Research on Freshwater Fishes

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Abstract

Studies on tilapias focused on the refinement of strain comparison methods, refinement and pilot-testing of broodstock improvement procedure, selective breeding and evaluation of red tilapias, genetic variability determination in hatchery-bred tilapia and the development of criteria for tilapia fingerling quality assessment.

On carps, feeding of bighead carp (*Aristichthys nobilis*) to enhance reproductive performance was done and stunting was applied as a technique in broodstock development. Studies on the tolerance of bighead carp fry to low salinities were conducted. Free-living nematodes were tested as alternative larval food. The culture potential of grass carp in lake-based cages was also determined.

Research on the native catfish (*Clarias macrocephalus*) focused on endocrine studies during the final stages of maturation. Hatchery techniques were refined by identification of factors that increase fry production. Practical diets were developed for broodstock, hatchery, nursery and grow-out phases. A collaborative project on the ecological impact of African catfish (*C. gariepinus*) introduction in natural waters was undertaken.

The occurrence of EUS (epizootic ulcerative syndrome) among wild fishes in Laguna Lake decreases the marketability of both wild and cultured fishes from the lake. Hence, the bacteria and virus associated with EUS and their virulence, modes of disease transmission, developmental stages of dermal lesions and hematological changes in severely affected fish were studied.

Laguna Lake, where fish catch and aquaculture production contribute significantly to the country's total freshwater fish production, has been the focus of extensive ecological research in collaboration with other local and foreign research and academic institutions.

Introduction

The species of freshwater fish that are the subject of research at SEAFDEC Aquaculture Department are the tilapias *Oreochromis niloticus* and *Oreochromis* spp. (red tilapia), bighead carp *Aristichthys nobilis*, grass carp *Ctenopharyngodon idella*, and the Asian catfish *Clarias macrocephalus.* The extent of African catfish *Clarias gariepinus* farming in the Philippines was assessed as a special collaborative project between SEAFDEC/AQD and the Bureau of Fisheries and Aquatic Resources (BFAR).

The tilapias are a major group of freshwater fishes produced in the Philippines, Thailand, and

Malaysia (FAO, 1999). They are second only in production to the common carp *Cyprinus carpio* in Indonesia and are equally important with other freshwater fishes in Laos and Brunei. In the Philippines, the bighead carp has become an important food fish cultured mainly in pens and cages in Laguna Lake, the country's largest lake. More recently, some fish farmers have started culturing grass carp. The native catfish, *C. macrocephalus*, is one of the favorite freshwater food fishes in the Philippines but it is fast becoming scarce in many natural habitats. Lately, there is a renewed interest in the culture of *C. macrocephalus* due to advances in its artificial propagation.

As a continuation of the reports on studies on freshwater fishes conducted prior to 1994 (Carlos and Santiago, 1988; Fermin, 1988; Basiao, 1994; Emata, 1995), the present review focuses on research activities over the past five years (1995-1999) that address persistent problems in the culture of some freshwater fishes. It also includes a project on lake ecology and other related studies implemented during the period.

Nile Tilapia *Oreochromis niloticus* and the Red Tilapia

Strain comparison procedures

The efficiency of using an "internal reference" population in detecting strain differences under different environmental conditions has been demonstrated (Basiao and Doyle, 1990a,b; Romana-Eguia and Doyle, 1992, Romana-Eguia and Eguia, 1993). Recently, the growth and growth depensation of three strains of Nile tilapia fingerlings under crowded condition showed that inclusion of an "internal reference" strain increased the sensitivity of the statistical test to detect a 7% significant difference among strains (Basiao *et al.*, 1996). The Israel and NIFI strains grew significantly faster than the CLSU strain under experimental conditions. The crowding effect was eliminated after 12 weeks of compensation. The reference strain improved the resolution of the strain-testing experiment as shown by an increase of r^2 from 0.06 to 0.91. Approximately 450 replicate families would have been needed to detect the significant strain differences if the reference strain had not been included in the experimental design. In aquaculture, the cost of making a Type II error, i.e. believing that no difference exists when there is a real difference, can lead to opportunity costs of forgone production.

Broodstock improvement procedure

In artisanal spawning, there is a high non-genetic size variation among fish that are nominally of the same age. Large size is therefore not an accurate indicator of breeding value. For this and perhaps other reasons the conventional mass selection on tilapia has not shown positive results. The collimation procedure or early size-grading and culling of large fry reduces the self-amplifying phenotypic variance in growth. Collimation increases the genetic to phenotypic variance or heritability at the time of selection. The usual mass selection procedure was modified by size-grading the fingerlings before a two-step directional mass selection procedure was applied on Nile tilapia stocked in net cages in Laguna Lake. One generation of collimated mass selection resulted in a significant positive response of 3% relative to the control (Basiao and Doyle, 1999). The realized heritability (h^2) of » 16% is comparable with other recent estimates of h^2 in aquaculture populations of tilapia.

Farm-based broodstock improvement

The collimated mass selection procedure developed at BFS was pilot-tested in a small hatchery farm in Laguna, Philippines. This collaborative, farmer participatory research aims to assist farmers develop their own tilapia broodstock. Results of the farm trial showed a 7 to 9% response to selection after three months of culture (ZU Basiao, unpublished data). An important implication of the collimated

mass selection is that fish farmers would have more control over their choice of good quality spawners. Dependence on a "franchise-dealer" type of seed production will be minimized and socio-economically self-sustaining genetic conservation of breeds will be achieved.

