to  $4.2 \times 10^5$  CFU/g.

Three to five dominant colonies were randomly selected from each sample

(pond water and sediment, tilapia gills and intestine). To date, a total of 685 bacterial isolates have been purified and stored at  $-80^\circ\text{C}$ . As for the water parameters in the source ponds, no significant variations were noted in water temperature ( $30\text{-}32^\circ\text{C}$ ), salinity (5-10ppt), and pH (7.9-8.7). But there were significantly wide variations in the levels of dissolved (5-9ppm), total dissolved solids (4- 8g/L),  $\text{NH}_4$  (0.14- 0.51ppm),  $\text{NO}_3$  (13-22ppm) and  $\text{NO}_2$  (0.01-0.04ppm).

Dissection of fish samples to get the gills and intestine [TOP]; bacteria are cultured in three types of agar plates (TSA, TCBS, GSP) [L-R, UPPER MIDDLE]; bacterial colonies are purified [LOWER MIDDLE] and stocked at  $-80^\circ\text{C}$  [BOTTOM] for reference and further analysis



PHOTOS BY LD DELA PENA

PHOTOS BY RV PAKINGKING JR

Biofilm on the surface of abalone larval rearing plates [MIDDLE]; sampling of biofilm on the plate surface was done in a 2x2 inch area using a sterile spatula [BOTTOM]



PHOTOS BY EA TENDENCIA

Where mud crabs are grown in the Philippines [L-R]: aquasilviculture ponds with mangrove trees inside the net/pen; monoculture in brackishwater ponds and mudcrab fattening using plastic containers [ARROW] in northern Samar

### Promoting the wider use of conventional and new diagnostic tools for emerging diseases

The epidemiological study and elucidation of the spread route of shrimp viral diseases in Southeast Asia is on-going. Shrimp samples, gills and pleopods were taken from 10 of 15 farms in the Zamboanga peninsula in southern Philippines for possible viral detection/analysis. A total of 14 shrimp farmers were interviewed: 11 are into *Penaeus monodon* extensive polyculture with milkfish/crab; two are into extensive monoculture one is into semi-intensive monoculture; one farm was experiencing mortality during the sampling/interview. None of the farmers interviewed observed yellow head disease in the present crop.

To identify and describe the diseases affecting farmed and wild mud crab, ten provinces in the Philippines were visited: Albay, Bataan, Cagayan, Camarines Sur, Camarines Norte, Capiz, Northern Samar, Pangasinan, Quezon, and Sorsogon. A total of 280 crab samples from three

types of culture environments (poly/monocultured pond, aquasilviculture and natural bodies of water) were processed for bacterial and fungal isolation, parasite detection and viral detection. External examination showed several abnormalities such as short abdominal flap; discoloration (blackish, rusty, reddish/pinkish) of the abdominal region; dark carapace; necrotized holes on carapace, claw and legs; presence of 'lumut'/fouling organisms; and presence of barnacles *Balanus* sp. on the carapace. Internally, observed abnormalities are the presence of stalked barnacles *Octolasmis* spp. on the gills, necrotic gills, black/brown gills, and discolored gonads (black, greenish). A few pure bacterial cultures were isolated from the sampled mud crab. Isolates are for identification, pathogenicity test and correlation with disease signs or abnormalities. No fungal infection was observed. Sample analysis for viral detection is on-going.

Mudcrab with normal, whitish abdominal region [BELOW]; crab with diseased, rusty abdominal region [BOTTOM]



PHOTOS BY EA TENDENCIA

### Finding effective and safe alternative drugs / chemicals / natural products to manage diseases

Immune parameters of sea bass *Lates calcarifer* are being determined after experimental exposure of juveniles to the parasitic *Amyloodinium ocellatum*. The parasite was isolated from a natural outbreak that occurred in AQD. The availability of infected fish was maintained by continuously cohabiting infected with naive fish in tanks. Serum samples from exposed fish were collected and stored at -80°C and head kidney primary cells were

isolated for assays of immune parameters. Several runs were conducted.

Experiments were designed to describe the infection of *A. ocellatum* on seabass. Dinospore infectivity or the probability that a dinospore infected a fish per day was estimated as the proportion of dinospore that result in trophonts (experiment 1); dinospore 48-h LD50 and trophont lethal loads at 48-h LD50

(experiment 2). Dinospore infectivity on pompano was done by using two replicates of one pompano placed in each aquaria containing 3L of water added with different numbers of dinospores: 1,000; 10,000; 100,000; 500,000; 1million. Parasites were allowed to attach to fish for 24 hours, and then fish were harvested. Trophonts were removed from fish body surface and gills, allowed to transform to tomites and counted to determine dinospore infectivity. Highest infectivity was 7% at 1million dinospores in 3L of water or 3,330 dinospores per L. For the LD50 experiment, the dinospores were added at 666; 1,332; 1,998; 2,664; and 3,330 d/L. Dead fish were recorded for 7

days post challenge. The results showed that 50% of the fish died between days 2 and 3 for all treatments and 100% on days 5 for 3,330 and 2,664 d/L, day 6 for 1,998 and 1,332 d/L, and day 7 for 666 d/L.

Optimization of *A. ocellatum* through *in vivo* passage procedure using pompano and siganids (2.5-5 cm long) was also conducted. This was done by cohabitation or adding naïve fish to tanks containing dinospores or tomonts. Pompano died within three days and siganids within six days. Their susceptibility made them ideal species to maintain the parasite. Dead fish were left in the tank for 3-8 h to allow trophonts to transform to tomonts and fall to the tank bottom. Dead fish were then removed, and new naïve fish added. Every two weeks, 2-4 infected fish were transferred to a new tank and were cohabited with naïve fish. This was to keep the parasite in water with good quality to prevent growth of contaminating microorganisms. The parasite has been successfully passaged for nine months with this procedure.

To examine *Amyloodinium* dinospore infectivity, 45 seabass larvae (23 mm, 0.14 g) were placed individually in aquaria containing 1L aerated seawater. Parasites were added at different concentrations: 500 dinospores per liter, treatment I; 1,000 for II; 1,500 for III and 2,000 for IV; the control treatment has no inoculum. Dinospores were allowed to infect the fish for 24 hours, the fish were harvested, and the number of trophonts that attached to the fish body and gills were counted. A method for quantifying attached trophonts was developed. Results showed dinospore infectivity as 0.22%, 0.19%, 0.15%, and 0.05% for treatments I, II, III, and IV respectively. The trend showed that the higher the concentration of dinospores, the lower the infectivity.

The trend was confirmed in another experiment where 2.54 cm sea bass were placed in 250 ml containers and allowed to be infected for 24 hours with 100,000 dinospores (25,000/L). Infectivity was determined to be 0.02%. The result suggested that for sea bass fry there is a certain threshold concentration for dinospores to be infective and increasing the dinospore concentration above this does not result in increased trophont attachment and infectivity rate. The lower infectivity rate of sea bass (0.02-0.22%) compared to pompano (7%) indicates that seabass is more resistant to *Amyloodinium*.

Additionally, to determine the genes expressed and the levels of expression during infection, tissue samples were

collected both from moribund naturally infected and experimentally infected fish for monitoring the expression of immune response genes by RT-PCR. Seabass (29 cm mean TL, 250 g mean weight) were challenged with *A. ocellatum* by cohabiting with 10 moribund pompanos with at least 800 trophonts attached in each fish (70 trophonts in each side of the gill) for 24 h. Fish were maintained in 250L tanks, and were sampled for gills, spleen, and head kidney at different time points: 0 h, 6 h, 12 h, 24 h, 2 d, 4 d, 8 d, 16 d, and 32 d. The tissues are stored in TRIzol at -80°C for RNA extraction. Infected and control cDNA library will be constructed and submitted for sequencing. From the sequences, primers will be designed to analyze changes in tissue-specific gene expression over the course of the infection cycle.

A new antiviral screening protocol was developed and modified based on published procedures to better investigate seaweed extracts and carrageenan. Instead of testing the antiviral activity directly during virus titer assay, virus suspension pre-treated or co-treated with test seaweed extract or commercial carrageenan were allowed to grow in susceptible fish cell lines first for at least 48 hours (or until sufficient CPE or cytopathic effect is observed in the positive control). After this, virus titer assay was conducted in the collected virus-extract suspensions.

For this modified protocol, several optimization tests were conducted in order to determine the appropriate virus dilution (that would induce the right level of CPE 48-72 h after inoculation) and extract concentration (that would not be cytotoxic to the cells). In addition, several methods to prepare and sterilize the seaweed extracts were also tested in order to ensure that the resulting extract for antiviral screening is free from bacterial and fungal contaminants but still retained most of its bioactive compounds with potential antiviral activity.

The first run of virus titer assay and anti-SVCV (spring viremia of carp virus) screening had been conducted but these need to be repeated to confirm results. It is noteworthy that significant virus titer reduction was observed in seaweed groups co-treated with mixed kappa, iota carrageenan and lambda carrageenan (0.65-0.85 log). However, it is not clear if the observed reduction is due to the action of bioactive compounds (i.e., carrageenan) or due to the reduced “opportunity” of the virus to access (and infect) the EPC cells due to the high viscous nature of the said carrageenan suspensions.

In search of novel approaches to control diseases in mud crab *Scylla serrata*, samplings to get and isolate putative probiotics from live crab and pond sediment were done in Pontevedra, Capiz;



PHOTOS BY E AMAR

Ajuy, Iloilo; Aklan; and Roxas City. Thirty-nine bacterial isolates from crab body surface and hepatopancreas, and pond sediments were submitted for genus level identification using conventional biochemical tests. Gram-staining yielded 29 gram-negative and 10 gram-positive isolates. For the gram(-) isolates, bacteria were all rod-shaped. Further tests such as glucose fermentation, Na<sup>+</sup> requirement for growth, and growth in TCBS and GSP agar media revealed that 27 out of 29 (93%) gram(-) isolates belonged to genus *Vibrio*, and the remaining two gram(-) isolates (7%) were *Aeromonas*. For gram(+) isolates, nine belonged to *Corynebacterium* and one was *Staphylococcus*. Pathogenicity testing will be conducted on these isolates to ascertain their suitability as probiotics for crab. Additional isolates from sampling areas in Sorsogon and Samar will be characterized.

Commercially-available natural products with reported antimicrobial activity were purchased from local suppliers. Leaves of terrestrial plants and mangrove trees with reported antimicrobial activity were also collected from Iloilo and Aklan, air-dried and ground into fine powder for solvent extraction of antimicrobial substances. Natural products and powdered leaves of plants were solvent extracted with either

90% or 70% ethanol and the crude extracts were tested for *in vitro* antimicrobial activity against aquaculture and human pathogens (ie. *Vibrio harveyi*, *Vibrio* sp., *Micrococcus luteus*, and *Escherichia coli*). Cell free supernatants from 24 h cultures of putative probiotics were similarly tested for antimicrobial activity. Crude extracts from ginger *Curcuma longa*, golden seal herb *Echinacea aungustifolia*, rambutan *Nephelium lappaceum*, and the tropical almond *Terminalia catappa* showed significant antibacterial activity against *V. harveyi* with inhibition zones of 10 cm or more. *C. longa* and *T. catappa* also showed antibacterial activity against *M. luteus* and *E. coli*.

In addition, 200 crablets (2.74 g ABW) were obtained from AQD's Dumangas Brackishwater Station and stocked at 10 crablets per tank in 20 60-L fiberglass tanks provided with shelters. Pathogenic *V. harveyi* was passed in live crab three times before injecting different dilutions of a 24 h culture to determine the median lethal dose (LD50). This dose will be injected to crablets that had been fed extract supplemented diets, and the crabs observed for mortality. *In vivo* efficacy will be assessed as the ability of the extracts to inhibit mortality due to *V. harveyi* infection.

## Regular and specialized training

Under the HWA program for 2013, AQD organized and conducted nine training courses, one of which was a distance learning course on fish nutrition and another an onsite course on fish health in Myanmar.

**Aquaculture technologies**, 7 January–8 February, held at AQD's Tigbauan Main Station (TMS). Mr. Benny Lim, a college student from Brunei Darussalam studying in Winchester, United Kingdom completed the specialized course. His training covered topics on natural food culture, culture of various species like mud crab & abalone, and fish health management.

**Aquaculture of selected marine species**, 24 October–19 November, TMS. Mr. Robert Ewald, a businessman from Ontario, Canada attended the specialized course. The



[CLOCKWISE] The specialized training course on aquaculture technologies; the GOJ-funded course on freshwater aquaculture where trainees try their hand at spawning bighead carp; and the bacteriology course for Palawan students



training covered lectures and practical sessions on rearing milkfish, grouper & shrimp, natural food production, and processing of milkfish and tilapia diet.

