

Research on Marine Fishes

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Abstract

Research on marine fishes at SEAFDEC/AQD from 1995 to date was mostly on milkfish *Chanos chanos*. Studies focused on the refinement of broodstock and seed production techniques to improve egg and larval production as well as to eliminate morphological deformities in hatchery-bred fry. A verification study with former shrimp hatchery operators demonstrated the technical and economic viability of the AQD-generated milkfish hatchery technology. Production and efficiency of semi-intensive grow-out in ponds were enhanced by the use of formulated feeds and appropriate feeding scheme. Milkfish farming in the Philippines was critically reviewed and recommendations to sustain milkfish culture production were made. Tobacco dust and metaldehyde formulation were proposed as alternatives to organotin-based pesticides in controlling the population of pond snail. The growth hormone of milkfish has been isolated and purified.

Addition of highly unsaturated fatty acid (HUFA)-rich oils in the diet did not improve the quality of spawned eggs of grouper *Epinephelus coioides*. A protocol for the intensive larval rearing of grouper was developed based on the results of several studies. A semi-intensive seed production method using copepod nauplii during the early feeding stages was also developed as an alternative to intensive method. Metamorphosis of larvae was significantly accelerated by exogenous thyroid hormones. Nutritional studies to reduce the amount of fish meal in grouper diets are in progress. Groupers grown in ponds or cages harbor a variety of parasites.

Biochemical criteria to assess the quality of spawned Asian sea bass *Lates calcarifer* eggs was characterized. *Diaphanosoma* or other copepods may be an alternative or supplemental live prey to *Artemia* during sea bass larviculture. A practical diet for sea bass culture was developed. Studies to determine the essential amino acid requirements of sea bass are about to be completed. The effects of immuno-stimulants in sea bass are presented.

Induced and natural spawning of mangrove red snapper *Lutjanus argentimaculatus* in concrete tanks or floating net cages has been documented. An improved larval rearing method has been developed using screened rotifers during the early feeding stage of the larvae. Exogenous thyroid hormones have advanced metamorphosis of larvae. A practical diet for snapper is under development.

Research on rabbitfish *Siganus guttatus* were geared to developing tools for growth enhancement. Pituitary growth hormone (GH) has been cloned, allowing

the production of recombinant rabbitfish GH. Rabbitfish prolactin, somatolactin have also been purified.

Studies on marine ornamental fish focused on two species of seahorses, *Hippocampus kuda* and *H. barbouri*, and on blue tang *Paracanthurus hepatus*. Progress on the biology, breeding, and seed production of seahorses are presented. Successive natural spawnings of blue tang in concrete circular tank have been recorded.

Introduction

Research on marine fishes at the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) from 1995 to date followed the list of priority species and recommendations of the Seminar-workshop on Aquaculture Development in Southeast Asia (ADSEA) in 1994. On top of the priority list was milkfish *Chanos chanos*, followed by grouper *Epinephelus coioides*, mangrove red snapper *Lutjanus argentimaculatus*, Asian sea bass *Lates calcarifer*, rabbitfish *Siganus guttatus*, striped mullet *Mugil cephalus*, and marine ornamental fishes. Except for striped mullet, SEAFDEC/AQD has addressed most of the recommended research areas in the other species. This paper presents the highlights on marine fish research at SEAFDEC/AQD from 1995 to 1999.

Milkfish

Broodstock management

Studies were continued on broodstock nutrition to further improve the quality of spawned eggs. More spawns and higher egg viability were observed in broodstock fed diets supplemented with vitamin C alone or in combination with Vitamin E than those fish fed without vitamin supplementation. Cumulative survival rates from eggs to normal larvae appeared to be higher in spawns of broodstock fed vitamin C alone or in combination with vitamin E (Emata et al., 2000).