Selective breeding and evaluation of red tilapia strains

The genetic improvement of a Philippine red tilapia strain through introgressive hybridization with Nile tilapia from Thailand and subsequent application of size-specific mass selection is in progress. Four distinct phenotypes e.g., bright red orange, orange with black blotches, pale pink and the grey color, were observed in the progenies of the mass-spawned introgressed red tilapia. One generation of introgression resulted in a significant positive response of 2.5% as measured by the percent differences in the lengths of introgressed red-orange offspring and the normal red tilapia (ZU Basiao, unpublished data).

A 2x2 factorial experiment to determine the reproductive efficiency of the introgressed red tilapia and the normal red (not introgressed with NIFI strain of Nile tilapia) fed a SEAFDEC-formulated diet and a commercial feed is another on-going study. Preliminary results showed that the number of spawning and seed (fry and egg) production were highest in the normal red breeders fed the commercial feed. The introgressed red and normal red tilapia fed the SEAFDEC-formulated feed showed comparable number of spawnings. However, fry production was higher in the normal red breeders, while egg production was higher in the introgressed red tilapia. The lowest spawning frequency and seed production were observed in the introgressed red breeders given the commercial feed (ZU Basiao, unpublished data).

The reproductive efficiency of three genetically diverse Philippine red tilapia strains (BFS, FAC and PF) and one Thai red tilapia strain (NIFI) in two seed production systems were compared with the objective of improving red tilapia fry production (Eguia, 1996). Results over a one-year period showed that seed (fry and egg) production in all strains was considerably higher in tanks than in cages. Mean daily seed production in tanks was highest for FAC (12.4 per spawner), followed by NIFI (11.2), BFS (9.5), and PF (5.6). Mean daily egg and fry harvests per female in fine-meshed net cages were 2.2 (BFS), 1.4 (NIFI), 1.2 (FAC), and 1.1 (PF).

In another study, the growth of three Philippine red tilapia strains and two imported Asian red tilapia strains (NIFI and HL) in freshwater, brackish water (17‰) and seawater (34‰) was evaluated (Romana-Eguia & Eguia, 1999). The five Asian strains grew best in brackish water. Likewise, the Taiwanese strain HL grew fastest in freshwater, and the Philippine strain PF in seawater, demonstrating that some Asian red tilapia strains can be developed for semi-intensive culture in brackish water ponds or marine cages.

Monitoring of genetic variation in hatchery-bred tilapia

The sustained growth of the aquaculture industry will depend largely on the development of a genetically promising seed with high level of genetic variation. Genetic drift, which can rob the hatchery population of genetic variance, is unavoidable in any selective breeding program. The genetic variation of hatchery-bred tilapia using protein and enzyme electrophoresis at BFS addresses this concern. Sixteen enzymes and 24 loci screened in three generations of hatchery-bred Nile tilapia using horizontal starch gel electrophoresis showed high levels of genetic variation in the enzymes SDH, GPI, PGM, CK, EST, and IDH. The six polymorphic loci detected in the parental population were also detected in the F_1 and F_2 generations (ZU Basiao, unpublished data), indicating that the level of genetic variation in the parental population was still present in the two succeeding generations.

Assessment of Tilapia Fingerling Quality

Research on tilapia fingerling quality evaluation is scarce. In fact, there is no standard method for assessing good quality tilapia fry and fingerlings. A quality assessment protocol is currently being developed and tested in several hatchery-bred tilapia fingerlings. A scoring index based on individual morphological, physiological and behavioral traits is used to rate tilapia fingerlings collected from ponds and lake-based farms around Laguna Lake. These fingerlings are also subjected to various stress and culture performance tests - i.e., handling and transport, salinity, growth and survival. From the results of the rating index and performance tests, a standard technique for fingerling evaluation will be established.

Bighead Carp *Aristichthys nobilis*

Broodstock development and management

The inconsistent number and viability of carp larvae from a female fish are among the major problems in bighead carp hatcheries. On the basis of previous results on supplemental feeding of bighead carp in cages in Laguna Lake (Santiago *et al*., 1991), a follow-up study was conducted to determine the effect of diets with or without vitamins A, E and C supplements on reproductive performance (Santiago and Gonzal, 1999). These vitamins were tested because of their known effects on reproduction in other fish species. The onset of gonad maturation was 2-3 months earlier in bighead carp given supplemental diet regardless of vitamin supplementation than in fish without supplemental diet. The test vitamins in the broodstock diets did not influence the number of gravid and milting fish monthly after sexual maturation has occurred. Results of induced spawning showed that fish fed the diet with supplemental vitamins A, E and C or vitamins A and C significantly increased the hatchability of eggs and the number of 3-day-old fry. The study further indicated that supplemental vitamin E was unnecessary, probably due to the high residual vitamin E in the diet ingredients.

High maintenance cost of bighead carp broodstock in cages, the relatively late sexual maturity (2-3 years), and unavailability of standard broodstock management techniques contribute to the problems encountered in carp hatchery operations. Stunting is being explored as a technique in carp broodstock development since stunted bighead carp demonstrates compensatory growth (AC Gonzal, unpublished data). An ongoing study compares the growth and reproductive performance of bighead carp stocked directly in cages in the lake from juvenile stage (control) and those of fish reared in tanks for multiples of 6 months prior to stocking in cages (AC Gonzal, personal communication). Preliminary results show that growth is faster in bighead carp reared in cages than in tanks, and some of the control fish have attained sexual maturity in 24 months.

Shortage of good quality broodstock hampers the commercial production of bighead carp. Population genetic studies using protein and enzyme electrophoresis have been initiated to document the genetic structure of bighead carp populations in Laguna Lake. This will be the basis for proper broodstock management and sound genetic improvement program. Initial result from one farm showed possible polymorphism only in a-GDH and IDH. The enzymes ADH, 6PGD, PGM, MDH, CK, GPI, HBDH and ME were all monomorphic (ZU Basiao, unpublished data).

