SEAFDEC-JIRCAS collaboration

The Japan International Research Center for Agricultural Sciences, based in Tsukuba City, collaborates with SEAFDEC on the project, *Studies on sustainable production systems of aquatic animals in brackish mangrove areas* (fiscal year 2001-2005). In 2004, JIRCAS Scientists Dr. Hiroshi Ogata and Dr. Ikunari Kiryu conducted two research projects at AQD, one on the effect of essential fatty acids and vitamins on reproduction of marine fishes, and another on viral nervous necrosis in groupers (results below). Another JIRCAS Scientist, Dr. Yoshimi Fujioka, conducted a study in Thailand–on the role of benthic organisms as food for marine resources – and he presented the results during the Regional Technical Consultation on the Sustainable Use of Mangroves for Aquaculture, held in Bohol, Philippines in August 2004. Through JIRCAS' Counterpart Researcher Invitation Program, AQD's Esteban Garibay underwent training on fatty acid analysis at JIRCAS and on vitamin E analysis at the National Research Institute of Aquaculture in Mie from 27 January to 7 March 2004 under the guidance of Dr. Hiroshi Ogata and Dr. Hirofumi Furuita.

ES Garibay prepares rotifer enrichment tanks at the AQD hatchery, analyzes fatty acids at the JIRCAS laboratory, and enjoys the plum blossoms in Tsukuba with Dr. Ogata

Reproductive and larval performance of rabbitfish *Siganus guttatus* given arachidonic acid supplements. Arachidonic acid (ArA) has recently been shown to improve reproductive performance and larval survival in tropical marine fishes. A feeding test was done on rabbitfish *Siganus guttatus* broodstock. Three diets were tested on groups of 5 males and 5 females (340-810 g body weight) in 5-ton concrete tanks. Diet 1 was a basal diet with 1% soybean oil + 6% cod liver oil; Diet 2 had the soybean oil replaced with 0.75% ArA; Diet 3 had all of the soybean oil and a part of cod liver oil replaced with 1.5% ArA. Fish were fed twice at 4% of biomass/day. The breeders were allowed to spawn naturally. The eggs of *S. guttatus* are demersal and strongly adhesive on the walls of the spawning tank, and it was difficult to collect and count the spawned eggs. Best spawning and hatching was obtained with the diet containing 0.75% ArA, and the poorest result with the diet containing 1.5% ArA. The optimum supplementation rate in diets for broodstocks of tropical marine fishes might be around 0.5% ArA, as determined earlier for mangrove snapper.

Experiments were also done to incorporate ArA in rotifers for rabbitfish larvae. Rotifers that were reared on either baker's yeast, *Nannochloropsis sp.*, or the diet Culture Selco, were enriched over 24 h with DHA (docosahexaenoic acid) Protein Selco with and without supplemental 5% ArA. Fatty acid analysis of the freeze-dried rotifers showed that dietary ArA can be incorporated into rotifers. Rotifers reared in culture Selco and then enriched with DHA Protein Selco showed the best balance of essential fatty acids, with ratios of 1.3 ArA/EPA (eicosapentaenoic acid), 2.2 DHA/EPA, and 1.7 DHA/ArA. In another experiment, rotifers reared on Culture Selco were enriched with DHA Protein Selco plus 5, 10, 15, and 20% ArA. Results showed that ArA incorporation in the rotifers was directly proportional to the ArA levels in the diet. However, EPA and DHA levels in the rotifers decreased as the ArA level increased. The optimum ArA level for incorporation into rotifers seemed to be less than 5% of the DHA; higher ArA inhibited the absorption and/or accumulation of EPA and DHA in rotifers.

Fatty acids and fat-soluble vitamins in milkfish larvae from the wild and from the hatchery. Three batches of wild milkfish larvae (4–7 mg) from different localities, and 12 batches of hatchery-reared larvae and juveniles (different ages and weights, 3 mg–26 g) were analyzed for ArA, EPA, DHA, Vit A, and Vit E. Wild larvae had about 3% ArA, 7% EPA, and 20% DHA (% of total fatty acids), 20 IU Vit A/g fish, and 28-130 µg Vit E/g fish. The corresponding values in hatchery-reared larvae were 3-9% ArA, 2-12% EPA, 2-25% DHA, 13-57 IU Vit A/g fish, and 16-229 µg Vit E/g fish. Most of the differences in values between wild larvae and hatchery-reared larvae and juveniles could be due to age, or weight, or prior feeding history. Hatchery-reared larvae had higher ArA, similar EPA, but much lower DHA than wild larvae of similar and age. These results indicate that milkfish larvae in the hatchery must be fed rotifers enriched with DHA. Marine fish larvae can not synthesize DHA, EPA, and ArA and must get these in the diet. EPA and DHA are essential components of phospholipids in cell membranes. DHA improves resistance to stress in larval and juvenile fish.

