



Southeast Asian Fisheries Development Center  
**AQUACULTURE DEPARTMENT**  
[www.seafdec.org.ph](http://www.seafdec.org.ph)

# SEAFDEC/AQD Highlights 2009





**On the cover**  
**Reef rangers** by Andrea Bagarinao,  
 a mural at AQD FishWorld

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*Accounting* Mr. Juan Garin Jr.  
 Ms. Renee Valencia / Ms. Amelita Subosa

RD, Research Division; TVDD, Technology Verification & Demonstration Division;  
 TID, Training & Information Division; AFD, Administration & Finance Division;  
 BFS, Binangonan Freshwater Station; DBS, Dumangas Brackishwater Station;  
 IMS, Igang Marine Station; GOJ-TF, Government of Japan Trust Fund

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## Acronyms used in this report

ABOT	Agree-build-operate-transfer
ACIAR	Australian Center for International Agricultural Research
AFMA	Agriculture and Fisheries Modernization Act
ASEAN	Association of Southeast Asian Nations
AQD	Aquaculture Department of SEAFDEC
BFAR	Bureau of Fisheries and Aquatic Resources
BW	Body weight
CHED	Commission on Higher Education
CATP	Community Agricultural Technology Program
DA	Department of Agriculture (Philippines)
DA-BAR	Department of Agriculture Bureau of Agricultural Research
DOST-SEI	Department of Science and Technology Science Education Institute
DOST-PCAMRD	Department of Science and Technology Philippine Council for Aquatic Marine Resource Development
FFRC	Freshwater Fisheries Research Center
GCRV	Grass carp reovirus
GOJ-TF	Government of Japan Trust Fund
HPV	Hepatopancreatic parvo-like virus
ICDSA	Institutional capacity development for sustainable aquaculture
ICLARM	International Center for Living Aquatic Resources Management (now The WorldFish Center)
IHHNV	Infectious hypodermal and hematopoietic necrosis virus
IMNV	Infectious myonecrosis virus
KHV	Koi herpesvirus
LGU	Local government unit
LIPASECU	Libertad-Pandan-Sebaste-Culasi (Antique)
NFRDI	National Fisheries Research and Development Institute
NGO	Non-government organization
MBV	Monodon baculovirus
MOU/MOA	Memorandum of understanding / agreement
OIE	Office International des Epizooties
PEMSEA	Partnership in the Environmental Management for the Seas of East Asia
PL	Postlarvae
R&D	Research and development
SVCV	Spring viremia of carp virus
SEAFDEC	Southeast Asian Fisheries Development Center
SL	Shell length
SMR	Sagay Marine Reserve (Sagay, Negros Occidental)
TSV	Taura syndrome virus
UNITAR	United Nations Institute for Training and Research
UIP-MSI	University of the Philippines Marine Science Institute
UIP-NSRI	University of the Philippines Natural Sciences Research Institute
VNN	Viral nervous necrosis

## AQD Chief's report



AQD Chief Dr. Joebert Toledo (below) signs new collaborative R&D agreements, including FFRC China (above left) and St. Paul's University. Fifteen collaborators came on board in 2009 while 16 collaborative projects continue



**T**his year, SEAFDEC Aquaculture Department (AQD) began the implementation of its *Strategic Plans 2009-2012*.

AQD strategy is three-fold: (1) develop, verify and transfer viable science-based aquaculture technologies and information appropriate to the region; (2) strengthen the capacities of the aquaculture sector; and (3) promote organizational & financial stability.

For the first goal, a total of 94 research and verification studies were proposed, funded and conducted. Researchers published 25 papers in science journals to ensure that research results continue to be made public and easily accessible. Five prestigious institutions commended AQD's work, earning AQD two best paper and three best researcher awards. More R&D collaborators joined AQD in its vision of responsible aquaculture for the industry.

For the second goal, AQD organized 40 training sessions (612 people attending in total), 35% of which was conducted on-site in SEAFDEC member countries, the rest in AQD stations. Petron Foundation funded the most number of trainees (20%) followed by ASEAN Foundation (18%).

### New agreements with AQD

- Freshwater Fisheries Research Center / Chinese Academy of Fishery Sciences (FFRC China)*: Renewal of MOU for research, training and staff exchanges (2 years beginning May 09)
- Worldfish Center (ICLARM) (Penang, Malaysia)*: collaboration on the project "Culture of sandfish in Asia-Pacific" (Dec 09 - Nov 2011)
- Evonik Degussa GmbH (Germany)*: Research contract for the study on amino acid supplementation in common carp feeds (Mar 09 -Sept 09)
- NOVUS International Inc (USA)*: Research contract for the study on growth, survival and nutritional composition of grouper fry fed formulated diet (Sept 09 - Sept 2010)
- SmithBucklin Corp. (USA)*: Research contract for the study on the use of soybean meal and soy protein concentrate for milkfish (Jan-Dec 09)
- Bari Aquatech Sdn Bhd (Kuala Lumpur, Malaysia)*: AQD to provide technical assistance for abalone farming (2009-2011)
- St Paul's University (Philippines)*: Collaboration in instruction, research, training and extension programs in fisheries, aquatic and related sciences (2009-2014)
- University of San Agustin (Philippines)*: Collaboration in instruction, research, training and extension programs in fisheries, aquatic and related sciences (Dec 2009-Dec 2012)
- Capiz National High School (Philippines)*: Agreement on research studies (starting Jul 09)
- Centro Escolar University (Philippines)*: Agreement on research collaboration (starting Oct 09)
- University of the Philippines Visayas (Philippines)*: Agreement on research studies on mangrove polychaetes (starting Oct 09); Indian white shrimp (starting Nov 09); the algae *Lyngbia majuscula* (starting Nov 09); and mud crabs (starting June 09)
- Finfish Hatcheries Inc (Makati City, Philippines)*: Marine fish culture at the Igang Mariculture Park (Aug 2009 - Aug 2012)
- Alfonso Valderrama of Iloilo City*: Marine fish culture at the Igang Mariculture Park (Aug 2009-Aug 2012)
- Carmelo Celis of Dingle, Iloilo, Philippines*: Farm-level verification/ production of seabass and black hybrid tilapia in net cages and comparing SEAFDEC-formulated and commercial feeds
- William Ching of Iloilo City (Philippines)*: Integrated mollusk production at the Igang Mariculture Park (Jun 2009 - Jun 2012)

### Continuing collaborations with AQD

- North Carolina State University (USA) and AquaFish Collaborative Research Support Program*: Alternative feeding strategies to improve milkfish production
- Australian Center for International Agricultural Research (ACIAR)*: Integrated fisheries resource management (Rinconada Lakes, Philippines and New South Wales, Australia): Aquaculture and Water Quality Component
- ACIAR: Community Agricultural Technology Program (CATP)*
- ICLARM (The World Fish Centre)*: Culture of sandfish in Asia-Pacific
- United Soybean Board*: Use of soybean meal and soy protein concentrate as alternatives to fish meal in practical feeds
- United Nations Institute for Training and Research*: Capacity development in sea and human security in Malalison Island and Masbate City
- Novus International Inc*: Growth, survival and nutritional composition of grouper fry fed formulated diet
- Petron Foundation Inc*: Milkfish cage culture as livelihood option for affected Guimaras fisherfolk-Phase II
- Action for Community Empowerment Association Inc*: Enhancing adoption of mud crab production technologies in Northern Samar
- Bureau of Fisheries and Aquatic Resources – Philippines (BFAR)*: Grow-out of white shrimp in Paoay Lake
- BFAR*: National abalone breeding and culture program
- BFAR-National Fisheries Research and Development Institute (NFRDI)*: Identification of emerging problems and economically significant diseases in hatchery and grow-out production systems of white shrimp
- BFAR-NFRDI*: Aquaculture biotechnology program
- Province of Misamis Occidental, Philippines*: Technical assistance to Misamis Occidental Aquamarine Park (MOAP) multi-species hatchery
- Philippine Australia Community Assistance Program-MOAVEC (Misamis Occidental)*: ICDSA on grouper cage culture livelihood venture for selected fisherfolk communities in Misamis Occidental
- Gata Daku Multi-Purpose Cooperative (Misamis Occidental)*: ICDSA on enhancing adoption of improved grouper production technologies in Misamis Occidental

## AQD's award-winners

*Dr. Ma. Junemie Hazel Lebata-Ramos* was



recognized as one of three recipients of the **Japan International Award for Young Agricultural Researchers 2009** that was given by Japan International Research Centre for Agricultural Sciences in a ceremony at the University of Tokyo on November 4.

*Dr. Lebata-Ramos* was also the recipient of the prestigious **Dr. Elvira O. Tan Memorial Award** for best paper in aquaculture and inland fisheries. The award was given by the Philippine Department of Science & Technology for her paper *Evaluation of hatchery-based enhancement of the mudcrabs *Scylla spp.* fisheries in mangroves: Comparison of species and release strategies*. She co-authored the paper with Lewis Le Vay, Mark Walton, Joseph Binás, Emilia Quintito, Eduard Rodriguez and Jurgenne Primavera. The award ceremony took place at the University of the Philippines Los Banos on July 24

*Ms. Maria Rovilla Luhan* was awarded the



**2009 AFMA Best R&D Paper** for basic research by DA-Bureau of Agricultural Research during its *21st National research symposium* at Quezon City on October 9. Ms. Luhan's paper was on *Growing the reproductive cells (carpospores) of the*

*seaweed *Kappaphycus striatum* in the laboratory until outplanting in the field and maturation to tetrasporophytes* which she co-authored with Hananiah Sollesta

*Dr. Joebert Toledo* was recognized as the **Outstanding Professional of the Year** (fisheries technologist category) by the Professional Regulatory Commission. He was cited for sharing his expertise & knowledge through training and extension activities, and for his contribution to science literature which were deemed the result of his determination and untiring search for innovation and new technologies. The award ceremony was held June 19 in Manila

*Dr. Jurgenne Primavera*, AQD's scientist emerita, was honored by the University of the Philippines (UP) Alumni Association as one of its **Distinguished Alumnus for Environmental Conservation and Sustainable Development**. The honor was conferred June 20 at UP Diliman

Farmer-friendly reading materials supports the need for information, and at least 20 titles were produced this year describing AQD technologies. In addition, at least 15 AQD staff were present at international meetings to share & exchange information.

For the third goal, AQD began regularization of its facilities-based staff (as opposed to fixed-term staff for research studies) to enable continuity in operations. Of the 152 regular positions approved by the SEAFDEC Council, 106 has been filled up with qualified personnel. As of 31 December 2009, AQD has a total staff complement of 294 staff: 141 in research, 57 in technology verification, 26 in training & information, 49 in administration & finance and 21 in management group. AQD budget from its host country, the Philippines, has remained steady,

at Php 150 million which was 50% more than the 2006 budget.

AQD thanks its funding and program partners especially the Philippine government for their help in achieving the 2009 goals. These partnerships reflected the trust and confidence in AQD's ability to develop and disseminate research-based technologies.

To aquaculture stakeholders in Southeast Asia, AQD remains strong in its commitment to continue helping achieve food security and holistic human development.

**Joebert D. Toledo**, *D. Agri*  
Chief, SEAFDEC/AQD

## International fellowship-meetings attended by AQD staff

- Fulbright Senior Scholarship (16 Jan – 23 June, Univ of Arizona - USA); and 2009 Fulbright Visiting Scholar Conference (19-21 Apr, Washington DC) courtesy of the Fulbright Foreign Scholarship Board
- First International Congress on Aquatic Animal Health Management and Diseases (27-28 Jan, Tehran, Iran) organized by Veterinary Council of Iran
- 2<sup>nd</sup> RESCOPAR Meeting (25-26 Feb, East Kalimantan-Indonesia) organized by Wageningen University
- Aqua VIV Asia (11-13 Mar, Bangkok) organized by Novus, Bayer, partners
- Asia-Pacific Conference on Chemistry Education (13-17 Apr, Bohol, Phil) by DOST
- International Training Program on Scientific and Technological Information Management in Universities and Libraries: an Active Training Environment 9 (STIMULATE 9) (4 May – 30 Jul, Brussels, Belgium) by VLIR-UOS; and the 30th Annual Conference of the International Association of Technological University Libraries (1-4 June, Leuven, Belgium) sponsored by STIMULATE 9 and VLIR-UOS
- WSSV Detection and Challenge Test (18-27 May, Can Tho Univ, Vietnam) sponsored by Wageningen Univ
- Symposium on Aquatic Animal Health and Welfare (6-12 June, China) at FFRC China
- 10<sup>th</sup> International Symposium on Genetics in Aquaculture (22-26 June, Bangkok, Thailand) by the International Association for Genetics in Aquaculture
- Asian Publishing Convention (16-17 Jul, Manila, Phil) by a media consortium
- 7th International Abalone Symposium (19-24 July, Pattaya, Thailand) by the Marine Science Association of Thailand, partners
- 14th International EAFP Conference on Fish and Shellfish Diseases (14-18 Sept, Prague, Czech Republic)
- UNITAR Workshop (27 Sept – 4 Oct, Hiroshima, Japan)
- International Seminar on Electronic Libraries as a Center of Collaboration (7-9 Oct, Baguio City-Phil) organized by Saint Louis University Libraries
- Training on Fisheries Data Collection and Analysis (2-20 Nov, Wageningen, Netherlands) by Wageningen Int'l
- Asian Pacific Aquaculture 2009 Conference Expo (3-6 Nov, Kuala Lumpur, Malaysia) organized by the World Aquaculture Society
- Symposium on Aquaculture, Biology and Management of Commercially Important Crabs (8-11 Nov, Shanghai, China) by the Chinese Crustacean Society, Shanghai Ocean University
- East Asian Seas Congress (23-27 Nov, Phil. Convention Center-Phil) organized by PEMSEA
- 26th Conference of the OIE Regional Commission for Asia, the Far East and Oceania (16-20 Nov, Shanghai, China)
- International Symposium on Aquaculture and Fisheries Education (26 Nov - 1 Dec, Bangkok, Thailand) organized by Asian Institute of Aquaculture and partners
- 8th NACA Aquatic Animal Health Advisory Group Meeting (2-4 Dec, Bangkok, Thailand)
- Regional Workshop on Harmonization of Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals (1-5 Dec, Kuala Lumpur, Malaysia) organized by ASEAN, DOF-Malaysia

# Research and development projects 2009

	STUDY TITLE	STUDY LEADER	Per cent completed	BUDGET (Php)		COLLABORATING AGENCY
				SEAFDEC	External	
<b>DEPARTMENTAL PROGRAMS</b>						
<b>Integrated mollusc</b>						
1	Isolation and cultivation of <i>Cocconeis</i> sp. for settlement, growth and survival of post-larval abalone <i>Haliotis asinina</i>	MR de la Peña	30%	351 674		
2	Refinement of hatchery techniques for the donkey's ear abalone <i>Haliotis asinina</i> I Evaluation of the spawning, fecundity and seed quality produced from wild and existing hatchery breeders II Verification and modification of existing transport techniques for abalone III Development of a strategy for genetic management of abalone	MR de la Peña	10%	312 489		
3	Socioeconomic factors influencing participation of fishers and other stakeholders in stock enhancement of abalone in Anini-y, Antique and Concepcion, Iloilo	ET Aldon	70%	60 000		
4	Experimental hybridization between Philippine native abalone species: <i>Haliotis asinina</i> , <i>H. glabra</i> , <i>H. ovina</i> and <i>H. varia</i>	MR de la Peña	70%	586 557		
5	Diet development and evaluation for juvenile abalone, <i>Haliotis asinina</i> Linne: Lipid and essential fatty acid requirements	MB Teruel	90%	210 000		
6	Nursery rearing and culture of the tropical abalone, <i>Haliotis asinina</i> , Linne 1758, in plastic trays	VC Encena II	60%	822 060		
7	Large-scale production of donkey's ear abalone, <i>Haliotis asinina</i> juveniles	NC Bayona	90%	630 318		
<b>Shrimp and mudcrab domestication</b>						
8	Domestication of the indigenous white shrimp species <i>Penaeus merguensis</i> / <i>P. indicus</i>	FDP Estepa	25%	374 548		
9	Effects of dietary tryptophan on the antagonistic behavior of mud crab <i>Scylla serrata</i>	JLQ Laranja Jr.	50%	473 992		
10	Development of practical feed for the grow-out culture of mud crab, <i>Scylla serrata</i> Forskal	MR Catacutan	80%	492 960		
11	Culture of marine annelid <i>Perinereis</i> sp. and its use as substitute for fishmeal, shrimp meal and squid meal in shrimp feeds	VR Alava	40%	264 500		
12	Comparative performance of pond-sourced <i>P. monodon</i> broodstock fed natural and artificial diets	MB Teruel	60%	198 000	240 000	Government of Japan (Kagoshima University)
13	Ensuring sustainability of the Philippine shrimp industry through effective health management of native and introduced species	CL Pitogo	95%		US \$ 16 250	Fulbright (University of Arizona)
14	Nursery production of mud crab, <i>Scylla serrata</i> in brackishwater ponds	JLQ Laranja Jr.	50%	849 580		
15	Seed production of mud crab <i>Scylla</i> spp.	ET Qunitio	90%	670 180		
16	Verification of <i>Penaeus indicus</i> grow-out diets in ponds using environment-friendly scheme	NV Golez	25%	1 404 855		
17	Culture of mud crab ( <i>Scylla serrata</i> Forsskal) in mangrove pens	DD Baliao	Deferred		357 168	Land Bank - Technology Promotion Center

	STUDY TITLE	STUDY LEADER	Per cent completed	BUDGET (Php)		COLLABORATING AGENCY
				SEAFDEC	External	
<b>Marine fish</b>						
18	Growth and survival of grouper <i>Epinephelus</i> sp. larvae: An energetics approach (PhD Dissertation)	YHP Tirol	50%		270 000	CHED / PCAMRD / DA-BAR / DOST-SEI*
19	Development of assay utilizing IGF-II mRNA expression as marker for egg quality in grouper and siganid	JB Gonzaga	90%	663 808		
20	Growth, survival and nutritional composition of grouper fry fed formulated diet	OS Reyes	10%		132 900	Novus International Inc.
21	Studies on the seed production techniques of high-value marine species such as grouper, red snapper, seabass and pompano	OS Reyes	65%	1 782 800		
22	Alternative feeding strategies to improve milkfish production	EGDJ Ayson	90%		1 463 500	North Carolina State University
23	Use of PUFA-rich thraustochytrids as enrichment diets for live foods (rotifers and <i>Artemia</i> ) in the larviculture of the seabass, <i>Lates calcarifer</i>	DG Estenor	80%	156 101		
24	Studies on the seed production techniques of milkfish and rabbitfish	OS Reyes	75%	1 295 400		
25	Formulated diets for adult seahorse	MR Catacutan	15%	308 060		
26	Effect of dietary vitamin C on reproduction, egg and larval quality and immune responses of grouper <i>Epinephelus fuscoguttatus</i>	VR Alava	3%	247 200		
27	Use of soybean meal and soy protein concentrate as alternatives to fish meal in practical feeds for milkfish, <i>Chanos chanos</i>	RM Coloso	30%		1 240 128	United Soybean Board
28	White cowpea meal as alternative source of protein for grouper, <i>Epinephelus coioides</i>	PS Eusebio	75%	330 800		
29	Improvement of the nutritional value of locally available feed resources for practical aquatic feeds by submerged fermentation and solid substrate fermentation using milkfish gut bacteria and/or selected fungi	RM Coloso	60%	471 600		
30	Assessment of the potential of mysid shrimps (Crustacea: Mysidacea) as live food in marine fish culture 2: Nutritional evaluation of mysid shrimps	PS Eusebio	80%	188 800		
31	Development of nursery culture techniques for siganids in ponds: Verification of growth performance using supplemental diet of SEAFDEC formulated feeds	JM Ladja	95%	475 417		
32	Supplementation of L-tryptophan and ginger in formulated diets: Effects on the nursery performance of grouper and seabass reared in cages in brackishwater ponds	RSJ Gapasin	80%	632 453		
33	Intensive production of red tilapia hybrid ( <i>O. mossambicus</i> - <i>O. hornorum</i> hybrid x <i>O. niloticus</i> ) in polyculture with white shrimp ( <i>Penaeus indicus</i> ) and mudcrab ( <i>Scylla serrata</i> ) in brackishwater ponds	RM Coloso	20%	372 292		
34	Milkfish fingerlings production in floating net cages	EB Coniza	100%	564 410		
35	Nursery culture of pompano ( <i>Trachinotus blochii</i> Lacepede) in floating net cages	AG Gaitan	50%	106 540		
36	Pompano ( <i>Trachinotus blochii</i> Lacepede) grow-out culture in floating net cages	AG Gaitan	65%	609 907		
37	The use of phased diets (starter, grower and finisher) for grouper ( <i>Epinephelus fuscoguttatus</i> ) cultured in floating net cages	RM Coloso	70%	521 680		
38	Refinement of intensive grow-out culture of sea bass ( <i>Lates calcarifer</i> ) in brackish water ponds using SEAFDEC-formulated diets with higher energy level	RM Coloso	30%	652 672		
39	Nursery culture techniques for groupers, sea bass and snappers in cages and in pond: Production of juveniles using formulated diets	JM Ladja	90%	788 917		
40	Refinement and dissemination of intensive grow-out technique for the polyculture of milkfish ( <i>Chanos chanos</i> ), white shrimp ( <i>P. indicus</i> ) and crab ( <i>Scylla</i> spp.)	NV Golez	20%	624 624		
41	Netcage culture of sea bass, <i>Lates calcarifer</i> (Bloch) in freshwater farm reservoir using SEAFDEC formulated diet and commercial feed	DD Baliao	25%	314 027		
42	Verification of grouper, snapper and rabbitfish grow-out diets in cages in pond	EB Coniza	95%	619 120		

