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Broodstock management and seed production of marine fishes

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In milkfish floating cages, the AQD-designed manually operated egg sweeper is rotated three to five times around the cage to gradually collect eggs to the detachable conical net bag. For the rabbitfish, *Siganus guttatus*, an egg collector or substrate (=plastic sheets) is placed at the bottom of the tank prior to spawning and is eventually transferred to the incubation or rearing tanks.

Eggs are transported from IMSS to the hatcheries at TMS in double-layered oxygenated plastic bags placed inside a styrofoam box or a flat binder bag. Packing density ranges from 90,000 to 300,000 eggs in 8-10 liter of water depending on the species. Spawned eggs are temporarily stocked in incubation tanks and viable eggs are isolated by their higher degree of buoyancy.

For grouper, the incubation of spawned eggs is either conducted in 400 to 500 liter fiberglass tanks or directly stocked in larval rearing tanks. Stocking density varies from 5,000 to 10,000 eggs per ton for semi-intensive larval culture or 30,000 eggs per ton for intensive larval culture. At TMS, seawater and freshwater are supplied from the pump house/reservoir. Moderate aeration is provided to each tank.

Rotifers are essential in the initial stage of rearing the various fish larvae because of their size and the ease of culture. Most marine fish larvae are fed with rotifers on day 2 at 10-15 rotifer per ml. Newly hatched brine shrimp nauplii are usually given on day 15 starting at < 1 individual per ml. Feeding rate is gradually increased as the larvae grow.

A combination of a microparticulate feed and rotifer can result to bigger milkfish larvae. On the other hand, an AQD formulated milkfish larval diet containing adequate nutrition (highly unsaturated fatty acids and vitamin mix) was found to be an effective supplement for rotifers and alternative or complete replacement for the expensive brine shrimp nauplii. Furthermore, the copepod *Pseudodiaptomus annandali* is a potential substitute for *Artemia* as larval feed for milkfish. It results to better growth than when fed *Artemia* and *Brachionus*.

Milkfish are also observed to be more robust and to have slightly higher survival rates when reared in open outdoor tanks.

For rabbitfish, snapper and grouper larvae, screened rotifer can be used during initial feeding in the absence of SS-rotifer strain because of their small mouth.

The mortality of grouper is lower when fed with *Artemia* starting at day 21 instead of day 14. Two to three day-old larvae fed with *Acartia tsuensis* copepod nauplii, a cheaper substitute for *Artemia*, grew significantly faster and showed higher survival rate compared to those fed with rotifer only.

Rabbitfish and grouper larvae are reared initially in static water system for 5 to 7 days, otherwise, partial water change from 30-50% during rotifer feeding days and 50-75% on brine shrimp feeding period are followed. Larviculture of milkfish in open outdoor tanks requires greater volume of water to be changed, if not feasible, a flowthrough system is allowed for 1-2 hours until the water becomes clear of diatom bloom.

The initial stocking density used for most of these fish species is 30 larvae per l. For grouper, a stocking rate of 10-20 larvae per l is optimum.

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By JR Paniza

Since 1973 when SEAFDEC/AQD was established, its pool of experts carried out a regularly renewed comprehensive program of research, training, and information dissemination activities on five marine species: grouper (*Epinephelus coioides*), sea bass (*Lates calcarifer*), milkfish (*Chanos chanos*), rabbitfish (*Siganus guttatus*), and the mangrove red snapper (*Lutjanus argentimaculatus*). AQD has also verified in actual field conditions the technical, environmental, and socioeconomic considerations of the technologies it developed from research.

Following its first research breakthrough in 1974, the completion of the tiger shrimp life cycle by eyestalk ablation, AQD has kept on refining developed technologies to improve industry practices through innovative approaches like the application of biotechnology in aquaculture.

**Rabbitfish**

This fish is prized as much as other high-value fish such as groupers and snappers. However, the slow growth of rabbitfish hampers the expansion of its culture. This problem is now being addressed with the use of growth hormones produced by the rabbitfish itself.

AQD researchers were able to obtain the growth hormone with the application of biotechnology. They first cloned the cDNA of rabbitfish growth hormone (GH) and the insulin-like growth factors (IGF I and II). This work was conducted at a laboratory in Japan.

The GH was tested at AQD’s Tigbauan Main Station in Iloilo Province, Philippines. When given as weekly injections, researchers say, GH significantly increased the body weight and length of the rabbitfish. This means that when the growth hormone supplementation, the normal culture period of rabbitfish to reach marketable size can be shortened.

Moreover, AQD researchers emphasize that unlike the genetically modified organisms (GMO), which is practically the development of new species, the cloned GH is endogenous or produced by the same fish.
Milkfish
Several milkfish production technologies have been developed at AQD and subsequently adopted by the industry. Yet, problems on fry availability still exists in the Philippines. Studies to better understand growth regulation and factors that influence development of larvae and juveniles are among the focus of AQD’s research on milkfish.

Research to address growth regulation, and develop methods to enhance growth in juvenile milkfish involve the isolation, and characterization of GH and IGF-I and II. Like the rabbitfish, milkfish GH and the IGF have also been cloned. Preliminary work to produce recombinant growth hormone is underway and studies to determine when GH and IGF genes are expressed in embryos and larvac is being done.