A protocol for the transport of milkfish broodstock was developed (Emata, 2000; Garcia, et al., 2000). All milkfish broodstock survived after 10 h of overland transport in sealed oxygenated plastic bags with chilled (20-24 C) and diluted (28 ppt) seawater. Spawning of sexually mature milkfish subjected to such transport stress was not impaired.

Seed production

Hilomen-Garcia (1997) characterized the morphological abnormalities in hatchery-bred milkfish fry and juveniles. Abnormalities predominantly appeared as a cleft on the branchiostegal membrane and as a deformed operculum. The occurrence of these abnormalities in hatchery-bred juveniles reared in commercial ponds was highly variable (3-26%). Further observations suggest that such morphological abnormalities do not only affect the appearance of the fish but also impair growth and survival.

Several studies were conducted to reduce if not eliminate morphological abnormalities in hatchery-bred milkfish. Eggs transported at the "eyed stage" had higher viability compared to those transported at the cleavage, blastula or gastrula stage (Toledo et al., 1996a). Although no significant difference in survival was observed, the percentage of 45 day-old larvae with apparent morphological abnormalities was lower in groups transported at the "eyed" stage compared to other stages tested. The shock sensitivity of milkfish eggs was highest during cleavage until the early segmentation stage, rapidly declined as segmentation proceeded until the head and tail started to separate from the yolk, but returned

to high levels when the embryo began twitching and the heart beating until hatching (Hilomen-Garcia, 1998). These results suggest that the sensitivity of milkfish eggs to mechanical shock varies during development and C-shaped embryos may be manipulated or transported with minimum risk or injury.

The aerobic bacterial flora of milkfish eggs and larvae was examined (Fernandez *et al.*, 1996). *Pseudomonas* was the dominant bacteria in eggs and incubation water. Bacterial number in larvae and in rearing water increased during the culture period. *Pseudomonas* was detected as the dominant bacteria in the yolk-sac larvae. *Vibrio* predominated in the intestines as soon as the yolk-sac was absorbed among Day 3 larvae.

Duray (1995) examined the effect of tank color and rotifer density on rotifer ingestion, growth, and survival of milkfish larvae. The number of ingested rotifer, growth and survival of larvae were higher in black tanks than in tan tanks.

Enrichment of rotifer and *Artemia* with highly unsaturated fatty acid (HUFA) before feeding to milkfish larvae enhanced growth and resistance to salinity stress but not overall survival (Gapasin *et al.*, 1998). Forty-day-old milkfish previously fed HUFA+ vitamin C-enriched live food had significantly lower incidence of opercular deformities (8-15%) compared with those given HUFA-enriched (16-24%) or un-enriched (27-34%) food.

A nutritionally balanced and cost-effective diet for milkfish larvae was developed (Borlongan *et al.*, 2000). This diet could be fed to milkfish larvae in combination with rotifers starting 2 or 8 days post hatching, and could be fed alone starting from Day 15.

Semi-intensive seed production of milkfish was developed as an alternative to the intensive system (JD Toledo, unpublished data). Milkfish larvae were stocked in 10-ton tanks at 5 to 10 individuals/l and provided *Acartia* and *Pseudodiaptomus* nauplii and rotifers as food. Supplementary artificial feed was given from Day 10 onwards. Survival at harvest (Day 18) ranged from 42 to 80%.

Four hatcheries formerly used in shrimp seed production were selected to verify SEAFDEC/AQD's milkfish hatchery technology and to assess its economic viability (Garcia *et al.*, 1999). Two hatcheries were classified as large scale and the rest as small scale. Survival rates at harvest ranged from 15 to 42%. Cost-return analysis showed high profits for both types of operation. Return on investments varied from 54 to 61% with a payback period of 1.5 years.