	STUDY TITLE	STUDY LEADER	Per cent completed	BUDGET (Php)		COLLABORATING AGENCY
				SEAFDEC	External	
<b>Seaweed strain improvement</b>						
43	Seed production of <i>Kappaphycus</i> : Protoplast isolation and sporulation	MRJ Luhan	70%	675 615		
44	Acadian marine plant extract powder from <i>Ascophyllum nodosum</i> induces resistance in <i>Kappaphycus</i> against <i>Neosiphonia</i>	AQ Hurtado	50%	774 290		
<b>Aquatic ecology</b>						
45	<i>Anodontia philippiana</i> and <i>Holothuria scabra</i> as bioremediators in an intensive cage culture system	MJHL Ramos	10%	210 000		
46	Biodiversity in the brackishwater ponds and the adjoining mangroves, rivers, and shores in Dumangas, Iloilo: species composition and volume of the pond by-catch in relation to natural seeding and farm management practices	TU Bagarinao	50%	20 000		
47	Identification of fish species suitable for polyculture with sea cucumber	J Zarate	10%	248 500		
48	Sediment quality monitoring in Igang Marine Station towards development of detailed sediment quality assessment techniques	SMS Santander	65%	439 500		
49	Water quality monitoring of mariculture park in Humaraon Cove, Igang, Guimaras	GM Anuevo	80%	297 533		
50	Determination of ammonium uptake of <i>Gracilariopsis bailinae</i> and its impact on the co-culture of abalone, <i>Haliotis asinina</i> , and <i>G. bailinae</i> in a recirculating system	TRC Mallare	85%	42 600		
<b>Small-holder freshwater aquaculture</b>						
51	Reproductive biology of the silver perch <i>Leiopotherapon plumbeus</i> (Teleostei: Terapontidae) from selected freshwater habitats in Luzon	LMaB Garcia	60%	236 500	304 642	UP-NSRI**
52	Refinement of broodstock and nursery technology for the commercial production of bighead carp <i>Aristichthys nobilis</i> (Richardson) fingerlings in cages in Laguna de Bay I Reproductive performance and fry production of bighead carp on different feeding regimes II Optimum stocking density at different climatic conditions (warm and cold)	MA Laron	80%	75 344		
53	Supportive breeding and restocking of indigenous freshwater fishes in selected Philippine inland water systems I Cage farming of the silver therapon, <i>Leiopotherapon plumbeus</i> in Laguna de Bay	MA Laron	50%	106 024		
54	II Cage farming of the catfish <i>Arius</i> spp. in Laguna de Bay, Philippines	AD Evangelista	30%	105 600		
55	Growth of climbing perch <i>Anabas testudineus</i> in cages using different farm-based feeds	RV Eguia	20%	68 800		
56	Grow-out of <i>Penaeus vannamei</i>	MLC Aralar	15%		117 600	BFAR
57	Tilapia cage farming in freshwater dams and reservoirs using SEAFDEC and commercial feeds	DD Baliao	95%	391 678		
58	Grow-out culture of Asian catfish <i>Clarias macrocephalus</i> (Gunther) in net cages in Laguna de Bay, Philippines	AD Evangelista	50%	228 200		
59	Mass production of freshwater prawn post-larvae using green water system	MA Laron	60%	234 310		
60	Modular cage culture of tilapia in 2x2x2 m cages in Laguna de Bay	RV Eguia	100%	123 200		
61	Mass production of <i>Tilapia nilotica</i> and red tilapia	DM Reyes Jr.	75%	496 576		
62	Freshwater aquaculture seedstock production in Laguna de Bay: Advance bighead carp fingerlings production in a small fishpen	EV Aralar	70%	288 000		

STUDY TITLE	STUDY LEADER	Per cent completed	BUDGET (Php)		COLLABORATING AGENCY	
			SEAFDEC	External		
<b>REGIONAL PROGRAMS</b>						
<b>Development of technologies and human capacity building for sustainable aquaculture - Promotion of sustainable aquaculture in the ASEAN region</b>						
<b>63</b>	Domestication and genetic stock evaluation of the indigenous giant freshwater prawn subspecies <i>Macrobrachium rosenbergii rosenbergii</i> : I Assessment of breeding and reproductive performance	MRR Eguia	95%	124 000	52 640	ASEAN-GOJ-TF
<b>64</b>	II Determination of larval rearing and seed production requirements	MA Laron	50%	119 373	54 584	ASEAN-GOJ-TF
<b>65</b>	III Evaluation of performance in grow-out	MLC Aralar	50%	95 200	54 450	ASEAN-GOJ-TF
<b>66</b>	Domestication of mud crab <i>Scylla serrata</i>	ET Quinitio	90%	473 721	308 850	ASEAN-GOJ-TF
<b>67</b>	Development of specific pathogen free (SPF) shrimp ( <i>P. monodon</i> , <i>P. vannamei</i> ) broodstock: I Genetic characterization of <i>P. monodon</i> broodstock	MRR Eguia	95%		319 130	ASEAN-GOJ-TF
<b>68</b>	II Studies on improvement of maturation of pond-reared <i>Penaeus monodon</i> broodstock	FDP Estepa	100%	95 950	361 900	ASEAN-GOJ-TF
<b>69</b>	Development of strategies to extend the spawning season of Asian sea bass ( <i>Lates calcarifer</i> ) in captivity	EGDJ Ayson	90%		264 100	ASEAN-GOJ-TF
<b>70</b>	Establishment of a land-sea-based seaweed nursery of new and improved strains of <i>Kappaphycus</i> for farming: Propagation of plantlets from callus-like structures by tissue culture	AQ Hurtado	85%	910 235	285 600	ASEAN-GOJ-TF
<b>71</b>	Socioeconomic impact of adoption of sustainable aquaculture technologies in selected fisherfolk communities	DB Baticados	100%	468 388	132 200	ASEAN-GOJ-TF
<b>Disease surveillance system for aquatic animals</b>						
<b>72</b>	Withdrawal periods and residues of different antibiotics used in cultured shrimp	MT Amaiz	90%		188 726	GOJ-TF
<b>73</b>	Parasite fauna of bivalves and gastropods in the Philippines	GE Pagador	85%		45 000	GOJ-TF
<b>74</b>	Pilot testing of the "indigenous probiotic" in grow-out shrimp ponds	GL Po	80%		220 500	GOJ-TF
<b>75</b>	Development of a vaccine against viral nervous necrosis in economically important marine fish	RV Pakingking Jr.	100%	511 650	93 600	GOJ-TF
<b>76</b>	Epidemiology of the white spot syndrome virus (WSSV) in different shrimp ( <i>Penaeus monodon</i> ) culture techniques in the Philippines	EA Tendencia	70%		288 000	GOJ-TF
<b>77</b>	Surveillance of emerging fish viral pathogens in some Southeast Asian countries	GL Po	90%		539 000	GOJ-TF
<b>78</b>	Monitoring and surveillance of transboundary pathogens in cultured shrimps and freshwater prawns	CL Pitogo	90%		423 000	GOJ-TF
<b>79</b>	Development of control methods for viral nervous necrosis (VNN) of marine fish	LD de la Peña	100%		282 000	GOJ-TF
<b>80</b>	Development of immunological preventive methods for shrimp: Immunostimulation and vaccination strategies for WSSV prevention	EC Amar	100%		329 800	GOJ-TF

	STUDY TITLE	STUDY LEADER	Per cent completed	BUDGET (Php)		COLLABORATING AGENCY
				SEAFDEC	External	
<b>R&amp;D on stock enhancement for threatened species of international concern</b>						
<b>81</b>	Management of seahorses <i>Hippocampus barbouri</i> , <i>H. comes</i> , <i>H. kuda</i>	SMB Ursua	80%	417 840	161 000	GOJ-TF
<b>82</b>	Seed production of the humphead wrasse, <i>Cheilinus undulatus</i>	H Ogata	20%		165 000	GOJ-TF
<b>83</b>	Survival, growth and environment impact of hatchery produced <i>H. scabra</i> juveniles and local broodstock in sea cages and pen cultures in Igang Cove	MFJ Nievaes	100%	168 000	149 700	GOJ-TF
<b>84</b>	Experimental release of hatchery-produced donkey's ear abalone, <i>Haliotis asinina</i> , in Carbin Reef, Sagay Marine Reserve, Negros Occidental	SMB Ursua	100%	281 960	161 000	GOJ-TF
<b>85</b>	Fisheries and seed production of the angelwing clam <i>Pholas orientalis</i> Gmelin 1790 for the rehabilitation of depleted wild stock	MJHL Ramos	70%	136 425	150 000	GOJ-TF
<b>86</b>	Growth and survival of hatchery-reared giant clams <i>Tridacna gigas</i> in ocean nurseries in Negros and Panay Island	MJHL Ramos	100%		160 500	GOJ-TF
<b>87</b>	Stock enhancement of abalone <i>Haliotis asinina</i> in Sagay Marine Reserve	MJHL Ramos	95%	136 425	160 000	GOJ-TF
<b>88</b>	Development of techniques for sea ranching of the donkey-ear abalone <i>Haliotis asinina</i> : Biophysical and socioeconomic assessment of Anini-y and Concepcion in Iloilo for stock enhancement of abalone	ND Salayo	100%		159 478	GOJ-TF
<b>89</b>	Socioeconomic analysis of stock enhancement of abalone and giant clams in Sagay Marine Reserve in the Philippines	ND Salayo	100%	37 849	162 480	GOJ-TF
<b>SPECIAL PROJECTS</b>						
<b>90</b>	Integrated fisheries resource management (Rinconada Lakes, Philippines and New South Wales, Australia): Aquaculture and water component	MLC Aralar	90%		208 600	ACIAR
<b>91</b>	Enhancing adoption of mud crab production technologies in Northern Samar	ET Quintio/ RF Agbayani	95%	28 000	679 227	ACIAR-CATP***
<b>92</b>	Capacity-building of BFAR-NFRDI in fish health management through training and collaborative research: Identification of emerging problems and economically significant diseases in hatchery and grow-out production systems of <i>Penaeus vannamei</i>	CL Pitogo	75%		3 731 613	NFRDI
<b>93</b>	Enterprise development options: Organizational, financial and market analysis in milkfish cage culture as livelihood option of fishers affected by Guimaras oil spill	DHM Tormon	20%		142 200	Petron Foundation CITI Foundation
<b>94</b>	Establishment of polyculture system of tiger shrimp <i>Penaeus monodon</i> and sand fish <i>Holothuria scabra</i>	S Watanabe	25%		220 160	JIRCAS

## Integrated mollusc program: abalone

**D**onkey's ear abalone (*Haliotis asinina*) is the species of choice for the integrated mollusc program because it is a high-value commodity that small-scale farmers can raise in mariculture parks. Although standard protocols developed by AQD for its hatchery and grow-out facilities have consistently produced seedstock and market-sized abalone, there is still a need to refine these technologies to optimize production and reduce overall costs.



In the hatchery, postlarval settlement (3.5%) and survival (0.3-1%) are still the bottlenecks in operations. For the nursery phase that produces juveniles for grow-out, land-based tanks are expensive to put up and operate. In grow-out, growth rate is slow, with abalone requiring a continuous supply of seaweeds and an 8-9 month culture period to raise 1.5 cm SL abalone to >5 cm.



Abalone broodstock at AQD (note the red tags that facilitate monitoring, top) and juveniles feeding on seaweeds

Solutions can come in the form of (1) nutritionally complete formulated diets that can partially replace the limited supply of seaweeds, and give growers better control of their operations; (2) hybridization studies (*H. asinina* crossed with other native abalone species) to improve growth and taste; (3) selective breeding and domestication to minimize inbreeding and genetic deterioration of stocks; and (4) refinement of transport procedures for larvae / juveniles to ensure survival of large quantities of ready-to-settle larvae that will be sent to and maximize the capacity of grow-out centers under the Philippines' national breeding program.

The following were the studies in 2009.

### Isolation and cultivation of microalgae for postlarvae

Two strains of the microalgae *Cocconeis* sp. were isolated from mixed diatom samples collected from abalone nursery tanks (strain ANC) and from diatoms attached to the seaweed *Turbinaria* sp. collected from AQD's marine station (strain TIC).

To cultivate them, strain ANC was exposed to three light intensities using 1, 2 and 3 fluorescent tubes. Results showed that cultures lit with 1 bulb had the best cell density ( $977.81 \times 10^4$  cells per sq cm) and cell multiplication (0.92 division per day) compared with the rest which were easily contaminated with blue-green algae. Three growth media - F/2, TMRL, and Commercial II - were also compared. Strain ANC (lit with 1 bulb) and strain TIC grew best in cultures enriched with F/2. For strain ANC, cell density of  $485.42 \times 10^4$  cells per cm and cell multiplication of 2.32 div per day were attained in F/2; for strain TIC, cell density of  $836.46 \times 10^4$  and cell multiplication of 0.57 were obtained.

### Large-scale production of juveniles

From 674 breeders (472 females and 202 males) in the abalone hatchery at AQD's main station in Iloilo, about 238 million eggs/trochophore larvae were produced from January to November. Out of these, 115 million grew into veliger larvae from where 226 thousand juveniles ready for grow-out (5-8 mm SL) survived, or a survival rate of less than 0.5% for each 90-day cycle. Total juveniles produced was lower than in 2008 (299 thousand pieces) probably due to erratic weather conditions.

To minimize inbreeding and genetic deterioration of stock, AQD hatchery had cross-bred wild males to hatchery-reared female breeders.



A significant size difference can be seen in abalone fed with (green-banded abalone above) or without (arrow) lipid and essential fatty acids



Abalone can be grown to market size on longlines (top) with hanging plastic fruit trays (a) or prefabricated trays (b). The latter can also be used for nursery rearing in addition to propylene baskets (c) and round mesh cages (d)



## Nursery and culture using plastic trays

As an alternative to expensive land-based tanks, growing small-sized juveniles (16-20 mm SL) in sea cages using cheap plastic trays hung on long lines was proven feasible. Trials showed consistently high survival (90-93%) and growth increment in terms of shell length (26-30 mm per month) up to 3 months. In comparison, bigger-sized juveniles (26-30 mm SL) reached harvest size of 50-55 mm SL after 6 months, or length increment of 2.8-3 mm per month and weight gain of nearly 4 g per piece per month. The fourth month of culture (abalone = 31-35 mm SL) is critical as it is the phase when the average daily growth gradually plateaus. This is attributed to the beginning of maturity where abalone expends more energy to egg/sperm production rather than growth.

## Lipid and essential fatty acid requirements

Feed stability of abalone diet with lipid was better in terms of percent feed recovery rates after the 12th and 24th hour compared with the non-lipid diet. Lipid and essential fatty acid requirements for abalone juveniles were found to be optimum (in terms of growth) at 3.7% and 1.6% level of incorporation, respectively.

Fatty acid analyses of lipid samples showed predominance of linoleic acid (18:2n-6) and high levels of polyunsaturated fatty acids (PUFA). When linoleic acid, linolenic acid (18:3n-3) and n-3 PUFA (mixture of 20:5n-3 and 22:6n-3) were added to the diet, abalone weight gain significantly improved. The effect of supplementation of either linoleic or linolenic acid was significantly lower compared with supplementation of 20:4n-6 or n-3 HUFA.

## For entrepreneurs: a low-cost, small-scale, abalone and multi-species marine fish hatchery



*BFAR Director and SEAFDEC Council Director for the Philippines Atty. Malcolm Sarmiento graces the opening of the new facility at AQD's Tigbauan Main Station in west central Philippines*

**AQD** opened a backyard-scale, abalone and multi-species marine fish hatchery in July 2009 to demonstrate profitable operations of abalone and marine fish requiring minimal investment.

The area measures 896 sq meters and has separate spaces for marine fish and abalone. The common facilities include harvest and work areas, store room, equipment room, and roof structure. Each area has its own independent pre-filtered seawater supply system (with 2 hp centrifugal pumps and 1 hp roots blower for aeration) and concrete drain pits and main canal for harvesting and drainage.

The marine fish area is capable of producing at least 100-200 thousand milkfish fry and 10 thousand fingerlings of high-value marine fish such as seabass during each production run. The area has 10-ton circular larval rearing tanks (LRT) (6 units), 10-ton circular natural food culture tanks (NFT) (10 units), and 2-ton circular nursery tanks (NT) (12 units). LRT and NFT are made of rubberized canvas with marine plywood support. NT is made of concrete.

The abalone area is capable of producing 20-25 thousand abalone juveniles (1-1.5 cm) per month. It has 34-ton reservoir (1 unit), 4-ton settlement tanks (16 units), 4-ton secondary nursery tanks (4 units), 2-ton diatom culture tanks (4 units), tanks made of canvas reinforced with bamboo frames for support, and a reservoir made of marine plywood siding.

Investment cost for marine fish and abalone is Php 1 million. Projected sales per run of 21-45 days is Php 100 thousand each for milkfish, seabass and pompano; Php 150 thousand for snapper and grouper; Php 60 thousand for rabbitfish; and Php 64-80 thousand for abalone.



## Experimental hybridization among Philippine native abalone species

Two hybrids were produced from cross-breeding trials that started in mid-2008. The first hybrid (code: HAFGM) resulted from crossing *H. asinina* female and *H. glabra* male. Two successful runs resulted in 1000 pieces of 7-month old juveniles (3.5 cm SL, 12.5 g BW) and 2000 pieces of 3.5-month old juveniles (1-1.5 cm SL).

The second hybrid (code: HAFPM) resulted from crossing *H. asinina* female and *H. planata* male. Due to the limited number of males, only one run was made which produced 75 pieces of juveniles, now 1 year-old and already mature (4-5 cm SL, 27-28.5 g BW). The HAFPM breeders were mated to increase the number of experimental materials and this produced two batches of  $F_1$  generations. The first  $F_1$  resulted in 1200 pieces of 90-day old juveniles, the second in 1588 pieces of 45-day old juveniles (0.12 cm SL).

HAFGM and HAFPM were each compared separately with *H. asinina* for growth, survival and settlement. Initial results showed that the hybrids were generally better.

HAFGM grew at 0.013 cm SL per day or 0.084 g BW per day in the first 345 days compared to *H. asinina* (0.012 cm or 0.070 g) but growth tapered off after 435 days of rearing. The sizes of HAFGM and *H. asinina* are now similar, nearly 5 cm. HAFGM survival (7%) was higher compared with *H. asinina* (0.34%)

after one hatchery cycle of 90 days. When settlement was compared, HAFGM was also better, 11% and 25% settling at days 5 and 10, respectively, compared with *H. asinina* at 0.14% and 0.04%.

HAFGM hybrid had spawned three batches of  $F_1$ -trochophore larvae. The first batch of 385 600  $F_1$  produced 2783 juveniles (0.5-1.0 cm). The second batch of 200 900 produced 1650 juveniles (2.5-3.0 cm SL) which are now maturing. The third batch had 216 000 larvae.

HAFPM also grew better than *H. asinina*, 0.0028 cm SL per day and 0.059g BW per day compared with 0.0016 cm and 0.027 g. The 99 pieces of 1.7 year-old HAFPM breeders produced three  $F_1$  generations of 12 050 juveniles aged 5-11 months. The first batch of  $F_1$  are now ready to spawn, while the second batch already did with settlement of 2% and survival of 0.94%. These figures were better compared to *H. asinina* (0.4% settlement; 0.36% survival). However, the hybrid's growth (0.39 g per day) was slower than *H. asinina* (0.48 g).

Results of small-scale triploid experiments showed higher survival of *H. asinina* treated with 250  $\mu$ mole 6DMP (6-dimethylaminopurine) (0.034%) compared to control (no treatment, 0.003%) and 200  $\mu$ mole 6DMP treatment (0.0023%). After 7.5 months of rearing, the shell length of *H. asinina* treated with 200  $\mu$ mole was higher (3.3 cm SL) followed by control (3.2 cm) and 250  $\mu$ mole (3.1). But in terms of body weight, the control was higher (8.9 g) compared to 250 (8.7 g) and 200  $\mu$ mole (8.7 g).

