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MARINE FISH BROODSTOCK DEVELOPMENT AT SEAFDEC/AQD: STATUS AND ADVANCES

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The success of a fish culture operation depends, in part, on adequate supply of seed for hatchery and grow-out. The culture and husbandry of many of Southeast Asia's marine fish species are constrained by the unreliability of seed supply which are seasonally gathered from coastal areas. To augment the natural seed supply and decrease the dependence on wild catch, SEAFDEC/AQD has undertaken studies to develop a captive source of breeders for some of the economically important marine fish species in the region.

This paper presents a brief update of the status and recent advances in marine fish broodstock development undertaken by SEAFDEC/AQD.

Grouper (*Epinephelus* spp.)

Initial work on broodstock development of grouper at SEAFDEC/AQD began in 1986. Spawning of mature *E. salmoides* following two injections of luteinizing hormone-releasing hormone analogue (LHRHa) or human chorionic gonadotropin (HCG) and carp pituitary extract has been reported (Kungvankij et al. 1986). The treatment protocol was not, however, further duplicated nor improved in recent years due to lack of captive spawners. Subsequent efforts, therefore, concentrated on obtaining wild spawners, which were then reared in concrete tanks or sea cages.

Since grouper is a protogynous hermaphrodite, captive young juvenile groupers reared until the onset of gonadal maturation are most likely females, which invert into males at an unknown age. Wild spawners were usually females and very rarely were male grouper caught. Methods to control sex inversion in this species was, therefore, initiated in 1989. Two synthetic androgens, methyltestosterone (MT) and mibolerone, were used. MT in silastic capsules failed to induce sex inversion in adult female grouper (3-9 kg body weight) after 7 monthly implantations (SEAFDEC/AQD 1989). However, 3 months after termination of biweekly MT injections (0.5-5 mg/kg), histological signs of sex inversion in the gonads were noted (Tan-Fermin et al. 1989). Fish weighing more than 1.2 kg underwent spermatogenesis, but fish receiving MT and the hormone produced milt. Similarly, results indicate that juvenile grouper fed a moist diet enriched with mibolerone did not invert sex (SEAFDEC/

AQD 1989,1990). Intra-specific interaction of juvenile grouper of varying sizes held in communal tanks did not effectively invert sex of the largest individual in the group (SEAFDEC/AQD 1990). Only about 4% of the females possessed a transitional ovotestis 11 weeks after social interaction commenced.

The first spontaneous spawning of adult *E. suillus* broodstock in the Philippines was achieved in 1990 among fish reared in 50-t concrete tanks (6 females to 4 males) and in sea cages (1 female to 2 males). Tank-reared broodstock spawned year-round from 6 to 14 times monthly (SEAFDEC/AQD 1990). Cage-reared broodstock, however, spawn only from July to October, coinciding with the known breeding season of grouper in the Philippines.

Milkfish (*Chanos chanos*)

Initial research on milkfish focused on the biology of wild adults *(sabalo)* in Panay Island. Ecological studies to determine the age, spawning sites, migration patterns, and external sex markers of adult milkfish were conducted (Juario et al. 1983). This information was critical in the effort to use wild adults as source of seed for hatchery production of milkfish fry.

The availability of wild milkfish adults caught by fish traps during their annual spawning migration along the Panay coastline provided material to test the effectiveness of hypophysation to induce spawning. Following the success of semi-purified salmon gonadotropin (SG-G100) to induce ovulation in milkfish (SEAFDEC/AQD 1976), the use of a salmon pituitary homogenate (SPH) and HCG was further standardized to determine the optimum hormone dose, injection, intervals, and initial egg size required to trigger spawning. About 20 mg SPH and 3000 IU HCG per kg administered in 2 injections at 12-24 h intervals were required to induce spawning in fish with an initial egg diameter of 0.66 mm (Juario et al. 1984). Vitamin B was also injected to "counteract captive- and handling-related stress" of *sabalo* (Liao et al. 1979). About 5000 IU HCG and a long-acting testosterone formulation maintained milt production for a week in contrast to only 3 days when HCG alone was used (Juario et al. 1980).