Grow-out culture

Bagarinao (1998) extensively discussed the alleged milkfish fry shortage in the Philippines and the supply from hatcheries and fisheries. SEAFDEC/AQD in collaboration with the Bureau of Fisheries and Aquatic Resources (BFAR) and the International Center for Living Resources Management (ICLARM) also conducted a milkfish fry assessment study in five selected sites in the country. Generally, the supply of milkfish fry from traditional fry grounds is decreasing. As perceived by the fry gatherers themselves, the decline in catch is likely attributed to environmental degradation, pollution, and illegal catching of milkfish spawners. With the increase in demand due to intensification of grow-out operations, hatchery production should therefore be intensified to supplement production from wild sources.

Artificial feeds for nursery rearing of milkfish larvae have been formulated (Alava and Kanazawa, 1996). Growth and survival of larvae were higher at lower salinity and dietary level but was not affected by lipid source (cod liver or coconut oil). Overall, diets with 9% lipid promoted better growth than those with 18% lipid content (Alava, 1998).

Sulfide toxicity can be a causative factor in fish kills of milkfish and tilapia in brackish and marine waters. Bagarinao (1998) showed that sulfide, hypoxia, and acidic conditions are each toxic to milkfish at particular levels and aggravate each other's toxicity. Recommendations to avoid fish kills were made such as the maintenance of fully oxygenated conditions to avoid build-up of sulfide, ammonia, carbon dioxide, and other toxic substances.

Sumagaysay and Borlongan (1995) determined the most economical combination of dietary protein and feeding levels for semi-intensive milkfish culture in brackishwater ponds. Partial budgeting analysis shows that bigger profits can be earned with a 24% diet with balanced amino acids at a feeding rate of 4% of body weight daily. However, supplemental feeding should not exceed 38 kg/ha/day to maintain good water quality (Sumagaysay, 1998).

In contrast, a feeding rate of 4% could be wasteful and may be reduced to 2% of body weight per day (SEAFDEC/AQD, 1998). About 60-70% of the particles in the gut of milkfish in semi-intensive ponds are detritus. Natural food such as *lablab* makes up 15-20% and formulated feeds make up the remainder. Because milkfish do not feed in the morning when the dissolved oxygen is low, feeds should be given only from mid-morning when oxygen level is adequate for feeding.

The pond snail *Cerithidea cingulata* are long considered as pests in brackishwater ponds. Research on the biology of pond snails shows that: 1) they are deposit feeders; 2) they are able to retract into their shell and survive anaerobic conditions for extended periods; 3) they are intolerant of fresh water, sun-drying, very high and low pH, and very high ammonia levels; 4) they grow fast and sexually mature in one year of age; 5) 60-90% of the snails have mature gonads and spawn between March and November; 6) they have high fecundity, high hatching rates, low dispersal, and high recruitment in the pond system (T. Bagarinao and I. Olaguer, personal communication). Recommendations for an integrated management of the pond environment to control snail population were made.

Organotin pesticides (Brestan or Aquatin) have been used to control the population of pond snails in milkfish ponds in the Philippines. Coloso and Borlongan (1999) demonstrated that tin and triphenyltin residues accumulate in the sediment of brackish water ponds and milkfish tissue samples. The ban on organotin usage in milkfish pond should be strictly implemented to reduce the threat of this pesticide to the environment, natural resources, and public health.

Viable alternative methods to the use of organotins in brackish water ponds were developed. Snail mortalities (86-87%) in experimental ponds did not vary significantly 7 days after application of 80 to 120 kg/ha of 10% metaldehyde formulation (Coloso *et al.*, 1998). In field trials, in a heavily infested pond (more than 2000 snails/m²), a dose of 120 kg/ha was effective under both dry and wet conditions. In a moderately infested pond (less than 2000 snails/m²), a dose of 80 kg/ha of 10% metaldehyde formulation was effective under dry conditions but a dose of 120 kg/ha was needed under wet conditions. As metaldehyde is rapidly degraded in the aquatic system, its efficacy in controlling pond snails depends on a high initial dosage.

