# 2003 Highlights

Annual Report
SEAFDEC Aquaculture Department
Tigbauan, Iloilo, Philippines

## Leadership in the aquaculture sector

# The AQD Chief reports

Aquaculture is a significant producer of fish and other fishery products needed for food security in Southeast Asia and the SEAFDEC Aquaculture Department continues to address the various constraints affecting aquaculture in the region. AQD carried out with greater vigor its mandate in research, technology verification and commercialization, and training and information dissemination. Of the departmental programs funded mainly by the host government, 40 research projects were implemented in broodstock management and seed quality improvement, responsible aquaculture technologies, and strategies for stock enhancement. Our researchers published 38 scientific papers this year and many won national awards. Farming technologies for tiger shrimp, seaweed, abalone, and freshwater fishes were demonstrated at AQD stations, government centers, and private farms, including the facilities in Jalajala, Rizal. Thirteen training courses, two of them conducted on-line, had 119 participants from 20 countries. AQD further served fish farmers and the general public through our hatcheries, service laboratories, library, web sites, manuals and textbooks, fairs and exhibits, and through FishWorld.

More region-wide projects were implemented by AQD this year under the three programs funded through the SEAFDEC-ASEAN Fisheries Consultative Group mechanism. The Fish Disease Program included several research studies, a training course, and publication of a laboratory manual and a flyer on the white-spot disease. The Mangrove-Friendly Shrimp Farming Program included verification and pilot demonstration in six Member Countries, some research studies, a training course, a seminar-workshop, a web site, and production of an extension manual and a video. The Integrated Regional Aquaculture Program started this year with site visits and surveys in nine Member-Countries, and has already conducted several training courses.

AQD celebrated its 30th anniversary in July with AquaBiz, a roadshow seminar of the commercially viable technologies AQD has generated from scientific research. The 270 participants included fish farmers and other entrepreneurs and members of government and the academe. During AquaBiz, AQD launched the extension manual *Biology and Hatchery of Mud Crabs Scylla spp*. Earlier in February, the Laboratory for Advanced Aquaculture Technologies, located at AQD, was turned over by Japan to the Philippines. AQD and DA/BFAR now jointly undertake an Aquaculture Biotechnology Program.

The year 2002 was particularly challenging for AQD. Without prior notice, the Philippine government imposed a mandatory retention of 15% in July and another 10% in December, which meant that PhP 37.5 M of AQD's budget for the year was withheld. This budget cut seriously affected AQD operations, and those of all government agencies. In October, the government also announced that AQD's budget for 2004 is going to be P100 M only. To ensure the continuance of operations and projects in 2004, AQD offered an attractive package for voluntary resignation and early retirement, which 82 employees availed of by December.

## Inside




Prepared by T Bagarinao (dorisb@aqd.seafdec.org.ph)

Training and Information Division

SEAFDEC Aquaculture Department

Published by the SEAFDEC Aquaculture Department

Tigbauan, Iloilo

April 2004

ISSN 1655-5228

# SEAFDEC/AQD responds to Southeast Asia


Cambodia


Cambodia


Myanmar


Cambodia


Vietnam


Lao PDR


Brunei


Vietnam

Malaysia


Thailand

# AQD in the context of SEAFDEC

## Programs of the Southeast Asian Fisheries Development Center in 2003

**Departmental Programs**

| | | |
|---|---|---|
| 1. | Center-Wide Information Network | SEC-IPC |
| 2. | Working Group on Regional Fisheries Policy | SEC-PPC |
| 3. | Exploration of Tuna Resources and By-catch of Tuna Fishing in the Indian Ocean | TD |
| 4. | Information and Communications Technology | TD |
| 5. | Broodstock Management and Seed Quality Improvement | AQD |
| 6. | Responsible and Sustainable Aquaculture Technologies | AQD |
| 7. | Strategies for Aquaculture-Based Stock Enhancement | AQD |
| 8. | Bilateral Projects of Philippine Government with AQD as Implementing Agency | AQD |
| 9. | SEAFDEC-JIRCAS Collaborative Program | AQD |
| 10. | Resource Assessment and Management | MFRDMD |
| 11. | Fishery Biology | MFRDMD |
| 12. | Marine Conservation and Stock Enhancement | MFRDMD |

**Programs under the ASEAN-SEAFDEC Fisheries Consultative Group Mechanism**

| | | |
|---|---|---|
| 1. | Regionalization of the Code of Conduct for Responsible Fisheries in Southeast Asia | SEC |
| 2. | Fish Trade and the Environment | SEC |
| 3. | Digitized Atlas | SEC |
| 4. | Management of Shark Fisheries and Utilization in Southeast Asia | SEC |
| 5. | Locally Based Coastal Fisheries Management | TD |
| 6. | Promotion of Mangrove-Friendly Aquaculture in Southeast Asia | AQD |
| 7. | Fish Diseases Diagnostic Methodologies for Aquaculture | AQD |
| 8. | Conservation and Management of Sea Turtles in Southeast Asia | MFRDMD |
| 9. | Information Collection for Sustainable Pelagic Fisheries in the South China Sea | MFRDMD |
| 10. | Application of HACCP in the Fish Processing Industry in Southeast Asia | MFRD |
| 11. | Seafood Safety Information Network | MFRD |
| 12. | Chloramphenicol and Nitrofuran Residues in Farmed Fish and Fish Products | MFRD |
| 13. | Special 5-year Program on Sustainable Fisheries for Food Security in the ASEAN Region | |
| | • Towards Decentralized Management for Sustainable Fisheries | SEC-PPC |
| | • Improvement of Fishery Statistical System and Mechanisms | SEC-PPC |
| | • Responsible Fishing Technologies and Practices | TD |
| | • Resource Enhancement | TD |
| | • Harvesting of Underexploited Resources | TD |
| | • Aquaculture for Rural Development | AQD |
| | • Supply of Good Quality Seeds | AQD |
| | • Identification of Indicators for Sustainable Development and Management of Fisheries | MFRDMD |
| | • Information Gathering for Inland Capture Fisheries | MFRDMD |
| | • Maximizing the Utilization of Fish Catch | MFRD |
| | • Fish Quality and Safety Management Systems | MFRD |

# Research

Abalone

Seaweed

Mudcrab

Scientific research is an important method for producing information and technologies for responsible aquaculture and sustainable development. The SEAFDEC Aquaculture Department approved a total of 56 research studies for 2003 (table on pages 6-7). Forty of these studies were conducted under five Departmental research programs with funding from AQD, the European Union through the European Commission, United States Agency for International Development, Japan International Research Center for Agricultural Sciences, International Foundation for Science, and private companies such as Rovithai Ltd. and Degussa Texturant Systems. The other 16 studies were conducted under the three programs of the SEAFDEC-ASEAN Fisheries Consultative Group, with funding from the Government of Japan Trust Fund and the ASEAN Foundation. The table on pages 6-7 shows the approved budgets and the actual expenses in 2003.

Tiger shrimp

# Research studies in 2003

| Year started | % done in 2003 | Title of Study | Study Leaders | Approved budgets (PhP) from AQD | External agencies | | Expenses (PhP) |
|---|---|---|---|---|---|---|---|
| **Program I. Broodstock Management and Seed Quality Improvement** | | | | | | | |
| 2002 | 70 | Culture and management of *Scylla* Species: Seed production | ET Quinitio, CL Torres, VR Alava | | 427,373 | EC-CMMS | 463,438 |
| 2002 | 70 | Culture and management of *Scylla* Species: Nursery | FDP Estepa EM Rodriguez | | 168,386 | EC-CMMS | 140,092 |
| 2000 | 90 | Semi-intensive seed production of grouper *Epinephelus coioides* | JD Toledo | | 70,200 | ACIAR | 200,005 |
| 1999 | 97 | The digestive system and enzymes of *Epinephelus coioides* larvae | GF Quinitio PS Eusebio | | 325,745 | ACIAR | 305,550 |
| 2000 | 95 | Lipid nutrition studies on grouper *Epinephelus coioides* larvae | VR Alava | | 20,000 | ACIAR | 59,076 |
| 2000 | 80 | Improvement of growth and survival in farmed rabbitfish *Siganus guttatus* | FG Ayson | | 2,933,870 | USAID | 1,579,003 |
| 2000 | 98 | Vitamin C requirement of milkfish (*Chanos chanos*) larvae | IG Borlongan | | 236,200 | Rovithai | 63,035 |
| 2002 | 100 | Molecular genetic tags for farmed Asian populations of Nile and red tilapia and their application in future selective breeding programmes (Thesis) | MRR Eguia | 40,500 | | | 34,453 |
| 2003 | 50 | Refinement of larval rearing techniques for *Macrobrachium rosenbergii*: Evaluation of live food for larvae | MA Laron | 42,475 | | | 35,993 |
| 2000 | 100 | Culture of *Ceriodaphnia cornuta* in tanks and its use in the hatchery production of freshwater fishes | AD Evangelista | 10,000 | | | 16,105 |
| 2000 | 40 | Evaluation of a recirculation tank system for milkfish broodstock | AA Delos Reyes | 33,000 | | | 3,393 |
| 1995 | 100 | Effect of supplemental feeding, stocking density and continuous lighting on growth and survival of red snapper larvae | MN Duray | 49,167 | | | 31,801 |
| 1999 | 100 | Intensive hatchery techniques for the grouper *Epinephelus coioides* and the rabbitfish *Siganus guttatus* | MN Duray | 244,940 | | | 210,376 |
| | | | **Total** | 420,082 | 4,181,774 | | 3,142,320 |
| **Program II. Technologies for Responsible and Sustainable Aquaculture** | | | | | | | |
| 2002 | 60 | Culture and management of *Scylla* Species: Refinement of mangrove-mudcrab pen systems | JH Primavera | | 387,204 | EC-CMMS | 280,552 |
| 2002 | 60 | Culture and management of *Scylla* Species: Fisheries of *Scylla* species in Western Visayas | JH Primavera | | 753,476 | EC-CMMS | 343,877 |
| 2003 | 60 | Culture and management of *Scylla* Species: Geography and hydrography of Ibajay and Kalibo mangroves (Thesis) | MJHL Lebata | | 8,000 | EC-CMMS | 2,015 |
| 2003 | 10 | Technical and economic feasibility of modular system vs. straight-run method of abalone farming in floating cages | WG Gallardo | | 150,250 | Triple M Abalone | approved Sep 2003 |
| 2000 | 85 | Diet for grow-out of the mud crab *Scylla serrata*: apparent nutrient digestibility of some feed ingredients and dietary energy sources | MR Catacutan | 20,500 | | | 20,805 |
| 2000 | 100 | The fate of the uneaten feeds in milkfish and tilapia farming | IL Olaguer | 10,000 | | | 8,012 |
| 2000 | 99 | Uptake of heavy metals by *Gracilariopsis bailinae* | NG Guanzon Jr. | 42,501 | | | 43,892 |
| 1998 | 90 | Grow-out of the Asian catfish *Clarias macrocephalus* in ponds | EB Coniza | 6,500 | | | 10,424 |
| 2003 | 0 | Farming of the prawn *Macrobrachium rosenbergii* in modular cages in Laguna de Bay | MLC Aralar | 39,540 | | ABCDEF | approved Nov 2003 |
| | | | **Total** | 119,041 | 1,298,930 | | 709,577 |
| **Program III. Strategies for Aquaculture-Based Stock Enhancement** | | | | | | | |
| 2003 | 5 | Release strategies for stock enhancement of the tropical abalone *Haliotis asinina* | WG Gallardo | | 103,000 | IFS | approved Sep 2003 |
| 2001 | 65 | Assessment of potential sites for release and stock enhancement of abalone, top shell and sea horse | WG Gallardo | 431,166 | | | 215,907 |
| 2000 | 65 | Refinement of breeding and hatchery techniques for abalone *Haliotis asinina* and top shell *Trochus niloticus* | RSJ Gapasin WG Gallardo | 250,000 | | | 176,522 |
| 1999 | 95 | Nursery and grow-out of juvenile seahorses in lighted sea cages | LMB Garcia | 149,044 | | | 169,974 |
| | | | **Total** | 830,210 | 103,000 | | 562,403 |
| **Program IV. SEAFDEC/AQD–DA/BFAR Aquaculture Biotechnology Program** | | | | | | | |
| 2001 | 75 | Cloning of growth hormone, growth factors and gonadotropins in mullet, grouper and snapper | EGT de Jesus | | 350,000 | DA-BFAR | 234,739 |
| 2002 | 5 | Phosphorus utilization from common feed ingredients in low-pollution diets for milkfish, tiger shrimp, and mangrove snapper | RM Coloso | | 90,000 | DA-BFAR | 44,920 |
| 2002 | 60 | Collection and cultivation of microalgae as potential live food and bioremediation agents | MR de la Peña S Suda | | 20,000 | DA-BFAR | 12,270 |
| 2002 | 45 | Evaluation of tropical Australian microalgae as feed for rotifers and other species in the hatchery | MCE del Castillo | | 36,500 | DA-BFAR | 21,762 |
| 1999 | 60 | DHA-producing thraustochytrid fungi as lipid source for fish larvae | MCE del Castillo | | 73,000 | DA-BFAR | 34,786 |
| 2001 | 100 | Protoplast formation and DNA polymorphism analysis of seaweeds | MRJ Luhan | | 213,834 | DA-BFAR | 314,057 |
| 1998 | 85 | Life histories of *Gracilaria* and *Gracilariopsis* | MRJ Luhan | | 116,583 | DA-BFAR | 112,203 |
| 2000 | 65 | Improvement of *Kappaphycus* strains for aquaculture | AQ Hurtado | | 397,806 | DA-BFAR | 282,830 |
| 2002 | 100 | Effect of epiphytes on the productivity of *Kappaphycus* | AQ Hurtado | | 942,250 | Degussa | 656,170 |
| | | | **Total** | | 2,239,973 | | 1,713,737 |