**Community-based freshwater aquaculture for remote rural areas of Southeast Asia**, 25

November–4 December, TMS and BFS. Nine trainees from Cambodia, Egypt, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines and Vietnam completed the course. The lectures and practicals included the overview of freshwater aquaculture in Southeast Asia; hatchery, broodstock management & grow-out of commercially important freshwater species like tilapia, bighead carp, catfish, and freshwater prawn; aquatic ecology; design & construction of tanks, cages, pens, and ponds; preparation of feeds; major diseases; and sustainable management of small-scale farms. Participants also visited BFAR–National Fisheries Biological Center in Batangas and a catfish farm in Bulacan. The training was initiated through the Government of Japan Trust Fund to help Southeast Asian countries adopt freshwater aquaculture.

**Principles of aquaculture nutrition**, 22 July–12 December, a distance learning course conducted through the internet. It had 17 learners, joining from Brunei Darussalam (1) Indonesia (1), Myanmar (1) – all three with GOJ fellowships – and the Philippines (14). The course discussed the advances in aquaculture technology that have made fast fish growth and high yields possible, in particular fish nutrition and adequate feeding. It covered: nutrient requirements & effects of nutrient deficiencies on aquatic species; feeding behavior, digestive physiology & digestibility; aquafeed formulation, processing &

evaluation; and management & economics of feeds & feeding. Emphasis was given to tropical species like milkfish, tilapia, tiger shrimp, and other species of commercial importance. Aspects of feeds & feeding related to the conservation of the aquatic environment were also dealt with.

Offered in 2002, 2003, 2011 and this year, *AquaNutrition Online* (ANOL) was developed by AQD and the University of the Philippines Open University (UPOU). The current platform is powered by the open-source eFront.

**Abalone cage grow-out**, 12–13 April, TMS. This was a course for 20 participants from Western Philippines University in Puerto Princesa, Palawan. It covered lectures on culture of tropical abalone and practical session on the fabrication of cages for abalone grow-out culture.

**Freshwater fish health management with emphasis on detection of fish-borne zoonotic parasites**, 20–25 October. This was an on-site course held in Tharketa, Yangon, Myanmar for 11 officers of DOF (Department of Fisheries) and one farmer. The course was held by AQD with funding and logistical support from GOJ-TF and DOF-Myanmar.

The course was meant to enhance the skills of previously (2011) trained fish health workers on detection of freshwater fish zoonotic parasites collected from various Yangon sites. With AQD’s 2010 textbook on *Health management in aquaculture* as reference, the lectures covered theoretical and practical knowledge on signs & epizootiologies of economically-important freshwater fish diseases; evaluation of fish epizooties; submission of diseased fish samples for diagnosis; and basic laboratory skills for detection of parasitic pathogen with emphasis on zoonotic parasites (fishborne diseases). The practicals included methods of detecting parasites and a one-day field trip to a freshwater farm for actual fish sample collection and hands-on performance of parasite detection techniques.

**Bacteriology**, 20–24 May, TMS. Five teachers from Tubungan National High School in Iloilo completed the specialized course which included practical sessions on the preparation of bacterial media, principles of sterilization & aseptic techniques, bacterial sampling from water samples, characterization of bacterial isolates for genus level identification and antimicrobial assay.

**Microbiology**, 27–29 May, TMS. Ms. Mary Faith Balanay, a quality assurance supervisor from General Santos City, Philippines, attended lectures and practicals on the preparation of media, culture of *S. aureus*, *Vibrio*, *Salmonella*, yeast & molds, decontamination procedure and counting bacterial colonies.

**Fish health management in aquaculture**, 11–15 November, TMS. Twenty-five veterinarians from BFAR (Bureau of Fisheries & Aquatic Resources) regional and central offices finished the course. Lectures and practical sessions covered topics such as sustainable aquaculture, major diseases of marine fishes & crustaceans, diseases prevention & control, and techniques in disease diagnosis. In his message, AQD scientist Dr. Rolando Pakingking Jr, who is the technical lead person, hoped that this BFAR-funded activity will bring more collaborative endeavors between AQD and BFAR

[SHADED ROW BELOW]The on-site course in Myanmar: trainees process fish samples on-site for detection of fish-borne zoonotic parasites



PHOTOS BY G PAGADOR



[BOTTOM SHADED ROW] BFAR veterinarians learn fish health: collection of fish samples from the cages at AQD’s Igang station for parasitological examination; demonstration of fish necropsy; and collection fish blood and organs

## 2013 production from ponds and cages...

AQD earned from its research by-products of more than 20 tons of table-sized milkfish, pompano and other species. Income from ponds in AQD's Dumangas station and cages in Igang and Binangonan stations amounted to >PhP 2.4 million.

Species	Production (kg)	
	Ponds	Cages
Milkfish	4,781	5,897
Pompano	1,777	3,078
Snapper	1,294	11
Grouper	938	-
Seabass	736	158
Rabbitfish	366	-
Abalone	7.5	2
Mud crab	51	-
Tilapia	-	2,440
Giant freshwater prawn	-	104
	9,950.50	11,690

## ...and feedmill

AQD produced >90,000 kg of various feeds, with 29% going to the private sector. Of the feeds, milkfish, siganids, pompano and grouper topped the list requested by the fishfarmers and used in AQD studies. Income from feeds amounted to Php 916,040 in 2013.

Species	Production (kg)		Total (kg)
	AQD	Private sector	
Milkfish	31,950	23,379	55,329
Siganid	15,305	-	15,305
Pompano	7,757	-	7,757
Grouper	2,635	1,515	4,150
Seabass	3,840	227	4,067
Tilapia	1,765	38	1,803
Snapper	1,605	-	1,605
Mud crab	370	355	725
Shrimp	51	584	636
Prawn	320	9.4	330
Abalone	15	30	45
Feed ingredients	279	94	373
Mixed feeds	-	201	201
Others	-	119	119
	<b>65,892</b>	<b>26,552</b>	<b>92,443</b>

## Fish health diagnostic services...

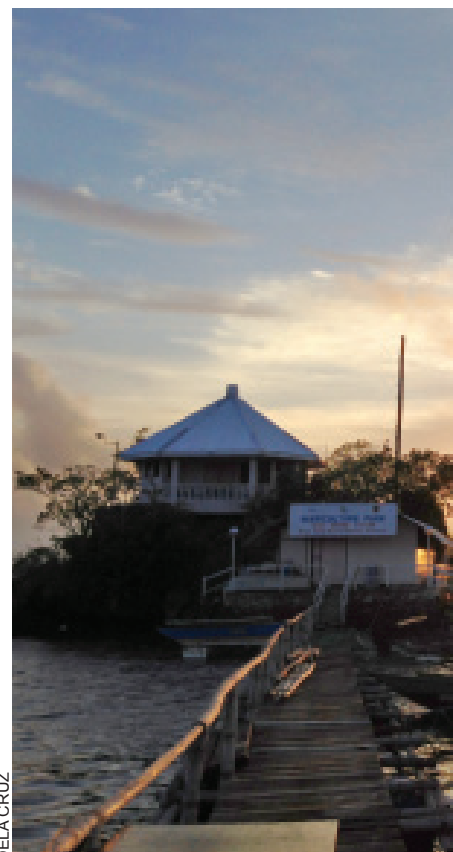
At AQD's fish health laboratory, a total of 318 diagnostic cases were examined consisting of shrimp (50%); fish (19%); crab (17%); mollusks (5%); water (5%); soil (0.6%); and other samples such as *Artemia* / seahorse / annelids / oyster / slugs / rabbit / white mouse (3.5%).

The eight viral diseases diagnosed by PCR were: (a) WSSV in shrimp (30 of 361 samples were positive) and in mud crab / others (2 positives of 112); (b) IHNV (15 positives of 119); (c) IMNV (107 samples all negative); (d) TSV (98 samples all negative); (e) YHV (3 samples negative); (f) VNN (20 of 54 samples positive); (g) RSIV (all 5 negative); (g) MBV (5 of 40 samples positive).

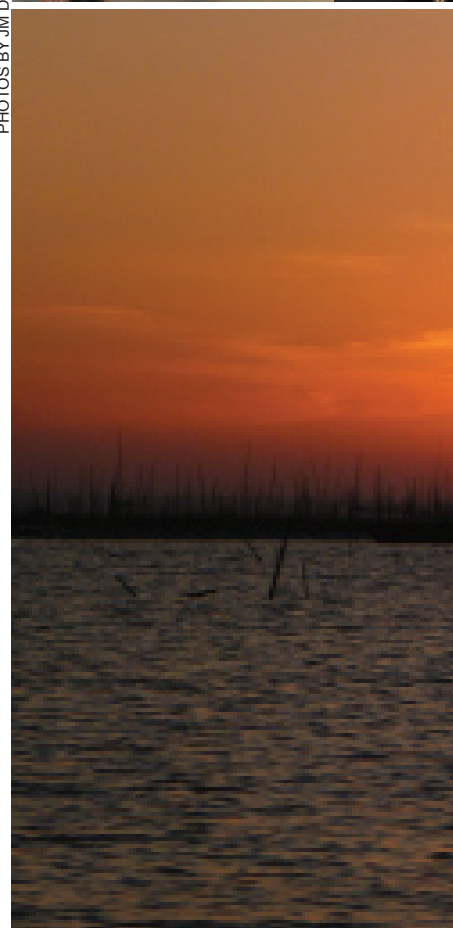
AQD also accepted samples for bacterial count (349 samples), bacterial identification (45), presence of parasites (6 of 16 samples positive), and histology for research / disease diagnosis (1,339 slides).

## ...and LFAAT

AQD's Laboratory Facilities for Advanced Aquaculture Technologies (LFAAT or the biotech lab), accepted for analysis a total of 4,220 samples (~11,438 analytes) from AQD research studies (94%) and the private sector (6%). LFAAT work included proximate analysis for crude protein, crude fat, crude fiber, calcium, phosphorus, moisture, and ash; fatty acid profile; water pH, alkalinity, ammonia-N, ammonium-N, chlorophyll, dissolved oxygen, nitrite, nitrate, phosphate, sulfide, total hardness, total suspended solids, and pesticide residues; soil pH, organic matter, available Fe / S / P, and CNS; microbiological analysis for APC, *Salmonella*, *Shigella*, *Staphylococcus aureus*, coliform, *Escherichia coli*, *Vibrio*, yeast & mold, and water potability; and scanning & transmission electron microscopy.



PHOTOS BY JIM DELA CRUZ





# Maintaining environmental integrity through responsible aquaculture (MEITRA)

The MEITRA thematic program addresses issues on the negative impacts of aquaculture on the environment and how these impacts will be minimized. The goal is to develop environment-based aquaculture technology by integrating environmental factors in SEAFDEC/AQD research activities and by promoting responsible aquaculture practices.

For 2013, monitoring of AQD's three stations and assessment of the fry fishery in Panay were well underway; studies on extractive species like sandfish, seaweeds and mollusk being done; modelling of Philippine lakes completed; and resource enhancement using giant clam continued.

## Assessing impacts on biodiversity, water-sediment quality in culture areas and adjacent ecosystems

As a first step to assess the impact of aquaculture, AQD has been regularly monitoring its stations:

**Igang Marine Station (IMS) and its surrounding area:** The bathymetric profile has been completed, and a monitoring system for the water and substrate quality established. For biodiversity, a total of 805 species in 292 families in 20 major taxa were identified.

**Tigbauan Main Station (TMS):** Sampling of shore areas is being conducted. Some 516 species in 199 families in 10 major taxa have been collected or photographed. Based on preliminary results, TMS intertidal area looks depauperate compared to IMS.

**Binangonan Freshwater Station (BFS):** A comparison of phytoplankton, zooplankton and fish and other vertebrate & invertebrate diversity in two sites around the station, the east cove (an aquaculture site) and the west cove (non-aquaculture site) was started in March 2013. Although total phytoplankton density was higher in the west, no significant differences in the diversity indices such as species richness ( $s$ ), Shannon-Weiner Index ( $H'$ ) and evenness Evenness ( $J'$ ) were observed. Zooplankton density did not differ between the two sites, although  $H'$  and  $J'$  were both significantly higher in the west cove. Based on the data from fish traps set-up in the two sites, much higher biomass of fish per day were caught in the east compared to the west cove. Significantly higher  $H'$  and  $J'$  were observed in east than the west cove. Dominant species included invasive alien species such as the knifefish, *Chitala ornata* in the west cove and janitor fish *Pterygoplichthys* sp. in the east cove have been identified.

A study on the biodiversity in the milkfish and shrimp fry ('semilya') fishery in the surf zones in southern and western Panay is also being implemented. Various fry collection sites in the Antique and Guimbal were sampled. During the fry gathering season, fry collection is done mostly during the flood tides (~2-4 h operation per day) of the 3 days around the new moon and full moon, when 2,000-15,000 fry could be obtained in one day's operation. The fry collectors pointed to a decline in fry catch from previous years, and blamed ricefield pesticides, oil spills, and fishing for 'sabalo'. More than 12 species of fish larvae and juveniles were identified in catches from Guimbal and Antique.



AQD's Igang Marine Station in Guimaras

## Identifying extractive species for IMTA (integrated multi-trophic aquaculture)

Various extractive species are being investigated. Among them the sandfish *Holothuria scabra*, the bivalve mollusk *Anodontia philippiana* and the seaweed *Gracilariopsis heteroclada*. The co-culture of these extractive species with other commercially important commodities is being investigated.