Tobacco dust is toxic to pond snails at certain levels (Borlongan *et al.*, 1998). The 72-h LC₉₉ (lethal concentration)₉₉ of tobacco dust with 2.8% nicotine content are 290,522 and 720 kg/ha for juvenile, sub-adult, and adult snails, respectively. LC₉₉ increased as the life stages or the size of the snails increased. The nicotine content of the tobacco dust directly affects its effective application rate. Milkfish juveniles can tolerate nicotine concentrations lethal to the pond snails.

Bagarinao (1998) revealed a disturbing trend in the milkfish industry in the Philippines: decline in production, continued low average yields, high production costs, and unfavorable market forces. During the past decade, the annual milkfish production declined and fluctuated to about 150,000 mt

from a peak of 240,000 mt in 1982, due to the shifting of farms to shrimp production, loss of ponds in Central Luzon to lahar, the non-renewal of Fishpond Lease Agreements, and low farm gate prices. The decline in milkfish production in fish pens in Laguna Lake was traced to domestic and agricultural pollution, and overcrowding of fish pens. Milkfish farming in marine pens and cages proliferated after it started in 1995 in Davao, Pangasinan, and Quezon. Harvests reached very high levels but fell drastically soon when the carrying capacity was exceeded in most sites and the cost of cages and feeds became prohibitive. Likewise, many pens and cages were ordered dismantled or were destroyed by storms.

The cDNAs encoding milkfish growth hormone (GH) and insulin-like growth factor-I (IGF-I) were cloned and sequenced (EGT de Jesus, personal communication). Milkfish GH and IGF-I cDNAs show higher sequence identity with carp and salmonid GH and IGF-I cDNAs than GH and IGF-I cDNAs of other teleosts.

Grouper

Broodstock management and seed production

The use of 17-alpha methyltestosterone (MT) to induce sex reversal in *E. coioides* was continued following the pioneering study by Tan-Fermin (1992). MT in silastic capsules was implanted to adult females at a dose of 4 mg/kg BW. Functional males were observed after 7-10 weeks of hormone implantation. Spermiation was maintained by implanting MT capsules every 3 months (Marte *et al.*, 1995).

Sex inversion in grouper can also be enhanced by manipulating the social environment of the spawners. A large female reared separately with smaller males in tank or floating net cage may naturally change to a male after 1-2 months (Quinitio *et al.*, 1997).

High variability of egg quality in *E. coioides* is partly due to the inconsistent quality of fish by-catch fed to broodstock. Enrichment of fish by-catch with oils rich in HUFA did not improve egg production, spawning frequency, fertilization and hatching rates, and egg viability (Quinitio *et al.*, 1996). Likewise, naturally spawned grouper eggs should be collected after neurulation when the optic vesicles are already formed to increase egg viability and hatching as well as to reduce mortality and occurrence of embryos (Caberoy and Quinitio, 1998).

Incorporating the results of previous studies (Duray *et al.*, 1995; Duray *et al.*, 1996b; Chavez *et al.*, 1995), a protocol for the intensive seed production of grouper was developed (Duray *et al.* 1997). Larvae are initially fed screened rotifers previously enriched with high HUFA boosters. Enriched *Artemia* nauplii or *Anemia* meta-nauplii are fed from Day 20 until metamorphosis when larvae are able to feed on minced fish.

Semi-intensive larval rearing was also developed (Toledo *et al.*, 1999). To propagate copepod nauplii in larval tanks, sub-adult and adult copepods, *Acartia tsuensis* and/or *Pseudodiaptomus amandalei*, collected from brackish water ponds are inoculated in the larval tanks 2-3 days before stocking of larvae (not more than 10 larvae/1). First-feeding grouper larvae preferred copepod nauplii than rotifer. Larvae fed copepod nauplii survived and grew better than larvae fed rotifers (Doi *et al.*, 1997; Toledo *et al.*, 1996b and 1997). *Acartia* contains high levels of HUFA particularly docosahexanoic acid (DHA), known as an essential fatty acid for marine fish larvae. Optimum densities of copepodids and adults of *Acartia* and *Pseudodiaptomus* to consequently produce nauplii for early feeding larvae were also determined (Toledo *et al.*, 1999). Studies to develop mass production techniques for *Acartia* and *Pseudodiaptomus* are ongoing.