Nursery

The culture of bighead carp in the Philippines is done mostly in Laguna Lake. Carp hatcheries in lakeshore communities support the nurseries and grow-out in cages, pens and ponds. However, the lake is subjected almost annually to saltwater inflow from Manila Bay through the Pasig River

when the lake's water level drops below that sea tide levels. A study was therefore conducted to assess the tolerance of bighead carp fry to a range of low salinities and determine the effect of salinity on growth (Garcia *et al*., 1999). Mean and median survival times of the fry (11, 18 and 35 days post-hatch) directly exposed to salinities ranging from 0 to l6‰ decreased as salinity of the rearing medium increased. Older fry were more tolerant to these salinities than younger fry. Survival in saline water also depends on the age of fry during initial exposure to low salinities. Median lethal salinity after 96 h revealed higher tolerance among 35-day old fry (7.6‰) than 18- (6.0‰) and 11-day old fry (2.3‰). The 18-day old fry reared in 0 and 2‰ for 3 and 4 weeks had higher growth than those reared in 4 and 6‰. The results demonstrate that bighead carp fry have some capability to osmoregulate that enables them to survive and grow in bodies of water subjected periodically to saltwater inflow.

Free-living nematodes constitute a portion of the diet of some fishes and crustaceans in natural environments. As part of a larger collaborative project funded by the European Union, a study on the suitability of free-living nematodes as alternative food for bighead carp larvae as well as those of several other fish species has been started. Preliminary results show that growth and survival of bighead carp larvae were lower than or comparable to those of larvae fed *Artemia* nauplii (CB Santiago, unpublished data). The ongoing study will test enriched nematodes as larval food for bighead carp and other fish species. The techniques for the mass production of free-living nematodes and a medium for their transport and storage in a semi-dry state will also be developed.

Grass Carp *Ctenopharyngodon idella*

Grow-out

Experiments were conducted to determine the culture potential of grass carp in Laguna Lake. Fingerlings with initial mean weight of 5 g were grown under different feeding regimes in net cages in the lake. After seven months, mean weight gain was highest (41 g) in the control fish fed rice bran (T Miharu and ZU Basiao, unpublished data). Fish fed the aquatic plant *Najas* sp. and the aquatic fern *Azolla* sp. had comparable weight gain (35.5 and 36.6 g).

Larger fingerlings with initial weight of 50 g were given two other aquatic weeds and rice bran as feeds. Preliminary results showed that the mean weight gain of fingerlings fed kangkong *Ipomoea aquatica* (21.4 g) and rice bran (20.8 g) did not differ significantly after six months. Fingerlings given water cabbage (*Pistia* sp.) had the lowest weight gain (15.4 g) (ZU Basiao and T Miharu, unpublished data).

Asian Catfish *Clarias macrocephalus*

Broodstock development

Endocrine studies during the final stages of maturation include the determination of optimum season for artificial propagation based on development of the gonads and serum steroid hormone profiles at different times of the reproductive cycle, and development of methods to minimize the sacrifice of male broodstock.

Captive *C. macrocephalus* contain mature oocytes (Tan-Fermin *et. al*., 1997a) and abundant spermatozoa (Tan-Fermin *et. al,* 1997b) year-round but do not spontaneously release the gametes. In spite of this, captive catfish show seasonality in gonad development during an annual cycle. Changes in reproductive parameters (gonadosomatic index or GSI, oocyte diameter, fecundity) and serum steroid hormone levels (testosterone or T and estradiol-17β or E_2) in female catfish suggest that the period January to April was not the best months to induce *C. macrocephalus* to spawn (Tan-Fermin

et. al., 1997a). Fluctuating mean values of GSI and serum steroid hormones (T and 11-ketotestosterone or 11-KT), lower levels of the maturation hormone (17α, 20β-dihydroxy-4-pregnen-3-one or DHP) and lower count of spermatozoa in January likewise indicated that, except in January, males can readily be a source of milt for artificial propagation any time of the year (Tan-Fermin *et. al*., 1997b).

Another study showed that luteinizing hormone releasing-hormone analogue plus pimozide (LHRHa+PIM)-injected fish during the off- and peak seasons similarly underwent oocyte maturation at 12 h post-injection followed by ovulation 4 h thereafter. However, mean values of the serum steroid hormones T, E_2 and DHP were lower during the off-season than peak season (Tan-Fermin et. al., 1999a). Egg production (20 eggs/g BW), fertilization (36%), hatching (20%) and larval survival (47%) rates were also much lower when gravid females were induced to spawn during the off-season than during the peak season (88 eggs/g body weight, 97%, 73%, and 95%, respectively; Tan-Fermin *et al*., 1997c).

Attempts to induce male catfish to spermiate and release milt by hormones (DHP, human chorionic gonadotropin, oxytocin) (JD Tan-Fermin, unpublished data) and pheromones (11ß-etiocholanolone-glucuronide, prostaglandin F2á) (LMaB Garcia, unpublished data) were unsuccessful. Hence, alternative methods to minimize the sacrifice of male catfish were pursued. An artificial seminal plasma was developed to immobilize sperm to conserve their energy. Milt diluted with the catfish artificial seminal plasma at 1:100 was activated with 0.6% NaCl to fertilize 5-10 g of ovulated eggs (Tan-Fermin *et. al*., 1999b). Activating the milt with 0.6% NaCl increased the number of motile sperm and duration of sperm motility. By this method, milt obtained from one male can be used to fertilize eggs from three to four females.