| Year started | % done in 2003 | Title of Study | Study Leaders | Approved budgets (PhP) from AQD | External agencies | | Expenses (PhP) |
|---|---|---|---|---|---|---|---|
| **Program V. SEAFDEC-JIRCAS Collaborative Program** | | | | | | | |
| 2003 | 0 | Pathogenesis and control of subclinical infection of grouper broodstock by viral nervous necrosis | I Kiryu | | not indicated | JIRCAS | |
| 2002 | 50 | Egg and larval quality of the mangrove red snapper fed improved broodstock diet | H Ogata<br>A Emata | 89,583 | 720,000 | JIRCAS | 645,973 |
| 2002 | 35 | Valuation of mangrove resources and services: Implications for the adoption of mangrove-friendly aquaculture in western Visayas | ND Salayo | | 198,600 | JIRCAS | 38,829 |
| 2002 | 50 | Property regimes in mangrove ecosystems: Implications for the adoption of mangrove-friendly aquaculture in the Philippines | SV Siar | | 224,765 | JIRCAS | 239,089 |
| 2003 | 30 | Property rights, governance and adoption of mangrove-friendly aquaculture technologies: the case of natural and reforested mangroves in the Philippines | DB Baticados | | 231,813 | JIRCAS | 78,702 |
| | | | **Total** | 89,583 | 1,375,178 | | 1,002,593 |
| **Program VI. Mangrove-Friendly Shrimp Farming** | | | | | | | |
| 2000 | 60 | Nutrient cycles in intensive shrimp culture (Phase II. Pond conditions) | NV Golez | | 793,180 | GOJ-TF | 634,283 |
| 2003 | 10 | Capacity of mangroves to process shrimp pond effluents. Phase II: Mechanisms of nutrient assimilation | AA Delos Reyes | | 780,395 | GOJ-TF | 273,879 |
| 2003 | 10 | Evaluation of probiotics and waste digesters used in the grow-out of the tiger shrimp *Penaeus monodon* | GL Po<br>TRC Mallare | | 796,471 | GOJ-TF | 110,836 |
| 2000 | 50 | Screening of probiotics for biocontrol/bioremediation in tiger shrimp farms I. Tank experiment | TRC Mallare | 84,500 | 130,600 | GOJ-TF | 146,570 |
| | | | **Total** | 84,500 | 2,500,646 | | 1,165,568 |
| **Program VII. Fish Disease Diagnostic Methodologies in Aquaculture** | | | | | | | |
| 2001 | 75 | Production of monoclonal antibodies against monodon baculovirus and hepatopancreatic parvo-like virus | ES Catap | | 443,500 | GOJ-TF | 234,868 |
| 2001 | 80 | Epizootiology of economically important viral diseases of wild *Penaeus monodon* | LD dela Peña | 169,166 | 799,000 | GOJ-TF | 591,309 |
| 2001 | 75 | Immunological indices for monitoring the health status of *Penaeus monodon* | EC Amar | | 212,000 | GOJ-TF | 51,414 |
| 2001 | 70 | Preventive measures against viral nervous necrosis in fish broodstock (grouper, milkfish, red snapper, sea bass) | LD dela Peña | | 699,000 | GOJ-TF | 164,127 |
| 2001 | 90 | Inhibition of luminous bacteria by tilapia 'green water' | EA Tendencia | 84,584 | 248,580 | GOJ-TF | 266,675 |
| 2000 | 90 | Use of bacteria as biological control agent against microbial diseases in shrimp *(Penaeus monodon)* and crab (*Scylla serrata*) hatcheries | CL Pitogo | 169,166 | 250,000 | GOJ-TF | 190,296 |
| 2000 | 92 | Parasitosis in marine and freshwater fishes: diagnosis, pathology, prevention and control of infection | EC Lacierda<br>GE Pagador | 70,136 | 353,657 | GOJ-TF | 279,753 |
| 2000 | 95 | Detection of pesticide and antibiotic residues in aquaculture products Phase I: Pesticide residues | IG Borlongan | | 131,500 | GOJ-TF | 42,534 |
| 2000 | 90 | Antibacterial metabolites in the microbial and phytoplankton flora of the 'green water' cultured *Penaeus monodon* | GL Po | | 125,000 | GOJ-TF | 82,124 |
| 2000 | 70 | Detection and identification of viral pathogens in farmed marine fishes | GL Po | | 778,000 | GOJ-TF | 266,074 |
| | | | **Total** | 493,051 | 4,040,237 | | 2,169,174 |
| **Program VIII. Integrated Regional Aquaculture Program** | | | | | | | |
| 2003 | 0 | Use of larval diets in the hatchery of milkfish and rabbitfish and early nursery of hormone-induced juveniles | FG Ayson | | 106,726 | GOJ-TF | approved Nov 2003 |
| 2003 | 0 | Practical diets for first feeding and older larvae of carnivorous fish species: snapper and sea bass | IG Borlongan | | 54,000 | GOJ-TF | approved Nov 2003 |
| | | | **Total** | | 160,726 | | |
| | | | **Grand Total** | 2,036,467 | 15,900,464 | | 10,465,372 |

EC-CMMS European Commission–Culture and management of *Scylla* spp.
JIRCAS Japan International Research Center for Agricultural Sciences
ACIAR Australian Center for International Agricultural Research
USAID United States Agency for International Development
IFS International Foundation for Science
GOJ-TF Government of Japan Trust Fund

# Improvement of broodstock and seed

The mud crabs *Scylla serrata*, *Scylla olivacea*, and *S. tranquebarica* have been the focus of much research at SEAFEC AQD over the past five years, much of it funded externally (earlier by the Australian Center for International Agricultural Research and recently by the European Union). In 2003, several studies were done on broodstock management and improvement of larval survival.

**Broodstock and larval quality.** Ovarian maturation in wild-sourced and pond-grown *Scylla serrata* was studied in terms of gross morphology, histological appearance, and nutrient profiles. Six ovarian stages from immature to spent can be discerned. During maturation, protein, lipids, minerals, and ascorbic acid were accumulated in the ovaries, which suggests that these nutrients are needed in reproduction. In wild-sourced crabs, the essential amino acids arginine, leucine, methionine, and valine increased between the immature and mature ovaries; triglycerides and phosphatidylcholine were accumulated; and the concentrations of essential fatty acids (docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid) were high. Lipids decreased as embryos developed and eggs hatched into zoeae. Information on nutrient profiles serves as basis for the improvement of diets for mud crab broodstock and larvae.

**Functional and physiological maturity of mud crabs.** Hatchery-reared *S. serrata*, *S. tranquebarica* and *S. olivacea* were grown to maturity, meristic measurements were made, and the gonads and external sexual characteristics were monitored. Abdomen width, carapace length, and propodus height, thickness, and length correlated with carapace width in different ways according to species and sex. Mating readiness occurs at smaller carapace width in *S. olivacea* (7.9 cm females, 7.8 cm males) than in *S. tranquebarica* (9.1 cm females, 8.7 cm males) and *S. serrata* (9.9 cm females, 9.4 cm males).

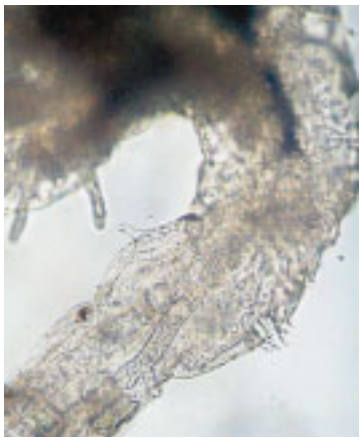
**Control of diseases in mud crabs.** Diseases in mud crab broodstock held for 3 months in tanks include shell disease due to a combination of fouling organisms and chitinolytic bacteria. Shell disease was first manifested by off-white and black patches on the shell and progressed to become perforations through which nematodes and other saprophytic organisms entered. Fouling affected the integrity of the shell. Disease problems during mud crab larval rearing included severe fouling of eggs and larvae with filamentous bacteria, protozoan infestation, and sporadic fungal infection in the egg mass. Mortality of larvae in the hatchery was due to systemic bacterial infection and occasionally, fungal infection. Based on several runs, the main sources of bacterial pathogens in the hatchery were developing eggs, untreated water supply, and natural food (rotifers and brine shrimp). Chlorination of the water supply (10-15 ppm active chlorine) effectively eliminated pathogenic bacteria in the water but not from other sources identified.

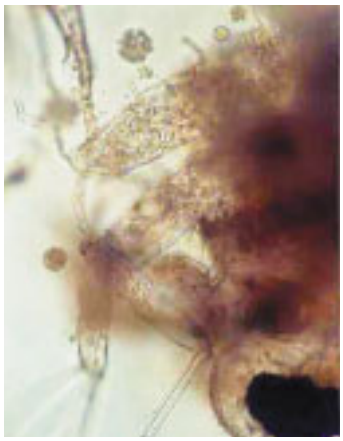
Fungi that were isolated from mud crab eggs and larvae are of two general types, vesicle-formers (*Lagenidium*-like) and non-vesicle-formers (*Sirolpidium*-like). Spore formation and release in both types of fungi were inhibited by a 10 ppm formalin bath for 24-40 h, but the fungi remained viable and grew when replanted on peptone yeast extract agar. Fungal hyphae exposed to 50 ppm and 100 ppm formalin for 24-40 h did not produce nor release spores and were not viable after exposure.

Crab with severe shell disease (large black patches) due to chitinolytic bacteria

Crab with perforations on the shell due to chitinolytic bacteria

Crab eggs with severe fouling due to sessile protozoans





Crab larvae with fungal infections

**Molting and growth of mud crab larvae at different salinities.** The effect of salinity on the molting interval and growth of early crab stages (from crab instar 1) of three species of *Scylla* was investigated. Among *Scylla olivacea*, survival rates (92 to 100%) and molt intervals (4 to 10 days) were similar among test salinities of 12, 16, 20, 24, and 32 ppt. However, molting at 20 ppt was more frequent, and the interval from molt 2 to molts 3 and 4 shorter at 12-24 ppt than at 32 ppt. After one month, crab weights were significantly higher at 12-16 ppt than at 20-24 ppt, and lowest at 32 ppt.

**Nursery of mud crabs.** Mud crab growers prefer larger juveniles and nursery techniques are being tested to grow small juveniles (0.25 g, 1 cm carapace width) to larger sizes (10 g, 3 cm). One experiment showed that *S. serrata* juveniles stocked in hapa nets had higher survival (77 ± 7%) than those stocked directly in earthen ponds (40 ± 4%). Stocking densities of 1, 3, and 5 individuals/$m^2$ did not affect growth and survival after one month in hapa net or pond. Another experiment compared the effect of different feeds (pellets, mussel meat, or mix of both) on *S. serrata* megalopae stocked in hapa net nursery. Low survival of 11-12% was obtained because of very low salinity (due to a typhoon) and difficulty in water replacement.

**Importance of HUFA to larvae of grouper *Epinephelus coioides*.** This study measured the amounts of highly unsaturated fatty acids (HUFA) in grouper eggs and larvae and in the plankton used in larval rearing, particularly the concentrations of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) in the tissues. Neurula eggs spawned by grouper fed fish biomass contained 0.18, 0.07, 0.10 µg/egg of polar DHA, EPA, and ARA, and 0.58, 0.22, 0.16 µg/egg of neutral DHA, EPA, and ARA. Both neutral and polar DHA and ARA were conserved in starved day 4 larvae, but EPA was depleted. With continuous feeding of larvae, both neutral and polar lipids increased with larval age (day 8, 15, 25, 35). Three-day starvation of these larvae significantly reduced the neutral DHA, EPA and ARA in older larvae, but not in younger day 11 larvae.

Rotifers reared on *Chlorella* and those enriched with the commercial products, HUFA Enriched and Ratio HUFA, contained high EPA, whereas those enriched with the products AG Adv and Alg 3050 contained the highest DHA. Polar lipids were higher than neutral lipids in *Chlorella*-reared rotifers but both declined with starvation time (3, 6, 12, 24 h). Thus, to make use of the best DHA, EPA, and ARA content, the rotifers should be fed to grouper larvae within 3 hours of harvest. *Artemia* nauplii enriched with AG Adv, AG F15, and Alg 3050 contained the highest DHA, whereas those fed Alg 3050 and the emulsions HUFA Enriched, Ratio HUFA, and Super HUFA, contained the highest EPA.

Grouper larvae fed rotifers enriched with various HUFA boosters showed better survival to day 14 than those fed unenriched rotifers. Larval growth rates were better with rotifers enriched with Alg 3050 and AG Adv. Metamorphic larvae (day 25-35) fed enriched *Artemia* had significantly higher growth, pigmentation, and survival than those fed unenriched *Artemia*. *Artemia* enriched with HUFA Enriched, Alg 3050, and mixed HUFA improved larval growth and survival. Total lipids and fatty acids were higher in enriched *Artemia* than in the control, and similarly so in the grouper larvae.

**The digestive system and enzymes of *E. coioides* larvae.** The activity of different enzymes was determined in the tissues of grouper larvae from hatching to day 60. Maltase activity was weak in all organs until day 20 and could not de detected thereafter. The brush border of the intestine had moderate enzyme activity on days 2-12 and high activity thereafter. The brush border of the pyloric caeca had high activity on days 25-60. Lipase activity in the intestine was low from day 14 and high from day 25; high in the pyloric caeca starting day 25, low in the stomach and liver from day 30, and was observed in the pancreas only from day 60. Trypsin activity was detected in larvae as early as hatching, and chymotrypsin activity on day 4. Trypsin activity was higher than chymotrypsin on days 8-60. However, chymotrypsin activity increased from day 40 to 45, and trypsin activity decreased from day 40 to 50. Leucine aminopeptidase activity was nearly zero on days 0-8, low on days 16-60, and high on day 40 at the start of metamorphosis of grouper

larvae. Acid phosphatase activity increased from day 12 to day 60. Alkaline phosphatase activity increased from hatching to day 60, highest at metamorphosis.

**Use of copepods in the hatchery of *E. coioides*.** Use of pond-grown zooplankton, particularly calanoid copepods, in the semi-intensive hatchery rearing of grouper increased larval survival from 3 to 10% at day 35-40. Thus, various techniques were tested for the mass production of zooplankton in earthen ponds. After 9-12 days in standing water, the zooplankton population in different ponds increased from 86-148 individuals/L to 1,524-3,186 ind/L. The major zooplankton components were rotifers, copepods, and cladocerans. The copepod *Acartia tsuensis* can be propagated in one-ton tanks on mixed microalgae with or without bread yeast. Density of A. tsuensis increased from 60 ind/L at stocking to about 900 ind/L after 3-5 days. A prototype collector for copepod eggs and nauplii was tested. The average numbers of eggs and nauplii collected (2,300-117,600 ind/L) varied with the density of copepodids and adults (100-800 ind/L) in the holding container. Hatching rates of collected eggs ranged 34-89%. Collected eggs may be stored at 4-10 °C for up to 7 days, but the viability of eggs was affected by protozoans. Eggs at the cleavage stage can survive freezing (thus, cryopreservation may be feasible).

**Improvement of growth and survival in the rabbitfish *Siganus guttatus*.** Thyroid hormone ($T_3$) treatment given through the spawners and/or through the rearing water improved the survival of rabbitfish larvae. In a separate experiment, 3-week old larvae immersed for one week in 0.01 ppm $T_3$ metamorphosed earlier than in the other $T_3$ doses. After metamorphosis, the juveniles can already be weaned to seaweeds or artificial diets. The system for the laboratory-scale production of recombinant rabbitfish GH is being set up. Liposomes containing recombinant rabbitfish GH will be prepared. These liposomes will then be mixed in microdiets and fed to rabbitfish larvae to test their efficacy as vehicle for oral delivery of recombinant rabbitfish GH.

**Vitamin C requirement of milkfish *Chanos chanos* larvae.** Diets with supplemental L-ascorbyl-2-phosphate supported significantly better growth and higher survival among milkfish larvae than a diet without vitamin C. Ascorbic acid and collagen content in the larvae increased with the amount of supplemental phosphorylated ascorbic acid. The optimum dietary vitamin C level required for normal growth and survival of milkfish larvae is about 200 mg ascorbic acid equivalent per kg dry diet.

### Larval Food Lab

A total of 16,880 liters of starter suspensions of *Chlorella* sp., *Tetraselmis* sp., *Chaetoceros* sp., *Brachionus* sp., *Skeletonema* sp. and *Artemia* sp. were provided to AQD researchers and the private sector. Hatching efficiency and hatching rate of different *Artemia* brands were determined upon request of the private sector.

**Molecular genetic tags for farmed Asian populations of Nile tilapia *Oreochromis niloticus* and red tilapia.** One study determined the genetic marker variation and growth of four Nile tilapia stocks (designated by the acronym of the institution or project of origin) under different conditions. When the stocks were fed a poor diet of rice bran, the FAC tilapia grew only slightly better (length increment = 29 mm) than GIFT, NIFI, and SEAFDEC (26 mm), but survival of the GIFT stock (92%) was significantly higher than of FAC (77%), SEAFDEC (48%), and NIFI (5%). When the stocks were grown in brackish water, growth was not significantly different between FAC (31 mm) and the others (25-27 mm), but the GIFT survived significantly better (90%) than FAC (77%), NIFI (23%), and SEAFDEC (5%).

Another study determined the genetic diversity across four generations of Nile tilapia (Thai Chitralada strain) that have undergone size-specific mass selection. Two control (unselected) stocks (first generation C1 and fourth generation C4) and three selected stocks (first generation S1, second generation S2, and fourth generation S4) were analyzed for microsatellite marker variation to determine the effect of size-specific mass selection on the stocks' genetic variability. Genetic variation at five microsatellite loci showed a slightly higher allelic diversity in the selected stocks (7.4-10 alleles) than in the control stocks (6.8-8.8 alleles). C1 had a higher expected heterozygosity ($\underline{H}e = 0.762$) than C4, and S2 had a higher $\underline{H}e = 0.769$ than S1and S4. Reductions in the number of alleles and in $\underline{H}e$ were noted in successive generations of both the control and selected lines. Significant deviations from Hardy-Weinberg equilibrium indicated inbreeding in all the control and selected stocks. The estimated inbreeding levels were not significantly different among the selected and control lines (based on Welch's t-test), but the increase in the degree of inbreeding after four generations was slightly higher within the selected line than within the control line.