Studies on sandfish in polyculture with other marine species are on-going. Production runs with milkfish were started in IMS pens and in Igang pond in May and June but high mortality due to disease and salinity fluctuation (due to heavy rains) were experienced in pens with milkfish+sandfish and in pens with milkfish only. Experiments with pompano were started in August.

Co-culture of seaweed with seabass is also investigated. After 150 days of culture, the specific growth rate (SGR) of seabass in monoculture and co-culture with seaweed was 3.7 and 3.6%/day, respectively. FCR of seabass in mono and co-culture treatments was 2.4 and 2.2, respectively, with survival rates ranging from 96 to 100%. The SGR of seaweeds ranged from 1.71- 14.0%/day, with highest growth obtained in March. Among the water quality parameters monitored, ammonia ranged from 0.009-4.55mg/l (co-culture) and 0.008-6.56mg/l (monoculture); nitrite: 0.015-0.21 mg/l (co-culture) and 0.015-0.08mg/l (mono); and phosphate from 0.034-0.49 mg/l (co-culture) and 0.034-0.43mg/l (mono).

The bivalve mollusk *A. philippiana* locally known as 'imbao' (I) is known to assimilate sulfide. Its potential and that of *G. heteroclada* (S) to improve culture conditions of milkfish (M) was investigated. The treatments consisted of M only, M+I, M+S and M+I+S. From the start of the experiment, sulfide levels significantly increased over time but did not significantly differ between treatments, except during the last sampling when both treatments with *A. philippiana* (M+I and M+I+S) had significantly lower sulfide levels. Phosphate, nitrate and nitrite levels did not significantly differ over time and between treatments, except in three samplings (starting July) when both treatments with *A. philippiana* (M+I and M+I+S) had significantly lower phosphate levels. Ammonia significantly increased over time in treatment M.

## Developing and promoting efficient and suitable environment-friendly systems

Experiments were conducted to determine optimal conditions for the culture of *H. scabra*. In the previous year, substrate type, salinity, and stocking density experiments were done to assess growth and survival of sandfish juveniles. It was noted that the substrate preference is the sandy-muddy, not silty-mud type. For pond/pen culture, a survey of various pond sites in Iloilo has been done with Concepcion, San Dionisio and Ajuy showing potential based on substrate type. AQD's Dumangas ponds were not suitable due to thick anoxic mud. The Igang pond run failed due to high mortalities caused by factors such as improper positioning of net bags, water quality, and heavy rains. For the sea-ranching of this commodity, Concepcion was selected to be the initial pilot site. Panobolon also showed good results for sandfish growth. Community preparation for the project has been completed with the agreement between AQD, LGU-Concepcion, the resource management council and the fisherfolk association being finalized.

Information on the carrying capacity of some freshwater systems in the Philippines based on modeling through an ACIAR (Australian Centre for International Agriculture Research) study has been completed. The model and sensitivity analysis showed that fish carrying capacity tonnages within the range of literature values for Asia are slightly higher than annual production recorded for some Philippine lakes. The dominant parameters driving carrying capacity were initial nutrient loading then increasing lake surface area, mean lake depth and flushing rates. In general, polymictic lakes are more productive than monomictic ones with eutrophic systems being by far more productive than either oligotrophic or mesotrophic systems. Oligo and mesotrophic lakes showed little sensitivity to carrying capacity models, suggesting that either these systems are not suited to aquaculture without carefully managed feeding strategies or else the model is not capturing important parameters that might influence productivity in these systems.

## Studying species with potential for resource enhancement

A number of species are being studied for resource enhancement: giant clam *Tridacna gigas*, abalone *Haliotis asinina*, and three species of mud crab *Scylla serrata*, *S. olivacea* and *S. tranquebarica*.

The study on giant clam in the marine protected areas (MPAs) of San Joaquin, Iloilo has been completed. More wild clams are being discovered and those that were measured showed to have good growth and high survival. AQD's presence in the area also increased the awareness of local communities on environment protection and the benefits of MPAs.

Aside from its extractive value, sandfish can profit fishfarmers: eviscerated, iced and priced at PhP 900 per kg in a stall in Metro Manila's Chinatown



PHOTO BY M. CASTANOS



## New guide on mangrove

### *Field guide to mangrove identification and community structure analysis*

By Ma. Junemie Hazel Lebata-Ramos

The field guide provides a key and images of plants and plant parts for identifying Philippine mangrove species; instructions on how to lay out quadrats and solve equations needed for mangrove community analysis; and a glossary. The guide is intended for researchers, academicians, students and those in government and non-government organizations actively involved in mangrove research.

The author Dr. Ramos is a scientist at SEAFDEC/AQD and has worked on mangroves starting 1993. She has published papers on the mangrove clam *Anodontia philippiana* and mangrove crabs *Scylla* spp. She is a lecturer on mangrove ecology, conservation and management, coastal resource management and stock enhancement in AQD training courses. Dr. Ramos is currently working on the stock enhancement of some marine invertebrates and the impacts of aquaculture to the environment.



Mangrove species identification in Ibayo ecopark in Aklan [TOP] using a 2009 guide published by PewFellows+AQD+ZSL [MIDDLE]; outplanting of mangrove at a rehabilitated pond in Leganes, Iloilo [BOTTOM]

## Training on mangroves

### *Community-based mangrove rehabilitation*, 17 – 21 June, TMS.

The USA-based ELTI (Environment Leadership & Training Initiative) funded and partnered with AQD and ZSL (Zoological Society of London) for the conduct of this course. A total of 22 trainees from three clusters of people's organizations in the Philippines (Luzon, Palawan and Northern Samar) attended. Their feedback on the course was positive, with most participants indicating that their (technical) learnings can be applied within 3-6 months in their respective project sites.

### *Mangrove conservation, rehabilitation & management*, 18 - 22

November, TMS. Twenty-two trainees successfully completed the course. They had undergone a series of lectures that covers mangrove biology & taxonomy to community's roles in mangrove conservation & rehabilitation. There were practical exercises such as species identification & outplanting and site visits.

The trainees were mainly from local government units (LGUs), provincial environmental management offices (PEMOs) and people's organizations (POs) who, at the end of the course, assured the resource persons that they will replicate the techniques of initiating a mangrove plantation in their respective places.

The course is part of the agreement between AQD and ZSL to pool resources and technical expertise to attain the vision of conserving mangroves through community-based groups. It was funded by the GIZ (Deutsche Gesellschaft für Internationale Zusammenarbeit), a German development organization.



## New facility

Co-funded by JIRCAS with AQD, a mini wet laboratory was inaugurated at AQD's Igang Marine Station on 10 October. "I learned that researchers do sampling in the dining hall of the station to avoid strong winds," said Dr. Masayoshi Saito, JIRCAS program director. "The new facility is a more conducive space."

JIRCAS and AQD also collaborate on an IMTA (integrated multi-trophic aquaculture) facility that was inaugurated 12 October 2012. Both projects were initiated by AQD visiting scientist Dr. Satoshi Watanabe.



# Adapting to climate change (cc)

The changes in the climate are projected to impact broadly across ecosystems, increasing pressures on all livelihoods and food supply chains including aquaculture. The overall goal of the CC thematic program is to identify the changes affecting fishfarmers and ensure the continued operation of fishfarms and hatcheries. For 2013, AQD worked on determining the effects of increasing temperature and other parameters on fish broodstock & larvae, and natural food.

## Generating scientific information on the effects of increasing temperature on fish reproductive performance and embryonic larval development...

There is very little information on how gonadal maturation and spawning in tropical aquaculture fishes are affected by increased or elevated water temperature as can be expected with global warming / climate change. This was investigated in rabbitfish *Siganus guttatus*. The reproductive performance of rabbitfish maintained at constant water temperature of 33°C was very poor; for a 12-month period, spawning was observed only twice and in both cases, the eggs were not hatched. Spawning success was best in breeders maintained at ambient temperature (29-30°C) followed by those maintained at 31°C. However, when the water temperature in the broodstock tanks was set to follow the diurnal temperature fluctuation (day time temp is higher than night time temp and a difference of 2°C was set between day and night time temp), the reproductive performance of rabbitfish improved significantly. As expected, gonadal development and spawning success were good in breeders maintained at ambient water temperature and in the group maintained at 31°C (day time temp) and 29°C (night temp). Interestingly, gonadal development was also significantly improved in the group exposed to day time temperature of 33°C and night time temperature of 31°C (31-33°C temp cycle) compared to fish that were exposed to constant temperature of 33°C. However, the fish still did not spawn.

The effect of elevated water temperature [ambient (28-29°C), 31°C, 33°C] on embryonic development was examined in milkfish, Asian sea bass and rabbitfish. Embryonic development success and hatching rate were always high when embryos were incubated in ambient temperature of 28-29°C. Embryonic development was aborted in all three species when fertilized eggs were incubated at 33°C. Although embryonic development proceeded normally in embryos incubated in 31°C, hatching rate was lower compared with those incubated in ambient temperature.

The effect of elevated water temperature on larval survival after 10 days of rearing was also investigated in milkfish, rabbitfish and the Asian sea bass. For these species,

survival was always best when reared at ambient water temperature. Survival was significantly low (< 2%) in milkfish and rabbitfish when reared in constant water temperatures of 31 and 33°C. Among the three, sea bass larvae survived the best at higher temperatures of 31°C (18% average survival) and 33°C (6.5% average survival).

During larval rearing of rabbitfish where the water temperatures follow a diurnal fluctuation (higher temperature during day time and lower temperature during night time with a 2°C difference), survival was not improved.

The effect of elevated temperature was examined on the embryonic development and hatching success in abalone. Highest embryonic development success and hatching rates were observed in groups reared in ambient temperature and lowest at 33°C. Results also showed that survival and settlement rate was very low in 33°C and 31°C, and highest in ambient temperature.

## ... and effects of increasing temperature, pH, salinity on reproduction and growth of natural food organisms in the hatchery

A factorial experiment to test interactive effects of temperature (29, 31, 33°C), pH (7.5, 7.8 and 8.0 ± 0.10) and salinity (20, 30 and 38 ppt) on reproduction, growth and survival of rotifer and copepods was conducted. Both are important food organisms for hatcheries.

Population growth of rotifer was significantly higher at high temperature (33°C) and low salinity (20 ppt), and low at low pH (pH 7.5), although no interactive effects on population growth was observed in combined conditions. The size of rotifers was not significantly different among treatments. No abnormality in swimming or morphology was observed in all treatments.

Two species of copepod were used: *Pseudodiaptomus annandalie* and *Acartia tsuensis*. Survival of *P. annandalie* was significantly lower in low pH (7.5) and high salinity (38 ppt) while survival of *A. tsuensis* was significantly low in low salinity (20 ppt) and low pH (7.5).

Based on the above data, rotifer and copepods are more resilient to elevated temperature but acidic conditions have significant effect on their growth and survival.

Climate change is upon us: damage to the dikes in an experimental pond in Iloilo, Philippines caused by super typhoon Yolanda / Hainan in late 2013



# Meeting social and economic challenges in aquaculture (MSECAP)

The MSECAP aims to address the recommendations which were adopted during the June 2011 *ASEAN-SEAFDEC Fisheries conference* and these cover the following areas: (1) enhancing the role of aquaculture in addressing food, income and livelihood security through improved governance, multi-agency collaboration, and comprehensive and inter-disciplinary approaches; (2) promoting sustainable aquaculture through enabling policies that support the management of natural and environmental resources; (3) enabling mechanisms, institutions and infrastructure to encourage adoption of better aquaculture practices; (4) understanding and improving linkages from production to marketing and trade of fishery products to support small and medium enterprise (SME) development; and (5) strengthening the capacity of aquaculture stakeholders by mainstreaming specific rural and peri-urban aquaculture programs and policies in local, national and international development programs.

## Prioritizing collaborative R&D in aquaculture

Lake-based grow-out polyculture of hatchery-produced giant freshwater prawn (GFP) with tilapia was demonstrated in Laguna de Bay in collaboration with a fishfarmer cooperative. Co-culture of high-value GFP with tilapia aims to supplement income of fish farmers. However, environmental constraints, in particular algal blooms (*Microcystis* sp.) that affect water quality, and harsh weather conditions in Laguna de Bay during monsoon resulted to low survival rates (39% for tilapia and 43% for GFP) and limited economic benefits. Hence, a third run is proposed to re-evaluate grow-out operations, identify improved technology adoption strategies, and enhance community participation.

As in previous years, hatchery-bred seeds did not only contribute directly to food production through grow-out culture, but also in the restocking of overfished waterbodies. Small-scale fishers, organized into a Barangay Fisheries and Aquatic Resources and Management Council (BFARMC), continue to cooperate in an action-oriented research through community-based stock enhancement of threatened high-value species in Sagay Marine Reserve (SMR) in Negros Occidental. Release strategies

and baseline studies for the stocking of sea cucumber and sea horse are being studied. For abalones, the “stock-protect-partial harvest” protocol has been successfully demonstrated and practiced by the BFARMC. The organization has accumulated funds for the maintenance of the release site, emergency loans among members, and bank savings for the organization. The released abalones remained intact in the coral areas and periodic harvesting and trade of abalones continued in spite of the devastation caused by super typhoon Haiyan in November 2013. Some gleaners reported catching and selling untagged abalone or “spill-overs” outside the demo-site.