Catfish broodstock were usually given fish by-catch (trash fish) alone or in combination with an artificial feed for nourishment. Since fish-by-catch is scarce and supply is unreliable, nutritionally adequate diets were developed as alternative food and tested in feeding trials to determine their effect on reproductive performance (Santiago and Gonzal, 1997). Catfish fed some of the diets had high relative fecundity, hatching rate, and number of 3-day-old larvae demonstrating that artificial diet alone can enhance reproductive performance and that fish by-catch is dispensable in the daily ration of the catfish.

Hatchery

Hatchery techniques were refined by improving the hatching efficiency of the eggs, determining the appropriate food for first-feeding larvae, and conducting studies on zooplankton density, weaning, stocking density and feeding rates.

To improve the hatching efficiency of catfish eggs, the best rinsing solution following dry fertilization, optimum stocking density and water hardness during incubation were determined. Fertilization and hatching rates were high when the fertilized egg mass were rinsed with solutions containing salt or tannin, or upon stocking the eggs at 2000 - 4000/1. Total hardness of the incubation water (0-400 ppm) did not seem to be a critical factor for hatching catfish eggs in a flow-through water system (JD Tan-Fermin and P Subosa, unpublished data).

The best food for first-feeding catfish larvae was determined by comparing the efficiency of selected live organisms (*Artemia* sp., *Brachionus calyciflorus, Chironomus plumosus, Moina macrocopa, Tubifex* sp.) and an artificial diet (39% protein) given at 25% of fish biomass. In two feeding trials, best growth was consistently observed in *Tubifex*-fed larvae, indicating that *Tubifex* sp. can be a partial or complete substitute for the more expensive *Artemia* (Duller-Evangelista, 1996). However, the lack of techniques for sustained mass production of *Tubifex* sp. limits its use as live

food for catfish larvae on a commercial scale.

Catfish larvae fed newly hatched *Artemia* nauplii at 10 and 20 individuals/ml had significantly higher size, specific growth rate (SGR), and survival than those fed at lower prey densities of 0.5-5 individual/ml (Fermin *et al*., 1995). Catfish larvae reared exclusively on a dry diet had significantly smaller body size, and lower SGR and survival than those given *Artemia* during weaning or on *Artemia* alone (Fermin and Bolivar, 1991). Four-day-old catfish larvae stocked at 50-150 individuals/ l showed comparable growth and survival (Fermin *et. al*., 1995).

A feeding rate of 20% of body weight given twice daily was optimal for growth of Day 10 - 15 catfish fry when an artificial diet containing 40% crude protein was used. The same diet can be fed once daily at 5-10% to older fry (AC Fermin, unpublished data).

Nursery

Production of catfish fingerlings in the nursery was done by rearing the fry in fiberglass tanks and in net cages installed in 3-ton concrete tanks and 3000 m^2 ponds at different stocking densities for one month.

Twenty- to 25- day old catfish fry maintained with or without shelters in fiberglass tanks at 1 individual/l and 2 individuals/l both reached 3.5 cm total length. However, those stocked with shelters at 1 individual/l had higher survival than those reared at 2 individuals/l (AC Fermin, unpublished data).

Catfish fingerlings maintained at 200 and 300 had final total lengths of 4 cm and 5-6 cm when reared in tanks and ponds, respectively (AC Fermin unpublished data). Mean total lengths of 3 cm and 4 cm, acceptable sizes for stocking in the grow-out, can likewise be attained when fish were reared at 400-800 fry/m^2 in tanks and at 800-1200 fry/m^2 in ponds (R Bombeo *et al*., unpublished data). The faster growth of fingerlings in ponds than in tanks was probably due to the abundance of zooplankton, the preferred food of catfish fry during the early stage, in the rearing water. Furthermore, survival rates were high when fry were grown in net cages suspended in either tanks or ponds.

Grow-out

SEAFDEC-formulated diets containing 19% (Diet 1) or 34.2% (Diet 2) crude protein (CP), commercial fish pellets with 28.9% CP (Diet 3), and a combination of blanched chicken entrails and rice bran containing 31.8% CP (Diet 4) were tested as grow-out feeds for catfish (E Coniza *et al*., unpublished data). After 120 days of culture, fish fed Diet 2 showed the highest mean body weight (108.9 g), total length (23.3 cm), specific growth rate (2.85%), weight gain/day (0.88 g), apparent lipid retention (131.7%), and production (18.15 kg/25m^2). Fish on Diet 2 also had lower feed conversion ratio (2.5). Fish fed Diet 4 had the highest protein efficiency ratio (2.35) and whole body crude fat content (34.5%). Fish given Diets 3 and 4 had higher growth and production than fish fed Diet 1. Survival rates (67.7-81.2%) and results of sensory evaluation of the odor, flavor, and appearance of the flesh showed that these were not significantly different among the treatment groups.

Cost-return analysis on a per hectare per crop basis showed that catfish fed Diet 2 gave the highest net profit after payment of tax (P473,037), a return of investment (ROI) of 75% and lowest payback period of 1.3 years. Culturing catfish with Diets 4 and 3 resulted in similar ROI of 43%, while growing juveniles using Diet 1 was a losing investment.

African Catfish *Clarias gariepinus*

Based from the assessment of BFAR and SEAFDEC, African catfish farming in the Philippines is popular in Luzon, including the Bicol region, and some parts of Panay. Feeding experiments in the laboratory indicated that *C. gariepinus* consume a wide variety of live food, with high preference for shrimp and small fishes. Monitoring of fish catch and collection of African catfish proved that escapees in Laguna Lake can grow up to 12 kg. Examination of stomach contents of wild-caught *C. gariepinus* suggested that this species is omnivorous and can feed on almost any available food, especially fish and plant materials. Data indicate that the presence of African catfish in the lake can adversely affect the indigenous fish species in the wild and may contribute to biodiversity loss (AE Santiago and AC Gonzal, unpublished data).