**Live food for freshwater fishes.** The freshwater cladoceran *Ceriodaphnia cornuta* in Laguna de Bay was propagated and used, alone or in mixtures with *Moina*, *Tubifex*, and *Artemia*, as feed for bighead carp, Nile tilapia, and catfish. Survival was highest among larvae fed *Ceriodaphnia* alone, but growth was highest among larvae fed zooplankton mixtures, particularly 70% *Moina* + 25% *Ceriodaphnia* + 5% *Tubifex*.

**Live food for freshwater prawn *Macrobrachium rosenbergii*.** To refine larval rearing techniques for the freshwater prawn, three species of live food were tested: *Moina*, *Artemia* and the free-living nematode *Panagrellus redivivus*. Growth, survival, and larval production differed significantly among treatments. Larvae fed *Moina* were larger than those fed *Artemia*. Larvae fed *P. redivivus* survived until day 8 only.

# Technologies for responsible aquaculture

Under this program were studies on grow-out techniques for mud crabs, abalone, and catfish, uptake of heavy metals by of seaweeds, and related ecological studies on mud crabs and mangroves.

**Refinement of mangrove-mud crab pen system.** Wild-sourced juvenile *Scylla olivacea* were grown in net-pens provided with different food sources. A partial harvest showed the biggest crabs from pens where fish biomass was used as feed. Almost as big were the crabs given strips of cow or carabao hide. Crabs in pens with natural productivity alone, or given pellets alone were small. A 4-month rearing trial to compare the color varieties of *S. olivacea* showed that 'sinaw' (lighter, shiny, less aggressive) grew larger than 'morongsod' (dark, very aggressive). In another run, hatchery-bred juveniles of *S. tranquebarica* and *S. serrata* were stocked at 0.8/m$^2$ and fed either a low-cost pellet only, or pellet with fish biomass. Results showed that *S. serrata* grew larger on the mixed diet, and *S. tranquebarica* grew well on pellets only. The grow-out experiments showed the following results:

- To grow mud crabs in mangrove net-pens (mesh 2.5 cm), the minimum stocking size is 3.5 cm carapace width.
- Wild juveniles in net-pens survive for one month without being given food, but hatchery-bred juveniles do not, probably because they are not familiar with the natural food items in the mangroves.
- Juvenile crabs must be weaned to an all-pellet diet for at least 2 weeks in nursery ponds before transfer to net-pens.

**Grow-out diet for *Scylla serrata*.** Plant feedstuff, namely corn meal, copra meal, and rice bran were found to be digestible by mud crabs (3-5 g wet weight). These ingredients were used as carbohydrate sources in test diets for mud crabs, and the growth of crabs was compared. Frequent molting in crabs means faster growth, and newly molted crabs have high water content. Since molting does not occur at the same time, each crab was monitored to determine the number of days for each crab to reach an arbitrary endpoint, the 5th day after the 3rd molt. At this end point, the crab weight and carapace width were measured. The diets tested had similar effect on crab growth. The total number of days for each crab to reach the arbitrary date from the day dietary treatments were given averaged 160 ± 22 days. Crab flesh had 76% moisture and 74% protein on average.

**Modular versus straight-run net-cage culture of abalone *Haliotis asinina*.** A collaborative research with the private sector on the culture of abalone in net cages was started in September 2003. The first abalone stocks were fed mixed species of wild seaweeds available at the cage site in Panobolon, Guimaras, but the growth was inferior to that of abalone fed *Gracilaria* only. *Gracilaria* will therefore be used in all subsequent grow-out runs to compare the modular and straight-run culture methods.

**Grow-out of Asian catfish *Clarias macrocephalus* in ponds.** Juveniles (weight 0.3 g, length 3.3 cm) of the Asian catfish were stocked at 10, 20 and 30 fish/m$^2$ in nine 18 m$^2$ tanks lined with mud and were fed a SEAFDEC-formulated diet with 34% crude protein. After 195 days of culture, catfish stocked at 10 fish/m2 were significantly larger (85 g, 22 cm) and survived better (96%) than those at 20 and 30 fish/m$^2$. Feed conversion ratio ranged 2.6–3.2 but did not differ among the densities tested. Catfish production at 30/m$^2$ was higher than at 10 and 20 fish /m2. To study compensatory growth, different sizes of same-age juveniles (small 0.2 g, 2.5 cm; medium 1.1 g, 5 cm; and large 6.5 g, 9 cm) were stocked at 10 fish/m$^2$ in nine 3-m$^2$ tanks lined with mud and given the same SEAFDEC diet. After 180 days of culture, large juveniles grew to significantly larger sizes (108 g, 24 cm; weight gain 0.6 g/day) and yielded more at harvest than the medium and small juveniles, but survival was all high at 95-99%.

**Uptake of heavy metals by seaweeds.** Seaweeds are increasingly being used to clean up effluents in shrimp farms with integrated recirculation systems. Seaweeds do not absorb nutrients only, but also other pollutants such as heavy metals. The red seaweed *Gracilariopsis bailinae* is being tested for use in treating effluents in shrimp farms, and this study determined the capacity of *G. bailinae* to sequester heavy metals. The seaweed was exposed to copper, zinc, and cadmium at concentrations of 50-500 ppm for 6-72 h. Copper contents in the thallus and in the agar were directly proportional to concentration (highest at 500 ppm copper in the medium) and to exposure time (highest at 72 h). Zinc content in the thallus was highest after 72 h exposure to 400 ppm zinc; zinc in the agar was highest after 24 h in 500 ppm zinc. Cadmium in the thallus was highest after 36 h at 400 ppm; cadmium in the agar was highest after 60 h at 500 ppm. These results indicate that *G. bailinae* can effectively sequester heavy metals.

### Centralized Analytical Lab

A total of 688 water samples were analyzed for pH, nitrite, ammonia, phosphate, and dissolved oxygen; 67 soil samples for pH, organic matter content, lime requirement, available sulfate, and available phosphate; and 377 feed ingredient samples for moisture, crude protein, crude fat, fiber, ash, and phosphorus.

### Pilot Feed Mill

A total of 21,392 kg of various milled feeds and feed ingredients were dispensed.

**Mud crab fisheries in Western Visayas.** Mud crab populations in a replanted mangrove forest (Buswang, Kalibo) and in a natural forest (Bugtong Bato, Ibajay) were monitored twice a month since April 2002. Sampling in Kalibo and Ibajay, Aklan from Jan to Oct 2003 yielded a total of 6,792 crabs caught by four kinds of fishing gear: 'bintol' (baited lift net), 'tapangan' (bamboo trap), 'bakikong' (set net), and 'tonton' (baited line and scoop). Two-thirds of the catch was from Kalibo, where 'tapangan' gave the highest catch (both number and biomass) per unit effort (CPUE per trap per tide). In lbajay, 'bintol' caught the most crabs, but the 'tapangan' caught the largest biomass. The catch was mostly *Scylla olivacea* at both sites. *Scylla* tranquebarica was caught mostly by 'bakikong' in Kalibo and by 'tapangan' in lbajay. *Scylla serrata* comprised <1% of the catch in Kalibo and Ibajay. The catch consisted mostly of males (52-65%) almost throughout the year, except Jul-Aug in Kalibo and Jan and Jul in Ibajay when females were more abundant.

Sampling of mud crabs by 'bakikong' in Kalibo from Mar 2002 to Dec 2003 (21 months) yielded 71% *S. olivacea*, 28% *S. tranquebarica* and 0.04% *S. serrata*. This result suggests that *S. olivacea* is more mangrove-dependent than the other species. Mark-recapture data showed that *S. olivacea* moves extensively within the Aklan estuary, but not out to sea. Crabs caught by 'tapangan' and 'bintol' increased in average size over the 21-month period from 61 grams (6.5 cm carapace width) to 96 grams (7.6 cm). But CPUE decreased nearly to half from Apr 2002 to Nov 2003. The mean size of the captured crabs was 7.5 cm, smaller than the size of females at first sexual maturity (8.4 cm). Gravid females were caught every month; thus, reproduction was year-round.

**Geography and hydrography of Ibajay and Kalibo mangroves.** Mapping of the mangroves in Ibajay, Aklan was done in February and included the major and minor creeks; the total mangrove area was about 70 hectares. The plantation mangroves of New Buswang, Kalibo, Aklan were mapped in May. Formerly 50 ha, the reforested mangroves now measure 57 ha due to 7 ha of natural growth. Across the river from the reforested mangroves, the muddy coast is now also covered with 13 ha of natural growth. Including the nipa groves north of the reforested area, the New Buswang mangroves now cover a total of 75 ha. Plotted in the new mangrove maps are study sites for a mark-recapture experiment and for stock enhancement of mud crabs. Crab collectors were identified to help in the mark-recapture experiment.

**Information dissemination for mangrove conservation.** AQD researchers lectured in the nationwide training program of the Bureau of Fisheries and Aquatic Resources' Fisheries Resource Management Project. The lectures covered mangrove conservation, rehabilitation, and mangrove-friendly aquaculture. In the course of studies on mangrove nurseries and mangrove-friendly aquaculture, AQD researchers have built an impressive storehouse of information on Panay mangroves. A grant from the Man and the Biosphere Project of the United Nations Educational, Scientific, and Cultural Organization supported additional field work and documentation (morphological and ecological data, color photography) of mangroves all over Panay and the publication of *Handbook of Mangroves in the Philippines – Panay*. This *Handbook* will enable scientists, students, teachers, and the general public to identify 35 species of Philippine mangroves. Target date for release of the profusely illustrated handbook is early 2004.

## Disease Diagnostic Lab

A total of 43 diagnostic requests and 432 samples were processed. The samples included 315 shrimps, 34 fish, 32 mud crabs, one seaweed, and 50 water samples for disease identification or bacterial counts. The diseases diagnosed included white spot syndrome virus (WSSV), monodon baculovirus (MBV), viral nervous necrosis (VNN), luminous *Vibrio*sis (*Vibrio harveyi*, *Vibrio* spp.), and *Aeromonas* infection. The bulk (363) of the samples came from the private sector and 69 samples from within AQD.

## Microtechnique Lab

Processed for histological slides were a total of 1,435 samples including liver, gills, kidney, spleen, heart, skin, muscles, intestines, brain, eyes, hepatopancreas, gonads, and larvae from shrimps, mud crabs, abalone, and different fishes. A total of 2,491 slides were prepared for histological analysis.

# Strategies for stock enhancement

**Refinement of hatchery techniques for abalone *Haliotis asinina* and top shell *Trochus niloticus*.** Production of abalone veligers was improved when trochophore larvae were incubated in flow-through rather than in static sea water. Production was further enhanced with UV-treated rather than sand-filtered sea water.

For the rearing of juveniles, a continuous 24-hour flow-through system is used. To save on seawater pumping costs, an experiment was conducted to determine if flow-through sea water for only 10 hours daily or 10 hours every other day would affect growth and survival of abalone juveniles. Results showed no significant effect on growth and survival during the first month, but slower growth thereafter.

In contrast to wild top shell spawners, hatchery-reared top shell broodstock did not spawn according to the lunar cycle. Spawning could be induced by vigorous aeration and UV-irradiated sea water, but not always, even after 2-4 months' recovery between induction trials.

More than 33,000 abalone juveniles and 4,000 top shell juveniles were produced in 2003. These juveniles were used for stock enhancement and grow-out by AQD, government, and the private sector.

**Potential sites for release and stock assessment of abalone, top shell, and seahorse.** Buri island in Ajuy, Iloilo was assessed and found unsuitable for abalone stock enhancement because appropriate habitat and food for abalone were not available. Heavy siltation had adversely affected the corals, mollusks, and other organisms in the fringing reef. Run-off and floods from rivers probably caused siltation all along the coast of the municipality. No other sites could be surveyed due to lack of funds.

**Release strategies for stock enhancement of abalone.** Hatchery-bred abalone 1.5 cm in shell length were diet-tagged by feeding them an artificial diet for 3-4 weeks, then with the seaweed *Gracilaria* until they reached the desired larger sizes (three size groups of shell length 1.5, 3.0, and 4.5 cm). These diet-tagged abalones were behaviorally conditioned and trained to look for their own food and avoid predators in preparation for their release in rocky and coral reefs in a marine reserve.

**Nursery and grow-out of sea horse in lighted sea cages.** In separate experiments, juvenile *Hippocampus kuda* (10-15 days old) and *H. barbouri* (10 days old and younger) produced in the hatchery were grown with or without added feed (thawed shrimp *Acetes*) in lighted or unlighted sea cages at AQD's Igang Station. Lighted cages were lit from midnight until 0600 h; the photo-positive zooplankton became available to the juvenile sea horse. In cages where *Acetes* were given, the juveniles were fed *ad libitum* between 1400 h and 1600 h daily. After 12 weeks of rearing, *H. kuda* fed thawed *Acetes* in lighted cages grew significantly better than those in the other treatments, but survival was similarly low (9-25%) in all groups. After 41-42 days of rearing, *H. barbouri* had 0-19% survival.

**Training in stock enhancement.** Researchers in the AQD Stock Enhancement Program lectured in the nationwide training program of the Bureau of Fisheries and Aquatic Resources' Fisheries Resource Management Project. The lectures covered stock enhancement principles, genetic and socioeconomic considerations, seed production, and release and monitoring strategies for various marine species.

# Aquaculture biotechnology

The Laboratory for Advanced Aquaculture Technologies or Biotech Lab was constructed at AQD's Tigbauan Main Station from Mar 2002 to Feb 2003, as a fisheries grant-aid to the Government of the Philippines from the Government of Japan. The completed facilities of the Biotech Lab were turned over on 27 Feb, by First Secretary Eiji Ueno of the Embassy of Japan, and Resident Representative Osamu Nagaki of JICA, to Secretary Luis Lorenzo of the Philippine Department of Agriculture, UnderSecretary Cesar Drilon, and AQD Chief Rolando Platon. A SEAFDEC/AQD–DA/BFAR Workshop on Aquaculture Technology was held 17 March 2003 to discuss the priority research projects that might be done at the Biotech Lab. The 15th Meeting of the Philippine Technical and Administrative Committee for SEAFDEC/AQD programmed five projects for 2003-2005 under the Aquaculture Biotechnology Program. In 2003, DA/BFAR contributed PhP 2 Million to eight research projects in the Biotech Program, and Degussa Texturant Systems funded one study on seaweed epiphytes.

**Cloning of growth hormone, growth factors and gonadotropins in mullet, grouper, and snapper.** The full-length cDNAs for grouper and snapper growth hormones (GH) and grouper and rabbitfish gonadotropins (GtH-Ib and IIb) have been cloned and sequenced. Grouper insulin-like growth factor I (IGF-I) has also been cloned and sequencing is in progress. Partial sequences of snapper GtH Ib (internal region and 3' end) and GtH IIb (3'end) were also obtained. Cloning of the 5' end as well as the full-length cDNAs will be started shortly.

**Low-pollution diets for milkfish, tiger shrimp, and the mangrove red snapper.** To study *in vitro* and *in vivo* phosphorus digestibility of common feed ingredients, the assay method for phytase was standardized using the enzyme from the fungus *Aspergillus ficuum*. The Fiske Subbarow method for phosphate determination in biological samples was also standardized.