## Refinement of hatchery and transport techniques

To determine the effects of transport on survival of veligers and their settlement, abalone were transported at three stocking densities (25, 50 and 100 thousand per liter) and at four transit times (1, 3, 9, and 18 hours). Results of the first trial show that veligers transported at 25 thousand per l had the highest survival (66%) up to 18 hours compared to higher loading densities (50 thousand per l, 56%; 100 thousand per l, 31%). This suggests that, if hatchery operators need a shorter transit time of 1-9 hours, veligers can be transported at 50 thousand per l. In terms of settlement, veligers transported at 25 thousand per l in 9 hours had the highest settlement (4%) at day 5 of rearing. This result is comparable to 50 thousand per l (3%).

Results also show that the highest loading density of 100 thousand per l can be used to transport abalone for 1 hour (74% survival) to up to 3 hours (86% survival). However, lower densities of 25-50 thou per l can extend transport time to up to 9 h (65-100% survival). Regardless of loading density, mortality was almost 100% when transport time was extended to 18 hours. Though dissolved oxygen was not significantly different up to 18 hours transit at different loading densities, the concentration of ammonia was high (0.6-1.36 ppm) starting at 9 hours up to 18 hours of transport. Settlement was also affected by transit time. After 9 hours, no settlement was observed at 100 thousand per l; at 25-50 thousand per l, settlement was 0.8-1.6%. At 18 hours, no veliger settled regardless of loading density.

## Participation of fishers in abalone stock enhancement

A household survey was conducted in March through April to determine the socioeconomic factors affecting the participation of fishers and other stakeholders in stock enhancement of abalone in Nasidman, Ajuy, Iloilo. Preliminary results showed that majority of respondents (n=53) were hook & line and net fishers, had no secondary occupation, had stayed in Nasidman for 30-35 years, and belong to the Aglipay church. Nearly half perceived their living condition as average while 30% thought they were poor. The establishment of Nasidman Island Sanctuary was deemed beneficial because it had shown improved fish catch. Majority expressed their willingness to participate in a stock enhancement project (83%). This was validated during the focus group discussions conducted in May and August, and the community's active participation in the action plan development workshop conducted in October.

# Shrimp and mud crab domestication

The long term objective of the shrimp and mud crab program is to develop a viable technology for the production of good quality seed and captive broodstock of native species, particularly *Penaeus monodon*, *P. indicus* and *P. merguensis* for shrimps and *Scylla* spp. for crabs, that can be genetically selected for desired heritable characteristics, particularly disease resistance and/or fast growth.



N GOLEZ



Adult and juvenile *P. indicus*

## SHRIMP

### Domestication of indigenous white shrimp

Production runs for *P. indicus* were monitored to assess performance of wild broodstock from Tigbauan, Iloilo. Weekly samplings from March to October showed that only 6-29% of females matured and at least 65% of these spawned. Estimated nauplii per female was about 4500-10 000 per female and increased to about 9000-30 000 after 3 months. Percentage maturation and spawning were similar to last year where one female produced about 20 000 nauplii. No spawning was noted in November and December of 2008 and 2009.

Third-generation white shrimp had reached broodstock size. Males were separated from females so that reciprocal matings between  $F_3$  and wild stocks from Aklan and Tigbauan were facilitated. Since October, monitoring of ablated stocks showed only one batch of nauplii and this was from an  $F_3$  female and a Tigbauan wild male. Fecundity was low (14 000 NI), but survival to PL 15 was 30%.



Marine annelids and the formulated feed incorporating them



### Marine annelid as animal protein source in feeds

Two species of marine annelids were collected near AQD's main station in Iloilo. Majority (99%) of this was the *Perinereis* sp. which are commonly used as live food for crustacean broodstock. After dissection, preliminary results showed that 61% of the marine annelids were oocyte-bearing females and 39% were not. Wet weights were, respectively, 1.4 and 1 g. Nuptial dance was observed inside the holding tank during the new moon phase.

Feeding tests evaluated substitution levels (0% or control, 20, 40, 60, 80 and 100%) of animal protein sources with annelids in formulated diets for white shrimp *P. indicus* juveniles and *S. serrata* crab instar. Percentage weight gain in all tests were consistently highest in control diets.

### Reproductive performance of pond-sourced tiger shrimp

Results of the first feeding study [natural (control) vs. formulated diets (with polychaete extract)] in floating cages showed that tiger shrimp from ponds matured and spawned once. Mortalities were noted after 1-2 weeks in both treatments although initial response of shrimp to the polychaete extract diet was good.

### Verification of white shrimp diets in ponds using environment-friendly schemes

White shrimp was reared with 500 pieces of siganids to attain the desired stocking density based on the carrying capacity of an experimental pond in AQD's Dumangas Brackishwater Station. The stock was harvested (289 kg) before the target date after a WSSV outbreak. The average body weight was 12-14 g for the first batch of shrimps, the rest was only 6-9 g. Results indicate that the SEAFDEC-formulated diet gave good growth, and that the addition of siganids can improve feed utilization efficiency, resulting in better economic return and less environmental pollution.

### Health management of native and introduced species

Shrimp samples with known viral infections previously detected at AQD were re-analyzed for MBV, HPV, TSV, and IHNV at the University of Arizona, an OIE reference laboratory for crustacean diseases. Results confirmed the original diagnosis.

Twenty seven samples were tested for IHNV using two primers -- IHNV389F/R and IHNV309F/R. Interestingly, the IHNV isolates obtained from *P. monodon* post-larvae from Myanmar and *P. indicus* from Iloilo, Philippines



Preparation of crabs for taste test

tested negative with primer IHHV309F/R, showing their non-infectious nature, and that the IHHNV-related sequences that were detected are part of the *P. monodon* genome. IHHNV DNA from 13 samples of *P. monodon* and *P. vannamei* that were chosen based on their sources and geographical origin were amplified and submitted for sequencing. Phylogenetic analysis showed high degree of similarity of most IHHNV isolates with the type IHHNV strain derived from Hawaii, although some strains showed closer relationship with type strains from Taiwan/China group. A phylogenetic tree was derived from the sequence results that showed three major strain groupings based on their clustering with Hawaii, Taiwan/China/Thailand, and Madagascar/Australia/India strains.

## MUD CRAB

### Seed production of mud crab

Mud crabs, *Scylla serrata*, *S. tranquebarica* and *S. olivacea* were procured from Capiz, Iloilo and Samar. In general, their egg development was synchronous while embryonic development was similar and classifiable into 10 developmental stages. *S. serrata* (10 days) exhibited a longer incubation period than *S. tranquebarica* and *S. olivacea* (9 days). Egg sizes for all three crabs ranged from 0.28-0.35 mm. Zoea 1 of *S. serrata* had the longest measurements in most of the morphological characters.

The time from full maturation to spawning was shorter in Capiz (11-22 days) than Samar (27-38 days) broodstock, likewise, the duration from spawning to hatching (Capiz, 6-8 days; Samar, 8-11 days). The number of zoeae produced ranged from 2.2 to 4.35 million per female in broodstock from Capiz and from 1.09 to 3.9 million per female in broodstock from

Samar.

The number of megalopa/crab instar produced as of November was 238 262 with survival of 1-6%.

### Practical feed for mudcrab grow-out

In the tank experiment, survival of mud crab ranged 67-83% after 26 weeks. Weight, FCR, and carapace width were similar in the six dietary treatments (factorial design: 38% and 45% crude protein; 5%, 7% and 10% crude fat levels). Analysis of crab body showed that ash was 33-39% of total dry weight while crude fat was not more than 1.5%. Chitin in the carapace of crab fed diet with 38% protein and 10% crude fat was highest among treatments. Levels of Ca and P in the carapace in all treatments averaged 6.85% and 0.875%, respectively, with a Ca:P ratio of 7.8:1.

In the pond experiment, two diets similar in gross energy (16.8 MJ per kg diet) but different in protein levels (37% and 45%) were fed to mud crab. Feeding was twice daily at 5-8% BW and consisted of 80% pellet and 20% trash fish. Male and female crabs were stocked at a 1:1 ratio with initial average weight of 20 g (range, 7-70). After 20 days, average weight of crab in each pond ranged 68-130g, and after 40 days, it was 110-140g. At termination on day 100, one pond where 80% survived had crabs weighing 334 g on average. Survival in ponds with escapees (hole on the pond wall) was 35-50%.

Mud crab fed the high protein diet had little size variation. Result of analysis of crab exoskeleton showed high levels of Ca (12%) while P (0.83%) was similar to those crabs reared in tanks though the Ca to P ratio (14.3:1) was higher. Analysis of fat body and protein in crab flesh is not yet complete. A taste test with 22 respondents using the hedonic rating was conducted at harvest with results showing pellet-fed mud crabs to be better than wild crab.

### Dietary tryptophan to reduce antagonistic behavior

Different dietary levels of tryptophan (trp) were successfully incorporated in the mud crab diet. The trp levels were 0.3% (control), 0.5%, 0.75% and 1% of the diet, equivalent to 0.78% (control), 1.22%, 1.83% and 2.44% of protein, respectively. After a month of feeding, mud crab juveniles (1-7 g) were set up for a fight experiment (antagonistic behavior). Results showed a significant decrease in attack intensity by crabs fed 1% trp compared with those fed the control or 0.5% trp. In addition, there were more retreats than attacks observed with those fed 0.75 or 1.0% trp. A longer latency between attacks and less number of attacks were observed in crabs fed higher trp levels.

Serotonin (5-HT) concentration in the hemolymph was also measured in mud crab after 15 (rest) and 30 days (rest and after fight). Concentrations did not vary significantly in crabs at rest although these were slightly higher for crab fed trp than control. However, 5-HT concentrations after fight were significantly higher in some crabs fed trp than control.

In terms of growth and survival, more of the crabs fed 0.5 to 1.0% trp survived than the control after 15 and 30 days. Weight gain, increments in internal carapace width and carapace length were higher, but not significantly, different than control.

### Verifying pond nursery

Eight production runs in two phases were made to raise hatchery-bred megalopae/crab instars in hapa cages (20 sq m) set inside a pond (600 sq m). Stocking density was 30-50 per m<sup>2</sup>. In total, 25 830 crablets were harvested and sold as early as 20 days after stocking. Sizes at harvest were 0.8-1.5 cm ICW for phase 1 and 1.6-3.0 cm for phase 2; survival ranged 25-50% and 64-71% respectively.

The feeding experiment where tryptophan is added to the diet of mud crab. This is meant to reduce the crabs' antagonistic behavior.

Crabs were first held and fed individually (bottom) before attack and retreat patterns were observed in an aquarium (below)



JL LARANJA JR



R COLOSO



Juveniles feeding on practical feed crumbles (top) in the feed (containing soybean) trial for milkfish

## Marine fish

**M**arine fishes such as milkfish, grouper, sea bass, mangrove red snapper, rabbit fish, pompano, hybrid red tilapia, and seahorse were among the species studied in 24 research topics in the marine fish program in 2009. The studies were done in laboratory, broodstock, and hatchery/nursery facilities at TMS, brackishwater ponds in DBS, and in floating net cages at IMS.

The objectives were to: (a) improve the technologies for broodstock management, seed production, nursery, and grow-out culture of groupers (*Epinephelus coioides* and *E. fuscoguttatus*), the Asian sea bass (*Lates calcarifer*), mangrove red snapper (*Lutjanus argentimaculatus*), rabbitfish (*Siganus guttatus*) and milkfish (*Chanos chanos*); (b) develop breeding and seed production technologies for Asian pompano (*Trachinotus blochii*); (c) develop practical feed for Barbour's seahorse (*Hippocampus barbouri*) broodstock; (d) develop polyculture techniques for saline-tolerant, hybrid red tilapia (*O. mossambicus-hornorum* hybrid x *O. niloticus*) in brackish water ponds

### BROODSTOCK

#### Developing a marker for egg quality

Previous studies have shown that insulin-like growth factor II (IGF-II) mRNA expression could be used to predict percent development of early embryo stage in marine fishes. This information will be used to develop a non-invasive, simple, and rapid assay for egg quality in marine fishes like grouper and siganid. The present work involves the standardization of the technique using real time-polymerase chain reaction (RT-PCR).

#### Formulated diet for seahorse

The acceptability of formulated diets for subadult seahorse *H. barbouri* was first tested prior to nutrient requirement studies. Results showed that seahorse stocked in tanks (3-4 pairs in each tank) did not feed on the microbound diet form (over-dried or freeze-dried), the soft feed bound by carrageenan/cornstarch, nor the feed with squid or *Acetes* (similar to the seahorse food in the wild). After 20-day feeding trials, seahorse decreased in weight. A gummy sort of feed will be tried next.

Sampling for snub nose pompano juveniles (below) and water exchange for grouper larval tank at the marine fish hatchery



O REYES



### HATCHERY AND SEED PRODUCTION

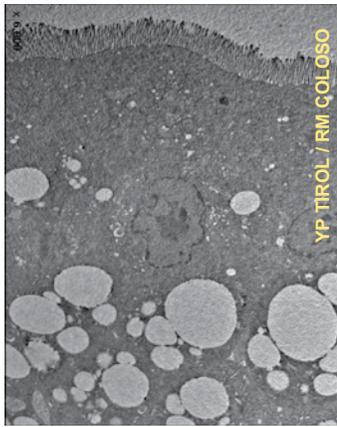
#### Seed production of milkfish and rabbit fish

Several strategies are being used to improve the seed production techniques for milkfish and siganids which include (1) the feeding of the milkfish and rabbit fish broodstock fortified diet; (2) the early introduction of SEAFDEC larval diet; and (3) the enrichment of rotifers with SEAFDEC formulated enrichment media. In milkfish broodstock, the fortification of the diet with vitamins and lipids improved the spawning

frequency, egg production and fertilization rate. However, survival rate of milkfish fry was lower in 2009 compared with that obtained in 2008. The early introduction of larval diet and emulsion to enrich the rotifer also reduced the incidence of abnormal larvae. The advancing age and nutritional status of milkfish broodstock are factors that could affect their performance in captivity. Survival rates of siganid fry were also improved.

AQD's integrated broodstock and hatchery complex where most production runs are conducted





Transmission electron microscopy (6000X) of day 33 grouper larvae fed natural food (top) and co-fed formulated diet (above). The small intestinal cell of the latter shows bigger and more lipid vacuoles, indicating enhanced lipid absorption for their energy and essential fatty acid needs



Morphological changes in post-hatching mysids: a developing brood pouch (encircled) is observed on day 13

## Seed production of grouper, red snapper, sea bass and pompano

The interventions used to improve the seed production techniques for high value marine fish are as follows: (1) the supplementation of grouper and red snapper broodstock diets with vitamins and arachidonic acid (ARA) in capsule form; (2) the use of larval diet at an early stage; and (3) the use of locally produced enrichment media for rotifer. Survival rates for sea bass, grouper (*E. coioides*), and pompano fry were 30, 5, and 10 %, respectively. Spawning frequency for grouper (*E. fuscoguttatus*) was low. More trials are needed to demonstrate consistently improved survival and spawning frequency of marine fishes as a result of the interventions.

## Nutritional evaluation of mysid shrimps

Previous results indicated that mysids *Mesopodopsis orientalis* (Crustacea: Mysidacea) are potential live food substitute for *Artemia* in grouper larval culture because they increased the levels of highly unsaturated fatty acids (HUFA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and digestive enzymes in larval grouper. Mysids were superior than enriched *Artemia* biomass as live food for grouper larvae. Thus, an intensive culture system is needed to mass produce mysids.

A laboratory scale, indoor culture system for mysid mass production has shown promising results. A total of 1000 brooder mysids with a stocking density of 50 mysids per liter in a 25-liter aquarium containing 20 liters water (salinity, 22 ppt) in an air lift aerated system gave about 100 hatchlings daily within 3 weeks and the production increased

for almost 4 weeks until only 50% of the brooder mysids survived in 6 weeks. The average length of mysids a day after hatching (DAH) was 225  $\mu$ m to 831  $\mu$ m at 18 DAH. Brood pouch started to appear on the 14<sup>th</sup> day from the day they were collected (14 DAH - 18 DAH), which suggested that mysids were already sexually mature two weeks after hatching.

## Thraustochytrids as enrichment diet for live food

The study has shown that rotifer and *Artemia* fed endemic PUFA-rich thraustochytrids had increased DHA levels. The enriched rotifer and *Artemia* nauplii could then be used as natural food for sea bass larvae to increase the levels of DHA in the larvae. A feeding experiment showed that survival rates of sea bass fry fed thraustochytrid enriched rotifer and *Artemia* (74 %) were comparable with those fed DHA Selco enriched rotifer and *Artemia* (78 %), proving to be much better than those fed the unenriched rotifer and *Artemia* (control, 50 %).

## Bioenergetic studies on grouper larvae

Diurnal measurement of respiration rate (oxygen consumption) of Day 3 unfed grouper larvae showed initially about 2-3 times higher levels of oxygen consumption than those that were fed for the first 12 hours of monitoring. This reflected the increased catabolism in unfed larvae as they searched for food when the yolk and oil globule were about to be depleted. The maintenance or routine metabolic requirement for small (0.16 mg per liter O<sub>2</sub> per g per hour) and big (0.21 mg) grouper juveniles was estimated. Size-grading apparently increased the maintenance requirement for those given

natural food alone. In addition, feeding natural food or co-feeding with artificial diet increased oxygen consumption about 7.5 fold over the maintenance requirement. Furthermore, fish fed natural food and size-graded had a maintenance requirement that was as high as those fed natural food or co-fed artificial diet but not size-graded. Highest oxygen consumption (2.7 mg) was observed in non-size graded, bigger juveniles co-fed artificial diet. Available data suggest that size grading and/or co-feeding increase basal metabolism in grouper juveniles.

Transmission electron microscopy (TEM) to visualize the ultrastructure of the gut of grouper larvae during development showed larger sizes and higher densities of lipid vacuoles in the intestinal epithelial cells of Day 33 co-fed grouper larvae compared with those of larvae given natural food.

## NURSERY

### Supplementation of L-tryptophan and ginger in formulated diets

Previous culture trials in nursery cages in brackishwater ponds showed that L-tryptophan (Trp) supplementation (0.65%) significantly increased growth and survival in marine fish juveniles (grouper, *E. fuscoguttatus*; mangrove red snapper, *L. argentimaculatus*; and sea bass, *L. calcarifer*). In addition, disease resistance against *Vibrio* sp. was enhanced in grouper and sea bass fed diets supplemented with ginger compared with those fed the basal diet (control). Supplementation of formulated diets with ginger only (2%) or L-trp (0.65%) + ginger (2%) enhanced the immune response of seabass juveniles. However, in trials done on grouper in 2009,

no significant differences in growth and survival were observed among the different diet treatments. Survival was low for this batch of fish, thus no challenge experiment was conducted.

### Nursery of grouper and snapper in cages in pond

Mangrove red snapper *L. argentimaculatus* (1 g ABW; 2.5 cm ABL) were reared in black netcages inside the pond at 100 fish per m<sup>2</sup> for 90 days. Fish were fed formulated diets as follows: Treatment 1, SEAFDEC diet; Treatments 2 and 3, two different commercial feeds (CF-SF and CF-OF). At 90 days of culture, mangrove red snapper fed SEAFDEC diet showed the best growth, survival, and specific growth rates. Between the two commercial feeds used, CF-SF gave better growth and survival.

In grouper, *E. coioides*, experiments showed that stocking initially bigger fish resulted in a higher survival rate than using smaller fish. Overall, fish fed SEAFDEC formulated diet showed better growth, survival, and specific growth rates than those fed commercial feed.

### Nursery of siganids using SEAFDEC feeds as supplemental diet

Siganids were stocked at a density of 1.9 fish per m<sup>2</sup> and grown on natural food in brackishwater ponds. After 30 days, they were given seaweeds *ad libitum* or SEAFDEC formulated diets (39-40% crude protein) of 1:1 fish-to-plant protein source ratio (Diet 1) or 1:2 ratio (Diet 2). At 77 days of culture, fish

fed formulated diet had better growth rates than those given seaweeds. Feed conversion ratio (FCR) was better in those given formulated diets (FCR of 1.7 and 2 for diet 1 and diet 2, respectively) than in those given seaweed (FCR of 11.43). The data also revealed the possibility of inclusion of more plant protein source in the practical feed for siganids.

### Milkfish fingerling production in floating netcages

Several batches of hatchery-reared milkfish fry (*hatirin*) were stocked at 600 or 200 per m<sup>3</sup> in fine-meshed marine floating netcages and reared for 30 or 60 days. The first batch of *hatirin* reared at the higher stocking density suffered from slow growth and low survival (35%). After 30 days at the lower stocking density of 200 per m<sup>3</sup>, the second batch of *hatirin* attained a higher average body weight but even lower survival (21%). The third batch reared at the same stocking density for 60 days had improved growth, were sorted into two sizes (38 g ABW and 11 g), but had a low survival of 20%. Low survival might have been due to parasitic infestation (*Brooklynella* sp.) and inadequate acclimation from brackishwater to seawater.