EUS in Some Freshwater Fishes

The first confirmed outbreak of epizootic ulcerative syndrome (EUS) in the Philippines occurred in 1985 (Llobrera and Gacutan, 1987). EUS affected only some wild fishes in Laguna Lake such as the snakehead *Ophicephalus striatus*, Thai catfish *Clarias batrachus,* gouramis *Trichogater pectoralis* and *T. trichopterus,* white goby *Glossogobius guirus,* and climbing perch *Anabas testudineus.* The market value of both the wild and cultured fishes in the lake decreases whenever EUS-infected wild fishes are present.

Total bacterial counts of skin and muscle lesions increased with the severity of lesions in EUS-affected snakehead and Thai catfish (Lio-Po *et al*., 1992). Moreover, bacterial isolates were predominantly *Aeromonas hydrophila,* which, through injection, induce dermo-muscular necrotic lesions in fish. *A. hydrophila* isolated from normal, apparently normal, and EUS-affected catfish were all virulent to the catfish juveniles when injected intramuscularly (Leaño *et al*., 1996). However, only seven out of 16 extracellular protein (ECP) preparations induced dermo-necrotic lesions in the catfish, indicating a lack of correlation between virulence of *A. hydrophila* and ECP production.

Co-habitation with EUS-positive fish was a faster way of transmitting EUS in the snakehead than exposure to EUS-enzootic environment (Cruz-Lacierda and Shariff, 1995). Different modes of entry (intra-muscular injection, gastric gavage, fish food, and immersion of injured fish in water inoculated with the test bacteria) for A. *hydrophila* were tested, but only intra-muscular injection of at least 10^6 cfu/ml induced dermo-muscular necrotic lesions in *C. batrachus* (Lio-Po *et al*., 1996). In another study, *A. hydrophila* isolated from snakehead in the Philippines and *Aquaspirillum* sp., *Pseudomonas* sp., and *Streptococcus* sp. isolated from snakehead in Thailand were injected intramuscularly into healthy snakehead and catfish. Differences in the susceptibility of the two fish species to the bacteria were evident, with *A. hydrophila* inducing the most severe lesions in both species (Lio-Po *et al*., 1998).

Based on gross and histopathologic features, the developmental stages of dermal lesions in snakehead were characterized (Cruz-Lacierda, 1995). Histopathologically, all stages exhibited chronic, necrotic, and mycotic granulomatous inflammatory response to infection. A highly invasive, broad, branching, aseptate fungal hyphae were associated with all stages. Ultrastructural examinations showed that the fungal hyphae contained mitochondria with tubular cristae, which are morphologically consistent with Class Oomycetes. The stomach, spleen and kidney of fish with advanced stages of EUS also exhibited the mycotic granulomatous response to the infection.

Hematological changes in snakehead severely affected by the EUS include a significant decrease in hematocrit values and in serum protein and hemoglobin concentrations compared to those of

normal and apparently normal fish (Cruz-Lacierda and Shariff, 1994). In addition, granulocyte counts were significantly higher among severely affected fish than in normal and apparently normal fish.

Viruses have also been associated with EUS. A rhabdovirus (65x175 nm) from EUS-affected snakehead was isolated in snakehead spleen cells (SHS). The virus induced dermal lesions or caused high mortality in young snakehead (Lio-Po, 1998). Related studies on mixed infection, histopathology, and viral protein characterization were likewise conducted.

Lake Ecology and Related Studies

Water quality, nutrients, and plankton in Laguna Lake

As part of a bigger collaborative project funded by the European Union to develop a scientific base for a sustained and rational management of Laguna Lake especially for fisheries and aquaculture, the Lake was monitored from 1995 to 1997. Four institutions collaborated to monitor water quality as well as nutrients and plankton, fish biota, sediments, and watershed. Data gathered were used in calibrating water quality models developed for the lake by two other collaborating agencies.

Data gathered by the SEAFDEC/AQD team showed that temperature, dissolved oxygen, pH, alkalinity, and total hardness in Laguna Lake are favorable to lake aquatic biota and comparable with conditions for fish culture in ponds (AC Gonzal, unpublished data). Because of its shallow depth (average depth = 2.8 m) and vast surface area (90,000 ha), the lake is subject to wind-driven mixing of water, which disturbs the sediment thereby causing its generally turbid condition. Soil erosion from the watershed also contributes to lake turbidity. Field and laboratory data confirm earlier reports that turbidity in the lake can be controlled naturally by allowing saltwater inflow from Manila Bay through the Pasig River, usually during the peak of summer. The generally high turbidity of the lake limits primary production and, when water clears up following saltwater intrusion, the next limiting factor is nitrogen (N). Results of high frequency samplings after a typhoon, during algal bloom or during saltwater intrusion demonstrated rapid changes in water quality and nutrient levels, and the succession of diatoms and blue-green algae in the plankton community. Overall, primary production in Laguna Lake is low compared with other lakes having similar nutrient levels.