**Studies on new species of natural food.** Adequate amounts of good-quality natural food (microalgae and zooplankton) are essential to successful hatchery operations and the production of good-quality seed for grow-out. Several studies were conducted to find suitable plankton species, mass cultivation techniques, and feeding combinations for different species of marine and freshwater larvae. In one study, five species of microalgae were tested through a multi-step cultivation technique. The outdoor production of *Nephroselmis* sp. (Igang strain), *Rhodomonas* sp. (Boracay strain), and *Chlorella* sp. (Palawan strain) was found erratic. Two species from Dumangas, Iloilo were found easy to cultivate outdoors: *Minutocellus polymorphus* (Dumangas strain) until one-ton capacity, and *Chlorella* sp. (Dumangas strain) until 10-ton capacity. The population growth curves of the above strains were determined. *Chlorella* sp. from Palawan, Dumangas and Oton are highly acceptable food for the rotifer *Brachionus* sp.

In another study, five newly isolated cryptomonad species or strains were successfully produced using F2 medium in scale-up semi-axenic culture in the laboratory (27-28°C, 400-500 lux continuous light). Maximum cell count attained at late exponential phase was 4.5-5x$10^6$/mL after 5-7 days in 600-900 mL culture with adequate aeration. Growth of the five isolates was similar to those of tropical microalgae in Australia.

*Schizochytrium mangrovei* is a marine fungus that produces lipids rich in the essential fatty acids DHA and EPA. The expensive peptone-yeast-glucose culture medium, using analytical grade D-glucose, is the standard culture medium for *S. mangrovei*. However, locally available off-the-shelf glucose can replace analytical grade D-glucose when used at a concentration of 40 g/L of medium, and can reduce costs by 50%. In a related study, rotifers were enriched with freeze-dried *S. mangrovei* for 5 h or 10 h. The enriched rotifers had the highest lipid content when *S. mangrovei* was given at 200 mg/L of rotifer suspension. However, the fatty acid composition was best (37mg DHA/g and 7-17 mg EPA/g dry weight rotifers) in rotifers fed *S. mangrovei* for 5 hours at 400 mg/L of rotifer suspension.

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# SEAFDEC-JIRCAS collaboration

**Improved diet for mangrove snapper broodstock.** The study showed that a semi-extruded dry diet supplemented with soybean oil, tuna orbital oil and arachidonate had the highest egg production (9.3 million from 11 spawns), normal larvae (74±5%), and survival to 3 weeks (36±7%), compared with diets lacking arachidonate. Broodstock fed diets with soybean oil and squid oil produced 3.6 million eggs from 8 spawns and 56±15% normal larvae, of which 24±8% survived to 3 weeks. The poorest results were from broodstock fed diets with soybean oil and cod liver oil (2.4 million eggs in 6 spawns, 39±14% normal larvae, 18±8% survival). Egg diameter, egg viability, hatching rate, larval size at hatching, and larval activity index were not significantly different among the three treatments.

**Property regimes in mangrove ecosystems.** A survey was conducted on a random sample of men and women in five barangays in Carles, Iloilo and one barangay in Escalante City, Negros Occidental. The free-listing exercise done in Carles showed that for both men and women, 'pakinhason' (shells) is the most frequently mentioned item, as well as the item mentioned first most of the time. Shells were the most important mangrove resource from the point of view of the resource users. A pattern emerged from the correlation analyses between socio-demographic variables and the mangrove resources mentioned by the respondents. The bivalve 'tuway' was a resource for older men and women who did not fish, whereas fish and the bivalve 'bug-atan' were important for younger respondents who fished. Fish and 'imbao' (mangrove clam) were important to island dwellers dependent on fishing for livelihood. To men, 'alimango' (mud crab) was clearly an important resource.

**Valuation of mangrove resources and services.** Fishery and mangrove resources and services, on-site and off-shore, were identified through focus group discussions and interviews with key informants. Surveys of the mangrove community structure were done in seven barangays in Carles, Iloilo. Surveys were also done to obtain information on how households in coastal communities use and value mangrove resources and services. Altogether, 168 respondents were interviewed in two weeks – 24 heads of families (or alternates involved in the decision-making in the household) in each barangay. A structured questionnaire was used with flash cards of leaves, flowers, or fruits of the 18 local mangrove species, and flash cards with statements that gauge the value attributed by respondents to different mangrove goods and services.

**Property rights and governance in mangrove ecosystems.** Aside from the resource-users, the local government units and two national government agencies, the Department of Environment and Natural Resources and the Department of Agriculture-Bureau of Fisheries and Aquatic Resources are involved in the governance of mangrove areas. In reforested mangrove areas, there is active participation of community members because of the presence of organized groups tasked to manage and protect the resources. Focus group discussions in six barangays in Carles, Iloilo revealed that mangrove destruction started to become obvious to the community in the 1960s. Mangrove destruction became more widespread in the 1970s to 1990s due to the expansion of fishpond operations. In the 1980s, the collective action of three barangays against a fishpond operator enabled them to stop the expansion of the pond towards their resource base. But the collective action of oyster farm operators in another barangay against a fishpond operator in the 1990s was not successful. It was found that most owners of fish ponds are not residents of the municipality. Key informants felt that a community-based mangrove forestry project is good for the community. However, others felt that the granting of a stewardship contract to project members may deprive the majority of the community access to the mangrove resources. Setting up community-based forestry management projects may be difficult where local governments have other development priorities.

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**Improvement of *Kappaphycus* strains.** Since the 1970s, farming of the seaweed *Kappaphycus* has made use of vegetative branches, and this method has led to unstable production, declining crop quality, and poor carrageenan quality. To address the problem of inferior seedstock, AQD scientists used tissue culture to produce plantlets from sliced up callus tissue at the ends of broken branches of seaweed. Three color varieties of *Kappaphycus alvarezii* var. *adik* have been generated from tissue culture. The regenerating tissue sections have been grown in the lab to be outplanted in net cages.

**Life history of seaweeds.** The life histories of *Gracilaria firma* and *Gracilariopsis heteroclada* were documented. *Gracilariopsis heteroclada* spores were grown at salinities of 15, 20, 25, 30 ppt for 60 days; the resulting plants were biggest at 15 ppt and are being grown in tanks for outplanting in low-salinity areas. *Gracilaria firma* is also grown in tanks at 10 ppt for mass production.

**Epiphytes on seaweeds.** In February, May, and November, *Kappaphycus* farms in Calaguas Island, Camarines Norte were visited to assess the occurrence of epiphytes and 'ice-ice' disease. Calaguas Island has seen low *Kappaphycus* production since 2000, decreasing number of planters, and persistent occurrence of 'goosebumps' (*Polysiphonia* epiphytes). *Kappaphycus alvarezii* variety *tambalang* was farmed using the fixed off-bottom, raft, and hanging long-line methods at stocking densities of 1 or 2 kg per meter of line for periods of 45, 60, or 75 days. *Kappaphycus alvarezii* variety *adik* and *K. striatum* variety *sacol* are less prone to thallus whitening and fragmentation than *K. alvarezii* variety *tambalang*. They can also grow at water depths down to 1 m below the surface, where epiphytes are less abundant.

# Research publications

AQD researchers produced 38 scientific papers in 2003. As of December 2003, SEAFDEC AQD has a total of 1082 publications: 567 (53%) in ISI-covered journals, 196 (18%) in Philippine journals, and 319 (29%) in books and conference proceedings.

In 2003, 20 AQD papers won the ***DA Secretary's Award*** from the Department of Agriculture, Bureau of Agricultural Research. Another three papers won the AFMA R&D Award, also from the same agency.

The *DOST-PCAMRD, **17th Elvira O. Tan Memorial Award** for Best Published Paper in Aquaculture* went to AQD Senior Scientist AQ Hurtado for the paper:

Hurtado AQ, Agbayani RF. 2002. Deep-sea farming of *Kappaphycus* using the multiple raft, long-line method. Bot. Mar. 45: 438-444.

AQ Hurtado also delivered the Dean DK Villaluz Memorial Lecture during AQD's 20th anniversary on 9 July. The title of the lecture was "Seaweed industry and research: partners towards globalization."


Baldia SF, Conaco MCG, Nishijima T, Imanishi S, Harada KI. 2003. Microcystin production during algal bloom occurrence in Laguna de Bay, the Philippines. Fish. Sci. 69: 110-116.

*Bautista-Teruel MN, Eusebio PS, Welsh TP. 2003. Utilization of feed pea, *Pisum sativum*, meal as protein source in practical diets for juvenile shrimp, *Penaeus monodon*. Aquaculture 225: 121-131.

*Bautista-Teruel MN, Fermin AC, Koshio SS. 2003. Diet development and evaluation for juvenile abalone *Haliotis asinina*: animal and plant protein sources. Aquaculture 219: 645-653.

*Borlongan IG, Eusebio PS, Welsh T. 2003. Potential of feed pea *(Pisum sativum)* meal as a protein source in practical diets for milkfish (*Chanos chanos* Forsskal). Aquaculture 225: 89-98.

*Catacutan MR, Eusebio PS, Teshima S. 2003. Apparent digestibility of selected feedstuffs by mud crab, *Scylla serrata*. Aquaculture 216: 253-261.

Catap ES, Lavilla-Pitogo CR, Maeno Y, Traviña RD. 2003. Occurrence, histopathology and experimental transmission of hepatopancreatic parvovirus infection in *Penaeus monodon* postlarvae. Dis. Aquat. Org. 57: 11-17.

Coloso RM, King K, Fletcher JW, Hendrex MA, Subramanyam M, Weis P, Ferraris RP. 2003. Phosphorus utilization in rainbow trout *(Oncorhynchus mykiss)* fed practical diets and its consequences on effluent phosphorus levels. Aquaculture 220: 801-820.

Coloso RM, King K, Fletcher JW, Weis P, Werner A, Ferraris RP. 2003. Dietary P regulates phosphate transporter expression, phosphatase activity, and effluent P partitioning in trout culture. J. Comp. Physiol. B 173: 519-530.

*Coniza EB, Catacutan MR, Tan-Fermin JD. 2003. Growth and yield of Asian catfish *Clarias macrocephalus* (Gunther) fed different grow-out diets. Israel J. Aquacult.-Bamidgeh 55: 53-60.

De la Peña LD, Lavilla-Pitogo CR, Namikoshi A, Nishizawa T, Inui Y, Muroga K. 2003. Mortality in pond-cultured shrimp *Penaeus monodon* in the Philippines associated with *Vibrio harveyi* and white spot syndrome virus. Fish Pathol. 38: 59-61.

Delos Reyes AA Jr. 2002. Recirculating aquaculture systems: Experiences, concepts, designs, and equipment, pp. 65-82. *In:* Tansinsin LG, Halos PM, Joson LM, Sonido EP, Anglo PG, Rabago LM, Sevilla FB III, dela Peña FT (eds) Harnessing Science and Technology to Meet the Global Challenge. Philippine Association for the Advancement of Science, Manila.

Emata AC. 2003. Reproductive performance in induced and spontaneous spawning of the mangrove red snapper, *Lutjanus argentimaculatus*: a potential candidate for sustainable aquaculture. Aquacult. Res.34: 849-857.

Emata AC, Borlongan IG. 2003. A practical broodstock diet for the mangrove red snapper, *Lutjanus argentimaculatus*. Aquaculture 225: 83-88.

*Estudillo CB, Duray MN. 2003. Transport of hatchery-reared and wild grouper larvae, *Epinephelus* sp. Aquaculture 219: 279-290.

Gallardo WG, Bautista-Teruel MN, Fermin AC, Marte CL. 2003. Shell marking by artificial feeding of the tropical abalone *Haliotis asinina* Linne juveniles for searanching and stock enhancement. Aquacult. Res.34: 839-842.

Gallardo WG, Buen SMA. 2003. Evaluation of mucus, Navicula, and mixed diatoms as larval settlement inducers for the tropical abalone *Haliotis asinina*. Aquaculture 221: 357-364.

Garcia LMaB, Marte CL, Agbayani RF. 2002. Mararison Island, pp. 83-85. In: Aliño PM, Miclat EFB, Nañola CL Jr., Roa-Quiaoit HA, Campos RT (eds) Atlas of Philippine Coral Reefs. Goodwill Trading Co, Quezon City.

Golez MSN, Ohno A, Toledo JD, Tanaka Y, Ishimaru T. 2002. Population dynamics of the calanoid copepod, *Acartia tsuensis* in a brackish-water pond in the Philippines. Fish. Sci. 68: 341-344.

Hilomen-Garcia GV, Baldevarona RB, Lacanilao F. 2002. Milt production of sea bass Lates calcarifer Bloch administered an analogue of luteinizing hormone-releasing hormone and 17±-methyl-testosterone. Israel J. Aquacult.-Bamidgeh 54: 173-182.

Hilomen-Garcia GV, de los Reyes R, Garcia CMH. 2003. Tolerance of seahorse *Hippocampus kuda* (Bleeker) juveniles to various salinities. J. Appl. Ichthyol. 19: 94-98.

*Hurtado AQ, Cheney DP. 2003. Propagule production of Eucheuma denticulatum (Burman) Collins et Harvey by tissue culture. Bot. Mar. 46: 338-341.

Hurtado AQ. 2003. Seaweed research at SEAFDEC Aquaculture Department, 65-68. In: Hurtado AQ, Luhan MRJ (eds) Proceedings of the National Seaweed

Symposium, 3-4 September 2002. Seaweed Industry Association of the Philippines, Cebu City.

Hurtado AQ, Luhan MRJ (eds). 2003. Proceedings of the National Seaweed Symposium 3-4 September 2002. Seaweed Industry Association of the Philippines, Cebu City.

Kim SR, Nonaka L, Oh MJ, Lavilla-Pitogo CR, Suzuki S. 2003. Distribution of an oxytetracycline resistance determinant tet(34) among marine bacterial isolates of a *Vibrio* species. Microb. Environ. 18: 74-81.

Kimura S, Dunlap PV, Peristiwady T, Lavilla-Pitogo CR. 2003. The *Leiognathus* aureus complex (Perciformes: Leiognathidae) with the description of a new species. Ichthyol. Res. 50: 221-232.

Leaño EM, Gapasin RSJ, Polohan B, Vrijmoed LLP. 2003. Growth and fatty acid production of thraustochytrids from Panay mangroves, Philippines. Fungal Divers. 12: 111-113.

Lio-Po GD, Lim LHS. 2002. Infectious diseases of warmwater fish in fresh water, pp. 213-282. In: Woo PTK, Bruno DW, Lim LHS (eds) Diseases and Disorders of Finfish in Cage Culture. CABI Publishing, Massachusetts, USA.

Marte CL. 2003. Larviculture of marine species in Southeast Asia: current research and industry prospects. Aquaculture 227: 293-304.

Peñaflorida VD. 2002. Evaluation of plant proteins as partial replacement for animal proteins in diets of *Penaeus indicus* and *P. merguiensis* juveniles. Israel J. Aquacult.-Bamidgeh 54: 116-124.

Primavera JH, Lebata MJHL, Gustilo LF, Altamirano JP. 2002. Collection of the clam Anodontia edentula in mangrove habitats in Panay and Guimaras, central Philippines. Wetlands Ecol. Manage. 10: 363-370.

Primavera YH. 2002. The coral reef fisheries of Malalison Island, west Central Philippines two years after fish sanctuary protection. UPV J. Nat. Sci. 7: 120-132.

Reyes OS, Fermin AC. 2003. Terrestrial leaf meals or freshwater aquatic fern as potential feed ingredients for farmed abalone *Haliotis asinina* (Linnaeus 1758). Aquacult. Res. 34: 593-600.

*Rodriguez EM, Triño AT, Minagawa M. 2003. Diet and harvesting regimen for the production of mudcrab *Scylla olivacea* in brackish water ponds. Fish. Sci. 69: 37-42.

Santiago CB, Gonzal AC, Ricci M, Harpaz S. 2003. Response of bighead carp Aristichthys nobilis and Asian catfish *Clarias macrocephalus* larvae to free-living nematodes *Panagrellus redivivus* as alternative feed. J. Appl. Ichthyol. 19: 239-243.

Siar SV. 2003. Change in aplaya: Resource use and responses to changing markets among fisherfolk in Honda Bay, Palawan. Philipp. Quart.Cult. Soc. 31: 226-239.