### Nursery of pompano

Pompano were raised in eight units of 2 x 2 x 1.5 m B-net nursery cages at IMS. Stocking density was either 250 or 500 fish per cubic meter and pompano were fed commercial diet. After 60 days, survival rate was 98% in both densities. Fish weighed 17-18 g.

## GROW-OUT

### Alternative feeding strategies for milkfish

The effect of alternative feeding regimes on growth of milkfish in tanks provided with flow-through water was determined. Milkfish fed a ration equivalent to 10% of ABW on alternate days did not grow as well as those fed the same ration daily. In contrast, growth of fish subjected to two-week alternate starvation and refeeding cycle was comparable with that of the control group that was fed daily, suggesting that compensatory growth mechanisms might be at work. Growth of fish subjected to a 4-week starvation and refeeding cycle was lower than that of the control. In addition, growth of fish was not affected by reduction of ration from 10 to 7.5% of ABW.

The effect of different feeding regimes on growth of milkfish in a simulated marine cage environment was also determined. Results generally reflected the result of the tank experiment. However, fish subjected to a 2-week alternate starvation and refeeding cycle did not exhibit compensatory growth as much as that observed in tanks. This could have been due to fish feeding on natural food (algae growing on nets and plankton in the water) in the floating net cages. Furthermore, prolonging the starvation period to 3 weeks did not enhance the compensatory growth response.

### Verification of grouper, snapper and rabbitfish grow-out diets in cages in pond

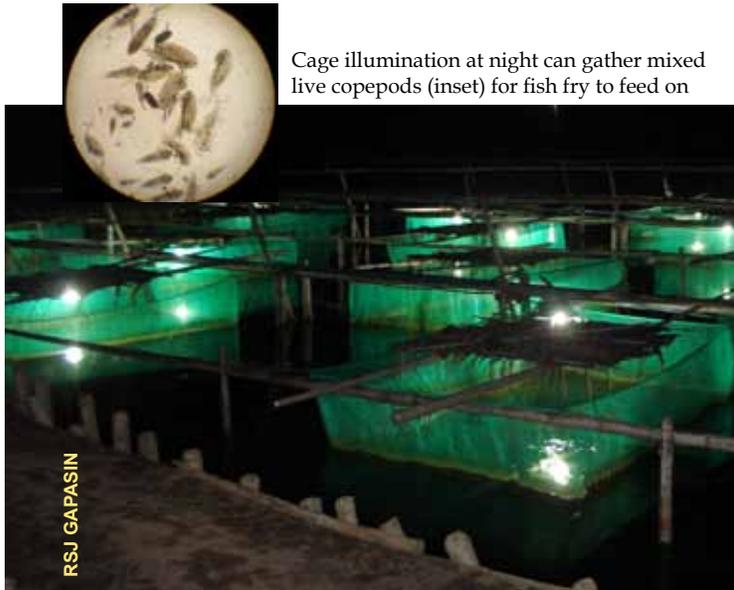
Verification studies on the effectivity of SEAFDEC formulated diet compared with commercial diet in the culture of marine fishes continue to show that fish fed SEAFDEC diet had consistently comparable, if not better, growth and survival than those fed the commercial diet. At initial ABW of about 30 g (stocked at 6 per m<sup>2</sup>), market size snapper could be harvested in six months with high specific growth rates (SGR) of about 1.5% and high survival (97%). Market size grouper, *E. fuscoguttatus*, at initial ABW of about 70 g (stocked at 5 per m<sup>2</sup>), could be harvested in eight months, but the SGR is lower at 0.8 % with high FCR of 3.2. In contrast, market size sea bass, at initial ABW of about 50 g (stocked at 0.5 per m<sup>2</sup>), could be harvested in six months with high SGR of 1.3%, high survival (99%) and FCR of 1.8. Market size rabbit fish, at initial ABW of about 70 g (stocked at 8 per m<sup>2</sup>), could be harvested in three months with high SGR of 1.3 %, high survival (97 %), and good FCR of 1.6. Results also showed that more plant protein source could be included in the diet of rabbitfish without adversely affecting growth and survival.

### Refining intensive pond culture of sea bass

Sea bass, grown at a stocking density of 1 per m<sup>2</sup> and given SEAFDEC diet, compared well with those given commercial feed. Harvested at six months, seabass on the SEAFDEC diet had ABW of 361 g while those on commercial feed had ABW of 304 g. FCR (about 2.6) and survival (99%) between the two treatments were also comparable. Another trial run is ongoing.

Sea bass harvest at AQD's Dumagas Brackishwater Station





Cage illumination at night can gather mixed live copepods (inset) for fish fry to feed on

### Refining polyculture of milkfish, white shrimp and mud crab

Milkfish were fed SEAFDEC diet (28% crude protein) starting at day 35 after the natural food (*lumot*) in the pond was depleted. At day 45 when the fish were bigger, the feed crude protein was adjusted to 32% which increased the daily growth rate to 2.8 g per day. At harvest after 120 days, average body weight (ABW) was 394 g, survival rate was 95%, FCR was 2.0, and production was 2.9 tons. White shrimp and crab suffered heavy mortality due to WSSV infection. A new trial is ongoing.

### Netcage culture of sea bass in freshwater

Sea bass in floating net cages were grown in Lake Vito in Dingle, Iloilo and fed SEAFDEC diet or commercial feed to compare their growth performance in freshwater. Preliminary results showed fish growth as comparable in both diets but survival was low even though bigger juveniles (20-25 g initial ABW) and a longer acclimation period from sea water to freshwater to minimize stress were used.

### Phased diets (starter, grower, and finisher) for grouper cultured in floating netcages

Grouper (*E. fuscoguttatus*) were cultured in marine floating net cages and fed SEAFDEC starter, grower or finisher diets. At initial ABW of about 38 g (stocked at 17 per m<sup>3</sup>), fish were harvested after 5 months when they attained about 430 g ABW with an overall FCR of about 2.7. Total harvest was 242 kg.

Sensory evaluation was done on grouper prepared two ways. Steamed grouper was rated moderately liked or 3.0 on a hedonic scale of 6.0. For grouper prepared as tangy fish sour soup (*sinigang*), the rating was 2.0 or very much liked. Odor, flavor, and texture were described as marine, fresh oil, and firm, respectively, for both cooking preparations.

### Improving the nutritional value of locally available feed resources

Two isolates from milkfish gut, presumptive *Bacillus sp.* and *Lactobacillus sp.*, were assayed for their ability to ferment carbohydrates, and the

activities of the extracellular enzymes of supernatants from broth cultures of the isolates were determined. DNA was also extracted from pure isolates of *B. cereus* and *Lactobacillus sp.* and subsequently subjected to PCR analysis of the 16S rRNA gene to confirm the identity of the isolates. Bacterial genomic DNA isolated from *B. cereus* and *Lactobacillus sp.* were sent to Macrogen Inc. in South Korea for sequencing the whole 1500 bp PCR product. Comparison of sequences of PCR products with known sequences in Gene Bank confirmed the identification of *B. cereus*. However, the *Lactobacillus sp.* PCR product of the 16S rRNA did not align well with known *Lactobacillus* gene sequences, suggesting that another strategy needs to be implemented to confirm the species of *Lactobacillus* isolate.

Fermentation trials were done on *Lactobacillus sp.* isolate using ipil-ipil leaf meal as substrate. After several fermentation trials, rice bran-molasses, starch, or nutrient broth seemed to be suitable media for *Lactobacillus sp.* For nutrient broth, the bacterial count peaked after 48 h, then decreased after 72 h. For both rice bran-molasses and starch, bacterial count peaked at 72 hours. Specific activities were high for carbohydrase, alpha-amylase, and protease in both rice bran-molasses and starch containing media, while low in nutrient broth.

### Soy bean meal as alternative to fish meal in milkfish diets

Milkfish juveniles were reared in tanks with flow-through sea water given nine test diets with varying protein to energy ratios (protein – 30, 37.5, 45 %; fat – 5, 10, 15 %). At the end of the culture period, growth, survival, SGR, FCR, HSI, liver protein, whole body composition, and PER were determined. Growth data

suggest that the optimum protein and fat levels in the practical diets containing fishmeal and soy bean meal are 34.7 and 9.8%, respectively.

### White cowpea meal as alternative plant protein source

In finding alternatives to fish meal in aquaculture feed, white cowpea meal was tried to partially replace fish meal in grouper diets. White cowpea meal (20%) in grouper diet was comparable with fishmeal-based diet based on the growth performance, FCR, survival and flesh quality evaluation. Regardless of batch of fish, the growth performance of grouper between two dietary treatments was comparable based on % body weight gain (BWG), survival rate, and FCR for the control and cowpea containing diet fed fish, respectively. Flesh quality evaluation showed that both control fish and fish fed cowpea containing diet were rated “moderately liked” or generally acceptable by 20 evaluators.

The gastric evacuation time in groupers fed practical diets was 12 hours (only 50% of ingested feed had evacuated within 12 hours) and the fish stomach was 50% full at night time suggesting that feeding can be done once a day every morning. The feed should be given to fish gradually until they finish the ration for the day.

### Pompano in floating net cages

Pompano were reared in IMS floating net cages in four units 5 x 5 x 3 m grow-out cages at a stocking density of 28 fish/m<sup>3</sup>. The fish were fed commercial pompano diet (starter grower to finisher) at 4% of body weight per day in two rations. After 120 days, the fish in four cages had an average mean body weight of 304 grams and survival rate of 92 %.

# Seaweed strain improvement

**S**eed production of seaweed (*Kappaphycus*) plantlets developed by AQD from spores and tissues continues, as with the establishment of a land- and sea-based nursery. The aim is to eventually provide the industry with a fast-growing strain that is also disease-resistant and yields good carrageenan.

The tambalang variety (purple) of *Kappaphycus alvarezii* free-floats in a fiberglass tank; this variety is developed from tissue culture technique at AQD



*Kappaphycus* (Ks1-TP) sporelings were grown at different temperatures (15, 25, 35°C) for 30 days. Results showed that the strain could survive and adapt to higher temperatures of 25 and 35°C.

Physical characterization of carrageenan of the different stages of *Kappaphycus* Ks1-CP was also done. Carrageenan yield was highest in vegetative diploids (18%) followed by tetrasporophytes (13%) and carposporophytes (3%). The vegetative sample had also the highest gel strength (1596 g per cm<sup>2</sup>) and was the most viscous (99.50 mPa.s).

*K. alvarezii* and *K. striatum* exhibited variation in gross morphology when grown in natural conditions. To distinguish one from the other, distinct and unique markers were identified using restriction enzyme PCR.

Growth of five varieties of *Kappaphycus* -- "vanguard" variety grown in Cabalagnan, "vanguard" that came from TMS, brown sacol from Calatagan in Batangas, green sacol and Ks1-CP -- were compared by growing

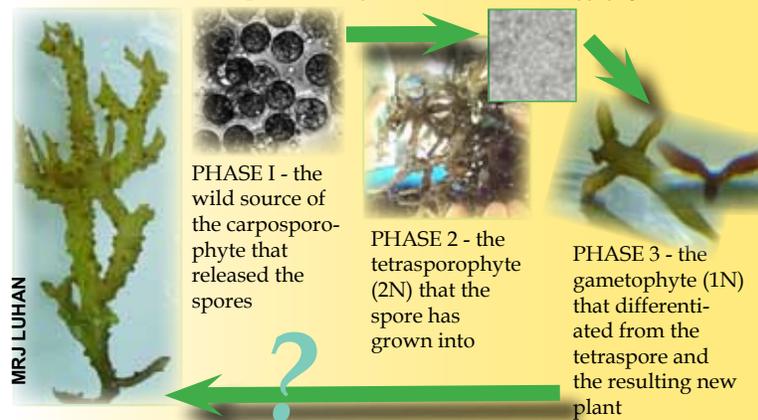
Children loop propagules of tambalang for outplanting in Tigtabon island off Zamboanga City (below); the harvest in June 2009 (right)



AQ HURTADO



AQD documents the tri-phasic life cycle of the seaweed *Kappaphycus*



two kg of each variety onto 10-meter long lines in Panabulon Island, Guimaras for 60 days. Results of three replicates showed the "vanguard" Cabalagnan variety as growing the best, 20% per day, while the rest ranged 3-10%. However, when culture was extended by a month, only Ks1-CP and *K. striatum* from Calatagan survived.

A study was conducted to determine whether AMPEP (Acadian marine plant extract powder) from *Ascophyllum nodosum* can induce resistance in seaweeds against the epiphyte *Neosiphonia*. Two *K. alvarezii* varieties, "tungawan" and giant "tambalang", were raised at different water levels -- 0, 50, 100, and 150 cm below the surface -- with or without prior dipping in AMPEP. Results of the 8-month grow-out showed that both varieties cultured at 150-cm water

depth and treated with AMPEP had the lowest percentage of *Neosiphonia* infestation and coverage. This depth also had the highest salinity and lowest temperature, dissolved oxygen, and light intensity. When native carrageenan was extracted, seaweed cultured at 150-cm also had good yield, gel strength, cohesiveness, rigidity and viscosity.

The recovery of *K. alvarezii* ("tungawan" variety) infected with *Neosiphonia* was also tested. AMPEP (0.1 g per liter) was applied at four frequencies (3x, 2x, 1x, and control) within the 45-day culture period. Results of two runs showed that seaweeds treated with AMPEP twice had the best recovery.

In preparation for the establishment of a land- and sea-based *Kappaphycus* nursery, several studies were conducted on tis-

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# Aquatic ecology

The program aims to assess the impact of aquaculture on the environment and develop bioremediation measures to mitigate such impacts.

## Water quality of Humaraon Cove, Guimaras

Water quality in Humaraon Cove, Igang, Guimaras was monitored every month to assess the impact of continuous mariculture activities. Salinity, temperature and dissolved oxygen did not change notably between sampling sites and sampling months. Ammonia, phosphate, nitrite and chlorophyll *a* were all below the critical level set by the Department of Environment and Natural Resources (DENR) for Class C (fishery water for propagation of fishes) sea water (0.5 mg per liter ammonia, 1 mg per liter phosphate). However, total suspended solid (TSS) measurements (88.25-322.75 mg per liter) did not only increase but were also much higher than the critical value

A two-year monitoring (bottom) of sediment quality in AQD's Igang Mariculture Park shows sediment becoming progressively lighter from 2008 to 2009 (below, left to right), indicating the area's gradual recovery from the impact of mariculture because of prudent management



set by DENR at <100 mg per liter. The consistent high values of TSS suggested that waste products from uneaten feeds or feces coming from mariculture were not totally flushed out by the system, thus it stayed in the water column and accumulated with time.

## Sediment quality monitoring at IMS

Sampling points (3 fish cages and 3 non-cages) that were established in 2008 were continuously monitored this year to determine the recovery of sediments from the impacts of mariculture. Data obtained from February-December 2008 and 2009 were compared. Phosphate and ammonia concentration of pore water in March and April 2008 were highest for both cage and non-cage areas. There was also a constant increase in the number of gastropods especially in cage areas from February to May 2009 but this decreased from June to November. Color of sediment also improved from black to brown in 2009 in cage areas. Results suggest that the sediment in Igang Marine Station is gradually recovering from the negative impacts of mariculture activities.

## Biodiversity in AQD ponds and adjoining mangroves and shores

As part of the study on the changes in faunal diversity after mangrove forests and swamps have been converted into brackishwater ponds, the by-catch or non-crop species in AQD ponds was documented.

Between April and December 2009, seven ponds in DBS were harvested and totally drained, and the by-catch identified and enumerated.

Pond 1 (area~ 1 ha, at the west end, nearest freshwater Talaugis River) was used for polyculture of milkfish, white shrimp, and mudcrab. Harvest over four days yielded about 26 kg and 35 species of by-catch, mostly tenpounder, tarpon, tilapia, seabass, eels, gobies, and freshwater shrimps.

Pond 2 (0.89 ha) was used to grow seabass in one compartment and milkfish in another. By-catch was obtained only on the last day during total drain; 21 species amounted to 10 kg. The volume and number of species in the by-catch was 2x greater in the milkfish pond than in the seabass pond, presumably because the carnivorous seabass ate some of the by-catch-to-be.

Pond 4 (0.54 ha, divided into 6 compartments) used for rabbitfish nursery was drained over three days and yielded 27 kg of 30 by-catch species.

Ponds 9 and 11 (each 0.87 ha) were meant to be biosecure shrimp ponds but were hit by WSSV. The 2-day harvest in Pond 9 yielded 55 kg of by-catch, mostly small gobies. Pond 11 by-catch was obtained only during the last day of a week-long harvest, about 13 kg of mostly gobies. The number of bycatch species was drastically reduced to about 10, presumably by the pretreatment undergone by the seawater allowed into both ponds - chlorination in a settling pond, then



**The price that aquaculture pays:** loss of fauna diversity in mangrove areas that have been converted into brackishwater ponds

conditioning in a reservoir pond with biomanipulator fishes. The volume of 'dalodalo' (gobies) was high since the shrimps did not (could not) eat them.

Pond 10 (0.82 ha divided into 6 compartments) was first sampled when two compartments used to grow sea bass yielded about 2 kg of by-catch, mostly gobies, shrimps, native mudcrab, and jellyfish. Pond 10 was later used for milkfish growout; about 20 species constituted the bycatch and the volume ranged 0.5-4 kg per compartment.

Pond 15 (0.89 ha, at the east end, nearest seawater Pulau River and mangroves) was sampled in May when it was used to hold mangrove snappers in nursery cages. When Pond 15 was drained after 3 months, about 15 kg of bycatch was collected, including 60 species of fishes, crustaceans, and mollusks, most of which also occurred in the adjacent mangroves.

## Polyculture of sandfish and marine fishes

Two preliminary trials were conducted in aquaria and tanks to determine which fishes (milkfish, sea bass, rabbitfish, grouper and/or pompano) are suitable to culture with sea cucumber (*Holothuria scabra*, sandfish). Initial results showed that juvenile rabbitfish were not compatible with sandfish, as some of the latter were bitten and subsequently died. By the end of the trials, the remaining sandfish had lost weight and had a shorter average length. The sandfish also remained hidden under the sand in the rabbitfish tanks throughout the trials. This behavior was not observed in the milkfish, sea bass, grouper or pompano tanks; instead, sandfish would emerge from the sand in the afternoon, as they normally do. The effect of initial size and stocking density of sandfish in culture with grouper will be done next.

## Polyculture of sandfish and tiger shrimp

To get baseline information on the feeding habits and nutritional condition of sandfish, carbon & nitrogen stable isotope ratio analysis and coelomic fluid analysis were conducted. Although sandfish unselectively ingests sediment for feeding, comparison of stable isotopic signatures between sediment and sandfish collected from AQD's Igang Marine Station showed that sandfish assimilated only certain fractions of the organic matters contained in the sediment.

A method to ascertain the nutritional condition of sandfish juveniles based on protein & carbohydrate concentration in a small amount (c.a. 10 µl) of coelomic fluid (circulatory fluid filling the body cavity) was established.

With sandfish



Three-month old juvenile sandfish. The sandfish can be used as bioremediator in fish culture because they can subsist on uneaten feeds and feces excreted by fish in a polyculture system



Feeding studies for sandfish are ongoing with benthic diatom, macroalgae, shrimp feed, shrimp feces and shrimp pond detritus being tested

constantly changing size, two anaesthetization methods were also tested to make measurements reliable. While potassium hydroxide at 0.5% concentration was found ineffective, 2% menthol anaesthetized sandfish within 25 minutes with body wall longitudinal and circular muscles relaxing and the coefficient of variation of body length & weight significantly reduced.

## Culture of abalone and seaweeds in a recirculating system

To find out if seaweeds (*Gracilariopsis bailinae*) can take up the ammonia (NH<sub>4</sub>) excreted by abalone

(*Haliotis asinina*) and clean up the recirculating water system, experiments were continued. This time using 1 ppm NH<sub>4</sub>, stocking density of 1 kg seaweeds per m<sup>2</sup> and water volume exchange 4x a day. Results showed that ammonium removal by seaweeds was 48%. Specific growth rates, agar yield, and crude protein content were 1.4-1.8% per day, 4% and 17%, respectively, for *Gracilariopsis* receiving 1 ppm NH<sub>4</sub>; and 1.4-1.7%, 3.5%, and 13% for the control. Wet-to-dry weight ratios of seaweeds ranged 8-10; temperature, salinity and pH were 26-29°C, 34-35 ppt and 8.0-8.5, respectively.

## SEAWEED from page 19

sue culture, outplanting, mutagenesis and analysis of genetic variability by AFLP (amplified fragment length polymorphism). The objective was to determine the most optimal conditions for the mass production of *Kappaphycus*.

Results showed that the optimal temperature for the three *Kappaphycus* varieties was 25°C, while pH could range from 6-7 to 9. The density of 1 section seaweed per 2 ml of culture media (AMPEP + plant growth regulator) had the highest percentage of shoot formation.