Effect of N/P ratios on phytoplankton from Laguna Lake

Changes in phytoplankton communities in lakes may be influenced by the quality and quantity of nutrient inputs, and by the ratio of N and phosphorus (P) in the water. When natural phytoplankton from Laguna Lake were batch cultured at three different N/P ratios (2N:1P; 6N:1P; 12N:1P) with each ratio containing two levels of phosphorus (0.5 and 0.25 mg P/l), all cultures were dominated by members of Chlorophyceae and Bacillariophyceae (M Cuvin-Aralar, unpublished data). The N/P ratios did not influence the number of species. However, the Shannon-Wiener diversity index (*H'*) and Index of Evenness (*J'*) were significantly low at the highest N/P ratio, demonstrating that this level of nutrient enrichment has led to the dominance of a few species. The proximate composition of the phytoplankton harvests did not differ significantly among the N/P ratios, but crude protein as percentage of organic matter was significantly higher in phytoplankton cultured in the two higher N/P ratios. Moreover, Nile tilapia fed with the phytoplankton grown at the highest N/P ratio had significantly better growth than fish fed harvests from the two lower N/P ratios (M Cuvin-Aralar, unpublished data).

Growth of dominant algal species from Laguna Lake

Because of the algae's importance to the development of a fish model, studies on growth of the

dominant algal species in Laguna Lake were conducted. Three algal species each representing the blue-greens, greens, and diatoms (*Microcystis aeruginosa, Pediastrum duplex* and *Cyclotella meneghiniana*) were isolated from lake water, grown in appropriate media, and established in axenic condition. In a study to determine optimum light intensity, highest growth of *Microcystis* was observed at a light intensity of 1000 lx; *Cyclotella* at 3200 lx and *Pediastrum* at 6400 lx (S Baldia, unpublished data). Moreover, experiments on the kinetics of nitrogen and phosphorus utilization using starved *Microcystis* cultures were conducted. Similar experiments on *Pediastrum* and *Cyclotella* are in progress.

Toxins in Microcystis aeruginosa

The role of toxins from the blue-green alga *Microcystis aeruginosa* in fish kills that sometimes occur in Laguna Lake has not been demonstrated. However, preliminary analyses showed that *Microcystis* harvested from Laguna Lake in 1996, 1998, and 1999 blooms were positive for mycrocystin (Fastner, personal communication). An ongoing study further identified and quantified the toxins in *Microcystis* at different stages of algal growth and determined the environmental conditions favoring blooms and toxin production (S Baldia, personal communication). Feeding trials will also be conducted to determine their toxic effects on fish.

Feeding ecology of cultured and wild fishes

Commercial feeds are used more often in the culture of Nile tilapia, especially during periods of high turbidity and low phytoplankton production in Laguna Lake. To optimize the benefits derived from this food source, a study was conducted to determine the feeding periodicity and daily ration of Nile tilapia in cages. Weights of stomach contents (expressed as percentage of body mass) were determined in tilapia juveniles obtained at 3-h intervals over a 24-h period and analyzed by the computer model MAXIMS (Richter *et al*., 1999). The model predicted that larger fish (mean weight = 31.5 g) on natural food alone fed continuously from dawn to dusk, ingesting an equivalent of 5.1% of fish body weight. Smaller fish (mean weight = 9.8 g) had two distinct feeding periods within daylight hours, ingesting 13.7 % body weight equivalent. Fish (mean weight = 81.7 g) given supplemental feed once in the morning when water was turbid fed intensely until the feed ran out before mid-day after which ingestion of natural food took place later in the day. The fish ingested supplemental feed equivalent to 5.8% of body weight and natural food equivalent to 5.1% of body weight. The feeding periodicity of milkfish *Chanos chanos* Forsskal in pens was likewise studied (H Richter, unpublished data).

Unlike cultured tilapia that feed mainly on phytoplankton when the lake is clear and on detritus when turbid (H Richter *et al*., 1999), wild fishes in Laguna Lake consume large amounts of other natural food items. The silver perch *Terapon plumbeus* were observed to feed on zooplankton, benthic crustaceans, benthic algae, fishes, and insects (M Koch *et. al*., in press). Fishes and benthic organisms were important food components in silver perch sampled close to a fish pen, while zooplankton and insects were more important for the fish in open water. In the case of the Manila catfish (*Arius manillensis*), zooplankton was the major food source at both stations (M Koch, unpublished data). In addition, molluscs were important in the diet of *A. manillensis* in the open water while crustaceans were more important in fish near the pen. Other food components (fish, insects, detritus and others) were equally important in fish at both stations. Small *A. manillensis* (<100 mm SL) fed mainly on zooplankton, while larger fish (>200 mm) fed nearly exclusively on fish. The above results demonstrate that aquaculture structures (the pens) enhance the food spectrum of wild fishes by forming a substrate for epiphytic algae that attract other potential food items. The pens also provide sanctuary to wild

fishes and benthic organisms. Results also revealed that there is little competition for natural food sources between the wild and cultured fishes in Laguna Lake.

Fish feeds and their efficiency

About 50% of the cage operators and 30% of the pen operators in Laguna Lake use commercial artificial feeds in growing fish. It was estimated that fish farmers in the lake use about 1,339 tons of commercial artificial feeds/yr. Assuming the feeds contain 30% crude protein and about 40% of nitrogen intake is retained in fish, the amount of nitrogen released to the lake due to feeding is estimated at 38.6 tons nitrogen annually. This is much lower than the estimated annual load of total Kjeldahl nitrogen from the watershed inclusive of two typhoons and occurrence of saltwater intrusion. However, improved management of feeding can further reduce nitrogen load due to feeds.