Siar SV. 2003. Knowledge, gender, and resources in small scale fishing: the case of Honda Bay, Palawan, Philippines. Environ. Manage. 31: 569-580.

*Sumagaysay-Chavoso NS. 2003. Nitrogen and phosphorus digestibility and excretion of different-sized groups of milkfish (*Chanos chanos* Forsskal) fed formulated and natural food-based diets. Aquacult. Res. 34: 407-418.

*Sumagaysay-Chavoso NS, San Diego-McGlone ML. 2003. Water quality and holding capacity of intensive and semi-intensive milkfish (*Chanos chanos*) ponds. Aquaculture 219: 413-429.

Tendencia EA. 2003. Iodine disinfection of grouper *Epinephelus coioides* eggs. Bull. Eur. Ass. Fish Pathol. 23: 191-196.

*Tendencia EA, de la Peña MR. 2003. Investigation of some components of the greenwater system which makes it effective in the initial control of luminous bacteria. Aquaculture 218: 115-119.

## *DA-BAR DA Secretary's Award

*Bombeo RF, Fermin AC, Tan-Fermin JD. 2002. Nursery rearing of the Asian catfish, *Clarias macrocephalus* (Gunther), at different stocking densities in cages suspended in tanks and ponds. Aquacult. Res. 33: 1031-1036.

*Cuvin-Aralar ML, Fastner J, Focken U, Becker K, and Aralar EV. 2002. Microcystins in natural blooms and laboratory cultured Microcystis aeruginosa from Laguna de Bay, Philippines. Syst. Appl. Microbiol 25: 179-182.

*Eusebio PS, Coloso RM. 2002. Proteolytic enzyme activity of juvenile Asian seabass, Lates calcarifer is increased with protein intake. Aquac. Res. 33: 569-574.

*Fermin AC. 2002. Effects of alternate starvation and refeeding cycles on food consumption and compensatory growth of abalone, *Haliotis asinina* (Linnaeus). Aquac. Res. 33: 197-202.

*Fermin AC, Buen SM. 2001/2002. Grow-out culture of tropical abalone, *Haliotis asinina* (Linnaeus) in suspended mesh cages with different shelter surface areas. Aquacult. Int. 9: 499-508.

*Madrones-Ladja JA. 2002. Salinity effect on the embryonic development, larval growth and survival at metamorphosis of Placuna placenta Linnaeus (1758). Aquaculture 214: 411-418.

*Peñaflorida VD. 2002. Evaluation of plant proteins as partial replacement for animal proteins in diets of *Penaeus indicus* and *P. merguiensis* juveniles. Isr. J. Aquacult. Bamidgeh 54: 116-124.

*Tendencia EA, de la Peña LD. 2002. Level and percentage recovery of resistance to oxytetracycline and oxolinic acid of bacteria from shrimp ponds. Aquaculture 213: 1-13.

*Triño AT, Rodriguez EM. 2002. Pen culture of mudcrab *Scylla serrata* in tidal flats reforested with mangrove tress. Aquaculture 211: 125:134.

## DA-BAR AFMA R&D Paper Award

Tan-Fermin JD, Garcia LMB, Quinitio GF, Toledo JD. Reproductive biology, embryonic development, larval morphology, and larval rearing trials in the blue tang Paracanthurus hepatus (Linnaeus).

Lebata MJHL, Primavera JH, Altamirano JP, Doyola EFC, Gustillo LF. Induced spawning and larval development of the mangrove clam Anodontia edentula.

Luhan MRJ. Growth and agar quality of *Gracilaria heteroclada* Zhang et Xia grown in filter tank of the finfish broodstock tank.

# Fish diseases diagnostic methodologies

The Program aims to establish a disease control system in aquaculture and help ensure that only healthy and wholesome aquaculture products, including hatchery-bred seed, are traded in Southeast Asia. AQD hosts the Program, but implementors include the Aquatic Animal Health Research Institute (Thailand), the Samut Chakhorn Coastal Aquaculture Development Center (Thailand), and the SEAFDEC Marine Fisheries Research Department (Singapore). In 2003, AQD continued and completed several research studies under the Program (below). In addition, the Program co-sponsored the training course Viral Diseases of Shrimps and Marine Fishes held at AQD Tigbauan for 11 participants from 10 Asian countries. Information materials were also produced: the proceedings volume *Disease Control in Fish and Shrimp Aquaculture in Southeast Asia – Diagnosis and Husbandry Techniques*, the flyer *Living with White Spot Disease in Shrimp Farming*, and the *Laboratory Manual of Standardized Methods for the Analysis of Pesticide and Antibiotic Residues in Aquaculture Products*.

## Research in aquatic health management

**Bacteria as biological control agent against microbial diseases in tiger shrimp and mud crab hatcheries.** Bacterial strain C1 can be grown in practical medium (10% rice bran extract) but reaches only 10% of the maximum cell density attained in nutrient broth ($10^8$ cfu/ml) in 24-96 hours. Strain C1 bacteria was added to a recirculation filter with oyster *Placuna sella* shells, but with poor results. An experiment using plastic beads (4 mm diameter) showed that effective colonization of the beads by strain C1 occurred after 24 hours and that the C1 population was maintained at levels of $10^5$-$10^6$ cfu/ml for 8 days when the colonized beads were transferred to sterile seawater. Another treatment showed that although the colonized beads did not totally eliminate luminous bacteria, the latter were maintained at its inoculation level of $10^2$ cfu/ml for two weeks. Colonized beads that were transferred into unfiltered sea water showed a markedly reduced C1 population from $10^6$ to $10^3$ cfu/ml in two days.

To study the ability of strain C1 to associate with mud crab larvae, megalopae were immersed for three hours in a C1 suspension with $10^6$ cfu/ml, and then transferred to glass aquaria with either sterile or non-sterile sea water with aeration. The associated bacterial flora of megalopae before and after immersion, as well as 24 hours after were grown on nutrient agar and TCBS agar. Total plate count of bacteria in megalopae after immersion in strain C1 was 10x higher than the non-immersed group, but presumptive *Vibrio* counts in both groups were the same. Immersed megalopae transferred to sterile sea water had a stable bacterial population after 24 hours, but those transferred to non-sterile sea water had higher total counts and presumptive *Vibrio*. Significantly, no luminous bacteria were isolated from treatments containing C1-immersed megalopae. Water in glass jars containing non-immersed megalopae harbored luminous bacteria ($10^1$ to $10^3$ cfu/ml).

**Effect of tilapia culture water on luminous bacteria and tiger shrimp.** Experiments were all done in covered 3-ton concrete tanks filled with micro-filtered sea water (24 ppt) and stocked with tiger shrimp (80-110 grams/tank) and inoculated with luminous bacteria *Vibrio harveyi* to an initial concentration of $10^3$ cfu/ml. One experiment compared the effect of grouper, milkfish, and Nile tilapia, stocked at 500 g/tank, on luminous bacteria, where tiger shrimp was stocked at 110 g/tank. Luminous bacteria were not detected in tanks stocked with Nile tilapia from day 22 until end of experiment at day 28. Grouper or milkfish at biomass of 500 g/$m^3$ did not inhibit luminous bacteria in a culture system with shrimp biomass at 110 g/$m^3$. When the shrimp biomass was kept at 80 g/tank in another run, the luminous bacteria were significantly reduced at days 5-21 in the presence of Nile tilapia, and at days 10-21 in the presence of grouper and milkfish. Another experiment compared the inhibitory effect of *Chlorella* sp., *Tilapia hornorum*, and a combination of *Chlorella* sp. and *Tilapia hornorum*. All treatments reduced the counts of luminous bacteria at day 6-21, but especially in tanks with tilapia only. Another experiment determined the effect of feeding of tilapia and shrimp on luminous bacteria. In tanks with fed tilapia and shrimp, no luminous bacteria could be detected at days 4-21. Tanks with unfed tilapia and shrimp had lower counts of luminous bacteria than tanks with fed or unfed shrimps only.

**Antibacterial metabolites in 'green water' culture of tiger shrimp.** An indigenous probiotic developed in this study was found effective in inhibiting luminous *Vibrio* in soil treatment experiments. The probiotic bacteria showed stability in pond water for more than 45 days. A large tank experiment is being prepared. In the meantime, test tube experiments where diatoms were inoculated with luminous *Vibrio* showed an initial increase in luminous *Vibrio* counts 10-100 times after three days, then a reduction to undetectable *Vibrio* levels by day 7. Similar experiments done in aquaria with aeration showed a reduction in luminous *Vibrio* after only 24 hours.

**Epizootiology of viral diseases of wild tiger shrimp.** Sampling for wild tiger shrimp was conducted during the dry and wet seasons in Capiz, Negros Occidental, Bohol, Quezon, Palawan, Surigao del Sur and Misamis Occidental (at least 100 shrimps from each site). Body weights were measured and tissues were processed for the detection of DNA viruses and RNA viruses. Polymerase chain reaction (PCR) protocols were optimized for the detection of white-spot syndrome virus (WSSV) and monodon baculovirus (MBV). One-step PCR and nested PCR showed that 26% of all shrimps during the dry season and 8% of those during the wet season were positive for both WSSV and MBV (see chart).

| Sampling sites | Dry season | | | Wet season | | |
|---|---|---|---|---|---|---|
| | Shrimps sampled | % positive after nested PCR | | Shrimps sampled | % positive after nested PCR | |
| | | WSSV | MBV | | WSSV | MBV |
| Capiz | 100 | 3 | 32 | 103 | 0 | 12 |
| Negros Occidental | 105 | 3 | 23 | 101 | 0 | 0 |
| Bohol | 102 | 0 | 2 | 101 | 0 | 0 |
| Quezon | 103 | 9 | 18 | 106 | 0 | 8 |
| Palawan | 102 | 11 | 0 | 103 | 2 | 5 |
| Misamis Occidental | 100 | 16 | 33 | 100 | 0 | 25 |
| Surigao Sur | 105 | 25 | 30 | 101 | 0 | 8 |
| *Average* | | *10* | *19* | | *0.3* | *8* |

**Immunological response of tiger shrimp to WSSV.** Shrimps that were screened and found negative for WSSV by PCR and histopathology were used to determine the immunological response to the virus. Forty shrimps were fed tissues from the cephalothorax of WSSV-infected shrimp found positive by nested PCR. Handling stress caused some early mortality, but no shrimps died thereafter. Inoculation was repeated using WSSV-infected shrimps found positive by one-step PCR. Three groups of five shrimps each were either (1) fed the cephalothorax of infected shrimps, or (2) immersed in filtrates from the shrimp tissue homogenates, or (3) injected with the tissue filtrate. All shrimps that were fed or injected died after 7 days, and 80% of those that were immersed died after 14 days. Based on this information, filtrates prepared from shrimps that died (and tested PCR-positive) were injected to healthy shrimps to monitor changes in lymphoid organ histology and in haemolymph indices (hemocyte count, phenoloxidase activity, superoxide anion production, and plasma total protein) at different times post-inoculation.

**Production of antibodies against MBV and HPV.** Two batches of tiger shrimp postlarvae positive for monodon baculovirus (MBV) were obtained and several MBV infection experiments were done to produce materials for the purification of virions and screening of polyclonal antiserum and hybridoma supernatants. Purified viral particles were confirmed by transmission electron microscopy of negatively stained samples. For monoclonal antibody production, purified virions were prepared for immunization of mice. Balb/C mice were injected intraperitoneally with 200 µl immunogen (with adjuvant) per mouse. Three booster injections were given at 100 µl/mouse (without adjuvant) at 21-day intervals. Cell fusion was done 3-5 days after the last booster shot. Immunized spleen cells were isolated and mixed with myeloma cells at a ratio of 5:1 in polyethylene glycol. The resulting hybridomas were grown for 14 days in culture medium with hypoxanthine-aminopterin-thymidine and then in medium with hypoxanthine-thymidine. Supernatants from growing cells were collected for screening.

Work on monoclonal antibodies against hepatopancreatic parvo-like virus (HPV) was continued. Supernatants collected from the hybridomas produced last year were screened. The immunofluorescence test using impression smears of infected hepatopancreas and paraffin-embedded tissues was optimized. Fluorescein isothiocyanate-conjugated secondary antibody (goat anti-mouse immunoglobulin) was used at a dilution of 1:100. Of the 72 supernatants, 31 had strong fluorescence, 5 had slight, and 36 had none.

For polyclonal antibody production, white New Zealand rabbits were injected subcutaneously with 250 µl of purified immunogen (with adjuvant) at each of four sites. Two booster shots were given at 21-day intervals before collection of blood for determination of antibody titer. Immunofluorescence test was optimized using the polyclonal antibodies produced. These were tested using impression smears and paraffin-embedded tissues. Fluorescence was produced by a 1:100 dilution of antiserum and 1:50 dilution of flourescein isothiocyanate-conjugated anti-rabbit immunoglobulin. ELISA (enzyme-linked immunosorbent assay) was optimized by checkerboard titration. Anti-HPV serum (primary antibody) diluted at 1:2000 and secondary antibody (peroxidase-conjugated anti-rabbit IgG) diluted at 1:2000 gave optimum reading at 490 nm for HPV-positive samples. A 1:800 dilution of anti-MBV antiserum and 1:2000 dilution of secondary antibody were optimum for MBV-positive samples.

**Viral nervous necrosis in fish broodstock.** SEAFDEC/AQD's fish broodstocks maintained in concrete tanks in Tigbauan and in net-cages in Igang were all screened for viral nervous necrosis (VNN). Samples of eggs, milt, gills, and blood were taken and two viral assay passages of the samples in E-11 cells were done. Harvested cell suspensions were subjected to RT-PCR and nested PCR. Of 32 groupers *Epinephelus coioides* in Tigbauan, 19 were found positive and 13 were negative for VNN. The VNN-positive grouper were separated from the VNN-negative grouper. Of five *E. coioides* and nine sea bass in Igang, all were found negative during the first passage in E-11 cells, but nested PCR showed that 4/5 grouper and all nine sea bass were positive for VNN. Another group of sea bass in Tigbauan were screened by direct nested PCR and 8/9 found positive for VNN. The detection rate for VNN is higher with the combination of cell culture and PCR than with the direct PCR only.

Cell culture and PCR assays also showed 12/15 *E. malabaricus* and 5/14 *E. coioides* in Tigbauan positive for VNN. Significantly, the fish fed to these grouper were also found positive for VNN. Thus, nine species of fish used as feed for grouper broodstocks were sampled from Iloilo Fishing Port and screened for VNN to determine the extent of contamination. Two species were found positive for VNN namely, 'bulaw' *Rastrelliger* sp. (22%) and 'sapsap' *Leiognathus* spp. (80%), but the others were negative. This result indicates that some wild fish do harbor the VNN virus and that VNN infection of fish broodstock at SEAFDEC/AQD may be due to wild fish used as feed.

Eggs from *E. coioides* broodstock in Igang were monitored for VNN during 14 consecutive days of spawnings. Eggs from the first 6-7 days of spawning were negative for VNN but those from the succeeding spawnings were positive for VNN. Survival of larvae from VNN-positive broodstock ranged 1–7.5%, but PCR tests revealed that some larvae were VNN-negative. Eggs from VNN-positive broodstock were disinfected with iodine.

Additional sampling of pre-spawning broodstocks in Tigbauan showed 29/52 *E. fuscoguttatus*, 2/10 red snapper, and 0/4 new *E. coioides* positive for VNN. VNN-positive *E. fuscoguttatus* were segregated from the VNN-negative fish. Also found VNN-positive were many of the pre-spawning broodstocks in Igang: 13/39 *E. coioides* and 5/20 sea bass.

**Detection and identification of viral pathogens in farmed marine fishes.** Tissue filtrates of grouper larvae from SEAFDEC and of juveniles from a private hatchery were positive for virus by cell culture and confirmed positive for VNN by RT-PCR. Tissue culture assays found milkfish fry negative for virus but positive for VNN by RT-PCR. Catfish samples were negative for virus. Red snapper injected intraperitoneally with tissue filtrate from diseased rabbitfish did not show significant histopathological changes and the inoculated virus was not recovered from red snapper tissue filtrates.