Grouper larvae show an ontogenetic shift to changes in salinity and temperature (GF Quinitio and NB Caberoy, personal communication). Day 20 larvae preferred salinities of 8-32 ppt at 25 C but these were narrowed to 8-18 ppt at 30 C. At Day 40, survival were similar between 8 and 40 ppt at 25 and 30 C despite changes in gill Na⁺,K⁺-ATPase activity and chloride cell morphology. No major changes were observed in enzyme activity, chloride cell morphology as well as plasma osmolality in Day 60 juveniles.

The process of metamorphosis in grouper requires more than two weeks to be completed. Thyroid hormones, triiodothyronine (T₃) or thyroxine (T₄) accelerate metamorphosis in grouper larvae in a dose-dependent manner (de Jesus *et al.*, 1998). Three-week old larvae immersed in 1 ppm T₃ or T₄ completed metamorphosis within 2 days. Larvae treated with 0.01 ppm of the hormones completed metamorphosis in 5-6 days whereas, untreated controls took 10-21 days to complete metamorphosis. Thyroid hormone treatment after collection and during transport did not improve the post-transport survival of wild-caught pre-metamorphic grouper larvae. Post-transport mortalities were associated with the sudden shift of diet from live prey to minced trash fish or formulated feeds and the sudden confinement of un-domesticated larvae to smaller space (MC Estudillo, personal communication).

Grow-out

Nursery and grow-out for hatchery-bred juveniles were developed in earthen-ponds (IB Tuburan *et al.*, personal communication). Survival ranged from 50 to 67% and from 72 to 85% in nursery and grow-out, respectively. Economic analysis shows both culture systems as highly profitable with a payback period of 1.7 years. Morphological abnormalities in hatchery-bred grouper such as open operculum, depressed antero-dorsal fins, and deformed lower jaw were noted.

Nutritional studies to eliminate or decrease dependence on trash fish to feed groupers were undertaken. Best growth and survival of grouper fry were obtained in diets containing 42% protein, 10% lipid, and a supplementation of 1% HUFA. Poorest weight gain was observed in diets without HUFA supplement (OM Millamena, personal communication).

Research aimed at reducing the fish meal component of artificial diets for grouper is currently being undertaken. The apparent digestibility of locally available protein sources and its acceptability in a compounded diet for grouper juveniles is presently examined. Preliminary results indicate that white cowpea meal can be used as a partial replacement for fishmeal in grouper diets (PS Eusebio, personal communication). Other protein sources such as slaughterhouse by-products are being tested. Up to 60% of fish meal may be replaced by blood meal in artificial diet for grouper juveniles (OM Millamena, personal communication).

Disease among cultured grouper is an emerging problem. Higher diversity of parasites and intensity of infection were observed in groupers grown in floating net cages than in brackishwater ponds (E Cruz-Lacierda, personal communication). Cage- and pond-reared grouper harbor various species of protozoa, parasitic worms, and nematodes. Research on the life cycle of a predominant monogenean infecting grouper is now ongoing (GE Pagador, personal communication).

Mangrove Red Snapper

Broodstock and seed production

Wild-caught mangrove red snapper adults reared in floating net cages or in concrete tanks are sexually mature from April to October. Successful spawning of spermiating males and mature females with mean oocyte diameter of at least 0.42 mm injected 1,000 IU hCG/kg or 100 µg LHRHa/kg body

weight occurred from June to October but not on April or May. Latency period was 32-40 hours after hormone injection. Egg and larval quality were highly variable (AC Emata, personal communication). Broodstock from wild-caught or hatchery-produced fry reared in floating net cages or concrete tanks were sexually mature after 5 years among males and 6 years among females. Natural spawning was observed in cages and tanks stocked at 1:1 or 3:4 (female:male) sex ratio. Spawning occurred up to 4 consecutive days with egg collection ranging from 250,000 to 1 million eggs per spawning. Egg larval quality was more superior than those produced from induced spawning (AC Emata, personal communication).