Pathogenesis and control of subclinical viral nervous necrosis (VNN) in grouper broodstock. The major infection route of VNN is considered to be vertical transmission via gonad of subclinically infected broodstock. Investigation regarding such broodstock is needed in order to prevent the disease. Five healthy *Epinephelus coioides* (4-12 kg body weight) were used, two from AQD Tigbauan and three from the Inland Sea Ranching Station in Puerto Princesa City, Palawan. The fish were aseptically dissected, and 14 different tissues were collected from each. For cell culture using E-11 cell line and RT-PCR methods, the tissues were stored in −80°C. Samples for histopathology were fixed in 10% buffered formalin. Additionally, 11 wild juvenile groupers (8 *E. coioides* and 3 *E. malabaricus,* 2-4 g body weight) were purchased from a grouper supplier in Roxas City, Capiz. Five different tissues were collected from each fish, and stored in −80°C for RT-PCR assay.

In order to detect VNN virus from fish tissues, the RT-PCR, nested PCR, cell culture, and combination of cell culture and RT-PCR were used. A suitable method for virus detection in the tissues of asymptomatic fish was determined. The latent virus was difficult to detect by cell culture or even by the combination method. These methods also required longer period and were not suitable for handling many samples. Random hexamers were used in the synthesis of cDNA, and additional two new primer sets were used which resulted in the improvement of both the RT-PCR and nested PCR. The method of virus detection using PCR was standardized.

Analysis using an improved PCR assay detected VNN in in the 5 breeders and 9 of 11 juveniles sampled. The highest ratio of virus detection was in brain and eye, 14/16 brains and 8/11 eyes positive for VNN. But other tissues were also found positive for VNN: 6/16 gills, 4/13 spleen, 4/10 kidney, 3/5 blood, 5/5 intestine, 5/14 liver, 3/4 gonad, 4/5 swim bladder, and 4/4 skin. Histological analysis will also be done using fluorescent antibody technique. Once the distribution of the virus is clarified, virus transmission may be understood, and preventive measures may be developed.

Brackishwater Aquaculture in Western Visayas

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Total aquaculture production in Western Visayas was 88,711 mt in 1995 and rose to 99,258 mt in 2001 due to increase in seaweeds. The value, however, dropped from PhP 8.5 B in 1995 to PhP 3.4 B in 2001 due to reduction in tiger shrimp. The commodities in descending order of market prices in 2001 are tiger shrimp, grouper, mudcrab, milkfish, oyster, seaweed, and mussel. Tiger shrimp, grouper, and seaweed are for export, but grouper prices were low in 1999-2000 due to economic weakness in Taiwan, Hong Kong, and other importer countries.

All tiger shrimp are produced in brackishwater ponds, and production in Western Visayas reached a high of 33,958 mt in 1995, but has stabilized around 1,000 mt since 1998. The main reason for this huge decline was the outbreak of luminous bacterial disease and various viral diseases that forced many intensive farms in Negros Occidental to stop operations. Farms now operate at the low-cost extensive level, or shift to environment-friendly intensive systems using reservoir ponds for intake water, where tilapia is raised to produce 'green water' which effectively controls bacterial diseases.

Milkfish is mainly raised in brackishwater ponds, but there has been an increase in production from cages and pens since 1999, when fish prices were high. The national production rose to 232,000 mt in 2002 from 170,000 mt in 1999. The glut caused milkfish prices to fall in 2002 and forced some intensive farm operators to quit the business.

A variety of species comes from small-scale aquaculture. Oyster production has been increasing since 1999 but mussel production has had large annual fluctuations. Grouper production in ponds was highest in 2001. Production of mud crab has been increasing since 1997. Seaweed production volume has increased so high as to more than compensate for the 50% fall in seaweed prices.

Brackishwater ponds are mainly operated by business people or merchants engaged in businesses other than fishing, who live inside or outside of the pond locality. Small-scale farms are mostly operated by local residents and coastal fishers, many of whom are 'poor'. Milkfish cages and pens require commercial pellets and the operators are middle-class.

Mangrove replanting is now being widely encouraged by the central and local governments alongside small-scale aquaculture to produce a variety of fishes, crustaceans, and mollusks for food and income.

A survey was conducted in Ivisan and Carles in northern Panay. In Ivisan, primary income is from fishing and secondary income from aquaculture. About 29% of the farms started in 1997-1999, and 71% started in 2000-2003. Carles has households engaged in a variety of other work and businesses, but many have turned to aquaculture for their primary and secondary incomes – 36% started before 1989, 21% in 1997-1999 (mostly small-scale farms), and 43% in 2000-2003 (mostly brackishwater ponds). Most of those currently engaged in aquaculture want to continue in their businesses and seek help from government, researchers, and fellow farmers.