**Parasitosis in marine and freshwater fishes.** The most prevalent parasites of pond-reared mangrove red snapper were the gill monogenean *Haliotrema* and the copepod *Caligus*. In the rabbitfish, the most prevalent was *Caligus*. Other parasites found were two species of digeneans in red snapper and the nematode *Procamallanus* in rabbitfish. In tank-reared native catfish, the most prevalent was the skin monogenean *Gyrodactylus*, and pond-reared catfish harbored nematodes and cestodes. Another 19 species of fish caught in brackishwater ponds and water supply canals yielded the monogeneans *Benedenia* and *Pseudorhabdosynochus lantauensis*, digeneans, acanthocephalans, and copepods. Scanning electron microscopy was done on selected parasites.

Healthy juvenile grouper were exposed to eggs of the gill monogenean *P. lantauensis* at 0, 500, 1000, 2000 and 5000 eggs per 20 fish. After 15 days, none of the control fish died, but mortality in the treatments increased from 35% to 85% with the intensity of infection. At day 5, the hematocrit in grouper infected with 5000 eggs/20 fish was significantly lower than in the controls and in fish infected with 500 eggs/20 fish. At day 10, the hematocrit of fish infected with 5000 eggs/20 fish was significantly lower than all other treatments. Photomicrographs and measurements of taxonomic characters were taken of fixed and stained adult *P. lantauensis*.

**Pesticide residues in aquaculture products.** Ten species of brackishwater and eight species of freshwater farmed species (100 samples) from both urban and rural/agricultural sites were sampled during the dry season. No significant levels of pesticide residues were detected. Sampling, processing and analyses of wet season samples are ongoing.

## Research on probiotics (Mangrove-Friendly Shrimp Farming Program)

**Mode of action of probiotics used in tiger shrimp grow-out.** Two commercial probiotics A and B were tested under laboratory conditions for their biocontrol effects against luminous *Vibrio harveyi*. The total bacterial count in the water with probiotic A did not differ from that without A (control). Probiotic A did not inhibit luminous *Vibrio* during the 7-day treatment. With probiotic B, the total bacteria increased 100x in 24-48 hours, but declined significantly by day 7. Probiotic B inhibited luminous *Vibrio* 24 hours after treatment, but did not sustain the inhibition.

Commercial probiotics B and C lowered the ammonia concentrations in UV-treated sea water with 0.5–2.0 ppm ammonia with and without aeration. Probiotic B lowered the ammonia by 30-50% (aerated) and 2-32% (unaerated) after 24 hours, and by 65-95% (aerated) and 17-50% (unaerated) after 72 hours. The total bacterial count in sea water with ammonia and probiotic B reached $10^5$ cfu/ml after 24 hours. Similarly, probiotic C lowered the ammonia by 60-90% (aerated) and 50-70% (unaerated). In the absence of probiotics B and C, ammonia also declined by 42-50% (aerated) and 0-33% (unaerated) in 48 hours. Probiotics B and C had no effect on nitrite. Bacterial counts in sea water with nitrite and probiotic B increased from $10^3$ to $10^4$-$10^6$ cfu/ml in 24-72 hours.

Probiotics A, B, and C were inoculated into deoxygenated sea water with 5, 50, 200 and 400 mM sulfide, and the suspensions were sampled every two hours for 48 hours for sulfide concentrations and bacterial counts. Probiotic B removed low sulfide concentrations within 12 hours and higher concentrations within 48 hours. Probiotic C lowered all sulfide concentrations after 24 hours, and probiotic A after only 18 hours. Controls without probiotics had minimal change in sulfide after 48 hours. For all three probiotics, the total bacterial counts increased from $10^3$ to $10^6$ cfu/ml during the 48 hours incubation in the four sulfide concentrations.

**Use of probiotics in rearing tiger shrimp.** Commercial probiotics B and C were tested in the larval rearing of tiger shrimp postlarvae (stocked at 30/$m^2$) in 250-liter tanks with a closed-recirculating water system. The probiotics were either incorporated in the shrimp diet, or added to the water; controls had no probiotic. In the test with probiotic B, the shrimp postlarvae (4.4 mg) were grown for 84 days; 41-47% survived and reached average weights of 591-860 mg (not different among treatments). Probiotic C was tested on shrimp postlarvae (25 mg) for 84 days. Average weights and survival were significantly different between the control (37%, 664 mg), with probiotic in the feed (47%, 1065 mg), and with probiotic in the water (53%, 2026 mg). For probiotics B and C, survival of shrimps after exposure to *Vibrio harveyi*i was similar between the treatments and the control.

**Use of probiotics in a closed-recirculation pond system.** Tiger shrimp were stocked in a closed-recirculation pond system. Water samples for physico-chemical analysis and plankton and bacterial counts were taken before and after stocking of shrimps and also two days after application of probiotics. Water lost by evaporation or seepage was replenished once a week. The study is ongoing.

# Mangrove-friendly shrimp farming

The Program included verification and pilot demonstration, research, training, and information dissemination geared at promoting mangrove-friendly shrimp farming. Improved shrimp farming practices using two water management systems, the low-discharge and the closed-recirculation systems, were verified in Thailand and the Philippines, and the experiences documented and published in 2001 as State-of-the-Art compilations. These compilations were updated in 2002 into the extension manual *Best Management Practices in Mangrove-Friendly Shrimp Farming*. The technology has been demonstrated in several shrimp farms in the Philippines, Thailand, Vietnam, and Myanmar with remarkable success. Similar demonstrations were being conducted in Cambodia and Malaysia.

Research studies were conducted to define the use of probiotics (see previous page) in shrimp ponds.

Nine government officers and fisherfolk representatives from eight ASEAN countries undertook the third formal training course on Mangrove-Friendly Shrimp farming at AQD Tigbauan in October 2003. Information was also disseminated through the mangrove website www.mangroveweb.net. The videos on environment-friendly shrimp farming are nearing completion. The Regional Seminar-Workshop on Mangrove-Friendly Shrimp Aquaculture was held in Bangkok in June 2003. The workshop recommended, among many things, the crafting of a *Regional Code of Practice for the Responsible Use of Mangroves in Aquaculture*.

**Pilot demonstration in the ASEAN Countries in 2003**

| | |
|---|---|
| **Thailand** | In Songkhla, the integrated physical and biological technologies for water recycling in shrimp farms to improve the quality of effluents from shrimp farms, was conducted. Here the effluents pass through bivalve filtration to remove suspended matter, then to the biological filtration facility (trickling filter), then on to seaweed ponds. In Phuket, mitigation measures for effluents from shrimp farms in mangroves and coastal areas were evaluated and verified. In Kung Krabaen Bay, Chantaburi, the seawater irrigation facility for sustainable shrimp farming was showcased. |
| **Philippines** | Greater involvement of BFAR Demonstration Training Centers, the private sector, and banks; on-the-job training at the AQD site and other sites in the country. |
| **Vietnam** | Verification of technology for a semi-intensive shrimp farm with production of at least 1,500 kg/ha/crop. |
| **Myanmar** | Successful demonstration and harvest at a site near Yangon; another site being considered. |
| **Cambodia** | Project site for the pilot demonstration was identified during the site visit in Sep 2003; pond design and construction started in Nov 2003 with technical assistance from AQD. |
| **Malaysia** | Project site for the pilot demonstration was identified during the site visit in Sep 2003; pond design and construction soon to start. |
| **Brunei Darussalam** | Discussions have been made for a pilot demonstration project in 2004. |
| **Indonesia** | Discussions have been made for a pilot demonstration project in 2004. |

# Integrated Regional Aquaculture Program

The Integrated Regional Aquaculture Program (IRAP) promotes sustainable aquaculture through demonstration and dissemination of technologies that are environment-friendly and beneficial to rural communities. IRAP is implemented through site visits and surveys, pilot demonstration, research, training, and information dissemination in all ASEAN and SEAFDEC Member Countries except Singapore and Japan. IRAP comprises two projects:

1. Aquaculture for Rural Development – Vietnam as the Lead ASEAN Country
   The Project verifies aquaculture technologies in various aquatic ecosystems, disseminates appropriate technologies to small-scale fish farmers.
2. Supply of Good Quality Seeds – Indonesia as the Lead ASEAN Country
   The Project identifies, verifies and disseminates appropriate technologies in broodstock management and seed production of farmed freshwater and marine species.

In 2003, site visits and surveys were done in nine participating countries – Indonesia, Brunei Darussalam, and Malaysia on 12-23 May; Myanmar, Lao PDR, and Cambodia on 28 June to 7 July; and Vietnam, Thailand, and the Philippine on 29 July to 8 August – and yielded valuable on-the-ground information. Based on the survey's findings and the country's needs and in consultation with potential implementors, projects (and sites or venues) were chosen for research, training, technology verification and pilot demonstration. For example, Thailand, Brunei Darussalam, Indonesia, and the Philippines are all interested in seed production, genetic improvement, as well as grow-out of the freshwater prawn *Macrobrachium rosenbergii*. These countries will then collaborate to achieve similar objectives. In support of these countries, AQD's Binangonan Freshwater Station has resumed research on the seed production and grow-out of *M. rosenbergii*.

Under IRAP, three types of training were conducted: (1) on-site training in beneficiary country on cost-sharing basis and using technical expertise from another ASEAN country; (2) training at AQD in the Philippines for technologies generated at AQD; and (3) attachment training in another ASEAN country for specific technologies in which the country is expert. All participating countries have availed of the training opportunities provided under IRAP. In particular, IRAP funds supported 14 trainees in AQD's training course AquaNutrition on-line, 12 trainees in AquaHealth on-line, five trainees in freshwater fish hatchery, and two trainees in marine fish hatchery. A 2004 calendar has been produced to disseminate information about IRAP and aquaculture around Southeast Asia.

Also under IRAP, two research studies were approved in Nov 2003 on the use of larval diets in the hatchery of milkfish, rabbitfish, red snapper, and sea bass.


Farm supplies


Giant prawn


Giant catfish

# Technology verification and commercialization

SEAFDEC/AQD continued to implement activities geared at the commercialization and promotion of developed technologies, initially in the Philippines, and later in the ASEAN Countries under specific financial arrangements. The SEAFDEC/AQD–DA/BFAR Joint Mission for Accelerated Nationwide Technology Transfer Program (JMANTTP) was launched in 1999 to intensify techno-transfer for sustainable aquaculture to improve fish production, employment and livelihood, and export revenues. In 2003, environment-friendly shrimp farming was carried out in BFAR Demonstration and Training Centers and in 16 private farms, in cooperation with local government units and LandBank. Several seminars on environment-friendly intensive shrimp farming were provided by AQD upon the request of farmer groups.

Aside from the ABCDEF and JMANTTP projects, AQD also assisted several small livelihood projects of people's organizations and government agencies, such as grouper farming in cages in Sibunag, Guimaras, and seaweed farming in Parara Norte, Tigbauan, near AQD.

With the vision to bring more aquaculture to the countryside, SEAFDEC/AQD entered into an agreement with the Meralco Foundation Inc. (MFI) to operate the defunct MFI Tilapia Hatchery and Family Farm School in Jalajala, Rizal, on the north shore of Laguna de Bay. AQD staff moved into the Jalajala facility in August 2002 and put the place in order (AQD advanced about PhP 2.9 Million in operating funds). In October 2002, AQD and MFI organized the Aquaculture-Based Community Development Enterprises Foundation, Inc. (ABCDEF) to fully operate a commercial hatchery for freshwater fishes. ABCDEF received in April 2003 a grant of PhP 2 Million from the Countryside Development Fund of Senator Ramon Magsaysay through the Department of Science and Technology. ABCDEF has since maintained a standing stock of 1.5 million juveniles of SEAFDEC-strain (Sst) tilapia at any one time, and sold about 2.5 million juveniles worth PhP 1.3 Million to 41 buyers all over the Philippines. Once the hatchery income is able to sustain operations, ABCDEF will pay back the funds advanced by AQD.

In October 2003, Senator Magsaysay, Secretary Luis Lorenzo and other officials of the Department of Agriculture, and officials of the local government units and farmer organizations visited the Jalajala facility. During that visit, a Memorandum of Understanding was signed between the ABCDEF Chairman, the Provincial Agrarian Reform Officer, and the Mayor of Jalajala to promote rural aquaculture as a means to increase farmer incomes and the local fish supply. Thus was launched the 'Stock now, pay later' plan, in which SSt tilapia juveniles are provided to selected farmers to the extent of PhP 7,000 worth of juveniles per family. The farmers raise the tilapia and pay ABCDEF from the sales of the harvest. Eight farmers availed of the 'Stock now, pay later' plan in 2003.

The SSt tilapia happens to be salt-tolerant (it was NOT selected for salt tolerance), more so than other Nile tilapia strains in the Philippines. The SSt tilapia is used by JMANTTP as biomanipulators in brackishwater shrimp ponds. Biomanipulators generate 'green water' in reservoirs and 'green water' effectively prevents luminous *Vibrio*sis. ABCDEF markets two types, the greenish SSt-Jade and the reddish SSt-Ruby. In addition to tilapia, the seed of bighead carp, native catfish, and snakehead may be produced by ABCDEF on demand.

The ABCDEF Jalajala facility served as venue for training in commercial freshwater hatchery. Among the trainees in 2003 were 21 students from three universities, several local fish farmers and technicians, and five government officers from Lao PDR and Cambodia under AQD's Integrated Regional Aquaculture Project.


ABCDEF Jalajala


JMNTTP


Stock now, pay later


Parara, Tigbauan

# Training

AQD conducted 13 short-term training courses in 2003 on a cost-recovery basis – with fellowships from the Government of Japan Trust Fund and other external agencies, or personal funds. Two of these were on-line courses (e-learning and e-training) funded under the Integrated Regional Aquaculture Program. In all, 119 trainees from 20 countries finished the 13 courses. In addition, 18 trainees from around the Philippines and students from other countries did internships (for a fee) with AQD scientists. Another 140 students from around the country served out their school-mandated hours of practicum and on-the-job training at AQD's library, laboratories, hatcheries, ponds, cages, and pens.