AMPEP-fertilized seedlings (five different *K. alvarezii* varieties) were grown first in a 3-ton tank with vertical and horizontal lines before they were moved to sea-based nursery. Seaweed on improvised vertical lines had bigger branch diameters with very stubby shoot growth while those on horizontal lines grew longer but not thicker. Growth shoots in both lines developed normally in length and size. These were then transferred to the sea-based nursery in Panyam, Zamboanga City. Abrupt increases in shoot length and overall body mass were observed in all generated plantlets. The clear waters and sufficient current in the area were advantageous, resulting in an average daily growth rate of 2.4 g per day.

# Small-holder freshwater aquaculture

The program aims to generate, verify and transfer science-based technologies on the breeding, hatchery and farming of selected freshwater fishes (giant freshwater prawn, Asiatic carps, tilapia and clariid catfishes) for rural development and improved livelihood.

## Giant freshwater prawn

A series of larval rearing test runs using the green water system was done in 2009. Newly hatched freshwater prawn larvae were stocked in two 1-ton fiberglass tanks at 50 larvae per liter and reared in 12 ppt green water. Larvae were fed *Artemia* nauplii from day 1 to 7 and *Artemia* plus egg custard from day 8 until larvae reached post larval stage. Based on the results of three larval rearing runs, the average survival was 37%. A total of 97 202 post larvae were produced, which took 36 days to metamorphose.

## Bighead carp nursery

The optimum stocking densities for rearing bighead carp *Aristichthys nobilis* fry during the cold and warm months were determined. The fry were stocked in 12 hapa netcages set in Laguna de Bay, and fed commercial fry mash at 50% body weight twice a day. After a month, results showed that, during the cold months (22-25°C), fry grew (to 0.42 g) and survived (93%) best if stocked at 200 fry per m<sup>3</sup>. In contrast, during the warm months (>25°C), stocking density may be optimum at 300 fry per m<sup>3</sup> (50% survival and 0.71g final weight).

Three-month old catfish fingerlings grown in tanks

In another study, production of advanced sizes of bighead carp fingerlings in a lake-based fish pen was verified. About 26 700 fingerlings (1.5-3g initial body weight, also known as sizes 12-17 in the local selling lingo based on net mesh sizes) were stocked in a small fishpen in Laguna de Bay. Out of this, 8000 "advanced" fingerlings (100-300 g) were harvested while the remaining fish were allowed to grow bigger.

## Nile and red tilapia

To promote tilapia (*Oreochromis* spp) farming among fishfarmers near Laguna de Bay, the technology for a modular system of intensive tilapia culture in lake-based netcages was verified. Tilapia were stocked at 75 per m<sup>2</sup> (in two units of 2 x 2 x 1 m netcages) and fed commercial diet at 3% fish biomass for four months. SEAFDEC-selected Nile tilapia strain was used in the first six cages, red tilapia in the next four and a commercial Nile tilapia strain from Calauan, Laguna in the last two cages. Results indicated that size at stocking and strain differences had an effect on growth. Tilapia that were 50 g or more grew better than <30 g fish. SEAFDEC-selected strain grew better compared with the commercial farm strain from Laguna. FCR ranged 1.8-2.1; weight at harvest, 195-297g.

Another verification study on tilapia cage farming was conducted in a freshwater reservoir in Dingle, Iloilo. Nile tilapia fingerlings were stocked at 15 per m<sup>3</sup> and fed SEAFDEC diet and two commercial diets. Feeding tilapia the SEAFDEC diet resulted in better growth (absolute growth rate or AGR=1.97g per day), survival (95%), FCR (1.29), production (154 kg per cage) and income (PhP 11,574) compared with feeding the first commercial feed (1.8g per day, 92%, 1.80, 136 kg per cage, PhP 9,535) after 87 days of culture. SEAFDEC diet was comparable with the second commercial diet.

To support the tilapia industry in Visayas and Mindanao, AQD continued its technology demonstration and fry production runs. [Luzon growers get their tilapia fry from BFS and elsewhere.] For 2009, the TMS hatchery produced 400 000 Nile and 50 000 red tilapia fry, and reared them further in canvass, fiberglass and concrete tanks until the fish reached 1-2 inches long. The average recovery of fry per female was 300-500 fry for Nile and 290 fry for red tilapia, higher than the optimum 150-200 fry per female. Recently, sex-reversed Nile tilapia fingerlings were produced using a methyl-testosterone-lined formulated diet.

Experimental set-up at AQD's Binangonan Freshwater Station along Laguna de Bay



A EVANGELISTA



### Asian catfish

Nursery rearing trial of Asian catfish *Clarias macrocephalus* (Gunther) in 2 x 2 x 1 m tanks showed that fry stocked at 100 per m<sup>2</sup> grew to an average weight of 21 g after 3 months of rearing. Cage trials have just started.

### Indigenous species

#### SILVER PERCH

Eight oocyte maturation stages and four different types of spermatogenic cells were described from histological sections of silver perch gonads. The oocyte development stages were chromatin nucleolus, early and late perinucleolus, oil droplet, primary oocyte, secondary oocyte, tertiary oocyte, and migratory nucleus stages. In males, four developmental stages of spermatogenic cells were observed, namely spermatogonia, spermatocytes, spermatids, and spermatozoa. Morphological features of these gonad development stages were typical of other bony fishes.

Isometric growth was revealed from the length-weight relationship (LWR) of 202 male and 209 female silver perch collected from the Candaba wetland in 2009. Peak gonad maturity shown by monthly variations in the

gonado-somatic index or GSI (i.e., gonad weight relative to gutted body weight) in both sexes occurred in June or the start of the wet season, reached lowest levels late during the year (or start of the dry season) but gradually recovered at the start of the year. Similarly, a seasonal variation in the gut index (i.e., gut weight relative to gutted body weight) observed in both sexes indicate that silver perch, after gonad maturity peaked in June, increased their food intake in July to reach a peak in December. This observation coincides well with the seasonal flooding of the Candaba wetland which occurs in July-August and recedes in November-December.

#### CLIMBING PERCH

A preliminary study on the growth of climbing perch *Anabas testudineus* in tanks using different farm-based feeds [ground janitor fish (JF), JF+rice bran, JF+corn starch and tilapia feed] was conducted. Crude protein content of feeds ranged 19-30%. After four months of rearing, results showed no significant differences in growth (range in weight gain: 0.95-1.3g) among treatments. But on the fifth month, the perch gained more weight from the tilapia feed treatment followed by raw JF treatment.

### MANILA CATFISH

Preliminary results of a 6-month culture trial showed that Manila catfish stocked at 50 per cage (2 x 2 x 1.5 m) had a length increment, weight gain, specific growth rate and survival rate of 2.8 cm, 27 g, 0.3% and 100%, respectively, when fed a diet formulated for Asian catfish. Highest growth was attained when fish were fed daily at 15% of fish biomass.

### Other studies

#### WHITE SHRIMP FARMING

To compare the farming potential of white shrimp, simultaneous studies were conducted in Laguna de Bay and Paoay Lake (Ilocos Norte). Two batches were stocked in nine cages at 90, 180 and 270 per cage. The first batch had larger (32 mm) and the second smaller (27 mm) juveniles. Results of two runs showed very poor final length (30 mm) and survival in Paoay, probably due to adverse weather conditions, lack of appropriate feeds and improper management of stocks. In Laguna de Bay, growth (123 mm and 14 g) and survival (90%) were much better after 3 months.

#### INTEGRATED FISHERIES RESOURCE MANAGEMENT

Training and briefing on tilapia cage culture (based on the results of the ACIAR project) were conducted for local government unit representatives, private sector, and BFAR V staff in Camarines Sur. Resource persons included AQD and BFAR-ACIAR project staff. After the training, advanced Nile tilapia fingerlings were stocked in cages in Lake Buhi and Lake Bato as part of techno-demo runs. Two more runs, at Ambuklao reservoir and Laguna de Bay, are currently underway to determine if the results obtained in Buhi and Bato can be applied to other lakes with similar trophic status.



Yolk-sac larvae of Manila catfish from the wild (top), first-feeding larvae (middle) and three-month old fingerlings grown on a ratio of 15% BW/day

A boatload of silver perch *Leiopotherapon plumbeus* in Candaba Wetland



## Development of technologies and human capacity building for the ASEAN region

**R**egional programs are funded by the GOJ Trust Fund (TF) under the ASEAN-SEAFDEC Fisheries Consultative Group (FCG) mechanism. For 2009, a total of 32 studies comprising three projects were continued. However, this set of studies under TF-IV ended this year after a 5-year cycle. Another set of studies (TF-V) begins in 2010 until 2014.

*The first regional program is "Development of technologies and human capacity building for sustainable aquaculture: Promotion of aquaculture technologies in the ASEAN region". Following are updates of the studies.*

During the TF-IV review from 9 to 10 December 2009 at AQD, the three external reviewers [top to bottom: Dr. Motohiko Sano and Dr. Koichi Okuzawa of the Fisheries Research Agency - Japan and Mr. Tadahiro Kawate of the SEAFDEC Secretariat] expressed their pleasure at the progress of the studies, noting that research is a difficult process but that AQD has done it in the "correct, step-by-step" process. Dr. Okuzawa singled out the development of the vaccine for viral nervous necrosis as the result he was most happy with.

*Rightmost:* The team working on aquaculture technologies. Study leaders for the three programs are from AQD, UP Visayas, Development of Fisheries Administration (Cambodia), Department of Fisheries (Myanmar), Research Institute for Aquaculture 2 and 3 (Vietnam), Research Institute for Freshwater Aquaculture (Indonesia), and Inland Aquatic Animal Health Research Institute (Thailand)

### Domestication of giant freshwater prawn

The study aims to refine existing breeding and husbandry techniques for the successful domestication of the giant freshwater prawn like *Macrobrachium rosenbergii rosenbergii* and *M. rosenbergii dacqueti*, stocks that are indigenous to the Philippines. Domestication is important because natural populations of native prawns have dwindled due to fishing pressure.

Results showed that, for *M. rosenbergii rosenbergii*, the most

successful larval production was observed when berried females (from Zarraga, Iloilo) were held in 0 and 6 ppt, and the larvae subsequently reared in 12 ppt. However, only 1% of the hatched larvae metamorphosed to postlarvae (PL), and in follow-up larval rearing trials (four runs), larval survival was also low, ranging 0.75 -7.5% after 36-50 days. The green water culture system at 12 ppt was used in rearing, and larvae were fed *Artemia* & egg custard.

Results also showed that juvenile prawns grown in hapa netcages in the lake grew better than those stocked in indoor tanks; 0.17 g weight gain per day after 151 days compared with 0.02 g after 60 days. Moving the indoor stock outdoors improved prawn growth, to 0.13 g per day after 50 days. The starting weight for the outdoor stock was 8.5 g, smaller compared with those in tanks (10.5 g).

After the juvenile prawns metamorphosed to PL in April 2009, they were raised in three

size groups. The large group yielded females that had undergone pre-mating molt after 82 days. This group averaged about 20 g in weight after growing 0.18 g per day, with survival of 85%.

For *M. rosenbergii dacqueti*, initial larval rearing trials also had poor survival but those that survived as the first generation hatchery-reared prawns can be raised to maturity and eventually complete their life cycle in captivity, the first step in domestication.

### SPF tiger shrimp: improving maturation of pond-reared broodstock

Before a SPF (specific pathogen free) broodstock can be developed, it is a prerequisite to first close the life cycle in captivity (which AQD did in the 1980s) and then domesticate the species. For tiger shrimp *Penaeus monodon*, steps toward domestication were undertaken by rearing two batches of 50 g pond-reared shrimp in concrete tanks provided with sand bottom. These stocks were selected after screening for morphological abnormalities and presence of five viruses (WSSV, YHV, IHNV, TSV and HPV).

After about 7 months, four unablated females reached maturation (stages III-IV) but spawned only after ablation, resulting in the production of



three batches of  $F_1$ . Fecundity and hatching rates were very low, and only 2500-5500 nauplii were obtained from each female. To augment the stocks, wild postlarvae (PL) from Palawan and Antique were collected and grown to broodstock size. These stocks and the  $F_1$  from ponds were monitored each month to compare growth. Wild PL reached 50-60 g size within 10-12 months while  $F_1$  reached this size two months later.

Another batch of nauplii was produced from crossing a tank-reared female (wild PL grown to brood size) and a  $F_1$  male. Around 7500 PL were grown from this batch.

To maintain the stocks, feeding management was studied, in particular, the use of marine annelids (*Perinereis nuntia*) which was previously shown to improve reproductive performance. Results showed that incorporation of annelids at 6% increased the diet's crude protein to 58% from the basal diet's 48%. Crude fat (14%) didn't change. When the annelid diet was compared with the control (basal diet + live annelids fed once weekly), and both diets given in combination with mussel meat, results showed that 60-100% of ablated females in both treatments reached at least Stage 2 maturity within four months.

The incorporation level of annelids was increased to 10 and 20% in a follow-up trial with all diets (including the control basal diet) given in combination with mussel meat, live annelids and squid meat. Before ablation, females attained at most Stage II maturity; after 3 months and after ablation, 50 -100% matured but no spawning occurred in all treatments.

### Genetic characterization of wild mud crab and tiger shrimp

To determine the potential sources of quality stocks for the domestication or selective breeding of mud crab *Scylla serrata* and tiger shrimp *Penaeus monodon*, wild populations were genetically assessed using microsatellite DNA markers. For crab, 30 samples each were collected from areas considered as having geographically distinct wild populations: Cagayan and Camarines Norte in Luzon, Samar in the Visayas, and Surigao in Mindanao.

For shrimp, samples were collected from eleven sources namely: Antique, Aklan, Bohol, Kolambugan, Roxas, Palawan, Negros, Digos and Panabo (both in Davao), and Magallanes and Masao (both in Butuan).

Crab antennae & leg muscle tissues and shrimp pleopods were preserved in 95% ethanol and subsequently processed at the Onagawa Field Science Center, Tohoku University in Japan for phenol chloroform-based DNA extraction, multiplex PCR amplification and microsatellite marker analysis using an ABI 3130 sequencer.

Results from the mudcrab microsatellite marker analysis show that the number of alleles in the three loci ranged 5-24 and that the Camarines Norte stock had the highest mean expected heterozygosity at 0.867,

followed by Cagayan (0.830), Surigao (0.824) and Samar (0.812) stocks. Although analysis of molecular variance showed no significant stock differences in genetic variability, high levels of genetic diversity in all four stocks imply that mud crab from any of these sources can be used for domestication and selective breeding.

In contrast, genetic variability indicators in tiger shrimp stocks showed that the number of alleles at three shrimp microsatellite loci ranged from 5 to 13 and that the highest mean expected heterozygosity was noted for Digos (0.826) followed by Negros (0.816), Bohol (0.798), Antique (0.797), Roxas (0.793), Kolambugan (0.787), Panabo (0.775), Magallanes (0.751), Masao (0.721), Palawan (0.668) and Aklan (0.642). AMOVA and principal component analysis on the shrimp gene frequency data showed that the Palawan population was significantly different from the other stocks.

### Domestication of mud crab

Mud crab *S. serrata* from Cagayan, Camarines Norte, Samar and Surigao were obtained for domestication. As mentioned earlier, results from the genetic characterization study showed that *S. serrata* from each source had high levels of genetic diversity but were not significantly different at the stock level, hence, stocks from any of the four sites could be used for domestication and selective breeding.

For base population ( $G_0$ ), the interval between 1<sup>st</sup> and 2<sup>nd</sup> spawnings was 34-52 days, and between 2<sup>nd</sup> and 3<sup>rd</sup> spawning, it is 33-34 days. Spawning to hatching takes 7-11 days for 1<sup>st</sup> hatching, 6-11 days for 2<sup>nd</sup> hatching and 7 days for 3<sup>rd</sup> hatching. A captive female spawning thrice in a single mating is not common.

Mud crab *Scylla serrata*



The number of zoeae per g BW of female ranged from 1241 to 8157. First generation ( $G_1$ ) families were produced from Cagayan (1 family), Camarines (6 families), Samar (8 families) and Surigao (9 families).  $G_1$  crablets (1.14-1.85 cm carapace width) attained market size in 6-7 months with survival of 32-47%. Two  $G_2$  families from Samar have been produced 11-12 months after hatching to zoea 1.

### Extending the spawning season of sea bass

One of the strategies to extend the spawning season of sea bass in captivity was to evaluate the effects of temperature on reproductive performance. Generally, higher percentage of mature fish at 29-31 °C was observed compared with those maintained at ambient temperature. Fecundity was comparable between the two treatments but hatching rates were better in the heated tanks.



**AQD trainees hard at work:** GOJ-TF supported three international training courses, namely: (1) *Marine fish hatchery* (19 May-24 June), (2) *Abalone hatchery and grow-out* (03-23 July) and (3) *Seaweed farming* (16-23 November). It also funded two extension manuals released in 2009: (1) *Seed production and grow-out of mud crab in Vietnam* and (2) *Philippine freshwater prawns (Macrobrachium spp.)*. The proceedings of the SEAFDEC international workshop on emerging fish diseases (held in 2007 in Thailand and funded by GOJ-TF) has been published in the *Israeli Journal of Aquaculture - Bamid'geh*, volume 61, number 3; this is guest edited by AQD's Dr. Gilda Lio-Po

### Tissue culture techniques to mass produce seaweed plantlets

A plant growing medium was first tested for its effectivity to raise explants of the seaweed *Kappaphycus*. AMPEP (Acadian marine plant extract powder) with and without plant growth regulators (PGR, phenyl acetic acid + Zeatin at 1mg per liter) were used to grow three color morphotypes (brown, purple and green) of *K. alvarezii* using adapted tissue culture techniques. The concentrations tested ranged from 0.001 to 5 ppm; the control was half-strength, enriched sterile seawater (ESS/2). Results showed that the brown morphotype required the highest concentrations of AMPEP (4-5 mg per liter, without PGR) to form shoots within 60 days. The purple morphotype required less, 3-5 mg per liter AMPEP, also without PGR, to have shoots in 21 days. The green morphotype produced shoots after 19 days with or without AMPEP. Survival of shoots ranged from 56-100% (brown), 100% (purple) and 83-100% (green).

The surviving plantlets (338 purple, 57 green and 170 brown morphotypes; total initial weight, 0.31g) were transported to a sea-based nursery in Panyam, Arena Blanco, Zamboanga City for test-planting. The accumulated weight after 266 days was 160 kg.

### Adoption of mudcrab culture technology

To evaluate the socioeconomic impact of the adoption of mud crab culture by fisherfolk in Northern Samar, a survey was undertaken in four sites (Laoang, Pambujan, Rosario, and Lavizares). The respondents (n=60) were mostly the beneficiaries of PACAP/CATP projects and trained by AQD in a season-long course on mudcrab culture.

Results showed that Northern Samar is a major supplier of *Scylla serrata* crablets in the country but not of market-sized crabs. Although crab fattening was introduced in 1998, it needed a re-introduction by LGUs and NGOs before it became widely known in 2004. Now, the practice is aquasilviculture as a supplemental livelihood as an individual, household or cooperative enterprise. Mud crab farms varied in size (140-2000 m<sup>2</sup>), culture practice (polyculture, monoculture or fattening) and area (ponds/ditches or in mangroves). Fattening method varied, too; crabs are communally reared in ponds and pens, individually reared in bamboo pens/plastic containers, or individually tied to poles.

Individual growers claimed their profits come from lowering the cost of their inputs by gleaned the seeds (match-box size) themselves and by finding substitutes for trashfish like *tusa* (a low-value crab), snails, cracked corn and others. They wanted to continue or expand their operations, but were constrained by lack of capital, lack of culture area, and absence of customary right over land use. For cooperative ventures, organizational problems had led to the disintegration of the groups, and former members claimed that there was not enough income to cover opportunity lost especially if the communal area was small.

# Development of fish disease surveillance system for aquatic animals

This project is successful in developing a vaccine for viral nervous necrosis (VNN) in marine fishes, while transboundary pathogens and emerging fish viruses are continually monitored. As always, prevention is better than disease control and most studies in TF-IV are along this line. The following are the updates.

## Control methods for VNN

To establish a strategy to maintain VNN-free stocks of economically important fishes like grouper, siganid and milkfish, studies on vertical viral transmission, susceptibility, and enhancing fish immunity through disinfection and DNA vaccination were conducted. Results showed that seawater treated with ozone (for 2.5 min), iodine (25 ppm) and Virkon (1.5) could effectively control infection in fish eggs. Groupers *Epinephelus fuscoguttatus* and *E. coioides* were susceptible to VNN while milkfish and siganids were not. The susceptibility of grouper was size-dependent; larvae weighing 8 g or less were highly susceptible (80-100% mortality after 4-8 days post-infection) while grouper weighing 90 g or more might not. DNA vaccination also worked; highest survival was for fish vaccinated with 100 ng compared with lower doses and unvaccinated group after a challenge test.