Relevant lessons from SMR are being applied in the shrimp (*Penaeus monodon*) stock enhancement study in New Washington, Aklan. The first trial stocking and intermediate culture of shrimps intended for release was conducted. However, low survival of postlarvae (12% after 6 weeks in nursery ponds) was observed and eventually destroyed by typhoon Haiyan.

These community-based stock enhancement studies have demonstrated the importance of synergy on the biological and social dimensions of fisheries management.



PHOTOS BY ND SALAYO

[TOP] Culture of prawn + tilapia in cages in Laguna de Bay is demonstrated to fish farming families and cooperatives. [ABOVE] Ponds for intermediate culture of tiger shrimp *Penaeus monodon* for release in New Washington estuaries (Aklan) are maintained by the Pinamuk-an Small Fisherfolks Association and LGU-New Washington in collaboration with AQD and Aklan State University

Tiger shrimp larvae tested in hatcheries prior to intermediate culture and release in estuaries

With partners Sagay Marine Reserve, Peace Corps Volunteer and AQD, the elected fourth set of Barangay Molocaboc's resource management council officers celebrate Christmas even after being devastated by typhoon Haiyan/Yolanda

## Allocating R&D resources to address emerging issues

The studies under MSECAP once again revealed some emerging issues in aquaculture, such as the need for reliable supply of breeders of aquaculture species in remote rural areas; enhancement of organizational solidarity and commitment among stakeholders, and improving financial management skills to enable participation of fish farmers in domestic and international trade. Hence, studies that demonstrate hatchery and grow-out technologies for freshwater and marine species were pursued to increase production of seeds for grow-out culture and for stock enhancement. The establishment of a small-scale abalone hatchery was proposed in Punta Roma, another coastal village in Sagay City, to complement stock enhancement.

Seaweed culture was also implemented to support abalone hatchery operations and cage culture. These seed production and grow-out activities on-farm benefited from the participation of organized fisherfolk and from some training on entrepreneurial skills development.

On-farm discussions with prospective fish farmer collaborator for the promotion of giant freshwater prawn culture in municipalities around Laguna de Bay



Multi-agency collaboration in coastal area capability development on shrimp stock enhancement: Aklan State University, fisherfolk cooperative, Japan's Research Institute for Humanity and Nature, LGU-New Washington and AQD

## Enhancing multi-agency collaborations

Considering the complexity of social and economic challenges in aquaculture, multi-agency collaboration is continually pursued.

For aquaculture technologies, demonstrations of culture methods are conducted through on-farm collaboration with potential technology adoptors such as fishfarming households and fisherfolk cooperatives. AQD support comes in the form of technical guidance and some farm inputs. For stock enhancement projects using hatchery-bred juveniles, the collaboration involves: (1) fishers organized as Fisheries and Aquatic Resources Management Council (FARMC) or as small-scale fisherfolk associations (SFA) who directly contribute manpower to the project; (2) local government units at the village and city level who provide logistical and regulatory oversight; and (3) AQD and partner local academic institution, such as the Aklan State University, as providers of technology and social support. The formulation of policies and regulations necessary to implement stock enhancement (i.e. establishment of protected areas) and the measures to sustain released stocks (i.e. abalone catch size regulation) were achieved in coordination with governance units such as the LGUs through the DENR-Protected Area Management Board (PAMB). Meanwhile, rewards system involving in-kind economic incentives such as the provision of hatchery-bred abalone juveniles for ranching and cage culture were implemented to encourage active participation of fishers in community-based stock enhancement. The involvement of traders was also critical in ensuring that market practices conform to fisheries regulations and policies formulated to ensure the sustainability of the fisheries.

## AQD at the meetings of stakeholders

### CONSULTATION WITH INDUSTRY

- 1st Meeting of the ASEAN Public Private Taskforce for Sustainable Fisheries & Aquaculture (27–28 March; Bali, Indonesia; organized by Indonesia's Ministry of Marine Affairs and Fisheries and ASEAN Secretariat)
- Meeting with Shrimp Hatchery Operators Regarding Importation of *Penaeus vannamei* from Countries Infected with EMS and IMNV (22 April; Quezon City; organized by DA-BFAR)
- Shrimp Industry Consultative Meeting (18–19 February; Quezon City, Manila; organized by DA-BFAR)
- National Marine Finfish Hatcheries Consultative Meeting (23–25 May; Cebu City; organized by BFAR-Inland Fisheries & Aquaculture Division)
- Tilapia R&D Program Review-cum-Industry Consultation (19-20 June; Munoz, Nueva Ecija; organized by CLSU, DOST, BFAR, CHED)

### CONSULTATION WITH HOST AND MEMBER-COUNTRIES

- WWF Grouper/Snapper Dialogue (9–10 October; Penang, Malaysia; organized by World Wildlife Fund)
- ASEAN-SEAFDEC Regional Consultation on Common/Coordinated Position of the Commercially-exploited Aquatic Species at the CITES-CoP16 (22 January; Bangkok, Thailand; organized by the SEAFDEC Secretariat)
- Consultation for the Finalization of *Joint DA-DILG Administrative Order on the Regulation for the Conservation of Blue Swimming Crab* (10–11 April; Cebu City; organized by DA-BFAR)
- Area-Wide Orientation on the ASEAN Economic Community (AEC) 2015 Focusing on Integration Initiatives Affecting the Agriculture & Fisheries Sector (13–15 November; Cebu City; organized by DA- National Agriculture & Fisheries Council)
- Communication Planning Workshop for Agricultural Biotechnology (22 March; Quezon City; organized by DA-Biotechnology Program)

### BOARD MEETING

- PhilFin Board of Trustees Meeting/s (30 January and 20 February; Manila; organized by University of the Philippines Visayas)
- DOST Governing Council Meeting (31 January–1 February; Cebu City; organized by the Philippine Department of Science & Technology)
- NACA 24th Governing Council Meeting (25–27 February; Vietnam; organized by the Network of Aquaculture Centres in Asia)
- NFRDI 21st Governing Board Meeting (13 June; Quezon City; organized by DA-National Fisheries Research & Development Institute)

## Stakeholders input into AQD work programs

There are plenty of chances for aquaculture stakeholders to input into AQD work priorities. National and regional consultations and meetings are periodically held on various topics, with AQD representatives taking note of reviews and inputs relevant to aquaculture or AQD operations and priorities.

But the formal yearly consultation is a four-step process.

**First step:** AQD holds an *In-house review and planning meeting* (19-20 September for this year) preceded by small group meetings in August on commodities and thematic research areas. Collaborators are invited here.

**Second step:** meeting of PTAC (Philippine Technical & Administrative Committee for SEAFDEC) which oversees AQD. The committee is composed of key representatives from the industry / academe / government institutions; the meeting in 2013 was held 29 October. The meeting is meant as an endorsement of AQD activities by the Philippine government.

**Third step:** *SEAFDEC Program Committee Meeting (PCM)* which is hosted by the Secretariat (25-26 November for 2013) and attended by representatives from member-countries and collaborating partners. This is held back-to-back with the meeting of the Fisheries Consultative Group of the ASEAN-SEAFDEC Strategic Partnership. The PCM reviews accomplishments and workplans and endorses the same to the SEAFDEC Council.

**Fourth step:** meeting of the SEAFDEC Council, the center's policy-making body made up of high-ranking officials from member-countries, usually held in the first quarter of the year. The Council now approves the year's work plan of AQD and its counterpart Departments in Thailand, Singapore and Malaysia.

Philippines, as represented by DA-BFAR National Director Atty. Asis Perez [LEFT with SEAFDEC Secretary-General Dr. Chumnarn Pongsri] is this year's chair of the SEAFDEC Council



AQD Chief Dr. FG Ayson, Dr. Gavino Trono, Dr. Paciente Cordero and Dr. Maribel Dionisio-Sese at the opening of the algae symposium



## AQD co-organizes and/or hosts the meetings of partners and collaborators

**International Seminar Workshop on Mudcrab Aquaculture and Fisheries Management** (ISMAF); 10 - 12 April 2013; Tamil, Nadu, India; organized by Rajiv Gandhi Centre for Aquaculture (the R&D arm of Marine Products Export Development Authority - India) and SEAFDEC/AQD. Six scientists (Dr. ET Quintino, Dr. MJH Leбата-Ramos, Dr. VR Alava, Dr. FD Estepa, Dr. MR Catacutan and Dr. JH Primavera) represented AQD during the seminar-workshop as paper presenters and workshop leaders. Over a hundred participants listened to eight country papers and 31 contributed papers in the technical sessions (22 oral and 9 poster presentations). There were also three workshops and two field trips.

**JIRCAS Workshop on Aquaculture;** 9 October; organized by Japan International Research Center for Agricultural Sciences; hosted by AQD at its Tigbauan Main Station, Iloilo. The workshop has about 30 participants from AQD, Fisheries Research Institute – Malaysia (FRI), Department of Fisheries Malaysia (DOF), King Mongkut's Institute of Technology Ladkrabang in Thailand (KMITL), Fisheries Research Agency Japan (FRA), and JIRCAS. It discussed the studies under JIRCAS' research project on *Development of aquaculture technologies for sustainable and equitable production of aquatic products in tropical coastal areas*. These studies with topics on integrated multi-trophic aquaculture, fishery management for blood cockles, and co-culture system of giant tiger prawns were done in collaboration with AQD, FRI, and KMITL respectively.

"It is AQD's hope that this joint undertaking (AQD and JIRCAS) will continue to become stronger as we work together towards the development of technologies for sustainable production of aquatic products that benefits the different sectors equitably," was AQD Chief Dr. Felix Ayson's message. In response, JIRCAS Program Director Dr. Masayoshi Saito said, "I hope our cooperation is not only bilateral but multilateral to get synergistic effects from our existing cooperation."

**5th Symposium and Scientific Meeting of PPSI;** 16 April '13; Tigbauan, Iloilo; organized by the Philippine Phycological Society Inc (PPSI) and AQD. "Our theme this year which is *Exploring algae diversity for health, environment and industry* reflects our continuing commitment to bring to the public mind the strategic importance and manifold usefulness of algae in our quotidian and long-term concerns." said PPSI president Dr. Maribel Dionisio-Sese during the opening ceremonies. AQD Chief Dr. Felix Ayson also pointed out that only a few of the algal species have been successfully cultivated, hence, there are still a lot of algal species that need to be explored and studied. Around 100 participants attended the symposium.

In addition, PPSI organized a training workshop on nutritional products of algae held 17-18 April at AQD's Tigbauan Main Station with about 15 participants. The training workshop included lectures and practical sessions on uses of agar, alginates & carageenan; agar & carageenan extraction; gel testing; growth kinetics; and lipid, protein & carbohydrates analysis.

**Taal Lake Aquaculture Forum;** 4 October '13; Talisay, Batangas; organized by AQD, DOST and De La Salle University - Manila; funded by B-MEG. AQD has been working with the private and public sectors to give technical advice to fishfarmers and to conduct seminars on good aquaculture practices. In the forum, two AQD scientists spoke on fish health and lake ecology.

**International Seminar on Environmental-friendly Aquaculture and Stock Enhancement in Southeast Asia;** 3 December 2013; SEAFDEC/AQD. Through the Japan Society for the Promotion of Science – Asia-Africa Science Platform (JSPS-AASP) program, Hokkaido University had been supporting the research done by some AQD staff.

During the seminar, four papers (3 AQD and 1 Hokkaido University) were presented. The topics included fatty acid profiles in abalone fed artificial diets supplemented with different enrichment products, identification of bacterial species in biofilm on settlement plates for abalone larvae, genetic studies on species identification & population structure of seahorse for stock enhancement and the various achievement in abalone and seahorse studies by the JSPS-AASP program.

The partnership of AQD and Hokkaido University will continue as Prof. Yasuaki Takagi presented three more programs that AQD staff can participate in, including the JSPS-DOST Bilateral program, Asia-Africa Science Platform program, and RONPAKU (dissertation Ph.D.) program.

[R-L] Hokkaido University's Prof. Katsutoshi Arai, Prof. Yasuaki Takagi, AQD Deputy Chief Dr. Teruo Azuma

# Promotion of sustainable and region-oriented aquaculture practices

Through trust funds provided by the Government of Japan-Trust Fund 5 (GOJ-TF5) since January in 2010, SEAFDEC/AQD has been continuously implementing a regional program entitled *Promotion of sustainable aquaculture and resource enhancement in Southeast Asia* under the ASEAN-SEAFDEC Fisheries Consultative Group Program, which, in collaboration with several research partners in the ASEAN region, comprise the following four projects:

- Promotion of sustainable and region-oriented aquaculture practices
- Resource enhancement of internationally threatened and over-exploited species in Southeast Asia through stock release
- Accelerating information dissemination and capacity building in fish health management in Southeast Asia
- Food safety of aquaculture products in Southeast.