## Training courses in 2003

| Training courses | Duration | Trainees (type, group) | Number: gender | Ages (yr) | Countries represented | Funding |
|---|---|---|---|---|---|---|
| Crab Seed Production | 23 Apr-22 May | Govt officers, private farmer | 6: 5M, 1F | 25-46 | Philippines | personal |
| Sustainable Aquafarming Systems | 7 May-5 Jun | Govt officers, private farmer | 3: 2M, 1F | 23-42 | Philippines | personal |
| Marine Fish Hatchery / Nursery | 2 Jun-16 Jul | Govt officers, private farmer | 6: 3M, 2F | 20-42 | Philippines, Myanmar | GOJ-TF, personal |
| Health Management in Aquaculture (On-line) | 2 Jun-12 Sep | Govt officers, private farmer | 17: 7M, 10F | | Myanmar, Indonesia, Vietnam, Malaysia, Thailand, Philippines, Oman, Saudi Arabia | GOJ-TF, personal |
| Principles of Aquaculture Nutrition (On-line) | 21 Jul-15 Nov | Govt officers, private farmer | 22: 9M, 13F | | Myanmar, Indonesia, Brunei, Iran, Vietnam, Malaysia, Thailand, Philippines, Nepal, Iran, Saudi Arabia | GOJ-TF, NACA, personal |
| Livelihood Opportunities in Aquaculture | 11-22 Aug | FARMC beneficiaries | 16: 15M, 1F | 24-69 | Philippines | DA-BFAR, NFARMC |
| Responsible Aquaculture Development | 15 Sep-13 Nov | Govt fisheries extension officers, researchers | 16: 13M, 3F | 24-43 | Cambodia, Indonesia, Philippines, Bangladesh, India, Pakistan, China, Kenya, Tanzania, Mozambique | JICA-TCTP |
| Crab Seed Production | 23 Sep-5 Nov | Coop members | 4M | 42-57 | Philippines | SKMPC |
| Tilapia and Carp Breeding | 30 Sep-19 Oct | Govt fisheries officers | 5 | | Lao PDR, Cambodia | ASEAN Foundation |
| Mangrove-Friendly Shrimp Aquaculture | 23 Oct-11 Nov | Govt officers, researchers, fisherfolk reps | 9M | 31-58 | Myanmar, Indonesia, Brunei, Cambodia, Vietnam, Malaysia, Thailand, Philippines | GOJ-TF |
| Rabbitfish Hatchery | 30 Oct-11 Nov | Govt officer | 1M | 29 | Vietnam | ASEAN Foundation |
| Viral Diseases of Shrimps and Marine Fishes | 5-21 Nov | Govt officers, researchers | 11: 5M, 6F | 28-50 | Myanmar, Lao PDR, Indonesia, Cambodia, Vietnam, Malaysia, Thailand, Philippines, India, China | GOJ-TF, NACA, OIE |
| Cage Culture of Abalone | 10-28 Nov | Govt officer, private farmers | 3: 2M, 1F | 28-49 | Philippines, Malaysia, Thailand | GOJ-TF, personal |

GOJ-TF Government of Japan Trust Fund; JICA-TCTP Japan International Cooperation Agency Third Country Training Program; NACA Network of Aquaculture Centers in the Asia-Pacific; OIE Office International des Epizooties; SKMPC Shariff Kabungsuan Multi-Purpose Cooperative; DA-BFAR Department of Agriculture Bureau of Fisheries and Aquatic Resources, NFARMC National Fisheries and Aquatic Resources Management Council

## Internships (for a fee) and on-the-job training in 2003

| Interns' Institution | Academic course | Assignment at AQD | Sessions | # by gender |
|---|---|---|---|---|
| **Iloilo and Panay** | | | | |
| Phil Science High School–WV | Years 3-4 | FishWorld, diff labs | 14 Apr-9 May | 13M + 12F |
| Univ Phil High School, Iloilo | Year 3 | different labs | 28 Apr-23 May | 1M + 3F |
| Univ San Agustin, Iloilo City | BS Chem | Chem lab | 08 Apr-15 May | 1F |
| Univ Phil–Visayas, Miagao | BS Chem | Chem lab | 21 Apr-26 May | 3M + 3F |
| West Visayas Coll Sci & Technol | Info Tech | Library, Data Bank | 21 Apr-22 May | 2M + 11F |
| Western Institute Technology | BS Biol | Mudcrab hatchery | 9 Jun-9 Jul | 1F |
| Iloilo State College Fisheries | BS Mar Biol | Abalone hatchery | 1 Apr-1 May | 1M |
| | BS Fisheries | Mudcrab hatchery | 4 Jun-4 Jul | 1F |
| | BS Fisheries | Fish health lab | Jan-Feb | 1M |
| | BS Fisheries | Natural food lab | 11 Nov-11 Dec | 1F |
| West Visayas State Univ | BS Info Mgt | Data Bank, Dev Com | 3 Nov-5 Mar | 3M |
| | BS Biol | Mudcrab hatchery | 22 Apr-22 May | 1F |
| | BS Biol | Abalone hatchery | 9 Jun-9 Jul | 1F |
| Aklan State Univ | DVetMed | Fish health lab | 24-28 Mar | 1F |
| | DVetMed | Fish health lab | 9-14 Jun | 4F |
| | DVetMed | Fish health lab | Apr-May | 1M |
| Individual, Buenavista, Guimaras | BS Fisheries | Mudcrab hatchery | 1-31 July | 1M |
| Individual, Jaro, Iloilo City | BS Buss Ad | Fish health lab | 11-15 Aug | 1M |
| Individual, Tigbauan, Iloilo | High School | Fish hatchery | 3 Dec-3 Jan | 2M |
| **Outside Panay** | | | | |
| Cagayan State Univ, Aparri | BS Fisheries | TMS hatcheries | 22 Apr-22 May | 1M + 1F |
| Don M Marcos Mem State U, Ilocos Norte | BS Fisheries | ABCDEF hatchery | 24 Apr–13 Aug | 7M + 4F |
| Central Luzon State Univ, Nueva Ecija | BS Fisheries | Abalone hatchery | 22 Apr-31 May | 1F |
| DA-BFAR Inland Fisheries Station, Batangas | BS Fisheries | Fish health lab | 6-17 Oct | 2F |
| DOST-PCAMRRD, Laguna | BS Fisheries | Farming Systems | 1 Apr-30 May | 1F |
| Individual, College, Laguna | BS Biol | Fish health lab | 16-19 Dec | 1M |
| Leyte State Univ, Tolosa | BS Fisheries, BS Educ | TMS labs, ABCDEF hatchery | 28 Apr-28 May | 10M + 4F |
| Southern Leyte State Coll Sci & Technol | BS Fisheries | Natural food lab | 27 Oct-14 Nov | 1M |
| Busuanga Mercantile Corp, Makati | M BA | Abalone hatchery | 5 May-5 Jun | 1M |
| Individual, Negros Oriental | | ABCDEF hatchery | 29 Apr–13 May | 1M |
| Mindanao State Univ, Marawi | BS Fisheries | different labs | 4 Apr-4 May | 1M + 2F |
| Mindanao State Univ, Naawan | BS Fisheries | TMS hatcheries | 10 Apr-31 May | 2M + 4F |
| Zamboanga Antonio Eustaquio College | BS ECEngg | Lab maintenance | 28 Apr–28 May | 1M |
| Zamboanga State Coll Mar Sci & Technology | Dipl Aquaculture | Mudcrab hatchery | 29 May-29 Jun | 1M |
| Marian College, Zamboanga | BS Commerce | Shrimp ponds | 6 Jun-6 Jul | 1M |
| Zamboanga State Coll Mar Sci & Technology | BS Fisheries | diff labs, hatcheries | 23 Apr-23 May | 6M + 4F |
| Davao del Norte State College | BS Fisheries | diff labs, hatcheries | 6 May-6 Jun | 9M + 8F |
| Sultan Kudarat Polytechnic State College | AB Educ | diff labs, hatcheries | 25 Apr-25 May | 4M + 2F |
| Individual, Isulan, Sultan Kudarat | BS RadTech | Catfish hatchery | 5-19 May | 1M |
| **Outside Philippines** | | | | |
| Dalhousie University, Canada | MSc (thesis) | Abalone hatchery | 24 Sep-19 Mar | 1F |
| Univ Reading, UK | MSc Ecol Econ (thesis) | Farming systems | 1 Apr-31 May | 1M |
| Univ Wales, Bangor, UK | MSc (thesis) | Mudcrab hatchery | 7 Jun-29 Aug | 1M + 1F |
| Memorial Univ, Canada | MSc (thesis) | Mudcrab project | 29 Sep-Nov | 1F |
| Stockholm Univ, Sweden | MSc (thesis) | Mudcrab project | 29 Sep-Nov | 1M |
| Cantho University, Vietnam | MSc (thesis) | Mudcrab project | 21-28 Nov | 1M + 1F |
| **Total 31 institutions** | | | **43 sessions** | **81M + 77F** |



AQD training courses

New information materials

# Information dissemination

Direct dissemination of aquaculture technologies was carried out during AquaBiz or the Seminar on Business Opportunities in Aquaculture, held in Iloilo City on 7-8 July 2003. AQD scientists presented 15 major aquaculture technologies to 270 fish farmers and other entrepreneurs, and members of government and the academe. More on AquaBiz on the inside back cover.

AQD scientists and researchers served as lecturers and resource persons in training programs and seminars conducted by the Department of Agriculture and other government agencies in the Philippines. In the nationwide training program of DA-BFAR's Fisheries Resource Management Project, JH Primavera lectured on mangrove conservation, rehabilitation, and mangrove-friendly aquaculture, and WG Gallardo lectured on stock enhancement principles, genetic and socioeconomic considerations, seed production, and release and monitoring strategies for various marine species. BFAR Region III tapped AQD staff for its techno-verification and extension seminars in Pampanga and Zambales on 9-12 April. FD Estepa, MN Duray, and GF Quinitio lectured on breeding and grow-out of fishes and crustaceans, and M de los Santos and CM Ganancial talked about environment-friendly shrimp farming. BFAR also held a Techno-Caravan in Baler, Aurora on 17-19 August, where ET Quinitio, ZU Basiao, FD Estepa, and M de los Santos lectured on shrimp hatchery management, biology and grow-out of mud crabs, biology and grow-out of tilapia, and environment-friendly shrimp farming. Similarly, the Department of Science and Technology Region 10 invited CL Torres, MTC Mallare, NV Golez, DR Chavez, and NR Jamon to lecture in four seminars held in 2003: Environment-Friendly Shrimp Aquaculture (Bonifacio, Misamis Occidental, 22-23 March), Environment-Friendly Shrimp Aquaculture (Cagayan de Oro City, 22-23 July), Technology/Investors Forum (Cagayan de Oro City, 29 October), and Seaweed Culture (Kolambugan, Lanao del Norte, 15-16 December).

AQD joined several fairs and exhibits in 2003: the Agri-Food Biotech Fair in Iloilo City, 4-8 March; the 1st Philippine Aquaculture Congress and Exhibit in Bacolod City, 7-10 May, the International Cooperative Month Fair in Guimbal, Iloilo, 22 October; and the Mindanao Cluster Science & Technology Fair in Cagayan de Oro City, 27-31 October. During fairs, AQD exhibits models of farm lay-out and posters of research findings, sells textbooks and extension manuals on aquaculture technologies, and gives away newsletters, brochures, and flyers.


Production of information materials from research findings continued in 2003. During AquaBiz, AQD launched its latest extension manual *Biology and Hatchery of Mud crabs Scylla* spp. by AQD Scientists ET Quinitio and FD Parado-Estepa. The extension manual *Best Management Practices for Mangrove-Friendly Shrimp Farming* by DB Baliao and S Tookwinas came off the press in December. Going to press is the colorfully illustrated *Handbook of Mangroves in the Philippines – Panay* by JH Primavera, RB Sadaba, MJHL Lebata, and JP Altamirano, funded with a grant from the Man and the Biosphere Project of the United Nations Educational, Scientific, and Cultural Organization. Two videos are nearing completion: *Environment-Friendly Intensive Shrimp Farming* and *Seaweed Farming*.

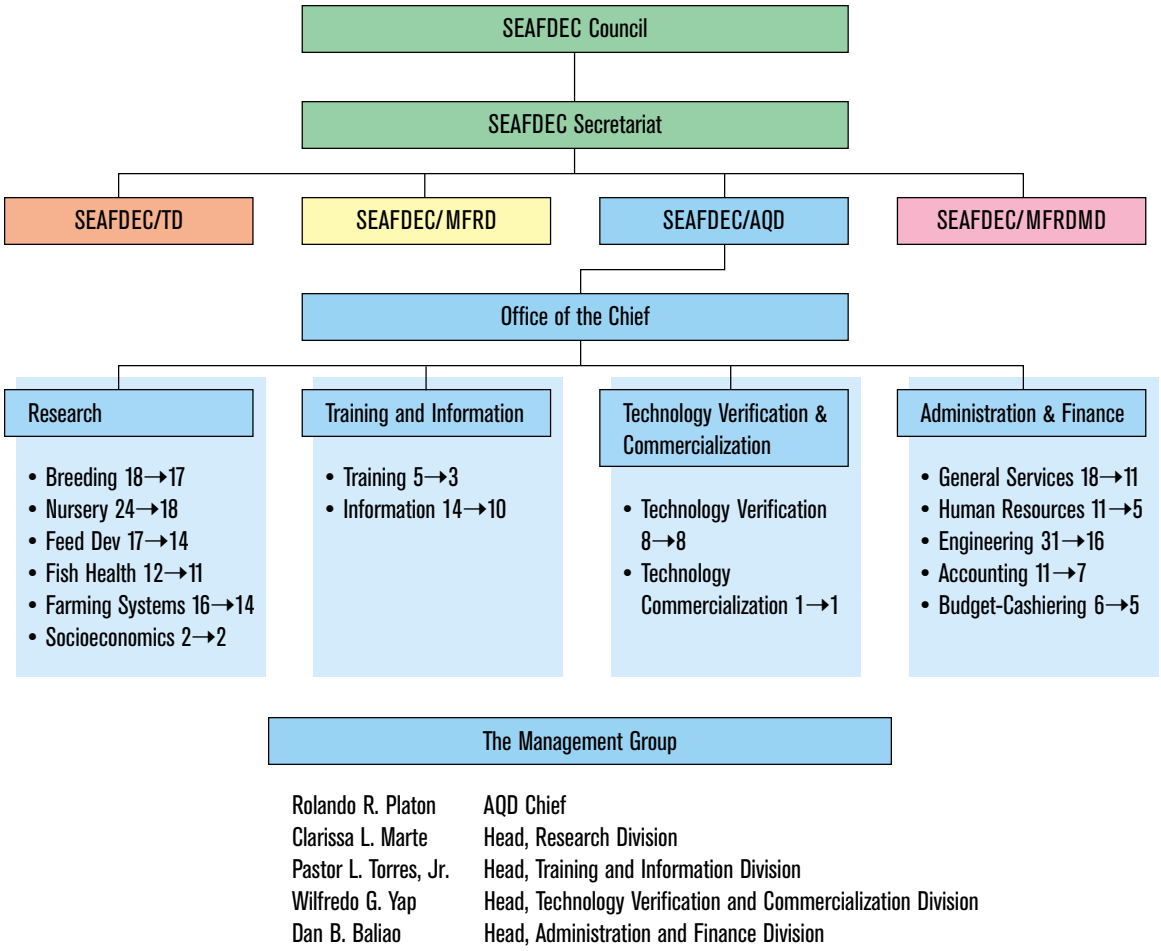
Several publications on aquatic animal health were produced by AQD. The conference proceedings volume *Disease Control in Fish and Shrimp Aquaculture in Southeast Asia – Diagnosis and Husbandry Techniques* was distributed early in 2003. A very timely flyer *Living with White Spot Disease in Shrimp Farming* was released at the end of the year and is available for free. The *Laboratory Manual of Standardized Methods for the Analysis of Pesticide and Antibiotic Residues in Aquaculture Products* by IG Borlongan of AQD and JNP Chuan of MFRD has gone to press.

AQD's annual report, the *2002 Highlights*, came out in time for the SEAFDEC Council Meeting in March. Three issues of the newsletter *SEAFDEC Asian Aquaculture* came out on schedule. There were 111 paid subscriptions to this newsletter in 2003, but about 1,300 copies of each issue were given free to government offices, fisheries schools, and libraries, and about 500 more to farmers and AQD visitors. AQD also released in 2003 the compilation volume, *Research Output of the Fisheries Sector Program Vol. 1, Articles published in scientific journals*, one-half of the work under a Memorandum of Agreement with the Philippine Department of Agriculture-Bureau of Agricultural Research.

AQD now maintains two websites, www.seafdec.org.ph and www.mangroveweb.net (the latter funded through the Mangrove-Friendly Shrimp Culture Program). AQD also hosts the website of the Fish Health Section of the Asian Fisheries Society at www.afs-fhs.seafdec.org.ph. The AQD web site has a discussion forum, Aquafarmers' Corner, where inquiries may be posted and answered and information disseminated on-line.

The holdings of the AQD Library now stand at 17,333 monographs, 8,880 journal volumes, 3,764 SEAFDEC publications, 8,880 pamphlets, 200 posters, 164 maps, 10,305 vertical files, and a variety of CD-ROMs. The AQD Library serves not only the AQD community, but also outsiders (at least 1,200 in 2003), mostly students from schools around Iloilo. In 2003, the Library answered 606 search requests and 167 queries from 19 countries.