A vaccine capable of preventing VNN would be a pivotal strategy to reduce significant economic losses in the fish industry. From June 2007 to November 2009, three studies on the efficacy of formalin-inactivated vaccine against VNN in sea bass (*Lates calcarifer*), grouper (*E. fuscoguttatus*), and pompano (*Trachinotus blochii*) were conducted. For the first study on seabass, results showed strong immune response against a single injection of the formalin-inactivated red-spotted grouper nervous necrosis virus (RGNNV, a betanodavirus), which was originally isolated in Japan. Sea bass produced neutralizing antibodies, with the highest titer at 60 days post-vaccination (mean titer 1:4480). When the vaccinated fish were challenged with the homologous RGNNV at day 54 post-vaccination, there were no mortalities on both the vaccinated and control fish. However, a quick clearance of the virus was observed in the brains and kidneys of the vaccinated fish, and followed by a significant increase in neutralizing-antibody titers. Furthermore, the vaccine-induced antibodies potentially neutralized the Philippine betanodavirus isolates (RGNNV) in a cross-neutralization assay. These results indicate the potential of the formalin-inactivated RGNNV vaccine against viral

nervous necrosis (VNN) of Asian sea bass.

For the second study on grouper, results showed that a single intramuscular vaccination of formalin-inactivated Philippine RGNNV induced potent immune responses and substantial protective immunity. Seroneutralization assay revealed the production of neutralizing antibody titers by the fish, with the highest titer observed at day 60 post-vaccination (1:5120). When vaccinated fish were challenged with the virus at 15, 30 and 75 days post-vaccination, significantly higher survival rates were obtained in vaccinated fish compared with control. There were also no detectable virus titers in fish brains and kidneys, and the levels of neutralizing-antibodies were high. Three and five months later, the surviving grouper at days 15 and 75 post-vaccination were re-tested (there was a 3-8 fold drop in antibody level), re-challenged, and tested again (increase in antibody level was 8-15 fold).

For the study on pompano, clinical signs of RGNNV infection were established in diseased fish - dark coloration, anorexia, abnormal swimming behavior - and later confirmed by histopathology, RT-PCR and viral cell culture. Histopathology showed pronounced vacuolations in

The team working on fish health. AQD's Deputy Chief and GOJ-TF program manager Dr. Teruo Azuma is on the first row, third from right



fish brain, spinal cord and retina. When the isolated betanodavirus was used to challenge pompano juveniles and fry by intramuscular injection and immersion, results showed significant mortality in challenged than in control fish. A vaccine of formalin-killed betanodavirus was then tested and delivered through intra-peritoneal injection (for juveniles) or immersion (for fry). Vaccinated juveniles produced neutralizing antibody titers ranging from 1:240, 1:2240, and 1:560 at days 15, 64, and 125 post-vaccination, respectively. Bath-immunized fry, in contrast, show higher survival at day 35 post-immunization compared with control.

### Immunostimulation and vaccination strategies against WSSV

With biosecurity in farms not enough to stop the threat of WSSV, the solution may be in strengthening shrimp with potential immunostimulants like oligonucleotides with CpG motifs (CpG ODN), reverse motif (GpC ODN) and bacterial (*Vibrio harveyi*) DNA. Results of tests on shrimp showed that CpG ODN and bacterial DNA enhanced immune parameters such as total hemocyte count (THC), plasma agglutination titer (PAT), and hemocyte lysate agglutination titer (HLAT). CpG ODN also significantly increased survival 15 days post-challenge with WSSV at challenge pressure of  $10^{6.45}$ . ODNs, in contrast, increased survival at lower challenge pressure ( $10^{7.45}$ ).

When vaccination / challenge schemes using formalin-inactivated vaccine were compared, results showed that bath-immersion vis-à-vis intramuscular injection gave higher survival rates and longer survival times. This

was probably because challenge conditions were closer to natural infection. Regardless of method, significant survival can only be obtained within 45 days post-vaccination. This indicates the need for several booster vaccinations to protect shrimp during the whole crop cycle (120 days). In addition, oral immunomodulators -- methyl sulfonate methane or wheat grass -- that were added to the vaccine before challenge enhanced the vaccine's effects; but not when these immunomodulators were incorporated (1 g per kg feed) to the feed.

In vaccination trials using recombinant VP28 (rVP28), produced as inclusion body protein, shrimp survived better with rVP28 compared with the mock-vaccinated control and to irrelevant *E. coli* proteins. This indicates the potential of recombinant VP28 vaccine to replace formalin-killed vaccine for field trials.

### WSSV epidemiology in tiger shrimp farming systems

To assess the WSSV outbreak in Negros, case studies were made of 11 ponds and 91 shrimp production cycles along with results of 174 interviews and 2000-2007 data on rainfall/atmospheric temperature in the island. Results showed that tiger shrimp *Penaeus monodon* grow-out farms had a three-stage progress to WSSV outbreak: hazard conditions, infection, then outbreak.

**Stage 1:** Hazardous conditions included a combination of more rainy days, high rainfall and low atmospheric temperature. These conditions meant lower salinity, cooler water, and lower pH; and water pH could determine toxic effects of substances including ammonia.

**Stage 2:** Infections occurred when shrimp were exposed to stress factors (ie, fluctuating temperature and pH) but these need not result in outbreaks.

**Stage 3:** Outbreaks occurred when WSSV-infected shrimp (especially younger shrimp) were further exposed to large fluctuations in salinity and high temperature, and when there was a higher number of yellow *Vibrio* colonies compared with green ones in pond water. The effect of different water physico-chemical parameters like water temperature, pH, and salinity could be single or synergistic. These stressors weakened shrimp immune response and increased viral replication. Outbreaks in more farms resulted from sharing infected water source and feeding with live molluscs which carried WSSV by ingesting viral particles from soil and water. Biosecurity measures and the use of probiotics appeared to have no significant effect on WSSV incidence.

From these results, shrimp farmers are advised to (1) avoid stocking during the rainy season and cold months, (2) use a reservoir and deep well to avoid contact with other farms or a re-circulating scheme like green-water technique which can eliminate vibrios (bacterial infection makes shrimp susceptible to WSSV, (3) proper

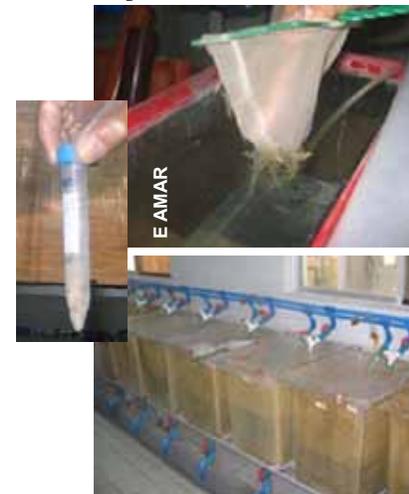
pond preparation (especially ploughing of pond bottom), and (4) plant mangroves in the receiving area of pond effluents to reduce organic pollution.

### Pilot testing of "SEAFDEC indigenous probiotic"

Although the "green water" system developed in Negros had been effective in preventing outbreaks, luminous vibriosis in shrimp is still persistent. This led AQD to isolate and identify bacteria -- collectively termed "SEAFDEC indigenous probiotic" -- from "green water". The tank trials were successful, with shrimp attaining higher biomass and survival than control. In the grow-out pond trials (three replicates), initial results showed that the mean survival rates of probiotic-treated shrimp were 100 and 97% at days 15 and 30, respectively.

"SEAFDEC indigenous

Clockwise: A tube of formalin-inactivated vaccine; transfer of shrimp to "vaccinating" tanks; and the set-up for the challenge test



Vaccinated (a) and unvaccinated (b) brown-marble grouper juveniles. The latter shows abnormal swimming behavior typical of VNN-infected fish

probiotic" were also mass-produced in the laboratory. Results showed that under aerated culture, the isolates yielded population one log higher than un-aerated culture. Growth was more stable at 22-28°C.

### Withdrawal periods and residues of antibiotics

Oxytetracycline (OTC) and oxolinic acid (OA) are two of the antibiotics that can be legally used in shrimp culture, and there is a need to establish the length of time it takes for their residues to be excreted out by shrimp to give consumers a safe food to eat. A feeding experiment was conducted where 50 g shrimp were fed SEAFDEC-formulated diet laced with 5g OTC or 4.5g OA per kg feed at 3% shrimp body weight for 7 days. Shrimp samples were taken at days 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 and 45 after the last feeding with antibiotic-laced feeds, and tested for antibiotic residues. OTC was extracted with 1% metaphosphoric solution with dichloromethane and analyzed by high performance liquid chromatograph (HPLC) with UV detector. OA was

Preparation, extraction and HPLC analysis of shrimp samples for antibiotic residue

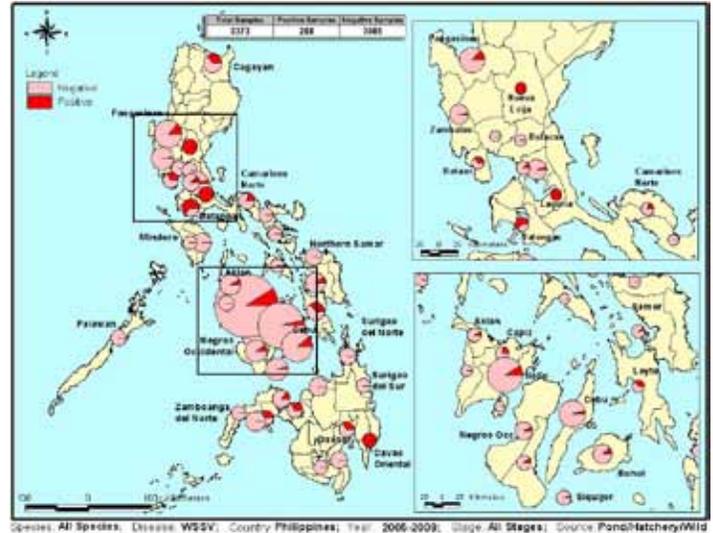


extracted with acetonitrile and analyzed in HPLC with fluorescence detector. Preliminary results showed zero residual concentrations at day 14 for OTC and at day 16 for OA.

### Monitoring and surveillance of transboundary pathogens

Disease is the biggest single threat to the sustainable shrimp aquaculture, and it's important to continue the monitoring and surveillance of transboundary pathogens affecting *Penaeus monodon*, *P. indicus*, *P. merguianesis* and *P. vannamei*, as well as freshwater prawn, *Macrobrachium rosenbergii*. Included in this study are WSSV, IHHNV, and significant diseases associated with *P. vannamei* such as TSV and IMNV; also the white tail disease of *M. rosenbergii*. Methods used to diagnose the viral diseases were based on standards prescribed by OIE. Results of viral analysis from 2005-2009 of samples submitted to SEAFDEC showed that of the 3,373 samples, 8.5% was WSSV-positive. This seems low considering anecdotal reports of widespread mortality in pond reared shrimps. Aside from shrimp, samples that tested WSSV-positive included freshwater prawn (15% of 172 samples), various crab species (10% of 276), marine annelids (24% of 17), and brine shrimp (4% of 23). There were 1,030 samples analyzed for IHHNV, 12% of which were positive. TSV (825 samples), IMNV (537 samples), and white tail disease (120 samples) were not found, and these are still presumed exotic to the Philippines. GAV and YHV were shown to have no significant strains in the Philippines as all 2005-2007 samples were negative. A significant result derived from the study was the absence of the 5 viruses in imported SPF *P. vannamei* broodstock.

Philippine map of the WSSV surveillance (CL PITOGO)



The three important outcomes of the study are as follows: (1) Mandatory postlarval analysis, most often with a battery of PCR tests for viruses, can prevent the spread of viral infection in shrimps, especially if batches that test positive for any of the viruses are rejected by farmers and destroyed. (2) Techniques are already available in order to prevent viral infection. The shrimp industry needs large numbers of healthy, SPF broodstock for their hatcheries and it is important to educate stakeholders about techniques to promote bio-security practices in order to avoid viral infections. (3) There is a need to develop in-country specific pathogen-free (SPF) (or at least high health) domesticated shrimp stocks to minimize the need for repeated importation. Keeping broodstock free of viruses will be a significant factor in breeding programs, especially that some diseases can be vertically transmitted.

### Surveillance of emerging fish viruses in five SEA countries

Active surveillance for the emerging viruses KHV, SVCV and GCRV among cultured carp were monitored annually

in Cambodia (2004 to 2008), Lao PDR (2005 to 2008), Myanmar (2005 to 2009), Philippines (2004 to 2008) and Vietnam (2005 to 2007) during the cool months of the year. To date, a total of 3659 fish samples consisting of common carp, koi carp, grass carp and silver carp were collected (531 from Cambodia, 353 from Lao PDR, 639 from Myanmar, 1478 from Philippines and 658 from Vietnam). Tests for the presence of KHV, SVCV and GCRV were made using PCR/RT-PCR, cell culture and bioassay. Results showed that the target viruses had not been present and the countries were consistently specific virus-free. Completed test results were forwarded to IFReDI, Cambodia; Department of Livestock and Fisheries, Lao PDR; Department of Fisheries, Myanmar; BFAR Philippines; and Research Institute of Aquaculture 2, Vietnam.

Biosecurity through vigilance is recommended to prevent introduction of potential carriers. In terms of diagnostic capability of the participating countries, Vietnam has the most rapid progress in upgrading of manpower and laboratory facilities. The Philippines has Level III diagnostic capability

except cell culture, while Myanmar lacks cell culture and histopathology. Fish Health diagnosis in Cambodia and Lao PDR are at Level II without histopathology and bacteriology; the former utilizes a common laboratory while the latter rides on the existing national veterinary laboratory.

## Parasitic fauna of bivalves and gastropods

A survey was conducted to get baseline information on parasite fauna associated with commercially important bivalves and gastropods.

For the green mussel *Perna viridis* (n=300) collected from Ivisan in Capiz, parasitic copepods (prevalence, 3.5%) were found in the mantle cavity. Its gill tissues had larval trematodes (prevalence, 26.5%) and ciliates (3%); its musculature, encysted tubellarian flatworm (4.5%); its digestive gland/mantle, trematodes (2.5%).

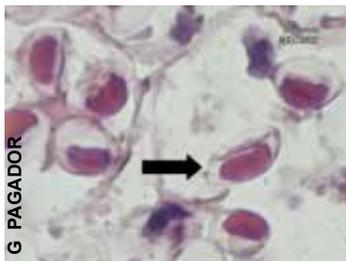
For nylon shell *Paphia semirugata* (n=200) obtained from a commercial growing area in Negros Occidental, no gross abnormalities were observed but light microscopy revealed uncysted metacercariae of a digenetic trematode in gonadal follicles and gonoduct with a prevalence of 9.5%. There were also cestodes encapsulated in the mantle and intestine (prevalence 6%) as were ciliate

protozoans (prevalence, 2.66%) attached to gill lamellae.

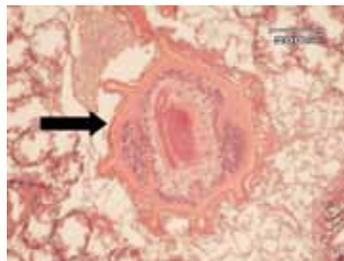
For slipper-shaped oysters *Crassostrea iredalei* (n=300) from Roxas in Capiz, there were barnacles, hooked mussel, boring sponge artifact and limy tube worm on its shell. The protozoan *Nematopsis* sp. (prevalence, 70%), cestode larvae *Tylocephalum* sp. (prevalence, 60%) and ciliates (prevalence, 13%) were also found in connective tissues of gills, mantle and digestive diverticulum.

For window-pane oysters (n=150) collected from Oton in Iloilo, barnacles and other oysters adhered to outer shells. Pea crabs (*Pinnotheres* sp.) were also found in the mantle cavity as were encysted larval cestodes (prevalence, 64%) in intestinal lumen and connective tissues. Ciliates (prevalence 26%) and metacercariae of trematodes (4%) were also observed in gills and connective tissues, respectively.

For abalone *Haliotis asinina* early juveniles (n=167) at the SEAFDEC hatchery, there were no evident parasites; for the bigger juveniles (n=240), there was a *Pseudoklossia*-like parasite (prevalence, 0.8%). Market-sized abalone (n=150) from AQD's marine station had barnacle *Pomatoceus* sp. on its shell, ciliates (prevalence, 23%) in the gills and digestive epithelium, and unidentified protozoa (prevalence, 10%) in foot muscle.



The protozoan *Nematopsis* sp. (arrow) found in the mantle and digestive diverticulum of slipper-shaped oyster



Encysted larval cestode (*Tylocephalum* sp.) (arrow) in the connective tissue of the intestinal lumen of window-pane oyster



The stock enhancement team



J RAMOS

Giant clam stock enhancement by AQD is in two sites in west central Philippines



MF NIEVALES



Hatchery-produced sandfish (inset) are reared in hapas serving as ocean nursery at AQD's Igang Marine Station



A village leader in Molocaboc, Sagay discusses with the community the importance of data gathering for resource management

# Stock enhancement of threatened species of international concern

The program covers five species - abalone *Haliotis asinina*, giant clam *Tridacna gigas*, sandfish/sea cucumber *Holothuria scabra*, angelwing clam *Pholas orientalis* and seahorse *Hippocampus*. There had been releases of abalone and giant clam hatchery-sourced seedstock in the wild, while the sea cucumber and angelwing clam activities focus on improving seed production to get enough stock for enhancement.

## Abalone in SMR

Wild and hatchery-reared abalone released at Sagay Marine Reserve (SMR) in Negros Island were monitored monthly for growth and survival. Abalone within the 2 m belt or 1 m to the left and right of ten 50-meter transects set 100 m apart were identified, counted, measured for shell length (SL) and body weight (BW); and their sexual maturation noted. Results of sampling in 2007-2008 showed wild abalone density to be positively correlated with dead branching coral with encrusting algae substrate. Mean SL (about 6 cm), mean BW (40-45 g), and highest percentage of partially and fully matured male and female abalone were significantly highest in April and September. But the wild abalone grew slower (0.1 cm per month) than the diet-tagged, hatchery-reared abalone (0.3 cm per month) that were released (n=1010) in the reef in July 2008. The latter were first acclimated to the reef for three days prior to release. Within a day after release, 13% died; in three days, <1% died; on the seventh day, no mortality and all had moved out of PVC shelters. Only 856 juveniles were eventually released at 86-88 individuals per transect. There were recaptures in Aug, Sep and Dec 2008 and in Feb and May 2009.

Important points were learned from the test release: (1) hatchery-reared abalone need to be weaned from their

dependence on PVC shelters; (2) they need to be acclimated in the area of release to improve survival; (3) their size at release is important; and (4) they move less if the habitat is favorable, ie. there is proper substrate, natural shelter and enough food.

## Abalone and giant clams in SMR

Whether stock enhancement is successful would depend upon the community, and hence, the ex-ante socioeconomic analysis of Brgy Molocaboc, a group of small islands within the Sagay reserve, was undertaken. There are about 5,000 small-scale fishers, fish processors and traders in the village. Two periodic follow-up (random and stratified purposive) surveys were conducted in 2009 as the baseline socioeconomic profile was already determined in 2007. The information, education and communication or IEC activities through seminars and participatory action planning were done in 2008.

Results showed a very slight improvement in the level of awareness and understanding of stock enhancement among fishers in 2009. Only 17% knew stock enhancement and cited their understanding of the SEAFDEC project in SMR and the seminars in Molocaboc. The respondents described stock enhancement as "sea ranching and culture activity in order to produce more abalone and giant clam

seeds in the sea" instead of being a "release activity to enhance wild stocks". Lack of livelihood and high dependence of fishers on gleaning activities persisted as majority (65%) of fishers either had no boats (57%) or had paddle boats only (9%). Although 35% did not reply to issues about expected compensation from participation in stock enhancement project, some fishers expressed that their participation in stock enhancement can be compensated by cash (39%) or by an assurance that they would benefit from "access to glean" in the future (26%). On community organizing, 87% agreed on the possibility of organizing fishers to facilitate income-generating projects.

## Giant clams in Negros and Panay islands

Like abalone, the giant clam *Tridacna gigas* is a species of international concern due to the overexploitation and depletion of wild stocks. While UP-MSI has been producing clam seedstock, releasing about 27 000 *T. gigas* in more than 40 sites across the Philippines, AQD was tasked to assist in the effort after the 2005 regional technical consultation on stock enhancement. On 31 March 2006, the first batch was stocked in cages in SMR's Carbin Reef, followed by a second stocking on 9 August 2006 in the Kawit Reef of Malalison Island in Antique, then a third batch on 28

February 2007 in AQD's Igang Marine Station in Nueva Valencia, Guimaras. Growth, survival and environmental parameters in all three sites were monitored monthly until December 2009. There were two class sizes of clams, 8 and 10 cm SL, at stocking. Results showed that clams reared in Igang had significantly the highest growth rate (0.80 cm per month) while Culasi had the lowest (0.5 cm). Highest survival was also observed in Igang at 86% after 945 days, followed by Sagay at 69% after 1268 days and lastly Culasi at 2.2% after 682 days. Mean size of clams as of September 2009 sampling was 345 cm SL in Igang, and 35 cm in Sagay. Both sizes were considered escape size ( $\geq 20$  cm SL). Culasi clams were mostly lost in June 2008 during Typhoon Frank while the remaining 11 clams were turned over to the local government and baywide management council. Correlating the parameters with growth rate, temperature and light intensity can be considered important factors. Salinity, total suspended solids and chlorophyll A did not significantly differ among the three sites. High survival in Igang may be attributed to absence of poaching since the station is well guarded.

