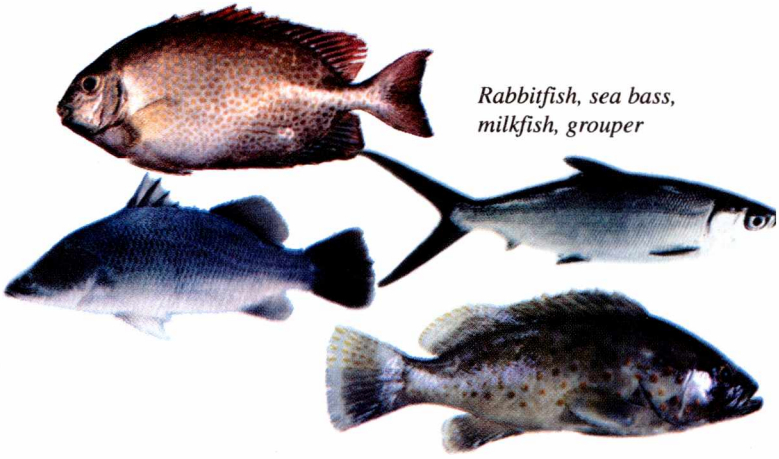

Rabbitfish, sea bass, milkfish, grouper

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 larvae culture or 30,000 eggs per ton for intensive larvae 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 annandalie* 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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Broodstock management and seed production of marine fishes

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 was 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 with 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.

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

Species	*Research focus*	*Research results*	*Expert involved*
Rabbitfish, *Siganus guttatus*	Seed production	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) *Chlorella*-fed rotifers supplemented with Nosan R–1, or (d) *Chorella*-fed rotifers	Marietta Duray
	Application of biotechnology in aquaculture	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	Dr. Felix Ayson, Dr. Evelyn Grace de Jesus
	Intensive seed production	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)	
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. annandalie* 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

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 larvae 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.

Species	Research focus	Research results	Expert involved
Mangrove red snapper, *Lutjanus argentimaculatus*	Broodstock management	Broodstock fed with formulated diet is observed to have similar egg production, viability, and hatching rate per spawn as those fed with trash fish	Dr. Arnil Emata
	Seed production	Newly hatched larvae stocked at 15,000 larvae per ton of water in 3-ton tanks have higher survival than those stocked at 30,000 (4.0%) or 45,000 (5.0%) larvae per ton	Marietta Duray
		Treatment with thyroxine either through bioencapsulation with *Artemia* or by immersion of 25-day old larvae did not improve survival in comparison with the control groups (96.5-98.4%)	
		Growth and survival of 30-day old larvae fed solely on *Artemia* were higher than those given with a mixed diet of *Artemia* and artificial diet (1:1), and artificial diet alone in three weeks	
		Feeding incidence for the newly hatched larvae was equally higher under ambient light rearing than under 24 or 16 light period conditions	
Grouper, *Epinephelus coioides*	Broodstock management	Advantage of using DHA in the diet over other treatments, as far as frequency of spawning and number of eggs produced are concerned	Dr. Veronica Alava, Dr. Gerald Quinitio
	Seed production	Best stage to disinfect eggs with iodine at 75 ppm for 10 minutes is when the embryo starts "twitching"	Eleanor Tendencia
		Survival of the larvae is highest in bigger tanks (3-5 tons) than in smaller tanks (0.5 ton)	Marietta Duray
		Larvae fed with HUFA enriched rotifer were found to be more stress-resistant	Marietta Duray
		Metamorphosis of larvae was enhanced with triiodothyronine, which is applied to the rearing water	Dr. Evelyn Grace de Jesus
	Larval food development	Two to three-day old larvae fed with *Acartia tsuensis* copepod nauplii at their first feeding grew significantly faster and showed higher survival rate compared to those fed with rotifer only	Joebert Toledo
		Acartia nauplii was found to have superior nutritive value than rotifer and is more appropriate for larvae at the early rearing stage	

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.

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SEAFDEC/AQD's R&D milestones on hatchery of marine fish species

Milkfish *Chanos chanos*

1976 first induced spawning of adults from the wild
1978 successful larval rearing in the hatchery
1980 maturation and natural spawning of captive broodstock in floating sea cages
1981 start of the National Bangus Breeding Program (NBBP) to raise broodstocks in floating sea cages at 12 regional sites in the Philippines
1982 extension manual on spawning and larval rearing
1983 completion of the milkfish life cycle in captivity
mass production of fry in the hatchery with eggs coming from natural spawning of captive adults
1984 start of regular training courses in milkfish hatchery
1986 maturation and natural spawning at four NBBP sites
1987 efficient egg collector developed for sea cages
1990 natural spawning of broodstock in concrete tanks
1991 refinement and verification of hatchery techniques
mass production of fry in a private commercial hatchery
1992 technology transferred to more private hatcheries
extension manual on broodstock and spawning
1995 formulation of effective diet for larvae in the hatchery privitization of NBBP stocks
1997 formulation of an effective broodstock diet
1998 improvement of fry quality through enrichment of live food
assessment of commercial milkfish hatcheries
improvement of egg handling and transport
1999 cloning of the GH, hormone the controls growth in the milkfish
development of broodstock transport technique
2000 production of GH using recombinant DNA technology

Grouper *Epinephelus coioides*

1989 broodstocks raised in floating cages and concrete tanks
1989 hormonal sex inversion of females to males
1990 maturation and year-round spawning and larval rearing
1992 first fry production in the hatchery
1994 completion of the grouper life cycle in captivity, intensive hatchery techniques, fry production
development of hatchery techniques
1995 sex-inversed males in natural spawning
1996 improved larval survival by use of copepod nauplii
1997 larval metamorphosis advanced by thyroid hormones
1999 refinement and verification of hatchery techniques

Rabbitfish *Siganus guttatus*

1983 first induced spawning of wild adults, first larval rearing
1985 year-round natural spawning of captive adults, mass production of fry in the hatchery
1986 completion of the rabbitfish life cycle in captivity
1988 refinement of hatchery techniques
start of training courses including rabbitfish hatchery
1989 formulation of diet for early juveniles in the nursery
1999 cloning of the GH, hormone the controls growth in rabbitfish
2000 production of GH using recombinant DNA technology
cloning of insulin-like growth factors (IGF I and II)

Snapper *Lutjanus sp.*

1993 first spawning in captivity
1994 production of fry in an experimental scale
1995 first natural spawning
1999 completion of the snapper lifecycle in captivity

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 plicaltis*, 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 *Pseudodiaptomus 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.