# Personnel and management




| | |
|---|---|
| Rolando R. Platon | AQD Chief |
| Clarissa L. Marte | Head, Research Division |
| Pastor L. Torres, Jr. | Head, Training and Information Division |
| Wilfredo G. Yap | Head, Technology Verification and Commercialization Division |
| Dan B. Baliao | Head, Administration and Finance Division |

SEAFDEC/AQD had 342 employees in 2003, including 248 permanent and 94 fixed-term. The Research Division with six Sections had the largest personnel complement of 126, which included 52 researchers and scientists with MSc and PhD degrees in various disciplines. In August, long-time fish hatchery stalwart, MN Duray, turned 60 and happily retired from AQD.

SEAFDEC/AQD continued its staff development program. Two AQD Scientists earned their PhDs in 2003 from Japanese universities through the Ronpaku Program: IG Borlongan from Tokyo University of Fisheries and MR Catacutan from Kagoshima University. On the other hand, MLC Aralar obtained her PhD from the University of Hohenheim (Germany) and NS Sumagaysay-Chavoso earned hers from the Marine Science Institute of the University of the Philippines, Diliman. Four junior researchers are currently abroad on Masteral programs: EV Aralar at the Universiti Putra Malaysia, TT Gonzales at Nagasaki University, and JP Bancaya at the Queensland University of Technology. Several researchers attended international and national conferences where they presented scientific papers.

Sixteen researchers earned promotions in 2003 based on scientific merit points from research publications. Virologist LD de la Peña became a regular employee after publishing several research papers.

AQD Senior Scientist JH Primavera was elected Foreign Member of the Swedish Royal Academy on Agriculture and Forestry. EG De Jesus won the ***Outstanding Young Scientist Award*** from the National Academy of Science and Technology of the Philippines. JD Toledo won the ***Gawad Pangisdaan Award*** *for Outstanding Fisheries Professional* from the Philippine Fisheries Association. NS Sumagaysay-Chavoso won an ***Outstanding Thesis/ Dissertation Award*** from the Philippine Council for Advanced Science and Technology Research and Development for her dissertation "Nutrient loading and effluents of intensive and semi-intensive milkfish ponds, and the environmental capacity of receiving water"

The AQD personnel distribution in 2003 is shown in the chart above for the period before and after 31 December, the end of all fixed-term contracts and the deadline for availment of the benefits package for voluntary resignation or early retirement from the service. Some 82 permanent employees opted for separation from the service, including AQD Scientists CL Marte, CB Santiago, ZU Basiao, and VD Peñaflorida.

# Finances

The host Government of the Philippines (GOP) approved for 2003 a contribution of PhP 150 Million to the SEAFDEC Aquaculture Department through the Department of Foreign Affairs' International Commitment Fund. Unfortunately, without prior notice, the GOP imposed a mandatory savings of 15% in July and another 10% in December, which meant that PhP 37.5 M of AQD's budget for the year was withheld. This budget cut seriously affected AQD operations (and those of all GOP agencies).

There has also been a shift in the funding mechanism of the Government of Japan (GOJ) for SEAFDEC – from core type to the project type funding. AQD and the other Departments used to receive regular GOJ contributions for operating expenses, equipment, fellowships, and special fellowship funds. Now, GOJ contributes to SEAFDEC through a Trust Fund, which is administered by the Secretariat in Bangkok. This GOJ Trust Fund provides the bulk of the budget for the many programs (list on page 4) undertaken by the Secretariat and the four Departments under the ASEAN-SEAFDEC Fisheries Consultative Group mechanism. Three of these programs were implemented by AQD as the Lead Department (pages 18-22).

Fortunately for AQD, non-GOP external sources of funds were also available for various Departmental programs and projects in research, training and information, and technology verification and commercialization.

The largest chunk of the AQD budget in 2003 went to Personnel Services (PhP 97,145,807) for 342 employees. Funds for Personnel Services all had to come from the GOP contribution of P112.5 Million. In October 2003, the GOP announced that AQD's budget for 2004 is going to be P100 M only. To ensure the continuance of operations and projects in 2004, AQD offered an attractive package for voluntary resignation and early retirement, which 82 employees availed of by 31 December 2003.

**Income (in Phil pesos) for the period 1 Jan to 31 Dec 2003**

| | |
|---|---|
| **A. Sources of Funds: General Operating Funds** | |
| Contributions | |
| Government of the Philippines (DFA Internatl Commitment Fund) | 112,500,000 |
| Government of Japan (thru ASEAN Foundation, for the ASEAN-SEAFDEC Special Five-Year Program) | 9,039,915 |
| | 121,539,915 |
| External grants | |
| Japan International Cooperation Agency | 3,348,498 |
| United States Agency for International Development | 2,731,307 |
| Dept Agriculture–Bureau of Fisheries and Aquatic Resources | 2,000,000 |
| Japan International Research Center for Agricultural Sciences | 1,045,000 |
| United Nations Educational, Scientific, and Cultural Organization | 650,345 |
| Degussa Texturant | 610,803 |
| International Foundation for Science | 500,031 |
| Rovithai | 241,157 |
| Australian Center for International Agricultural Research | 198,979 |
| Food and Agriculture Organization | 75,719 |
| International Union for the Conservation of Nature | 33,985 |
| | 11,435,824 |
| Income of Research Division | |
| Research service laboratories | 629,849 |
| Integrated fish broodstock & hatchery | 381,514 |
| Abalone hatchery | 77,150 |
| Mudcrab hatchery | 30,415 |
| Fish hatchery | 286,885 |
| Miscellaneous income | 28,156 |
| | 1,433,969 |
| Income of Training & Information Division | |
| Training and intership fees | 1,293,878 |
| Sales of publications & videos | 614,988 |
| Miscellaneous income | 200 |
| | 1,909,066 |
| Income of Administration and Finance Division, Office of the Chief | |
| Housing rentals | 2,422,687 |
| Vehicle rentals | 1,368,001 |
| Interest income | 837,057 |
| FishWorld income | 185,535 |
| Telephone income | 25,495 |
| Miscellaneous income | 919,188 |
| | 5,757,963 |
| **B. Source of Funds: Government of Japan Trust Fund** | |
| Fish Diseases Project | 4,970,934 |
| Mangrove-Friendly Aquaculture Project | 5,572,512 |
| Production income from mangrove-friendly shrimp farming | 3,021,976 |
| | 13,565,422 |
| **C. Source of Funds: DA/BFAR–JMANTTP** | |
| Production income from JMANTTP projects | 2,348,082 |
| Private sector collaborative grants | 339,000 |
| Department of Science and Technology | 166,350 |
| | 2,853,432 |
| **Total income** | **158,495,592** |

**Expenses (in Phil pesos) for the period 1 Jan to 31 Dec 2003**

| | |
|---|---|
| **A. General Operating Funds** | |
| Personnel services | 97,145,807 |
| Maintenance and operating expenses | 29,008,543 |
| Capital outlay | 1,857,995 |
| Project expenses | |
| Research | 2,458,253 |
| Training & Information | 1,202,424 |
| Technology Verification & Commercialization | 1,830,049 |
| | 133,503,071 |
| ASEAN-SEAFDEC Special Five-Year Program | 6,134,581 |
| Externally funded projects–research and training | |
| USAID | 1,579,003 |
| DA/BFAR Aquaculture Biotech Project | 5,407,238 |
| JIRCAS | 953,117 |
| Degussa Texturant | 656,170 |
| IFS 1,616 | |
| Rovithai | 147,687 |
| ACIAR | 622,645 |
| European Union-Nematode Project | 25,050 |
| EU-Mudcrab Project | 1,666,759 |
| Asia-Pacific Economic Cooperation | 5,644 |
| WorldFish Center | (2,482) |
| JICA Third Country Training Program | 3,440,477 |
| UNESCO–Mangrove Handbook | 545,426 |
| FAO–Regional Donors' Consultation Meeting | 5,545 |
| IUCN–Seminar Workshop | 4,031 |
| DA–Bureau Agric Research–FSP volume | 215,863 |
| University of Madagascar–training | 21,400 |
| | 15,295,189 |
| **B. Project expenses GOJ Trust Fund** | |
| Fish Diseases Project | 1,018,997 |
| Mangrove-Friendly Aquaculture Project | 4,496,868 |
| | 5,515,866 |
| **C. Project expenses DA/BFAR–JMANTTP** | |
| JMANTTP Projects | 4,269,413 |
| Private sector collaborative grants | 199,861 |
| Department of Science and Technology | 80,052 |
| | 4,549,325 |
| **Total expenses** | **164,998,031** |

# FishWorld matters

SEAFDEC FishWorld continues to serve the general public as AQD's visitor center, museum of aquatic biodiversity, and science and environment education center. In 2003, FishWorld received 16,620 visitors in 170 groups (129 schools) and earned P173,865 in entrance fees. The much-appreciated museum collection now has about 2,500 species of fishes, gastropods, bivalves, crustaceans, corals, echinoderms, and other aquatic animals and plants. FishWorld now offers a taxonomic identification (aquatic animals) service for a fee (P50 per species). In addition, FishWorld documented in 2003 the stranding or capture of four marine turtles (all were tagged then released), a melon-headed whale, a dugong, and a whale shark.

FishWorld held Aquaculture Week in July and Fish Conservation Week in October, with 15 contests that required studying and learning about aquaculture, aquatic ecosystems, and biodiversity (230 students, 117 coaches, and 35 schools participated). FishWorld also led the International Coastal Cleanup in southern Iloilo, where 3,135 volunteers removed about 10 tons of garbage from the beaches and waterways.

At the invitation of JT Biyo, Intel Outstanding Teacher, FishWorld Curator TU Bagarinao gave lectures at the Philippine Science High School on science culture in the Philippines, scientific research in Philippine high schools, and writing research proposals and publications. The Curator also presented papers on aquatic biodiversity research in the Philippines at workshops organized by the WorldFish Center and the DOST National Research Council of the Philippines.

**Participation in FishWorld events and contests in 2003**

| R&D Internships 14 Apr–9 May | | Students | Teachers | Schools |
|---|---|---|---|---|
| Hands on exposure to research in marine science and aquaculture | | 12 | | PhilSci |
| **Aquaculture Week 28 Jul–1 Aug** | | **Students** | **Teachers** | **Schools** |
| Painting Contest HS: "Life in my watershed" | | 16 | 16 | 16 |
| Ecology and Aquaculture Quiz | | 35 | 21 | 18 |
| Photojournalism: "Aquaculture in Panay: from the fish farm to the dinner table" | | 9 | 9 | 9 |
| Collect and Document Biodiversity: "Freshwater flora and fauna in Panay" | | 14 | 8 | 7 |
| Painting Contest ES: "Mangroves are important to me and my community" | | 16 | 15 | 15 |
| Nutrition and Aquaculture Quiz | | 30 | 16 | 15 |
| Bring, Show, and Tell: "My favorite freshwater creature" | | 13 | 8 | 8 |
| Aquarium Quiz | | 18 | 9 | 9 |
| Seafood Dish Contest | | 19 | 13 | 9 |
| | Total | 168 | 90 | 33 |
| **International Coastal Cleanup 20 Sep** | | **Elem Sch** | **High Sch** | **Univ/Coll** | **LGU, NGO** |
| Groups that returned ICC data cards to FishWorld | Total = 44 | 5 | 7 | 2 | 30 |
| Number of ICC volunteers | Total = 3135 | 750 | 968 | 81 | 1336 |
| **Fish Conservation Week 24-28 Oct** | | **Students** | **Teachers** | **Schools** |
| Bring, Show, and Tell: "All about shells" | | 9 | 7 | 5 |
| Aquarium Quiz: "Look, look at it!" | | 10 | 7 | 5 |
| Photojournalism: "We cleaned up our community" | | 6 | 6 | 6 |
| Science Clubs Collect and Document: "Freshwater fauna in Panay" | | 7 | 5 | 4 |
| Scientific Illustration: "The amazing shells: from common to endangered" | | 15 | 5 | 5 |
| Fish Conservation Quiz | | 16 | 7 | 8 |
| | Total | 63 | 27 | 13 |

# SEAFDEC/AQD responds to the Philippines


## AQUABIZ 2003

| Presenter | Technology |
|---|---|
| DD Baliao | Environment-friendly shrimp grow-out |
| ET Quinitio | Mud crab hatchery and nursery |
| DD Baliao | Mud crab grow-out in ponds and pens |
| MN Duray | Marine fish hatchery |
| NS Chavoso | Milkfish grow-out in ponds |
| JD Toledo | Investment opportunities in grouper farming |
| JD Toledo | Prospects in seabass farming |
| DD Baliao | Farming tilapia in cagec |
| RV Eguia | Tilapia hatchery and nursery |
| AC Emata | Aquaculture of the mangrove red snapper |
| AC Gonzal | Prospects in farming bighead carp |
| JT Fermin | Catfish seed production and grow-out |
| AQ Hurtado | Farming the seaweed *Kappaphycus* |
| LMB Garcia | Seahorses–a new species for aquaculture |
| WG Gallardo | Farming abalone–a new business opportunity |


AQD at fairs and exhibits

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

SEAFDEC conducts research on fisheries problems; generates appropriate fisheries technologies; trains researchers, technicians, fishers and aquafarmers, managers, and policy-makers; and disseminates information pertaining to the fisheries sector.

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

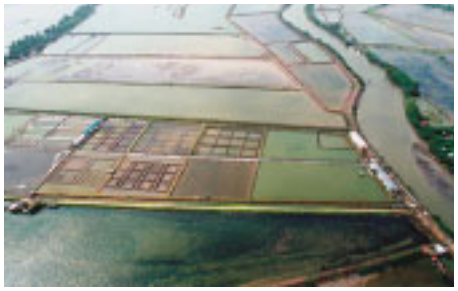
- The Training Department (TD) in Samut Prakan, Thailand (1967) for training in marine capture fisheries
- The Marine Fisheries Research Department (MFRD) in Singapore (1967) for post-harvest technologies
- The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development
- The Marine Fishery Resources Development and 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

SEAFDEC/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
- Disseminate and exchange aquaculture information

The Aquaculture Department in the Philippines maintains five stations: the Tigbauan Main Station and Dumangas Brackishwater Station in Iloilo; the Igang Marine Station in Guimaras; and the Binangonan Freshwater Station and ABCDEF Jalajala in Rizal.

Tigbauan Main Station



Dumangas Brackishwater Station

Igang marine Station

Binangonan Freshwater Station

**Aquaculture Department (AQD)**
5021 Tigbauan, Iloilo
Philippines
PO Box 256, 5000 Iloilo City
Philippines
Tel: (63 33) 511 9171; 336 2891;
336 2937; 336 2965
Cable: seafdec iloilo
Fax: (63 33) 335 1008; 511 8709
E-Mail: aqdchief@aqd.seafdec.org.ph
http://www.seafdec.org.ph

**Training Department (TD)**
PO Box 97
Phrasamutchedi
Samut Prakan 10290
Thailand
Tel: (66 2) 425 8040 to 5
Fax: (66 2) 425 8561
E-Mail: td@seafdec.org
http://www.seafdec.org

**Marine Fisheries Research Department (MFRD)**
2 Perahu Road off Limchukang Road
Singapore 718915
Tel: (65) 790 7973
Fax: (65) 790 7963, 861 3196
E-Mail: mfrdlibr@pacific.net.sg
http://www.asean.fishnet.gov.sg/mfrd.html

**Marine Fishery Resources Development and Management Department (MFRDMD)**
Fisheries Garden, Chendering
21080 Kuala Terengganu
Malaysia
Tel: (609) 617 5135
Fax: (609) 617 5136
E-Mail: seafdec@po.jaring.my
http://www.agrolink.moa.my/dof/seafdec.html

**Seafdec Secretariat**
Suraswadi Building
Department of Fisheries Compound
Kasetsart University Campus
Chatukchak, Bangkok 10900
Thailand
Tel: (66 2) 940 6326 to 940 6329
Fax: (66 2) 940 6336
E-Mail: secretariat@seafdec.org
http://www.seafdec.org