## Abalone in Nasidman Island

After a reconnaissance survey in February 2009 of six potential stock enhancement sites, Nasidman Island in Ajuy (Iloilo) was chosen based on habitat suitability, existence of abalone fishery, presence of fishers, gleaners and communities within the potential release site, level of protection of the fishery, enforcement of fishery ordinances, presence of peoples' organization and local government support.

Abalone population in Nasidman was surveyed by eight permanent transects in April, August and October. The socioeconomic profile of

fishers and acceptability of stock enhancement activities were determined, validated and conveyed by interview, short seminar, focus group discussions, and action planning workshop. Results showed that Nasidman as generally suitable for full-scale stock release and enhancement activities. The wild abalone population showed signs of overharvesting and very few wild samples were noted in transects despite availability of natural food. The Nasidman fishers (n=53) were generally young (<40 years old), male (92%) with average formal education (32% elementary and 32% high school), and married (79%) with small (1-4 members) to medium (5-8) household size. Majority (85%) fished 1-8 hours per trip, often daily. Only 17% considered their living condition good while 63% felt very poor to average.

Fishers perceived slight improvement in abundance status of fish, abalone, giant clam and sea cucumber when the fishery was regulated through municipal ordinances. However, abalone and sea cucumber were not reported as major catch. The catch of abalone ranged from 0-2 kg per fishing trip. Only 6% reported catching giant clams from 0-4 pieces per fishing trip, an obvious violation of regulations. About 32% reported catching 1-10 kg of sea cucumber.

During the participatory action plan development (PAPD) workshop in October, it appeared that relations among stakeholders were generally positive and solutions (like stock enhancement) to problems could be resolved through resource management plans that could be adopted by the municipal government and implemented with community members.

### Angelwing clam

With the aim of producing hatchery-reared juveniles and eventually releasing them to areas which need stock rehabilitation, several activities were

undertaken for the angelwing clam *Pholas orientalis*. Possible sources of broodstock in Panay and Negros islands were surveyed, the suitability of a site for future stock enhancement was assessed, seed production was done and larval development documented. Results showed that broodstock may be collected from Kalibo, Aklan having average clam density of 7 per sq meter of size 92 mm SL. The adult clams survived better in 20-40 ppt, and needed to be placed in upright position to be able to feed. They spawned naturally in captivity (Nov 2007; Nov 2008; Jan, Mar and May 2009) but no larvae survived because of contaminated algal food. Spawning induction were also made, 28 trials in all from May 2007 to Oct 2009. Male and female clams responded positively to serotonin at a minimum size of 9 cm SL. The Oct 2009 trials was the most successful in attaining early juvenile stage, and these are currently being reared in the hatchery and fed a mixture of two algal food.

### Sea cucumber

In Vietnam, sea cucumber (*Holothuria scabra*, sandfish) hatchery techniques have been developed successfully for commercial scale in hatcheries and ponds. However, the present technology demonstration is only done at the government's national hatchery facility, and more technology dissemination efforts needed to be done for SEAFDEC member-countries. In collaboration with Vietnam's Research Institute for Aquaculture 3, GOJ-TF funded the training of one AQD staff from 26 Sept to 5 Oct on sandfish hatchery techniques; the training covered management of broodstock, stimulation methods, larval rearing, settlement preparation and management of nursery systems. A manual of seed production is being completed.

**Cage and pen culture.** From May 2007 to 2009, AQD in collaboration with DOST produced several batches of

*H. scabra* juveniles in the hatchery, with the latest batch survival of over 3% from start of larval rearing to stage 1 juveniles (1-10 mm body length, BL). For the nursery phase, juveniles > 5 mm were moved to floating hapas/ocean nursery in AQD's Igang Marine Station. Transport trials of juveniles to the ocean nursery showed up to 100% survival of juveniles (< 10 mm BL, < 0.05g) after 6 hours. Three to 7 days after transfer, survival remained high at 89-97%. Juveniles grown to > 1 g in floating hapas subsisted on naturally growing biofilms for a month; these were later transferred to bottom sediments as they are naturally detritivorous and sediment ingesters. Juveniles that are 1-3 g were found to be best grown in protected enclosures at 25 per sq m. If grown in half-buried cages, growth rate could be up to 0.1 g per day. However, growth plateaued, indicating a need to thin out, release or transfer sandfish to larger enclosures. Biomass density did not exceed 100 g per sq m, suggestive of sub-optimal food conditions as evidenced by low organic matter content in sediments within cages.

There has been relative absence of wild sandfish population in seagrass meadows fronting IMS since 2008 to present. This makes it a potential site for stock enhancement. In preparation for larger scale stock release in Igang, low and open 100 sq m monitoring pens were established in March 2009. The pens were stocked with > 3 g sandfish juveniles at 1 per sq m. Growth increment was up to 1 g per day in the first 3 months. However, mean retrieval/survival was only 19%, 8% and 5% after 1, 2 and 3 months, respectively. Low retrieval survival suggests predation as a potential concern in IMS since survival in predator-protected cages was >50% even after nearly 2 months.

Broodstock sandfish collected in Guimaras for

spawning trials in TMS were periodically transferred into a 9 sq m pen in March 2008 then into a larger 150 sq m pen beginning Sept 2008 for holding/conditioning. Census within the broodstock pen since Sept 2008 revealed presence of recruit-sized (<10 g) juveniles in May and July 2009. This sign of recruitment shows the positive impact of broodstock clustering on stock enhancement.

### Seahorse

Copepods serve as live food for newborn seahorses (*H. comes*), but their high bacterial load could be a serious threat in the hatchery. To disinfect copepods, they were washed in flow-through UV-treated seawater and disinfected with 50 or 100 ppm formalin for 1 or 12 hours. Results showed that untreated copepods (control) had the highest survival (203%, adult copepods had by then shed eggs which grew into adults) while copepods subjected to 1 hour of 50 and 100 ppm formalin had 176% and 180% survival, respectively. The 12-hour formalin treatment at 50 and 100 ppm had 163% and 92% survival, respectively.

When formalin-treated (1-hr 30 ppm bath followed by repeated washing in UV-treated seawater) copepods were fed to infant seahorse stocked at 1 per liter in tanks for 10 days, survival was better starting at day 5 to 10 compared with non-formalin treated control. But the stretch sizes of seahorse were almost the same (2 cm, 0.03 g).

Three types of rearing water (sand-filtered, chlorinated, and UV-treated seawater) for newborn seahorse (8 mm, 0.004 g) were also investigated. Results showed that survival from birth up to 30 days were high in chlorinated and UV-treated (62-66%) but low in sand-filtered (41%) seawater. But stretch height (41 mm) and body weight (0.23 g) was highest in UV-treated seawater compared with sand-filtered (33 mm, 0.16 g) and chlorinated seawater (34 mm, 0.16 g).

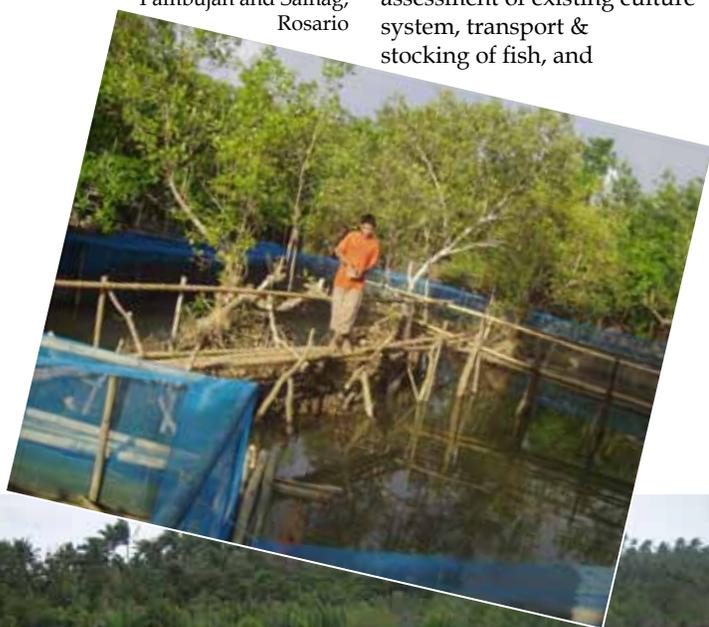
## ABOT AquaNegosyo and ICDSA

**A**quaculture continues to gain the interest of entrepreneurs as investment while local government units & grassroots organizations look at aquaculture as a means of providing additional livelihood and food security to their constituents. To respond better to stakeholders and partners, AQD has institutionalized two special projects that provide holistic technology assistance.

### ABOT AquaNegosyo

The *Agree-build-operate transfer aquabusiness* (ABOT Aqua-Negosyo) rendered technical assistance to six clients in 2009. The assistance included initial farm site assessment, assessment of existing culture system, transport & stocking of fish, and

Technology demonstration in Northern Samar: crab nurseries in Camparanga, Pambujan and Salhag, Rosario



monitoring of culture system and stocks. These entailed 19 man-trips to various farms in the Philippine provinces of Bataan, Batangas, Cebu, Pangasinan, Quezon, and Sorsogon; and about 59 man-days of direct technical assistance. Three (3) of these clients had signed formal agreements with AQD to avail of aquaculture business packages for multi-species pond grow out in Cebu, Quezon, and Sorsogon, respectively. The rest of the clients are in various stages of planning and other preparatory activities.

The project responded to queries of at least 100 more clients through email, phone or in person consultation. Queries ranged from technological and procedural concerns to marketing and financial aspects.

### ICDSA project

The *Institutional capacity development for sustainable aquaculture* (ICDSA) focused its efforts on four projects this year.

#### Enhancing the promotion of mud crab technology in Northern Samar

The mud crab nursery techniques that were disseminated to partner NGOs and fisherfolk organizations were proven to be financially

and technically viable. When done and managed properly, a family can earn an additional income of about P14 318 in six months in a 200 sq m pond as in Pambujan or as much as P25 533 for 700 sq m. About 150 persons were trained on mud crab culture.

**Nursery.** There were four nursery demonstration sites initially established in Northern Samar but only two sites (Rosario and Pambujan) had successfully carried out the nursery phase 1 where 0.3 cm crabs were grown to 2 cm carapace width or CW and nursery phase 2 where 1.5-2 cm crabs were grown to 3-4 cm. Stocking was 20 crabs per sq m in phase 1 and 7-10 in phase 2. The culture period for both was 21 days. Comparative cost-and-returns analyses were made of the nursery as a two-phase or as a straight run system. If seen as a two-phase run, analysis showed that Pambujan registered positive 6-run incomes of nearly P20 000 and return-on-investment (ROI) of 99% for phase 1; and over P24 000 income and 121% ROI for phase 2. In Rosario, however, there was a loss of P1783 with an ROI of -3% due to low survival (50%). Had survival increased by 5%, Rosario would have registered a 6-run income of nearly P4000 and 21% ROI.

If the nursery is seen as straight system where seed-

stock were reared from <1 to 4 cm CW for 42 days, results showed a 6-run loss of P1700 in phase 1. The first run of phase 2 in Pambujan and Rosario also posted a loss of nearly P5000 since survival was a low 41-43%. Survival needed to be increased to about 70% to be profitable. The second run of phase 2 posted better profits of P56 000 and P11 000, and ROIs of 66% and 23%, respectively, in Pambujan and Rosario. The high survival of 83% in Rosario more than compensated for the loss in phase 1; Pambujan, however did not fare well at 21% survival.

**Stock assessment and mangrove community structure.** The one-year stock assessment is in progress in three sites: Camparanga, Pambujan; Salhag, Rosario; and Palapag. Results in the mangrove study showed that the community structure of mangroves in Pambujan was dominated by *Avicennia marina* and *A. alba* (Avicenniaceae). Other species present were *A. rumphiana*, *Rhizophora apiculata*, *R. mucronata*, *Bruguiera gymnorhiza* (Rhizophoraceae), *Aegiceras corniculatum* (Myrsinaceae), *Ceriops tagal* (Rhizophoraceae), *Sonneratia alba* (Sonneratiaceae) and *Nypa fruticans* (Palmae). The average count of mangrove trees was slightly higher in the initial (24 stems per 100 sq m) compared with the final (21 stems per 100 sq m) sampling while total count of saplings and seedlings increased during the sampling year.

Rosario's mangrove stand was dominated by *A. apiculata* and *R. stylosa*. Other species present were *S. alba*, *R. apiculata*, *R. mucronata*, *A. officinalis* and *Nypa fruticans*. The average count of mangrove trees was higher in the initial (37 stems per 100 sq m) compared with the final (29 stems per 100 sq m). Saplings and seedlings likewise increased.

**Catch composition and effort.** Only *S. serrata* were collected during the entire sampling period in Pambujan. The dominant species in Rosario was *S. serrata*. *S. olivacea* were found from December to March with the peak in December to January. The catch composition using bamboo tube traps over 18 months in Pambujan was mud crab (*S. serrata*, 4%), other crabs which were mostly crenate crab *Thalamita crenata* (51%), prawn *Macrobrachium equidens* (39%), mollusc (3%) and fish (3%). For lift nets, the catch composition was (*S. serrata*, 5%), other crabs that were crenates (58%), mollusks (25%), prawns (10%) and fish mostly goby (1.24%).

In Rosario, most of the catch by bamboo tube traps was composed of mud crab (36% *S. serrata* and 1% *S. olivacea*) followed by prawn which were mostly *M. equidens* (31%), other crabs (22%), fish (6%) and mollusks (4%). For liftnets, most of the catch was non-*Scylla* crabs (68%) followed by mud crab (25%) and prawn (6%).

The mean catch per unit effort (CPUE) ranged 0.03-0.5 and 0.3-1 crabs per bamboo tube trap per day for Pambujan and Rosario, respectively. Mean CPUE ranged 0-0.08 in Pambujan and 0-0.8 crab per lift net per day in Rosario. In general, the relative abundance of mud crab over the 18-month period was higher in Rosario than in Pambujan due perhaps to the more strict enforcement of a provincial ordinance banning the collection of small crabs using the push net.

There were no mud crabs caught using the push net in Pambujan. The net substrate or shelter, a stationary crab collector, that was introduced as an alternate to push net collected mostly crenate crabs but no mud crab. In Rosario, however, crenate and mud crabs (10-25g BW; 3.9-5.6 cm CW) were obtained from the

net shelters.

**Size-frequency composition.** In Pambujan, the dominant size range collected for *S. serrata* was 8-9 cm CW in the bamboo tube traps, and 6-7 and 8-10 cm CW in the lift nets. In Rosario, dominant was size 3-5 cm CW for *S. serrata* and 4-5 cm ICW for *S. olivacea* in bamboo tube traps; and size 4-5 cm *S. serrata* in lift nets. The modal size class was larger for *S. olivacea* (4-5 cm ICW) than *S. serrata* (3-4) using the bamboo tube traps. There were no mature male and female crabs collected in any of the gear or site.

### Enhancing promotion of grouper culture in Misamis Occidental

The grouper cage culture disseminated in Panaon (PACAP-MOAVEC) and Sinacaban (Gata Daku Multi Purpose Cooperative) were proven to be technically and financially viable. The returns on investment on the two sites were 32% and 48%. A total of 120 rural folk were trained on grouper cage culture.

The MOAP multi-species hatchery, on the other hand, is now operational and has produced close to 10,000 fingerlings that were stocked in cages.



The multi-species hatchery at MOAP is designed by AQD

## Phase 2 of the pilot project on milkfish cage culture for oil spill affected fisherfolk in Guimaras

The first run in San Antonio posted a net income of about P44 000 which were shared equally by the two participating fisherfolk organizations. The season-long training course on milkfish cage culture attended by 122 fisherfolk was completed.

**Enterprise development.** Initial observation and interview of brokers and retailers in Iloilo's fish port showed minimal reporting on landings of traded milkfish from Guimaras. Most of the sources were from ponds in Panay. For the past two years, traders reported that the market for milkfish was promising. Milkfish from Luzon were even traded in Iloilo where prices were higher during lean months and typhoon seasons. Retailers preferred to market "pang-masa" sizes or 3 pieces to a kilogram (3:1), 4:1 and 5:1 in rural areas of Iloilo while city markets demanded bigger sizes 1:1 and 2:1. The existence

of milkfish post-harvest plants in Negros Occidental, Capiz, Aklan and Antique were perceived by brokers as one of the possible outlets in the Visayas. Though they did not perceive milkfish canning companies as rivals in terms of getting supply, they confirmed that these companies buy smaller 150 grams fish. The shorter period to culture this size is advantageous to growers.

Baseline data was also gathered on AQD trainees (n=117) who attended the second phase of the season-long course on milkfish culture. Results showed that the training opportunity was given to fishers across all age brackets. There were 58% who were 50 years and below while 42% constitute more experienced fishers aging 51 and above. Both long-time residents (92%) who had been in the community for 10 years and migrants (7%) were given the privilege of attending the training. There was also an equal participation of males (50%) and females (50%) with majority having reached secondary (43%) and elementary education (41%). Fishing was the major means

to support families as cited by married fishers (67%) or a means to feed extended families according to single fishers (22%). Most of the households was small (57%) to average (35%) but there were also big households (7%) having 11-15 members. Around 51% were Roman Catholics, 39% were Aglipayans and the rest belonged to other Christian religions (10%).

Fishing seasonality in Guimaras was investigated in the survey. Fishers perceived the dry and hot months of January to April as conducive for fishing. Rainy season w from May to October with July- August as typhoon months, and income from fishing dropped. Fishing fully resumed from October to December. Average monthly income from fishing peaked in March (around P1 400) and dropped in August (around P 800). There was no significant difference between the fishing area frequented by fishers before and after the oil spill in 2006. Twenty percent of the fishers spent 0-5 days per month fishing, 30% fished for 6-15 days, while around 20% fished almost every day

(26-30 days). The number of hours spent fishing ranged 1-4 hours for almost 56% of respondents, 31% spent 5-8 hrs while a minority spent more than 8 hrs. The way fishers managed their time fishing confirms that though fishing was their major livelihood, there were more than 60 other alternative and supplemental livelihood options all year round. Fifty percent of them spent 0-7 days working on these options,

25% the whole month (26-30 days), while the rest (25%) worked 8-25 days. In terms of coping time for fishers to adapt to stresses like the 2006 oil spill, more than 50% of fishers seemed resilient and coped within 1-6 months. Some fishers (40%) coped longer, about 6 months to a year, while the rest seemed not very resilient with coping time of more than a year.

The survey on business preference (n=100 trainee-respondents) showed that fishers were more comfortable with the cooperative set-up to run their business (61%) than other types of arrangement like sole proprietorship/ family run (15%), partnership (19%) or corporation (4.5%). When asked who they would want to be with in running their businesses, fishers chose to deal with family and relatives (35%) and any trusted individuals (20%) than deal with fellow fishers (18%) and people in their barangay (17%). The government (6%) and business entrepreneurs (4%) seemed the least trusted.

## Freshwater aquaculture in Dumarao, Capiz

Majority of the 22 beneficiaries in the municipality of Dumarao pursued tilapia culture as an additional livelihood. Some private operators have also invested on tilapia culture in the municipality.



One of the tilapia cage farms in Dumarao



Fisherfolk in Guimaras harvest milkfish from a sea cage

## Training

**T**raining and capacity building at the local and community levels are continuing activities at AQD. In 2009, 40 training sessions were conducted, a 5% increase over 2008. Thirty-five percent of these sessions were conducted on-site in SEAFDEC member countries and/or at the stakeholders' facilities; the other sessions were conducted at AQD's four stations.

A total of 612 national and foreign participants were trained, representing various stakeholder groups (government officers, fish farmers, fisherfolk, fishery school teachers, students and private sector practitioners). Of these trainees, 20% was funded by Petron/Citi Foundation, 18% by ASEAN Foundation, 14% by private sector, 7% by LGUs and 1% by GOJ-TF. The remaining were funded by other agencies.

### GOJ-TF and Japan-ASEAN training

There were three international training courses funded by GOJ-TF (details in the table below) in 2009.

AQD also assisted in the SEAFDEC-wide project on "Human resource development (HRD) for poverty alleviation and food security by fisheries intervention in the ASEAN region". This was funded by ASEAN Foundation through the Japan-ASEAN Solidarity Fund and aimed at enhancing the capacity of

fishers in selected rural communities and of fisheries officials working at the local level. AQD's tasks are focused on rural aquaculture as part of the overall intervention covering co-management, responsible fishing practices, backyard fishery post-harvest & processing, rural aquaculture and inland fisheries development.

Six countries conducted its HRD activities; the training topics were based on their needs.