Under controlled conditions, the efficiency and metabolism of two commercial feeds commonly used by fish farmers in Laguna Lake were determined in feeding trials using growth measurements, feed analyses, comparative carcass analyses, and respirometry to construct energy budgets. Growth of tilapia increased as feeding rate increased from 2.5% of fish body weight to 5%, but feed efficiency tended to decrease. Metabolizable energy was higher for the control diet than for the commercial feeds. Results of the bioenergetics approach of examining the response of the fish to the test feeds agree with the growth measurements. Tilapia given commercial feeds to satiation performed similarly, but fish given a standard laboratory diet had superior performance, indicating room for improvement for the commercial feeds (CB Santiago, unpublished data).

Future Activities

Continuing research studies on freshwater fishes will be completed and, if possible, commodities for research will be diversified to include other indigenous food fishes as well as aquarium fishes and prawns. The limnology of Laguna Lake will be further studied, with emphasis on toxic and hazardous substances in the water, sediment, and the food chain.

References

Basiao ZU. 1994. Tilapia, Carp and Catfish. In: F Lacanilao, RM Coloso, and GF Quinitio (editors), Proceedings of the Seminar-Workshop on Aquaculture Development in Southeast Asia and Japan and Prospects for Seafarming and Searanching. Aquaculture Development in Southeast Asia, 19-23 August 1991, pp. 24-31. SEAFDEC Aquaculture Department. Iloilo, Philippines.

Basiao ZU and Doyle RW. 1990a. Interaction between test and reference populations when tilapia strains are compared by the 'internal control' technique. Aquaculture 85:200-214.

Basiao ZU and Doyle RW. 1990b. Use of internal reference population for growth rate comparison of tilapia strains. I. In a crowded environment. In: R Hirano and I Hanyu (editors), The Second Asian Fisheries Forum. pp. 503-506. Asian Fisheries Society, Manila, Philippines.

Basiao ZU and Doyle RW. 1999. Test of size-specific mass selection for Nile tilapia, *Oreochromis niloticus* L., cage farming in the Philippines. Aquaculture Research 30: 373-378.

Basiao ZU, Doyle RW and Arago AL. 1996. A statistical power analysis of the 'internal reference' technique for comparing growth and growth depensation of tilapia strains. Journal of Fish Biology 49: 277-286.

Carlos MH and Santiago CB. 1988. Nursery and grow-out operation for tilapia and carp. In: JV Juario and LV

Benitez (editors). Perspectives in Aquaculture Development in Southeast Asia and Japan. Proceedings of the Seminar Workshop on Aquaculture Development in Southeast Asia, 8-12 September 1987, pp. 281-292. SEAFDEC Aquaculture Department. Iloilo, Philippines.

Cruz-Lacierda ER. 1995. Histopathology and hematology of epizootic ulcerative syndrome (EUS) - positive snakehead (*Ophicephalus striatus*). Ph.D. Thesis, Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia. 215 pp.

Cruz-Lacierda ER and Shariff M. 1994. The hematological changes in snakehead (*Ophicephalus striatus*) affected by epizootic ulcerative syndrome. In: LM Chou, AD Munro, TJ Lam, TW Chen, LKK Cheong, JK Ding, KK Hooi, HW Khoo, VPE Phang, KF Shim and CH Tan (editors). The Third Asian Fisheries Forum, pp. 361-364. Asian Fisheries Society, Manila, Philippines.

Cruz-Lacierda ER and Shariff M. 1995. Experimental transmission of epizootic ulcerative syndrome (EUS) in snakehead, *Ophicephalus striatus.* In: M Shariff, JR Arthur, and RP Subasinghe (editors), Diseases in Asian Aquaculture II, pp. 327-336. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Duller-Evangelista AT. 1996. Comparison of selected live organisms and artificial diet as food for the larval rearing of catfish, *Clarias macrocephalus* (Gunther). MSc. Thesis submitted to the College of Fisheries, University of the Philippines in the Visayas. 112 pp.

Eguia MRR. 1996. Reproductive performance of four red tilapia strains in different seed production systems. The Israel Journal of Aquaculture - Bamidgeh 48: 10-18.

Emata AC. 1995. Research on Marine and Freshwater Fishes. In: TU Bagarinao and EEC Flores (editors). Towards Sustainable Aquaculture in Southeast Asia and Japan. Proceedings of the Seminar-Workshop on Aquaculture Development in Southeast Asia, 26-28 July 1994, pp. 167-186. SEAFDEC Aquaculture Department. Iloilo, Philippines.

FAO. 1999. Aquaculture Production Statistics 1988-1997. FAO Fisheries Circular No. 815, Rev. 11, Food and Agriculture Organisation of the United Nations, Rome.

Fermin AC. 1988. Broodstock management and seed production of tilapia and carp. In: JV Juario and LV Benitez (editors). Perspectives in Aquaculture Development in Southeast Asia and Japan. Proceedings of the Seminar Workshop on Aquaculture Development in Southeast Asia, 8-12 September 1987, pp. 211-230. SEAFDEC Aquaculture Department. Iloilo, Philippines.

Fermin AC and Bolivar MEC. 1991. Larval rearing of the Philippine freshwater catfish, *Clarias macrocephalus* (Gunther), fed live zooplankton and artificial diet: a preliminary study. The Israeli Journal of Aquaculture - Bamidgeh 43: 87-94.

Fermin AC and Bolivar MEC. 1996. Weaning of the Asian catfish, *Clarias macrocephalus* (Gunther), larvae to formulated dry diet. In: CB Santiago, RM Coloso, OM Millamena, IG Borlongan (editors), Feeds for Small-Scale Aquaculture, pp. 83-87. Proceedings of the National Seminar-Workshop on Fish Nutrition and Feeds. SEAFDEC Aquaculture Department, Iloilo, Philippines.