Duray *et al.* (1996a) describe an improved hatchery rearing of mangrove red snapper larvae in large tanks using small rotifer and *Artemia*. The feeding regime consisted of *Chlorella*, *Brachionus*, *Artemia*, and minced trash fish. Best survival was achieved when larvae were fed screened *Brachionus* (<90 μm) during the first 14 days. Larvae fed *Artemia* at 2 per ml had highest survival when ration was given four times a day. Studies on the effects of supplement feeding, stocking density, and continuous lighting on the growth and survival of snapper larvae is ongoing (MN Duray, personal communication). Tolerance of *L. argentimaculatus* larvae to abrupt change in salinity was related with age (CB Estudillo, personal communication). Tolerance to abrupt salinity change increased remarkably starting on Day 28. Average survival was significantly higher at 16 ppt (7.6%) than at 40 ppt (4.3%) during the first 21 days of rearing. However, no significant difference in survival was observed among the salinities tested at the end of the second phase of rearing.

Thyroid hormones accelerate metamorphosis in *L. argentimaculatus* larvae. Three week-old larvae immersed in 0.01 ppm T_3 or T_4 completed metamorphosis after 1 week while no larvae metamorphosed in the thiourea-treated or untreated control groups. Five-week old larvae showed a dose-dependent response to both hormones (EGT de Jesus, personal communication).

Grow-out

Six diets with three protein (35, 42.5, and 50 %) and two lipid (6 and 12 %) levels were tested to formulate a practical diet for red snapper (MR Catacutan, personal communication). After 100 days of culture with 100% survival in all treatment groups, fish attained a final average weight between 116 and 165 gm from an initial mean weight of 25 gm. The optimum protein level was found to be 42.5%. Because fish carcass of samples from fish fed 6% lipid still contained 27-34% crude fat, it is likely that lipid level in the diet could still be lowered. The preferred protein-energy ratio was estimated at 130 mg protein/kcal.

After 98 days of culture, substitution of soybean meal to 30% of a control diet was found adequate. Substitution of defatted soybean meal higher than 30% adversely affected liver histology (MR Catacutan, personal communication). The formulated practical diet will be tested in pond- and/or cage-culture to further determine its bio-technical and economic feasibility.

Sea Bass

Broodstock and seed production

To establish a criteria for egg quality assessment, biochemical characteristics of fertilized sea bass eggs was correlated with egg quality. Positive correlation was observed among the following: total saturated fatty acids, phosphoserine, and aspartic levels with fertilization rates; DHA/EPA (eicosapentanoic acid) level with hatching rate; and DHA and aspartic acid content with normal zygotes. Arginine levels in spawned were negatively correlated with hatching rate (Nocillado *et al.*, 2000).

Mature sea bass readily spawn after injection or implantation of hormones such as luteinizing hormone releasing hormone analog (LHRHa). The spawning response of mature fish was significantly reduced after injection of dissolved LHRHa stored for more than 90 days in a refrigerator (4-10 C) or for more than 30 days at room temperature (28-30C). In contrast, mature fish spawned successfully after injection of an LHRHa solution subjected to alternate freezing and thawing or implanted with LHRHa in pellet-form stored at room temperature (Garcia, 1996).

Research to reduce dependence on *Artemia* in sea bass seed production was continued. Nursery rearing may be done in illuminated floating net cages (Fermin *et al.*, 1996; Fermin and Seronay, 1997). High zooplankton abundance at 300 lux consequently increased feeding incidence, gut content, specific growth rate, and survival of the larvae. Growth and survival were enhanced in larvae fed minced trash fish during the day. Partial replacement of *Artemia* by the brackishwater cladoceran, *Diaphanosoma celebensis*, in the larval rearing of sea bass was reported by dela Pena *et al.* (1998). Although *Diaphanosoma* contains higher crude protein and crude fat than *Artemia*, its percentage of n-3 HUFA particularly EPA and DHA was lower than *Artemia*.