HRD training in Myanmar (top and below) centers on mud crab and tilapia; in the Philippines, abalone is the focus



### GOJ-TF supported training

Venue: AQD's Tigbauan Main Station

#### Marine fish hatchery

18 May-23 June

Number of participants  
(female + male)

9  
(2F + 7M)

Countries represented by trainees

Brunei Darussalam (1), Cambodia (1), Malaysia (1), Germany (1), Myanmar (1) and Philippines (4)

#### Abalone hatchery & grow-out

02-22 July

5  
(2F + 3M)

Indonesia (1), Thailand (1), Singapore (1), Myanmar (1) and Philippines (1)

#### Seaweed farming

16-23 November

9  
(4F + 5M)

Malaysia (2) and Philippines (7)

### On-site HRD training on rural aquaculture

Venue: SEAFDEC member-countries

#### Freshwater aquaculture

01-07 September  
Vientiane Province, Lao PDR

25  
(6 F and 19 M)

Lao PDR

#### Abalone farming

28 September – 02 October  
Catbalogan, Samar

21  
(4 F + 17 M)

Philippines

#### Dumbo catfish farming

20-25 October  
Central Java, Indonesia

15

Indonesia

#### Freshwater prawn breeding and larval rearing

11 October – 14 November  
Department of Fisheries, Negara Brunei Darussalam

21  
(9 F + 12 M)

Brunei Darussalam

## On-site HRD training on rural aquaculture

<b>Marine fish culture</b> 11-17 November Sihanoukville, Cambodia	20 (M)	Cambodia
<b>Mud crab and tilapia farming</b> 18-22 November Ayeyarwaddy Division, Myanmar	15 (M)	Myanmar

## Client-driven training

*Venue: AQD's four stations (unless otherwise indicated)*

<b>Abalone nursery &amp; grow-out culture and seaweed farming</b> 16-21 February	8 (M)	Northern Marianas
<b>Abalone hatchery and grow-out</b> 05-19 October	4 (M)	Philippines
<b>Crab hatchery and grow-out</b> (three sessions) 27 April-01 May; 12-16 May; and 14 April-05 May	13 (2 F + 11 M)	Kenya (1), Malaysia (1), Singapore (2), Myanmar (1) and Philippines (8)
<b>Cage/pond culture of high-value species</b> 10-24 March	5 (2 F + 3 M)	Maldives (1) and Philippines (4)
<b>Grouper and pompano seed production</b> 03-14 November	1 (M)	Singapore
<b>Fish health management</b> (two sessions) 09-25 March and 09 November – 09 December	2 (1 F + 1 M)	Saudi Arabia and Tanzania
<b>Fish/shrimp parasite detection and identification</b> 18-21 February 2009	1 (M)	
<b>Freshwater prawn hatchery and grow-out operations</b> (three sessions) 19-23 January; 27-31 July and 07-11 December	8 (6 F + 2 M)	USA (1) and Philippines (7)
<b>Freshwater prawn farming</b> 12-16 October Isabela	27	Philippines
<b>Freshwater fish breeding and farming</b> (two sessions) 04-28 May 2009 and 16 November – 04 December	16 (1 F + 15 M)	Rwanda (9), China (1), Singapore (1) and Philippines (5)
<b>Induced spawning of catfish</b> 26-30 October	3 (1 F + 2 M)	Germany (1) and Philippines (2)
<b>Carp breeding and farming</b> 13-17 April	1 (M)	Philippines
<b>Tilapia hatchery and nursery operations</b> (three sessions) 23-27 February; 19-23 October and 14-18 December	8 (1 F + 7 M)	Singapore (1), Hawaii, USA (1), Germany (1) and Philippines (5)
<b>Basic techniques in the culture of natural food organisms</b> (two sessions) 26-30 January and 23-27 March	2 (1 F + 1 M)	Philippines
<b>Algal culture</b> 28 July – 13 August	3 (2 F + 1 M)	Philippines
<b>Algal isolation techniques</b> 09-13 November	2 (F)	Philippines
<b>Production of farm-based feeds for freshwater fishes</b> 26-27 January	1 (M)	Philippines
<b>Feed and nutrition</b> 24-25 September	3 (2 F + 1 M)	Philippines

Two of the client-driven training: crab hatchery & grow-out and parasite detection & identification





## Season-long courses and their modules; sessions dates; and number of participants (female, male)

### Grouper cage culture

For 10 fisherfolk associations covering four municipalities in Misamis Occidental: Sinacaban, Tudela, Jimenez and Panaon

<i>Third and last module:</i> Socioeconomics, business planning & management, marketing, harvest & postharvest, and monitoring of environmental impact	10 March 11 March	81 (15 F + 66 M)
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### Milkfish cage culture

For four barangays in Nueva Valencia, Guimaras: San Antonio, Igang, Magamay and Sto. Domingo

<i>Module 1:</i> Aquaculture production systems	30 June-01 July 25-26 June	122 (60 F + 62 M)
<i>Module 2:</i> Stock sampling, feed & feeding practices and cage maintenance	30-31 July 13-14 August	122 (60 F + 62 M)
<i>Module 3:</i> Fish health management, business planning, financial analysis & cooperative development & management	24-25 September 08-09 October	122 (60 F + 62 M)
<i>Module 4:</i> Harvest & post-harvest, handling, marketing and value-addition	20-21 May 26-27 October	124 (60 F + 64 M)



### Client-driven training

AQD organized 27 training sessions on various topics, from abalone culture to farm-based feeds (table overleaf). These were courses specifically requested by stakeholders to meet their needs. The courses were 80% practical sessions, and except for one, all were conducted at AQD stations.



### On-site season-long training

Two season-long courses for the ICDSA partners had been completed (table above). These were (1) *Grouper cage culture* for the partnership of AQD/PACAP/ACIAR-CATP/Misamis Occidental provincial government, and (2) *Milkfish cage culture* for the AQD/Petron Foundation/Citi Foundation project in Guimaras.

The modules of the training were conducted throughout the production cycle such that participants can learn as much technical knowledge and skills through actual exposure to site selection, cage construction and facility management, stocking, feeding, fish health management, harvesting and marketing. For the Guimaras run, profit from the harvest of the training/demonstration cage was given to trainees.

### On-the-job training, internship and study tours

There were 154 students (13-48 years old) from 21 colleges / universities in the Philippines who participated in the *Student on-the-job training (OJT)*. For Luzon, Don Mariano Marcos Memorial State University, Cavite State University (Naic campus), Central Luzon State University and University of Rizal System sent 27 students; in the Visayas, 89 OJTs came from Aklan State University, Southern Iloilo Polytechnic College, University of Iloilo, Northern Negros State College of Science & Technology, Central Philippine University, University of the Philippines Visayas, Negros State College of Agriculture, Capiz State University, West Visayas State University, and Western Visayas State College of Science & Technology. TESDA also sent four students to train. For Mindanao, 20 students were from Zamboanga State College of Marine Sciences & Technology, Mindanao University of Science & Technology, and Mindanao State University (Marawi and Maguindanao campuses).

Student OJT provides a mechanism for college

students to undergo actual work in aquaculture research and development; in the process, satisfy a school requirement for graduation.

In addition, there were 18 high school students who joined the *Summer internship*, and were from the University of the Philippines Visayas and Philippine Science High School Western Visayas.

AQD accepted 29 persons (13 females and 16 males) for *Internship*; three were from Germany, France and Myanmar and the rest were Filipinos. The interns were assigned according to their areas of interest, namely: abalone hatchery, crustacean hatchery, marine fish hatchery, fish health section, natural food laboratory, biotechnology laboratory, feed mill, Seahorse hatchery, brackishwater ponds, and training & information.

AQD also hosted 20 observation/study tours with a total of 164 visitors mostly from the Philippines. The other guests were from Australia (2), Micronesia (1), Turkey (1), USA (1), Kingdom of Saudi Arabia (3), Japan (2), Thailand (2), Malaysia (1) and Sweden (1). They toured the research facilities and met with the Chief and/or other AQD officials.

The season-long training enables participation in all practical aspects of culture, from making netcages and feeds to value-adding produce (milkfish)

# Publications and multi-media

To update stakeholders, AQD produced publications and video/DVDs (23 titles this year, most made available online); published a monthly newsletter; maintained a website ([www.seafdec.org.ph](http://www.seafdec.org.ph)); made press releases; and attended fairs & exhibits (five events).

## PROGRAM & INSTITUTIONAL PROMOTION

*AQD Highlights 2008*, a 40-page annual report on departmental and regional programs & activities

*Research and development for sustainable aquaculture in Southeast Asia: Strategic plans 2009-2012* outlines AQD's new strategic plans, mission statement, goals, objectives, and targets for its various R&D activities and administrative thrusts

*The Malalison Experience: empowering an island community in west central Philippines*, a 64-page booklet that describes AQD's community fishery resources management project from 1991 to 1998 with a post project assessment in 2009

*Binangonan Freshwater Station*, a 4-minute video showcasing the programs and activities of AQD's freshwater station on the shores of Laguna de Bay

*Compendium of aquaculture technologies*, a DVD containing descriptions and profiles of at least 11 technologies developed by AQD in the past 35 years, with notations on their financial analysis, technology adoptors, and the research publications behind these technologies

*Research collaboration at SEAFDEC/AQD*, 3-fold flyer on AQD's collaborative mechanism for potential partners

*Aquaculture training*, a single piece flyer listing AQD training, internship and student OJT programs

*AQD officials and commodity contact persons*, a bookmark-type publication containing contact persons for aquaculture commodities and programs

*AQD laboratory services*, a 2-leaf flyer listing various diagnostic services

*Hatchery seedstock*, a single piece flyer detailing fish seedstock available from AQD hatcheries

*Backyard hatchery*, a flyer on AQD's newest facility meant to demonstrate profitable operations of abalone and marine fish small-scale hatcheries

## TECH UPDATES

*Training handbook on rural aquaculture*, a 296-page book with chapters on concepts & approaches of sustainable rural aquaculture, importance & conservation of coastal ecosystems, aquaculture technologies, socioeconomics of fishing communities, policies concerning fisheries and aquaculture in Southeast Asia, business planning, and approaches and strategies of the technological transfer pathways at AQD



*Seed production and grow-out of mud crab (Scylla paramamosain) in Vietnam*, a 29-page manual on biology and techniques

*Philippine freshwater prawns (Macrobrachium spp.)*, a 50-page manual on the biology, morphology, natural distribution, habitat, and economic importance of selected freshwater prawns found in the country

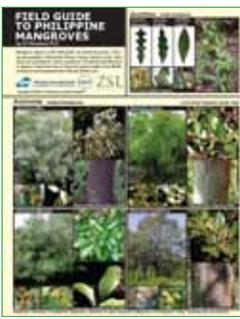
*Field guide to Philippine mangroves*, an 8-page guide with a user-friendly presentation of technical botanical description and illustrations of Philippine mangrove species

*Seed production of bighead carp*, a single piece flyer with basic information on the breeding and larval rearing of *Aristichthys nobilis*

*Grow-out production of bighead carp*, a single piece flyer on *A. nobilis*

*Seed production of giant freshwater prawn*, a flyer on the broodstock, larval rearing and nursery of *Macrobrachium rosenbergii*





**Grow-out production of giant freshwater prawn**, a flyer with basic information on the grow-out culture of *M. rosenbergii*

**Hatchery and nursery of grouper**, a flyer carrying technology profile and techniques in the hatchery and nursery of *Epinephelus* spp.

**Grow-out culture of grouper**, a flyer with basic information on site selection and culture techniques

**Mud crab culture**, a flyer on pond culture which includes site selection, culture conditions and methods, and financial indicators

**Milkfish culture in sea cages**, a flyer on rearing *Chanos chanos*, including site selection, cage design and construction, culture conditions and economics



## Newsletter

*AQD Matters*. Eleven issues (two shown at left) of AQD's internal newsletter were produced and distributed in print and electronically to AQD staff and friends, and select mass media.

## AQD website

AQD continued to retain its online adherents, with unique monthly visitors of not less than 3,000 per month. The website [www.seafdec.org.ph](http://www.seafdec.org.ph) was updated 10 times in 2009 (three leadstories shown below).

## Mass media

AQD assisted the mass media in the Philippines gather background information on at least five stories. As a result, AQD got at least 10 press coverage in the newspapers in 2009, and nearly 100 web or blog appearances.

**Viewership of the AQD website in 2009** (raw webhits by PHP Junkyard, line graph; and unique visitors by Google Analytics, bar graph)



## Library & DataBank

The AQD Library & DataBank has improved its accessibility to archived and updated information by keeping the online public access catalogue (OPAC) updated, acquiring new materials thru subscriptions and print/online services, continuing with the gifts and exchange program, and networking efforts with national and international libraries. It served 8 172 readers/visitors composed mostly of students from 93 different schools, government agencies and private sectors, and 8 194 search requests from OPAC, ASFA and other databases, and also responded to queries of at least 49 clients from five countries. Other activities were the digitization of AQD publications and documents and updating of list of the new research and techno-verification and demonstration studies, AQD's published research papers, and compilation of climate change literatures. The OPAC collection stands at 37 132 titles and 57 633 copies (4% is new).



New murals at AQD FishWorld

## Fairs & exhibits

AQD participated as exhibitor and its research staff served as resource persons in five fairs:

**Fisherfolk Advocacy Day**, February in Sibunag, Guimaras  
**Capiz Farmers' Day**, April in Roxas City  
**Manila Aquamarine & Aquatic Resources Exposition**, August at the World Trade Center (WTC), Metro Manila  
**Visayas Science & Technology Fair**, August in Bacolod City  
**AgriLink-FoodLink-AquaLink**, October at the WTC Manila

Over 1,000 people came to the AQD booths in these events and inquired about AQD publications, technologies and services.



Consultation with AQD in AgriLink, the country's largest agricultural fair

## AQD FishWorld

AQD FishWorld activities included: (1) visitor services ~ briefing, guided tour of facilities; (2) live animal exhibits in aquaculture, fisheries, and biodiversity (collection, identification, cataloguing); (3) rescue and rehabilitation of endangered marine animals; (4) R&D workshops and internships for high school science teachers and students; (5) *Aquaculture Week 2009* competitions among elementary and high schools students; and (6) research and workshop on *tangab* (filter net) fishery in Iloilo Strait for BFAR, fishers, and other stakeholders.

## Visitors

AQD had over 5,000 visitors in 2009, including these scientists/student-researchers who kindly gave short seminars to staff:

- Dr. Russell Borski**, North Carolina State University/Raleigh USA: *Compensatory growth: Mechanisms and applications for improving production efficiency of fish*, January 27
- Dr. Jacques Zarate**, University of Rhode Island: *The role of taurine and betaine transporters in osmotic adaptation of Atlantic salmon, *Salmo salar**, January 29
- Ms. Angeli Joyce Yap Dy**, Capiz National High School: *Screening and protein profile of milkfish (*Chanos chanos* Forsskal) bile for antioxidant, anticancer, antimicrobial and as a physicochemical defense system*, March 5
- Dr. Atsushi Yamamoto**, Kagoshima University: *Vaccination against *Lactococcus* infection and a re-emerging disease of cultured yellow-tails in Japan*, March 5
- Dr. Mudjekeewis Santos**, Tokyo University of Marine Science & Technology: *Characterization of polyclonal antibodies against Japanese flounder IgM derived from recombinant IgM constant region proteins*, April 29
- Dr. Yukio Maeno**, JIRCAS: *Pathological studies of infectious diseases in marine fish and shellfish*, May 20
- Dr. Eduardo Leño**, National Taiwan Ocean University: *Thraustochytrids: ecological role and potential commercial use*, May 27
- Dr. Christopher Marlowe Caipang**, Bode University College Norway: *Rapid detection of bacterial pathogens in fish by the loop-mediated isothermal amplification (LAMP) reaction*, June 22
- Dr. Hisashi Kurokura**, University of Tokyo: *Fisheries Policies in Japan*, June 30
- Dr. Hideo Mochizuki**, Borneo Marine Research Institute / Universiti Malaysia Sabah: *Effect of probiotics treatment on kuruma shrimp *Marsupenaeus japonicus* reared in a closed recirculating system*, September 24
- Dr. Fiona Pedroso**, Ehime University/Japan: *Molecular cloning, tissue distribution and hormonal regulation of IGFBP -1, -2, -3 and 5 in yellowtail, *Seriola quinqueradiata**, October 13
- Mr. Azzmer Azzar Abdul Hamid**, University Technology Malaysia: *Identification of *Pseudomonas* sp. strain S3 using polymerase chain reaction (PCR)*, October 21
- Dr. Salehuddin Hamdan**, University of Technology Malaysia: *Effect of effective microorganisms on catfish aquaculture*, October 21
- Dr. Fahrul Huyop**, University Technology Malaysia: *Use of 3-chloropropionic acid dehalogenase gene as selection marker for *Escherichia coli**, October 21
- Dr. Koichi Okuzawa**, Ishigaki Tropical Station/Fisheries Research Agency Japan: *Research and development for stock enhancement and aquaculture of fishes and turtle in Ishigaku Tropical Station, Okinawa, Japan*, December 8
- Dr. Motohiko Sano**, National Research Institute of Aquaculture / Fisheries Agency of Japan: *Current research topics in the National Research Institute of Aquaculture*, December 8

# Statement of source and application of funds, 2009

SOURCES OF FUNDS		APPLICATION OF FUNDS	
Government of the Philippines	Php 150 000 000	General administrative & non-project expenses:	Php 135 874 346
Government of Japan Trust Fund	9 370 192	AFD	45 316 552
Development of fish disease surveillance system	1 759 282	Management	16 493 598
Stock enhancement	3 939 654	RD	54 973 234
Development of technologies and human capacity building	3 671 256	TVDD	8 141 815
External grants	16 626 550	TID	10 949 146
NCSU: Alternative feeding strategies for milkfish	1 863 633	Program / project expenses	28 764 042
BFAR: National abalone breeding and culture program	250 000	RD	14 596 466
NACA: Live reef food fish trade (aquaculture study Phil)	156 218	TVDD	10 142 750
UNITAR: Capacity development in sea and human security	118 275	TID	4 024 826
ACIAR: Integrated fisheries resource management (aquaculture and water quality component)	175 529	Government of Japan Trust Fund projects	9 370 192
ACIAR4 / CATP	3 360 277	Development of fish disease surveillance system	3 197 748
Misamis Occidental: Aquamarine park	357 737	Stock enhancement	1 767 836
Misamis Occidental / Gata Daku Coop: Grouper project	819 032	Development of technologies and human capacity building	2 813 988
Misamis Occidental: Grouper project	135 813	GoJ committed funds / advances	1 590 620
Mindoro Oriental: ICDSA	57 868	Externally funded projects	11 058 315
BFAR: Biotechnology	5 000 000	SEAFDEC Secretariat: Regional technical consultation on sustainable aquaculture	16 406
JIRCAS	220 160	ASEAN Foundation: HRD for poverty alleviation and food security	141 376
Northern Samar: Mudcrab project	432 414	BFAR: National abalone breeding and culture program	187 076
USAID: Evaluation of sites, sustainable culture species	257 815	BFAR: Grow-out of white shrimp in Paoay Lake	107 379
Petron: Milkfish project	1 099 064	NFRDI: Aquaculture biotechnology	43 341
ASEAN Foundation: HRD project	462 226	NFRDI: Capacity building of NFRDI in fish health	336 469
Novus International Inc: Grouper study	362 367	DA-BAR: Publication of FSP funded research	328 496
ICLARM: Sandfish	1 498 123	PETRON 1	577 913
Internally generated funds	25 663 506	PETRON 2	1 606 369
Income - RD	6 246 062	ABOT: Collaborative projects	250 498
Income - TID	3 904 322	NCSU: Alternative feeding strategies for milkfish	1 578 692
Income - TVDD	6 077 309	USAID: Evaluation of sites, sustainable culture species	190 974
Income - AFD	8 887 781	UNITAR: Capacity development in sea and human security	120 667
Income - Management	548 033	USB: Use of soybean meal and soy protein concentrate	706 885
Committed funds from previous year	26 630 550	Novus International Inc: Growth, survival and nutritional composition of grouper fry	63 292
<b>Total sources of funds</b>	<b>228 290 799</b>	FAO: International workshop on farm feeding	7 112
		ACIAR: Integrated fisheries resource management	108 983
		JIRCAS	234 326
		Mariculture project in Aurora, Quezon	36 903
		ICDSA	4 415 161
		Committed funds	43 223 904
		Advances for on-going activities	2 931 558
		Capital outlay / repairs	40 292 346
		<b>Total application of funds</b>	<b>228 290 799</b>

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## About SEAFDEC

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

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

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

AQD is mandated to:

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

AQD maintains four stations (right, top to bottom): the Tigbauan Main Station and Dumangas Brackishwater Station in Iloilo province; the Igang Marine Station in Guimaras province; and the Binangonan Freshwater Station in Rizal province. AQD also maintains a Manila Office in Quezon City.

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