## Sustainable aquaculture

The main objectives of this project are to: (1) establish reliable mass seed production techniques for genetically improved strains of commercially important species through selective breeding and to develop hatchery technology for newly emerging species for aquaculture; (2) develop environment-friendly and cost-effective practical feeds using ingredients available in the region and establish guidelines on feeding management for sustainable aquaculture; (3) develop farming management strategies that eliminate the risk factors through epidemiological and environmental approaches to prevent and control diseases; and (4) verify and disseminate the project achievements especially in the lesser developed countries in the region through demonstration, training, lecture/seminar and publication activities.

### Selective breeding of mud crab *Scylla serrata*

First-generation juveniles (F1) were subjected to challenge test using luminescent bacteria *Vibrio harveyi* to evaluate the disease resistance of families from different sources. Juveniles raised from Samar broodstock showed the highest resistance. In terms of growth, juveniles among various families and generations reared either in ponds or tanks for two months did not differ significantly. F1 families seemed to have improved resistance to *V. harveyi*, but growth among various families was comparable.

### Selective breeding of tiger shrimp *Penaeus monodon*

Nine F1 groups were grown to broodstock / spawner size. Two of the groups produced 100,800 and 75,800 nauplii (hatching rate, 63 and 79%) while five re-matured. Of the latter, two resulted in nauplii production, another two in regression, and one spawned but eggs failed to hatch.

### Genetic improvement of giant freshwater prawn *Macrobrachium rosenbergii*

New prawn stocks (from Pampanga) were collected and have been set up for breeding. All other existing stocks (crossbreds, purebreds) have also been set up for spawning. Once same-age spawns are obtained from all five "strains", larvae will be on-grown until ready for the growth comparison run in cages.

### Hatchery technology of emerging species

Spawning trials for spotted scat *Scatophagus argus* was conducted to determine optimum hormone concentration to induce spawning. Spawning occurred after two successive injections of 50 µg of LHRH-a/kg of fish across a two-day period at 25 ppt, but the eggs released were unfertilized.

For the larviculture of pompano *Trachinotus blochii*, trials showed that the use of copepods promoted early metamorphosis, better survival and tolerance to hypoxic and high saline conditions.

### Replacement of fish meal in prawn diets

While the inclusion of up to 30% cowpea meal protein improved the reproductive performance of *M. rosenbergii* broodstock, larval quality and survival have yet to be determined in subsequent rearing trials.

### Disease tolerant and sustainable aquaculture environment

Extensive culture of *P. monodon* is being tried in ponds with and without tilapia in an effort to determine whether tilapia can reduce the incidence of disease in shrimp. Preliminary data showed that shrimp cultured with tilapia are bigger compared to those grown without tilapia.

## Resource enhancement

The main objectives of this project are to: (1) establish mass production technology and broodstock management, and to develop methodology of stock enhancement practice of internationally threatened species (species listed in CITES); (2) establish release strategies of regionally over-exploited species and verify the effectiveness of community managed sea ranching and socioeconomic strategies; (3) establish adaptive measures supporting resource enhancement for a changing environment; and (4) disseminate and demonstrate resource enhancement practices.

### Stock enhancement of seahorse and Napoleon wrasse

Monthly on-site assessment at Molocaboc Island, Sagay City was conducted to monitor baseline population of wild seahorses. Seahorses were collected from fringing coral reefs during night time at low tide (~1-1.5 m) and their gonad development graded. Partially and fully mature males and/or females were present every sampling with mean stretched length of 11.5 cm and mean body weight of 9.4 g. Samples were collected for further DNA study. Wild seahorses from Molocaboc Island were all identified as *Hippocampus comes*, while two species were identified in wild seahorses from Panal Reef (also in Sagay) as *H. comes* and *H. spinosissimus*. The population structure of wild and hatchery-reared *H. comes* showed 3 to 4 haplotypes and low to medium haplotype diversity ( $h = 0$  to 0.5) and very low nucleotide diversity ( $\pi = 0$  to 0.0015).

For the Napoleon wrasse *Cheilinus undulatus*, fin clip samples were obtained from juveniles captured in Guimaras, Tawitawi,

and Bohol for DNA analyses. Danajon, Bohol with its dual barrier reef was selected as a resource enhancement site. Authorization of the research activities were issued by ten municipalities that face the reef and satellite sites.

### Community managed sandfish sea ranching and stock release

Monitoring of wild stock in Sagay Marine Reserve (SMR) in northern Negros showed that the density of sexually mature sandfish was 4 per 100 m<sup>2</sup>. Spawning trials using SMR broodstock yielded 90,000 larvae.

A gratuitous permit is being worked out with the DENR-Protected Area Management Board to grant tenurial use rights over the sea ranching and nursery areas to AQD's local partner organization in the stock enhancement study.

### Managing released stocks

Monitoring of community-based resource enhancement was done every two months to train BFARMC (Barangay Molocaboc Fisheries & Aquatic Resources Management Council) to manage the project independently.

Partially harvested abalone were sold to a buyer in Vito at P270-300/kg live, while abalones captured outside the demo-site were sold at P230/kg by gleaners to buyer-stockers in Molocaboc who in turn sold these to the Vito buyer.

Fishers in Punta Roma were assisted in planting the seaweed *Gracilariopsis heteroclada*, an essential food for abalone. A 30 x 3 m enclosure to culture the seaweeds was fabricated and the test planting started. Abalone hatchery was also discussed and planned for Punta Roma.

### Adaptive measures for coral replenishment

Field surveys for coral reef substrates through line-intercept-transect and temperature profiling were done in coral reef areas off Nogas Island, Antique, Philippines. Preliminary data analyses of the three sampling depths (5, 10, and 15 m) showed that coral substrates by any type of organisms and by Scleractinia in particular decreased in the deeper layers. Among Scleractinia, *Porites* sp. occurred predominantly in all depths.

A new methodology for determining the density of zooxanthellae, symbiont of reef-building corals, was developed using *Porites* sp. fragments.

Negative effects of acidification and warming on growth and photosynthetic rates were confirmed in *Porites* sp. through the rearing experiments.

## Fish health

The main objectives of this project are to: (1) accelerate awareness about fish health management in resource-deprived countries through industry-wide capacity building; (2) guarantee food safety and sustainable production through innovative research; and (3) disseminate output of the project.

### Status and needs of primary aquatic animal health care in small-scale aquaculture

Surveys were conducted in Cambodia, Lao PDR, Myanmar, and Philippines to identify the gaps and needs in order to equip with capability to monitor diseases. Compared with Myanmar and the Philippines, small-scale farmers in Lao PDR and Cambodia had low level of awareness of fish health management and food safety, and other issues affecting fish production in ponds. There is a need to strengthen the expertise of government fish health staff in Myanmar, the Philippines, Lao PDR, and Cambodia in order to affectively disseminate available information on fish health management.

### Surveillance of parasites in freshwater fish

Tissue samples of tilapia collected from Dumangas, Oton and Arevalo (Iloilo, Philippines) in April 2013 yielded negative results for zoonotic parasites. Further, no significant parasitic infestations of the skin mucus and gills were observed.

### Molecular diagnosis and prevention of viruses in fish and shrimp

The plasmid positive control for WSSV was initially developed while optimization of q-PCR protocols for WSSV, RSIV,

KHV, VNN, IMNV and IHNV were already completed. LAMP assay for WSSV showed that the optimum incubation was at 61°C for 58 min.

### Immunization regimen to prevent NNV in high-value marine broodfish

Pompano broodstocks were intraperitoneally (IP) booster-vaccinated with inactivated Philippine strain of nervous necrosis virus (NNV). Determination of antibody titers in the sera of vaccinated/control fish collected is ongoing.

### Novel prophylactic and therapeutic methods for the prevention of viral infections

The seaweed *Ulva pertusa* extracts showed potent antibacterial activity against *Aeromonas hydrophila* and *A. sobria*. The minimum inhibitory concentration and minimum bactericidal concentration are being determined for *Vibrio alginolyticus*, *V. parahaemolyticus*, *Edwardsiella tarda*, and *Streptococcus* spp.

Mortality for *A. hydrophila*-challenged fish fed *U. pertusa* supplemented diet (100 g seaweed/kg feed, 5% BW) was not significantly different (40%) from *A. hydrophila*-challenged fish fed SEAFDEC-formulated diet (35%).

### Carriers for delivering vaccines to shrimp and other crustaceans

Carriers+vaccine were administered orally to the shrimp via the feed. The survival against WSSV challenge were 65%, 62%, 50% and 26% in inclusion bodies (IB)+chitosan, IB+alginate, naked IB, and inactivated recombinant bacteria groups, respectively, and 0% in the unvaccinated group. There were no apparent differences in survival among IB+chitosan, IB+alginate, and naked IB, but survival was significantly higher compared to inactivated recombinant bacteria and unvaccinated group. To determine appropriate dosages and vaccine-carrier ratios, a third trial is ongoing.

### Parasitic and shell diseases of abalone (*Haliotis asinina*)

Abalones are routinely infested by shell-infesting polychaete worm belonging to the family Dorvilleidae (prevalence, 20%). The condition indices of infested abalone consistently yielded significantly lower condition indices than uninfested abalone. In the presence of infested abalone, uninfested abalone became infested within 48-72 hours with an average of 5 worms (crawling larvae) per abalone.



PHOTOS BY SM URSUA

Night sampling of seahorses

## Food safety

The objectives of this projects are to: (1) contribute in the establishment of guidelines on the production of safe aquaculture products from Southeast Asia; (2) determine the presence and levels of commonly used chemicals in aquaculture products such as fish and shrimps; (3) compile and disseminate SEAFDEC guidelines on the use of antibiotics and chemicals in aquaculture to the ASEAN region; and (4) implement training course/workshop to promote food safety awareness in the ASEAN region.

### Withdrawal period of antibiotics in grouper

The estimated time to eliminate oxytetracycline (OTC) residue from the muscle of orange spotted grouper *Epinephelus coioides* was 21 days, while that for oxolinic acid (OXA) was 17 days.

Meanwhile, protocols for the detection of OXA and OTC using the micro-organism method in fish and shrimp muscles are being established.

### Guidelines on antibiotic/chemical usage

In the draft ASEAN guidelines, detailed recommendation coming from very recent studies such as withdrawal period for antibiotics should be included as with chemicals of recent concern in Southeast Asia such as ethoxyquin.

## Training fellowships

GOJ-TF granted 29 fellowships to government extension workers and fishfarmers in six of the 22 training courses or sessions organized by AQD in 2013:

Training course	Fellowship granted (number of trainees)
Abalone hatchery & grow-out	Indonesia (1), Philippines (1)
Marine fish hatchery	Myanmar (1), Philippines (1)
AquaNutrion Online	Brunei Darussalam (1), Indonesia (1), Myanmar (1)
Community-based freshwater aquaculture for remote rural areas in Southeast Asia	Cambodia (1), Indonesia (1), Lao PDR (1), Malaysia (1), Myanmar (1), Philippines (2), Vietnam (1)
Shrimp hatchery operation & management	Cambodia (1)
Freshwater fish health management with emphasis on detection of fish-borne zoonotic parasites	Myanmar (12)

## Scientists and stakeholders convene for food safety

The *International workshop on food safety of aquaculture products in Southeast Asia* was held from 8 to 9 May in Iloilo City. This workshop was organized by AQD with funding from the GOJ-Trust Fund. It focused on the status of food safety and traceability of aquaculture products including the standard protocols used for the detection of contaminants and regulatory mechanisms in SEAFDEC member countries.

SEAFDEC Deputy Secretary-General Mr. Hajime Kawamura was happy with AQD's successful promotion of sustainable aquaculture and he also noted that AQD should continue to extend support and enhance the capacity of small-scale aquaculture operators to adapt the necessary preventive and control measures.

AQD Chief Dr. Felix Ayson explained that this workshop served as the venue to address the concerns on food safety and to come up with recommendations on how to harmonize food safety standards. "AQD has been in the forefront of developing technologies for sustainable production of safe food products through aquaculture," he added.

A total of 150 aquaculture and food safety experts, scientists, representatives and observers from 11 countries attended the workshop. AQD Deputy Chief Dr. Teruo Azuma was the chair of the workshop organizing committee.

### Member countries report on food safety

It appeared that most SEAFDEC member countries have formulated legal frameworks to monitor and control chemicals used in aquaculture. Registration systems to control quality of fish produce are in place. For example in the Philippines, production and processing establishments are granted two-year permits; but once violation is found during the annual monitoring, the registration can be invalidated even before the expiration date. In all countries, only registered establishments and farms are allowed to export.

Exporting fish to the European market is the common target of member countries. Presentations included the requirements and qualities expected by importing countries; however, the workshop participants agreed that more research need to be conducted.