Grow-out

Research to ascertain the essential amino acid requirements of sea bass juveniles is almost completed. The essential amino acid levels for arginine, lysine, methionine, threonine, tryptophan, phenylalanine, and histidine was already determined while experiments for isoleucine, leucine, and valine is ongoing (RM Coloso, personal communication).

Catacutan and Coloso (1997) formulated a practical diet for sea bass containing 20% carbohydrate, 10-12 % lipid (1:1 mixture of cod liver oil and soy bean oil), and 42 % dietary protein with an energy level of about 337-358 kcal/100 gm diet and a protein:energy ratio close to 128 mg of protein per kcal. White cowpea and green mungbean meals can be used to replace 18% of the protein sources in practical diets for sea bass without affecting their growth (Eusebio and Coloso, 2000).

Experiments on disease prevention and control focused on the use of immuno-stimulants on the non-specific immune response of sea bass. Based on plasma lysozyme levels, betaglucans and levamisole enhance the nonspecific immune response while handling/transfer stress and poor water quality negatively affect the immune response of sea bass (JME Almendras, personal communication).

Rabbitfish

Research activities on rabbitfish were geared towards developing tools for growth enhancement in fish. GH was isolated from pituitary glands of rabbitfish by gel filtration and high performance liquid chromatography (Ayson *et al.*, 1999). The yield of pure GH was 1 mg/gm wet weight of pituitary glands. In the process of purifying GH, prolactin, and somatolactin, two pituitary hormones related to GH, were also purified. Antiserum against rabbitfish GH was produced in rabbits.

Rabbitfish GH cDNA was cloned for recombinant rabbitfish GH production (FG Ayson, personal communication). Excluding the poly-A tail, rabbitfish GH cDNA is 860 base pairs long. It contains a 588 base pair open reading frame encoding a signal peptide of 18 amino acids and a mature protein of 178 amino acid residues.

Marine Ornamental Fishes

Breeding and seed production of seahorses

Studies on the breeding and seed production of seahorses focused on two species, *Hippocampus kuda* and *H. barbouri*. After 90 days, parturition events (7-8/pair) and greater brood size (87-91 juveniles/gm female) were higher in *H. kuda* fed HUFA-enriched *Artemia* adults, mysids, and tilapia fry than those fed *Artemia* adults alone (2-3 parturition/pair and 18-26 juveniles/gm female). When groups of newly born *H. kuda* were fed *Brachionus* alone, copepods alone, or their combination, only seahorses on a combination diet survived at Day 10 (GH Garcia, unpublished data).

Results of experiments on the effect of illumination on daily feeding pattern of *H. kuda* indicate that seahorses may not eat for 12 h when food is not available but will compensate for the 12 h non-feeding period when food becomes available. Food consumption of *H. kuda* juveniles was significantly higher during daytime than during nighttime. Under natural photoperiod, a distinct diurnal feeding behavior was also observed in *H. barbouri* (GH Garcia, personal communication).

Simulated transport experiments on 33-day old hatchery-bred *H. kuda* showed that seahorses were grasping each other by the tail and had higher survival rates at higher loading densities (10 and 20 juveniles/500 ml) than those at lower densities (5 juveniles/500 ml). This result suggests the need of providing a holdfast during transport at low densities (GH Garcia, unpublished data).

Broodstock management of marine ornamental fish

Successive spawnings in the blue tang *Paracanthurus hepatus* have been documented. Spawning occurred for 5-12 consecutive days with egg collection ranging from 5,000 to 30,000 eggs per day. All larvae died 6 to 7 days post-hatching. Studies to determine the appropriate food and environmental conditions for the first-feeding larvae are going on.

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