In the hatchery, the cost of producing milkfish fry has been reduced through the development of larval feed for the larvae.

Mangrove red snapper
Recent developments in snapper aquaculture are focused on broodstock management and seed production to ensure fry availability.

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### AQD’s studies on broodstock management and seed production of marine fishes

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<td>Rabbitfish, <em>Siganus guttatus</em></td>
<td>Seed production</td>
<td>Feeding larvae fed with any of the following resulted to comparable growth: (a) HUFA-enriched rotifers at 15-20 individuals per ml, (b) HUFA-enriched rotifers supplemented with an artificial diet (NOSAN R–1) at 0.5 g per ton per day, (c) <em>Chlorella</em>-fed rotifers supplemented with Nosan R–1, or (d) <em>Chlorella</em>-fed rotifers</td>
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<tr>
<td>Application of biotechnology in aquaculture</td>
<td>Cloning and sequencing of the growth hormone (GH) Production of GH using recombinant DNA technology Cloning of insulin-like growth factors (IGF-I and IGF-II) Production of recombinant rabbitfish IGF-I GH mRNA was strongly expressed in the larvae from day 2 onwards while IGF-II seems to be expressed more than IGF-I during early development</td>
<td>Dr. Felix Ayson, Dr. Evelyn Grace de Jesus</td>
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<td>Intensive seed production</td>
<td>Faster growth compared to the control fish when given four injections of bGH once a week Fry treated with low dose of the hormone (0.01 µg per g BW) has faster growth than the group given the higher dose (0.1 µg per g BW) Juveniles grow better in dilute seawater than in full-strength water Survival of the larvae is highest in bigger tanks (3-5 tons) than in smaller tanks (0.0-0.5 ton)</td>
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| Milkfish, *Chanos chanos* | Application of biotechnology in aquaculture | Cloning and sequencing of growth hormone (GH) Cloning of the insulin-like growth factor (IGF-I) GH and IGF-I mRNAs were both detected in milkfish embryos but while GH expression increased as the larvae developed, there was no remarkable change in IGF-I expression from day 1 to day 10 | Dr. Felix Ayson, Dr. Evelyn Grace de Jesus |
| Larval food development | Larvae fed with *P. annandalei* gave better growth to the larvae than with *Artemia* and *Brachionus* | Romeo Caturao |
| AQD formulated diet is an effective supplement for rotifer and alternative for the expensive brine shrimp nauplii for milkfish larviculture | Ilda Borlongan |

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Following the completion of its life cycle in captivity in 1999, AQD documented the induced and natural spawning of snappers in concrete tanks or floating cages. It has also formulated a broodstock diet to ensure egg and larval quality and minimize the use of trash fish. Moreover, an improved larval rearing method was developed using screened rotifers during the early feeding stages of the larvae.

**Grouper**

The continuing refinement of developed culture techniques for the grouper addresses the limited production due to dependence on wild fry supply, fish-by-catch, and parasitic infestations and other diseases. Studies on the grouper hatchery technology is also focused on economic viability and sustainability.

In year 2000, initial results of the effects of the nutritional composition of diets on the productive performance of grouper indicated the advantage using DHA in the diet. A protocol for intensive larval rearing of grouper was also refined.

**Sea bass**

AQD modified the seed production technique for sea bass developed in Thailand to suit local conditions.

One of the studies, which ended in 1998, indicated the correlation of biochemical characteristics of fertilized eggs with egg quality. Another study suggests that mature sea bass can readily spawn by injection of frozen and thawed luteinizing hormone-releasing hormone analogue (LHRHa) solution or by implantation LHRHa pellets stored at room temperature.
On the other hand, the brackishwater cladoceran Diaphanosoma celebensis was tested as partial replacement of the expensive Artemia in larval rearing.

Marine ornamental fishes

The increasing demand for marine ornamental fishes has resulted in the exploitation of coral reef species and depletion of their habitats. To reduce the impact on wild population and ecosystems, AQD is carrying out breeding and seed production techniques for marine ornamental fishes. Methods for producing seahorse juveniles in the hatchery are being studied.

Meanwhile, improvement of captured broodstock and seed production of the blue tang Paracanthurus hepatus was conducted to characterize its spawned eggs and newly hatched larvae. Seed production studies are geared towards the improvement of water management and feeding schemes to increase larval survival.

Larval food

Cheaper substitutes for the expensive Artemia salina and Brachionus plicatilis, the two most commonly used natural food in fish larval rearing are being developed. These potential food substitutes are the copepods Acartia tsuensis (nauplii) and Psuedodiaptomus annandalie, and the AQD-formulated milkfish larval diet.

AQD’s active pursuit of aquaculture technology does not end in the research and development on broodstock management and seed improvement of cultured species. AQD also spearheads the recovery of overexploited wild stocks through the promotion of responsible aquaculture management. This program integrates environmental responsibility with existing aquaculture practices in order to make the industry more sustainable and to secure the region’s food resources.