Fermin AC, Bolivar MEC, Balad-on SBM and Vargas JB. 1995. Improved hatchery rearing techniques for the Asian catfish *Clarias macrocephalus* (Gunther). In: P Lavens, E Jaspers and I Roelant (editors), Proceedings of Larvi '95 - fish and Shellfish Larviculture Symposium, pp. 394-397. European Aquaculture Society, Special Publications No. 24, Ghent, Belgium.

Garcia LMB, Garcia CMH, Pineda AFS, Gammad EA, Canta J, Simon SPD, Hilomen-Garcia GV, Gonzal AC and Santiago CB. 1999. Survival and growth of bighead carp fry exposed to low salinities. Aquaculture International. 7: 241-250.

Koch M, Focken U, Richter H, Becker K and Santiago C. (in press). Feeding ecology of silver perch (*Terapon plumbeus*) and the impact of fish pens in Laguna de Bay, Philippines. Journal of Applied Ichthyology

Llobrera AT and Gacutan RQ. 1987. *Aeromonas hydrophila* associated with ulcerative epizootic in Laguna de Bay, Philippines. Aquaculture. 67: 237-278.

Leano EM, Lio-Po GD and Dureza LA. 1996. Virulence and production of extracellular proteins (ECP) of *Aeromonas hydrophila* associated with the epizootic ulcerative syndrome (EUS) of freshwater fish. UPV Journal of Natural Sciences. 1: 30-38.

Lio-Po G. 1998. Studies on several virus isolates, bacteria and a fungus associated with epizootic ulcerative syndrome (EUS) of several fishes in the Philippines. Ph. D. Thesis, Simon Fraser University, British Columbia, Canada. 247pp.

Lio-Po GD, Albright LJ and Alapide-Tendencia. 1992. *Aeromonas hydrophila* in the epizootic ulcerative syndrome (EUS) of snakehead, *Ophicephalus striatus,* and catfish, *Clarias batrachus*: quantitative estimation in natural infection and experimental induction of dermo-muscular necrotic lesion. In: M Shariff, RP Subasinghe and JR Arthur, (editors), Diseases in Asian Aquaculture I, pp. 461-474. Fish Health Section, Asian Fisheries Society, Manila. Philippines

Lio-Po GD, Albright LJ and Leano EM. 1996. Experiments on virulence dose and portals of entry for *Aeromonas hydrophila* in walking catfish. Journal of Aquatic Animal Health 8: 340-343.

Lio-Po GD, Albright LJ, Michel C and Leano EM. 1998. Experimental induction of lesions in snakeheads (*Ophiocephalus striatus*) and catfish (*Clarias batrachus*) with *Aeromonas hydrophila*, *Aquaspirillum* sp., *Pseudomonas* sp. and *Streptococcus* sp. Journal of Applied Ichthyology 14: 75-79.

Richter H, Focken U, Becker K, Santiago CB and Afuang W. 1999. Analysing the diel feeding patterns and daily ration of Nile tilapia, *Oreochromis niloticus* (L.), in Laguna de Bay, Philippines. Journal of Applied Ichthyology. 15: 165-170.

Romana-Eguia MRR and Doyle RW. 1992. Genotype-environment interaction in the response of three Nile tilapia strains to poor nutrition. Aquaculture 108: 1-12.

Romana-Eguia MRR & Eguia RV. 1993. Growth response of three *Oreochromis niloticus* strains to feed restriction. The Israel Journal of Aquaculture - Bamidgeh 45: 8-17.

Romana-Eguia MRR and Eguia RV. 1999. Growth of five Asian red tilapia strains in saline environments. Aquaculture 173: 161-170.

Santiago CB, Camacho AS and Laron MA. 1991. Growth and reproductive performance of bighead carp (*Aristichthys nobilis*) reared with or without feeding in floating cages. Aquaculture 96: 109-117.

Santiago CB and Gonzal AC. 1997. Growth and reproductive performance of the Asian catfish *Clarias macrocephalus* (Gunther) fed artificial diets. Journal of Applied Ichthyology 13: 37-40.

Santiago CB and Gonzal AC. 1999. Effect of prepared diet and vitamins A, E and C supplementation on the reproductive performance of cage-reared bighead carp *Aristichthys nobilis* (Richardson). Journal of Applied Ichthyology. 16: 8-13.

Tan-Fermin JD, Ijiri S, Ueda H, Adachi S and Yamauchi K. 1997a. Ovarian development and serum steroid hormone profiles in hatchery-bred female catfish *Clarias macrocephalus* (Gunther) during an annual reproductive cycle. Fisheries Sciences 63: 867-872.

Tan-Fermin JD, Miura T, Ueda H, Adachi S and Yamauchi K. 1997b. Testicular histology and serum steroid hormone profiles in hatchery-bred male catfish *Clarias macrocephalus* (Gunther) during an annual reproductive cycle. Fisheries Science 63: 681-686.

Tan-Fermin JD, Pagador RR and Chavez RC. 1997c. LHRHa and pimozide-induced spawning of Asian catfish *Clarias macrocephalus* (Gunther) at different times during an annual reproductive cycle. Aquaculture 148: 323-331.

Tan-Fermin JD, Marte CL, Ueda H, Adachi S and Yamauchi K 1999a. Effect of season on oocyte development and serum steroid hormones in LHRHa and pimozide-injected catfish *Clarias macrocephalus* (Gunther). Fisheries Science 65: 865-870.

Tan-Fermin JD, Miura T, Adachi S and Yamauchi K. 1999b. Seminal plasma composition, sperm motility, and milt dilution in the Asian catfish *Clarias macrocephalus* (Gunther). Aquaculture 171: 323-338.