A plenary paper and ten studies were also presented. The workshop report can be downloaded from the AQD website [www.seafdec.org.ph/](http://www.seafdec.org.ph/)



PHOTOS BY DEVCOM

[CLOCKWISE] AQD Chief Dr. FG Ayson, SEAFDEC Deputy Secretary-General Mr. Hajime and AQD Deputy Chief Dr. Azuma ceremonially open the food safety workshop exhibit; participants discuss issues in small groups, here with plenary speaker Dr. Mark Tamplin of the University of Tasmania, Australia [SECOND FROM RIGHT]; and representatives from SEAFDEC member-countries who reported on food safety measures



## TECHNOLOGY EXTENSION

# Technical assistance for fishfarmers and communities

Under its ABOTAquaNegosyo (*Agree-build-operate-transfer AquaBusiness*) program, AQD continued its three-year assistance to ACDI-VOCA (Cooperative Development International and Volunteers in Overseas Cooperative Assistance) in Timor Leste, providing technical assistance on three separate occasions. The assistance in 2013 included inspection of the newly constructed mud crab hatchery and nursery facilities; actual hatchery operation; and conduct of two training courses on natural food culture & hatchery operation (held 13-19 October with 14 participants) and feed formulation & preparation (held 20-27 November).

ABOT received 50 local and 11 foreign inquiries in 2013. These were mostly on the culture of seabass, grouper, milkfish, mudcrab, abalone, shrimp, tilapia and freshwater prawn. Eight local (Philippine) and three international clients were served. For local clients, the requests were mostly on the evaluation of sites and current pond operation on tilapia, milkfish and seabass. For international clients, technical assistance on sandfish hatchery and nursery was provided to Century Marine Products Inc. in Kota Kinabalu, Malaysia. Site assessment for tilapia farming was done for Microtend Enterprise LDA in Luanda, Angola (Africa).

AQD assessed the Tanjung Badak multi-species hatchery in Kota Kinabalu, Malaysia 5-7 November upon the request of a former AQD sandfish trainee Mr. Keith Ng of Century Marine Products. The facility was found to have high prospects for expansion into sea cucumber production



PHOTO BY J. ALTAMIRANO



PHOTO BY D. BALIAO

AQD assessed the 10-ha site in Angola from 4 to 10 June, and found it feasible, with a good freshwater source and some structures (eg. concrete gate) already in place. AQD's recommended design of farm operations is typical of a free-flowing impounded freshwater pond or reservoir for tilapia production in the Philippines. [ABOVE] Investor, local guide and officers of Microtend Enterprises of Angola

The ICDSA (*Institutional capacity development for sustainable aquaculture*) program seeks to promote aquaculture technologies developed by AQD in partnership with local government units, donor communities, fisherfolk/fishfarmers and other stakeholder groups. ICDSA provides a mechanism for the assessment of socioeconomic and environmental impacts of AQD aquaculture technologies and for building the capacity of beneficiary communities.

For collaborative projects with BFAR, AQD rendered technical assistance in the construction of multi-species marine fish hatcheries in different parts of the country. The multi-species marine fish hatcheries in Baler, Aurora; Sta. Lucia, Palawan; Bongabong, Oriental Mindoro have been completed and are already operational. The construction of the hatchery facilities in Sta. Cruz, Davao is almost completed; construction of the hatchery facilities in Sagnay, Camarines Sur and Laoang, Samar are in the early stages.

AQD also provided technical assistance for the operation of the hatcheries that are already operational (Baler, Sta. Lucia, Bongabong), the marine fish hatcheries in Ubay, Bohol; as well as the newly-established community-based hatcheries in CARAGA and Medellen, Cebu.

AQD will be working with Winrock International (an NGO) in implementing a development project for improved production efficiency in the CARAGA region. AQD's role is on capacity building in aquaculture through introduction of technologies that will enhance production of aquaculture species that are economically important to CARAGA. A consultation was organized with the provincial fisheries officers and key BFAR personnel.

As an offshoot of the USAID-ASEAN led dialogue on public-private partnership, AQD is working with MARKET (a USAID funded-initiative) on a project proposal to promote best feeding practices in aquaculture in selected SEA countries.



PHOTO BY C. GENZOLA

AQD Chief Dr. FG Ayson [LEFTMOST] visits the BFAR milkfish hatchery in Cortez, Surigao del Norte

## Training & information

# On-the-job training, internship and research mentorship

AQD has continued to demonstrate its significant contributions to aquaculture development in the region through building institutional capacities and developing a critical mass of experts on aquaculture technologies. The 22 training courses and/or sessions conducted in 2013 are reported under the thematic program areas.

It is to be noted that there were 200 trainees in total: 38% female, and 91% coming from SEAFDEC member-countries while the rest were from nine non-SEAFDEC countries. Ages ranged 18-78 years old.

AQD also continued with its internship program, availed of by 39 individuals; student on-the-job training was availed of

by 225 students from 31 schools/universities in Luzon (9), Visayas (17) and Mindanao (5).

Philippines, being the host country to AQD, had the most number of participants to the various capacity building programs. Overall, AQD has produced a large number of technical personnel who are now in the aquaculture business themselves or conduct or direct further R&D in their home countries. In view of the emphasis being given by AQD on building the critical mass of experts on aquaculture technologies, there is now a ripple effect that is created when these technologies are promoted and practiced.



AQD staff served as resource person on abalone culture training at Western Philippines University; OJT students from Sultan Kudarat State University (southern Philippines) during their briefing on marine fish hatchery operations

### AQD support to thesis students

From immunostimulation to sponge extracts, student theses get supported by AQD through consultation with research staff on experimental design & analysis and the actual implementation of the studies.

(1) **University of the Philippines Visayas:**

Ms. Maila Pan worked with AQD scientist Dr. VR Alava on "Effects of peptidoglycan immunostimulation on the growth of black tiger shrimp (*Penaeus monodon*)"

(2) **De La Salle University:**

Ms. Julie Añano worked with Dr. MRR Eguia on "Optimization of feeding and growth conditions for hatchery-bred larvae of indigenous Philippine silver therapon"

Mr. Mark Archei Javier worked with Dr. MRR Eguia on "Preliminary trials on the optimization of hormone dosages for induced breeding of Philippine silver perch"

Ms. Kristine Gonzales worked with Dr. MRR Eguia on "Growth and survival of Nile tilapia juveniles fed diets with varying levels of irradiated chitosan"

(3) **Ateneo de Manila University:**

Ms. Marie Noel M. Hernandez and Ms. Jonni Fay Teves worked with Dr. FA Aya on "Effects of replacing fishmeal with squash seed meal on the growth performance and blood chemistry of juvenile Nile tilapia"

Mr. Mark Gavin Dinsay and Mr. Barry Union Kayanan worked with Dr. FA Aya on "Partial replacement of fish and soybean meals with lysed cell wall *Chlorella* sp. for diets of Nile tilapia juveniles"

(4) **West Visayas State University:**

First year medical students worked with AQD's Ms. MR dela Pena and Dr. ET Quinitio on "Anthelmintic effect of holothurin extracted from sea cucumber (*Holothuria leucospilota*)"

(5) **John B. Lacson Foundation Maritime University (Arevalo) Inc:**

A group of four students worked with AQD's Ms. RM Albacete on "Effectiveness of different organic materials as alternative oil dispersant"

A group of five worked with AQD's Ms. MR dela Pena on "The effect of pea seed hull extract against rust"

(6) **University of San Agustin:**

Mr. Lennon Ponta-oy worked with Ms. MR dela Pena on "In vitro antioxidant activity of *Nannochlorum* sp. and its hypoglycemic effect on alloxan-induced white mice"

(7) **Polytechnic University of the Philippines:**

Mr. John Michael Castro and Ms. Liona Joy Calites worked with Ms.

Once enrolled in AQD's on-the-job training, students are first briefed and toured around the AQD stations before being placed in the laboratories or work stations of interest



MN Corpuz on "Growth and morphological development of hatchery-reared larvae and juveniles of Philippine silver therapon"

Ms. Armie Joyce Atterado and Ms. April Barbosa worked with Ms. MN Corpuz on "Effects of different salinity levels on the growth and survival performance of endemic juvenile silver therapon"

Mr. John Eric Garcia and Ms. Josephine Sumilang worked with Ms. MN Corpuz on "Morphometric and morphomeristic differences in the populations of *Arius manillensis* and *A. dispar* in Laguna de Bay"

Ms. Ren Divien Obeña and Ms. Jayrah Padilla worked with Ms. MN Corpuz on "A preliminary assessment of the stomach content of the clown knifefish *Chitala ornata* in the Laguna de Bay"

(8) **Iloilo National High School** (special science class):

A group of three students worked with AQD's Dr. MJH Ramos on "Assemblage of recycled containers: artificial coral reef." The thesis was an entry to the NYSTESC Research Congress 2013

Ms. Jann Lorryne Perez worked with Ms. Arnaiz on "Protein, calcium & omega-3 content of oil extracted from angelwing clam (*Cyrtopleura costata*)"

Mr. Raphael Ian Tan worked with AQD's Mr. D Catedral on "Lethal dose of copper sulphate pentahydrate on brine shrimps (*Artemia salina*)"

Three students worked with AQD's Dr. EC Amar and Ms. MR dela Pena on "Natural immunostimulants from *Citrus tangerina*, *Gracinia mangostana*, *Garcinia morella* and *Nephelium lappaceum* leaf and rind extracts on *Penaeus monodon* challenged by white spot syndrome virus"

Mr. Eli Gene Tisuela worked with Ms. MR dela Pena on "Effectiveness of different processes of drying thraustochytrids as an enrichment diet for *Brachionus plicatilis*"

Ms. Janine Origen worked with Ms. MR dela Peña on "The effect of 'apat-apat' (*Marsilea quadrifolia*) and 'lupo' (*Alternanthera sessilis*) extract in the platelet count of white mice (*Mus musculus*)"

Ms. Thea Angelica Nava worked with Ms. MT Arnaiz on "Wastewater microbiological treatment by lactic acid bacteria from rice washing"

Ms. Francine Tupas Garcia worked with Ms. MT Arnaiz on "Bioaccumulation of zinc in juvenile tiger shrimp"

Two students worked with Mr. D Catedral on "Effects of NH<sub>4</sub>-sulfur based fertilizer on soil microbial population"

(9) **Oton National High School:**

Mr. Michael Angelo Dejando worked with Ms. MR dela Peña on "Synergistic activity of marine sponge (*Ircinia* sp.) extract with ampicillin against *Staphylococcus aureus* and *Escherichia coli*"

(10) **Rizal Science High School:**

Three students worked with Dr. FA Aya on "Effect of varying levels of ipil- ipil seed meal on survival and growth of silver therapon"

# Information dissemination

## SCIENCE PAPERS

For the year 2013, AQD published 26 scientific papers in ISI-CC covered journals and conference proceedings which add up to a total of 1,417 scientific papers since 1973. These research results form the foundation of AQD's science-based technologies. The 2013 published papers are as follows:

- Abrogueña JBR, Bagarinao TU, Chicharo L. 2012. Fish habitats in a small, human-impacted Sibunag mangrove creek (Guimaras, Philippines): a basis for mangrove resource enhancement. *Ecology & Hydrobiology* 12: 311-319
- Alayon SB, Nemiz ES, Superio DL, de la Peña JG, Pacino LG. 2013. The development of an institutional repository at the Aquaculture Department of the Southeast Asian Fisheries Development Center, Philippines. Program: electronic library and information systems 47: 384-398
- Apines-Amar MJ, Amar EC, Faisan FP Jr. 2013. Growth, plasma cortisol, liver and kidney histology, and resistance to vibriosis in brown marbled grouper, *Epinephelus fuscoguttatus* fed onion and ginger. *Aquaculture, Aquarium, Conservation & Legislation – International Journal of the Bioflux Society – AACL Bioflux* 6: 530-538
- Bautista-Teruel MN, de la Pena MR, Asutilla AJ. 2013. Evaluation of agar-bound micro-particulate diet as alternative food in abalone hatchery: Effects of agar concentrations and feeding frequencies. *Journal of Shellfish Research* 32: 9-15
- Caipang CMA, Pakingking RV Jr., Apines-Amar MJS. 2012. Screening of vibriosis in Asian seabass, *Lates calcarifer* using loop-mediated isothermal amplification (LAMP) assay. *Human & Veterinary Medicine: International Journal of the Bioflux Society* 4: 52-57
- Corpuz MNC, Camacho MVC, Ocampo PP. 2013. Morphometric and morphometric variations in five populations of indigenous Celebes goby *Glossogobius celebius* (Perciformes: Gobiidae) from Southern Luzon, Philippines. *The Philippine Agricultural Scientist* 96: 75-85
- Cuvin-Aralar ML, Gibbs P, Palma A, Andayog A, Noblefranca L. 2012. Skip feeding as an alternative strategy in the production of Nile tilapia *Oreochromis niloticus* (Linn.) in cages in selected lakes in the Philippines. *The Philippine Agricultural Scientist* 95: 378-385.
- Cuvin-Aralar MLA, Lazartigue AG, Aralar EV. 2013. Net mesh size affects production of giant freshwater prawn *Macrobrachium rosenbergii* cultured in lake-based cages. *Journal of Applied Ichthyology* 29: 833-838
- Cuvin-Aralar MLA, Lückstädt C, Schroeder K, Kühlmann KJ. 2011. Effect of dietary organic acid salts, potassium diformate and sodium diformate on the growth performance of male Nile tilapia *Oreochromis niloticus*. *Bulletin of Fish Biology* 13: 33-40
- Encena VC II, de la Pena MR, Balinas VT. 2013. Evaluation of density and cage design for the nursery and grow-out of the tropical abalone *Haliotis asinina* Linne 1758. *Journal of Shellfish Research* 32: 21-27
- Gao J, Koshio S, Ishikawa M, Yokoyama S, Mamaug RE. Interactive effects of vitamin C and E supplementation on growth performance, fatty acid composition and reduction of oxidative stress in juvenile Japanese flounder *Paralichthys olivaceus* fed dietary oxidized fish oil. *Aquaculture* (online first)
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## MATERIALS FOR GENERAL READERSHIP

AQD publishes its own publications for timely dissemination of information and to help enhance its visibility to its various stakeholders.

AQD produced and disseminated its 2012 annual report, updated the set of flyers about its stations and programs, and made a folder to hold leaflets. It also designed and published a 2014 calendar-planner and bookmark & notepad sets, and made personalized birthday cards for employees to commemorate its ruby jubilee. Except for the latter, all these materials were made downloadable from the AQD website; around 3,109 downloads were logged by the site.

Regarding AQD's inventory of 41 manuals and books at the AQD Bookstore, a total of 281 queries from stakeholders were received, resulting to 142 book sales / transactions in 2013.

During the year, AQD regularly updated its stakeholders about events at its stations through these tools:

- (1) **monthly newsletter *AQD Matters*.** Each issue was mostly distributed through email to more than 800 stakeholders (171 were AQD employees) or as a download from the AQD website. Some of the 112 stories written in 2013 also appeared in the Secretariat's quarterly *SEAFDEC Newsletter*
- (2) **AQD website [www.seafdec.org.ph](http://www.seafdec.org.ph).** The site was updated 148 times in 2013, and had 61,013 unique visitors which continued the increasing trend in visitor number since the site was last overhauled in 2011
- (3) **social networks, Facebook's *Devcom Section page* and *YouTube account*.** The FB page had 1,014 likers; 165 stories were posted in total. The videos had 302,694 views (279 subscribers); five new videos of stakeholder testimonies on AQD's 40 years of R&D were made and posted
- (4) **weekly TV program *Mag-Agri Tayo*** ('Let's do agriculture'). Under an agreement with AQD, the show on PTV (People's Television Network) aired aquaculture technologies filmed by producers while visiting AQD for a total of 29 days in 2013. AQD also arranged for live interviews of its senior officials by two Iloilo TV programs to promote ruby jubilee activities. In addition, AQD was independently covered, with 43 stories appearing in the Philippine mass media and websites/blogs

## FAIRS & EXHIBITS

Fairs are good venues to meet stakeholders face-to-face. For 2013, AQD organized an exhibit for its ruby jubilee celebration and attended five fairs&exhibits, entertaining the queries of over 2,600 fishfarmers, entrepreneurs and students in its booths.

### ***40 years of aquaculture R&D: an exhibit of sustainable and viable fish farming technologies developed by SEAFDEC Aquaculture***

**Department**, 3-9 June '13; SM City Iloilo, Philippines. Iloilo City Mayor Hon. Jed Patrick Mabilog was the guest-of-honor at the opening-of-exhibit program on the first day. The mayor spoke of the importance of fish supply, with seafood restaurants very popular in Iloilo City, boosting tourism and giving employment. AQD Chief Dr. Felix Ayson, on the other hand, noted that aquaculture is now producing or supplying more than 40% of the worldwide fish requirement.

The exhibit featured posters on AQD accomplishments, fish aquaria, consultation, and souvenir give-aways. A slideshow can be found at: <http://www.seafdec.org.ph/2013/40-years-of-aquaculture-rd/>

**20th Panaad sa Negros Festival**; 8-14 April 2013; Bacolod City, Philippines. AQD showcased hatchery-reared seahorses and abalones, oriented visitors about AQD's stock enhancement project in the Sagay Marine Reserve in northern Negros, and gave out free flyers and sold its publications.

**4th Aquaculture Technology (AQUATECH) Expo & Convention**. 30-31 May; Tagaytay City, Phil. AQD co-organized the event, with four of its scientists tapped as resource persons / moderators: Dr. MR Eguia, Dr. ML Aralar, Dr. ND Salayo, and Dr. RV Pakingking Jr. talked, respectively, on: (1) genetic technologies for improved fish production; (2) culture of giant freshwater prawn in lake-based cages; (3) economics of fish farming, and (4) development & application of anti-nervous necrosis virus vaccine in marine fish hatcheries.

**AgriLink FoodLink AquaLink**; 10-12 October 2013; Pasay City, Phil. AQD offered technical consultation and promoted its programs. On the last day, AQD Chief Dr. FG Ayson and scientist Dr. FD Estepa lectured on *Aquaculture of pompano* and *Mud crab culture*, respectively. AQD has been a regular participant to the annual AgriLink.



AQD's ruby jubilee exhibit; AQD Chief Dr. FG Ayson and Iloilo City Mayor Hon. Jed Patrick Mabilog



PHOTOS BY DEYCOM

**DOST 2013 Visayas Cluster Science & Technology Fair**, 16-18 October '13; Iloilo City, Phil. The AQD booth featured the DOST funded research programs on mud crab and shrimp.

**Agri-Biotech Exhibit**; 21-24 Oct '13; Philippine Congress, Manila. AQD scientists Dr. Myrna Teruel and Dr. Maria Rowena Eguia provided free consultation on AQD's aquaculture technologies and training programs.



## AQD REGISTERS AS A BOOK PUBLISHER

AQD has registered as a book publisher with the National Book Development Board (NBDB), the government agency mandated to develop and support the Philippine publishing industry. Since 2002, AQD had produced at least 45 copyrighted publications categorized as applied science. All the books contain information on aquaculture technologies (hatchery, nursery, grow-out, among others) based on the results of the research conducted by AQD.

AQD at the fair [FROM TOP, L-R]: Panaad festival, biotech, AquaTech, AgriLink, DOST



PHOTOS BY G FAIGANI / MR EGUIA / GH GARCIA / RH LEDESMA / MB TERUEL





After SEAFDEC became an international partner of ASFA through an agreement with the SEAFDEC Secretariat, AQD became a collaborating center and sent one librarian to train on input procedures in Thailand, 18-22 March. ASFA or the *Aquatic Science & Fisheries Abstracts* is an international cooperative information system by the UN-FAO. Librarian Mr. Daryl Superio later attended the *ASFA Advisory board annual meeting 23–27 September* in Callao, Peru to report on AQD's progress

## LIBRARY

AQD became a collaborating partner in the FAO's ASFA program in 2013, submitting 224 records of which 67% are grey literature.

As the AQD Library moves towards integrating library systems and expanding its collection, it served in 2013 an increasing number of clients (26 per day) from various sectors, research staff, students, people from the academe and entrepreneurs interested in aquaculture, fisheries and related fields.

The library collection now stands at 20,639 monographs; 5,060 SEAFDEC publications; 11, 873 bound serial volumes; 10,320 pamphlets; and a variety of maps, posters, microfiche and CD-ROMs. Most of these are accessible through the online public access catalogue (webOPAC had 7,510 unique visitors in 2013) or downloadable through the institutional repository (SAIR had 16,469 unique visitors who made 300,390 downloads of free AQD e-publications numbering 1,816 files).

Alerts and bulletins were also distributed through email to stakeholders to publicize new book/serial arrivals. AQD also put-up mini-libraries in its stations.

AQD became the home to 93 librarians and staff for the *Summer conference on accreditation and compliance with standards*. This was organized by Philippine Librarians Association - Western Visayas Region Librarians Council and AQD from 24–25 May to update and prepare librarians for accreditation and professionalism.

In addition, the Association of Special Libraries of the Philippines in partnership with AQD held the workshop on *Organize IT digitally, open-source tools for your library* from 15 to 17 May; 88 librarians and information professionals attended



[LEFT] Green turtle captured and tagged MY(S) 8864Y in Malaysia then recaptured, retagged PH07051, and released in Guimbal Iloilo, Philippines, 20 Jul 2013. [RIGHT] Interns at FishWorld



## FISHWORLD

**Museum of aquatic biodiversity.** The museum reference collections now include about 5,000 marine species and continue to be used by students and teachers for their theses and for contests. Additional specimens were collected from Buyuan, Tigbauan; Lapu-lapu City; Dagupan, Pangasinan; Tacloban, Baybay, and Hinunangan, Leyte; Surigao City, and from around Siargao Island. A new 70 m<sup>2</sup> annex was built to house the formalin- and alcohol-preserved collections.

**Work on endangered megafauna.** A hawksbill, seven olive ridleys, and 23 green turtles caught by fishing gears between Iloilo and Guimaras were documented and released by FishWorld, including several that were sick or injured and nourished back to health in indoor tanks. Three stranded sunfish, a hooked tiger shark, and a large live sea snake were also received. Green turtle hatchlings were found at a beach in Damilisan, Miagao, Iloilo and many juveniles were captured in fish corrals in Hinunangan, Leyte.

**R&D internship and mentoring.** From 17 April to 15 May 2013, FishWorld conducted the R&D internships for 24 mostly high schools students in an effort to help build a core group of students with an enhanced awareness of, and appreciation for, marine ecosystems and biodiversity, aquaculture and fisheries, and the need for conservation.

**Sci-Art AquaWeek.** These annual contests (since 1995) require students, teachers, and parents to study about aquaculture, fisheries, and marine biodiversity. On 22-26 July, 191 students and 110 coaches from 18 elementary schools and 11 high schools participated in nine contests.

**Live animal exhibits.** The live animals in the aquaria, ponds, and tanks at FishWorld are important permanent exhibits that children and other visitors enjoy, despite the basic conditions. The live animals include about a hundred species of marine fishes and invertebrates, including the species that AQD does research on, and small animals saved from fishing gears.





[ABOVE] One-on-one consultation between fish farmers and AQD scientists/researchers. Farmers mostly asked about marine fish & abalone culture and small-scale/backyard freshwater hatcheries.  
[INSET] Dr. ML McGlone



## VISITORS

AQD entertained 20,938 visitors in 2013, with some of the scientists delivering research seminars:

- Dr. Sheila Mae Santander-de Leon**, University of the Philippines Visayas: *Aquaculture sediments, a sink waste of fish feeds*, 25 Jan
- Dr. Michiaki Yamashita**, National Research Institute of Fish Science, Yokohama, Japan: *The strong antioxidant selenoneine in tuna blood and its roles in selenium redox metabolism*, 7 May
- Dr. Mark Tamplin**, Tasmania Institute of Agriculture, Hobart, Tasmania: *The use of predictive tools to manage the safety and quality of food*, 7 May
- Mr. Kong Chee Leong**, Faculty of Applied Science, UCSI University, Malaysia: *Commercial jade perch-rockmelon aquaponic system*, 10 May
- Dr. Michele Nishiguchi**, New Mexico State University, Las Cruces, New Mexico: *You light up my life: How a luminous bacterium-squid mutualism can help decipher the evolutionary mechanism involved in shaping beneficial associations*, 7 June
- Dr. Mari Kawaguchi**, Sophia University, Tokyo, Japan: *Evolution of hatching enzyme genes in teleost fishes*, 26 June
- Dr. Michael Rice**, University of Rhode Island, USA: *Modeling of aquaculture and use of common water bodies: A research and extension program for Southeast Asia*, 26 June
- Dr. Tsuyoshi Sugita**, JIRCAS, Japan: *Effect of taurine supplementation in fish meal-based and soybean meal-based diets for fingerling red sea bream (*Pagrus major*) and yellowtail (*Seriols inqueradiata*)*, 15 Aug

## A FORUM FOR FARMERS

AQD welcomed more than 150 participants to its *Technology forum* on 12 July. The event started with five presentations by AQD: (1) use and culture of natural food by Ms. MR de la Peña; (2) sea cucumber production by Dr. JP Altamirano; (3) rabbitfish culture by Dr. FG Ayson; (4) seaweed culture by Ms. MR Luhan; and (5) emerging diseases by Dr. EA Amar. The presentations included new techniques / information that AQD would like to promote or see adopted as these can profit farmers. These were followed by one-on-one consultation.

## LECTURES ON SUSTAINABILITY

Dean Domiciano K. Villaluz (DKV) was AQD's first Chief, and for his very able leadership in AQD's formative years, a lecture series is held yearly in his honor. The ruby jubilee DKV speaker was Dr. Ma. Lourdes San Diego-McGlone who spoke on *Fish production and the environment* on 10 July. Dr. McGlone noted that the growing demand for fish to feed an increasing Philippine population has seen the expansion of fish culture to coastal waters (mariculture) as a government strategy to satisfy the demand for fish. "The case study of Bolinao, Pangasinan is a clear illustration of the link from expansion of mariculture activities to eutrophic waters, HABs or harmful algal blooms, and fish kills," she said. "The fish kills in Bolinao can occur in many other coastal waters," she further noted. "Mitigation measures such as monitoring from simple to high-tech, and modeling using hydro-dynamic & depositional models must be considered to prevent history from repeating itself."



PHOTOS BY DEVCOM

A question from the floor; [INSET, L-R] Dr. R Sadaba, Dr. T Abella, Dr. RE Mamauag, Dr. CL Marte

AQD also invited stakeholders to a lecture series for the ruby jubilee:

- (1) Dr. Resurreccion Sadaba of UP Visayas presented the *Short- and long-term impacts of oil spill* on 22 March. He highlighted UPV's oil spill response program covering 2006 until 2012 which dealt with the aftermath of the sinking of the Solar I tanker in the waters off Guimaras in 2006
- (2) Dr. Tereso Abella of the Central Luzon State University-Freshwater Aquaculture Center spoke on *Sustaining the quality of tilapia seed through genetic selection* on 25 April. He noted that "tilapia is the food of yesterday, food of today, and food of the future"
- (3) Dr. Roger Edward Mamauag of SEAFDEC/AQD discussed *Protein hydrolysates as alternative ingredient in marine fish diets* on 30 May. He presented the processing of alternative ingredients for fish diets through chemical or enzymatic degradation to yield hydrolysates
- (4) Dr. Clarissa Marte of the Integrated Services for the Development of Aquaculture & Fisheries Cooperative discussed how *We can make bangus mariculture a sustainable industry* on 20 June. She noted that cage farming within mariculture parks, if managed well, can provide a sustainable livelihood to fish farmer groups and small-scale operators that will not impact too much on the environment



## Staff development

In 2013, AQD supported the attendance of its research staff to various technical conferences where they presented science papers. Staff also learned from training courses.

Aquaculture America 2013 / WAS Triennial Meeting; 21-25 February 2013; Nashville, Tennessee, USA; organized by the World Aquaculture Society

10th Asian Fisheries and Aquaculture Forum (10AFAF) and 4th International Symposium on Cage Aquaculture in Asia (CAA4); 30 April - 04 May 2013; Yeosu, South Korea; organized by the Asian Fisheries Society

Student Exchange Program under the Japan Student Organization (JASSO) Scholarship; 07 July - 10 November 2013; Kagoshima, Japan; organized by Kagoshima University and University of the Philippines Visayas.

JSPS Ronpaku PhD Dissertation grant: 7 October-17 November study trip to Hokkaido, Japan

At the WAS meeting in Nashville: Dr. MJH Ramos [RIGHT] with Dr. Acacia Warren who worked with AQD on shrimp genetics in mid-'90s. Dr. Ramos presented a paper on giant clams in the *Tridacna* session, one of the 15 simultaneous sessions in the 5-day meeting



PHOTO COURTESY OF MJH RAMOS



PHOTO COURTESY OF SM URSUA

Ms. Shelah Mae Ursua is in the PhD dissertation program of Hokkaido University through a JSPS grant. She is working on *Genetic studies of seahorses for stock enhancement: species identification and population structure*

Follow-up Research Fellowship under the Japan Student Organization (JASSO); 22 August - 20 October 2013; Japan; With Prof. Dr. Atsushi Hagiwara

39th International Association of Aquatic and Marine Science Libraries and Information Centers (IAMSLIC) and 2013 SAIL Meeting; 20 - 24 October 2013; Florida, USA; organized by IAMSLIC

10th International Phycological Conference; 04 - 10 August 2013; Florida, USA; organized by the Phycological Society of America

International Federation of Library Associations and Institutions (IFLA) World Library and Information Congress (WLIC) 2013; 14 - 23 August 2013; Singapore; organized by IFLA

Visit Faculty of Fisheries Sciences, Hokkaido University; 06 October - 18 November 2013, 06 - 31 October 2013, 06 October - 01 November 2013; Hokkaido, Japan; organized by DOST and/or Hokkaido University under the framework of the Asia-Africa Science Platform Program of the Japan Society for the Promotion of Science

Training Course on Safety in the Use of Nuclear Equipment and Devices; 20-24 May 2013; Quezon City, Philippines; organized by Philippine Nuclear Research Institute

Training on Understanding and Establishing Laboratory Management System; 14 15 May 2013; Quezon City, Phil; organized by Philippine Metrology, Standard, Testing & Quality (PhilMSTQ) Center

Certificate Course on Fish Parasitology; 9-13 September 2013; Manila; organized by University of Santo Tomas, Manila

12th National Symposium on Marine Science; 24 - 26 October 2013; Tacloban City, Phil; organized by Philippine Association of Marine Sciences

21st Annual Meeting and Regional Scientific Convention of the Philippine Society for Microbiology (17-18 October 2013; Iloilo City, Philippines); organized by PSM-Visayas

Training-workshop on Measurement Uncertainty of Analytical Results (18-19 November 2013; Cagayan de Oro City, Philippines); organized by DOST-X, NorMin Laboratory Consortium Inc, Xavier University

Asian - Pacific Aquaculture (APA 2013) Conference: Positioning for Profit (10 - 13 December 2013; Ho Chi Minh City, Vietnam); organized by World Aquaculture Society



PHOTO COURTESY OF EB CONIZA

At the Asia-Pacific aquaculture conference in Hanoi: Dr. Eleonor Tendencia [SECOND FROM LEFT] talked about her study on *Management strategies employed by small-scale shrimp farmers in the Philippines in coping with economic crises due to shrimp diseases*. Mr. Eliseo Coniza [RIGHTMOST], on the other hand, discussed his paper on *Effect of liming on pond soil biota*. AQD scientists Dr. Mae Catacutan [LEFTMOST] and Dr. Relicardo Coloso [THIRD FROM RIGHT] were also present at the conference through grants from DOST



PHOTO COURTESY OF D CATEDRAL

Study-visit to Hokkaido University: AQD technical assistant Ms. Jilla Alcalde [FOREGROUND] and senior technical assistant Mr. Demy Catedral [RIGHTMOST] worked with Prof. Yutaka Itabashi and Prof. Tomoo Sawabe / Dr. Katsutoshi Arai [IN PINK]

## Statement of financial position and revenues & expenditures

STATEMENT OF FINANCIAL POSITION (US Dollar '000)		
	As of December 2013	As of December 2012
<b>ASSETS</b>		
<b>CURRENT ASSETS</b>		
Current and cash equivalents	2,358	1,874
Accounts receivables	525	418
Materials and supplies	79	62
Other current assets	-	28
Total current assets	2,962	2,382
<b>NON-CURRENT ASSETS</b>		
Cash investments	206	199
Other non-current assets	346	220
Total non-current assets	552	420
	3,514	2,803
<b>LIABILITIES</b>		
<b>CURRENT LIABILITIES</b>		
Accounts payable	479	416
Funds held-in-trust	338	212
Total liabilities	817	628
<b>NET ASSETS</b>		
Designated	2,697	2,172
Undesignated	-	-
Unrealized gain on AFS Financial assets		3
TOTAL NET ASSETS	2,697	2,175
TOTAL LIABILITIES AND NET ASSETS	3,514	2,803

STATEMENT OF REVENUES & EXPENDITURES (US Dollar '000)		
	As of December 2013	As of December 2012
<b>REVENUES</b>		
Contributions / Grants	5,068	5,055
Other income	633	464
TOTAL REVENUE	5,701	5,519
<b>EXPENDITURES</b>		
Research, verification & demonstration programs	3,148	2,808
Training & information programs	687	720
General administrative and non-projects	1,268	1,228
Total expenditures	5,103	4,756
<b>SURPLUS (DEFICIT)</b>	<b>598</b>	<b>763</b>

## The spirit of giving

In aid of its partner communities hard hit by super typhoon Yolanda / Haiyan, AQD employees, former trainees, and project partners raised a total of PHP 73,103 and US\$ 2,100 and this was given in December to AQD's six community partners in Panay and Negros islands: Concepcion and Ajuy, Iloilo; Dumarao, Capiz; New Washington, Aklan; Malalison Island and Caluya, Antique; and Molocaboc Island, Sagay. Other donations were made in kind.



## AQD personnel distribution

As of 31 December 2013, AQD had a total personnel complement of 193 (regular employees, 111; fixed-term employees, 82)

### AQD officers in 2013

*AQD Chief* Dr. Felix Ayson  
*Deputy Chief* Dr. Teruo Azuma  
*Head, RD* Dr. Relicardo Coloso /  
 Dr. Ma. Junemie Hazel Ramos  
*Head, TVDD* Dr. Emilia Qunitio  
*Head, TID* Dr. Evelyn Grace Ayson  
*Head, AFD* Ms. Kaylin Corre

*Head, BFS* Dr. Frolan Aya  
*Head, DBS* Dr. Emilia Qunitio  
*Head, IMS* Mr. Mateo Paquito Yap  
*Head, Manila Office* Dr. Ma. Rowena Eguia

#### Program leaders

*Producing quality seed for sustainable aquaculture* Dr. Ma. Rowena Eguia  
*Promoting healthy & wholesome aquaculture* Dr. Myrna Teruel and  
 Dr. Rolando Pakingking Jr  
*Maintaining environmental integrity through responsible aquaculture* Dr. Ma. Lourdes Aralar  
*Adapting to climate change* Dr. Fiona Pedroso  
*Meeting socio-economic challenges in aquaculture* Dr. Nerissa Salayo

*Regional programs* Dr. Teruo Azuma

#### Section heads

[RD]  
*Breeding & seed production* Dr. Fe Dolores Estepa  
*Fish health* Dr. Edgar Amar  
*Nutrition & feed development* Dr. Mae Catacutan  
*Farming systems & ecology* Dr. Jon Altamirano  
*Socioeconomics* Dr. Nerissa Salayo  
 [TVDD]  
*Demonstration & packaging* Ms. Jocelyn Ladja  
*Technology verification* Dr. Veronica Alava  
 [TID]  
*Training* Dr. Evelyn Grace Ayson  
*Development communication* Ms. Milagros Castañeros  
*Library & data banking services* Mr. Stephen Alayon  
 [AFD]  
*Engineering* Engr. Zaldy Suriaga  
*Human resource management* Ms. Nira Grace Llona  
*Budget-cashiering* Mr. Jiji Rillo  
*Accounting* Ms. Marivic Guevara /  
 Ms. Jasmine Gelvero

RD, Research Division

TVDD, Technology Verification & Demonstration Division

TID, Training & Information Division

AFD, Administration & Finance Division

BFS, Binangonan Freshwater Station

DBS, Dumangas Brackishwater Station

IMS, Igang Marine Station



# www.seafdec.org.ph

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization established in December 1967 to promote fisheries development in the region. The member-countries are Brunei Darussalam, Cambodia, Indonesia, Japan, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. The policy-making body of SEAFDEC is the Council of Directors, made-up of representatives of member countries.

SEAFDEC has four departments that focus on different aspects of fisheries development:

- **Training Department (TD)** in Samut Prakan, Thailand (1967) for training in marine capture fisheries
- **Marine Fisheries Research Department (MFRD)** in Singapore (1967) for post-harvest technologies
- **Aquaculture Department (AQD)** in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development
- **Marine Fishery Resources Development & Management Department (MFRDMD)** in Kuala Terengganu, Malaysia (1992) for the development and management of fishery resources in the exclusive economic zones of SEAFDEC member countries

AQD is mandated to:

- Conduct scientific research to generate aquaculture technologies appropriate for Southeast Asia
- Develop managerial, technical and skilled manpower for the aquaculture sector
- Produce, disseminate and exchange aquaculture information

AQD maintains four stations: Tigbauan Main Station and Dumangas Brackishwater Station in Iloilo province; Igang Marine Station in Guimaras province; and Binangonan Freshwater Station in Rizal province. AQD also has a Manila Office in Quezon City.

*Email:* aqdchief@seafdec.org.ph

*Tel.* (63 33) 330 7000 or 511 9170 *Fax* (63 33) 330 7031

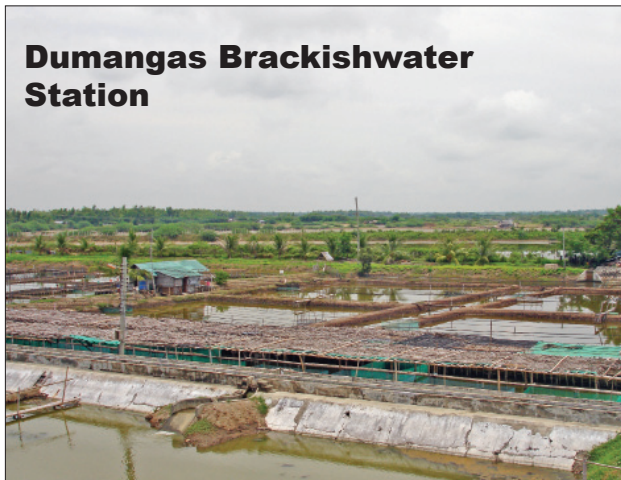
### Tigbauan Main Station



### Binangonan Freshwater Station



### Dumangas Brackishwater Station



### Igang Marine Station