In addition to hypophysation, LHRHa was also tested in mature milkfish. Initial results of LHRHa combined with HCG injection either failed to induce spawning when the initial egg size was 0.99 mm (SEAFDEC/AQD 1983) or fish underwent ovarian hydration to complete release of eggs (SEAFDEC/AQD 1986). Subsequent results, however, indicated that LHRHa administered by injection or pellet implantation to promote chronic release of the incorporated hormone can induce spawning of milkfish 18-36 h after administration (Marte et al. 1987). LHRHa contained in an osmotic pump was less effective than either pellet-implantation or injection.

Attempts to initiate and advance gonadal development of sexually immature adult milkfish and rematuration of spent *sabalo* by SPH, LHRHa, and estrogen failed (SEAFDEC/AQD 1980, 1981, 1982), in part, because "gonadotropin" remained in circulation for 1-2 days only (Marte and Crim 1983). However, hormone-implanted milkfish were sexually mature in a cage several months after termination of the hormone therapy (SEAFDEC/AQD 1980). Testosterone implantation of 3- and 4-year old fish resulted only in males

becoming mature (SEAFDEC/AQD 1984). Tank-reared 4-year old milkfish were mature after chronic administration of LHRHa and testosterone (SEAFDEC/AQD 1986; Marte et al. 1988). These variable results suggest that milkfish may have different hormonal requirements for maturation, re-maturation, and spawning.

Cryopreservation of gametes becomes an alternative when the supply of wild broodstock is depleted or when gametes which are not synchronously released are required for artificial fertilization. Milkfish serum remains as the best extender for cryopreservation of milkfish milt (SEAFDEC/AQD 1981; Hara et al. 1982). When used to fertilize eggs, frozen milt in glycerine had higher fertilization rates than milt with dimethyl sulphoxide as cryoprotectant.

Spontaneous maturation and spawning of cage-reared broodstock was first achieved in 1980 and has since been repeated yearly during the breeding season of milkfish in Panay and elsewhere in the Philippines (Marte and Lacanilao 1986; DA-BFAR 1989). Collection of naturally spawned eggs in sea cages has also been markedly improved by the development of an egg sweeper and a fine mesh *hapa* net to retain eggs inside the maturation cage (Garcia et al. 1988; Marte 1988). Likewise, adult milkfish reared in concrete tanks have also undergone spontaneous maturation and spawning since 1990 (Emata and Marte 1990). Breaking all previous records, a combined total of more than 70 million eggs from more than 50 spawnings by milkfish breeders in Igang and Tigbauan have been collected in 1992. Nutritional studies to improve the quality of milkfish seed suggest that a lipid-enriched diet may advance spawning or enhance egg production by cage-reared broodstock (SEAFDEC/AQD 1987).

Further, morphological and physiological changes occurring during sexual maturation of milkfish have been characterized. Although blood parameters appeared variable during maturation, pituitary cells showed distinct morphological changes during the annual gonadal cycle (Tan 1985). A female-specific protein (vitellogenin) characterized in milkfish plasma may be used to distinguish sexes (SEAFDEC/AQD 1989).

Snapper (*Lutjanus* spp.)

Wild snappers of unknown species caught in coastal waters off Panay Island are only recently being domesticated in sea cages and in concrete tanks. Fish have spontaneously matured in these holding structures, but failed to spawn naturally. Recently, mature snapper in circular concrete tanks have been induced to spawn 27 h after an injection of 1500 IU HCG/kg (ADN 1992). Eggs collected by air-lift and manual seining totalled about 1.3 million, of which 95% were fertilized.

Sea bass (*Lates calcarifer*)

Like milkfish, research on sea bass breeding was initiated by the successful spawning of hypophysed mature broodstock (SEAFDEC/AQD 1983). Several analogues of LHRHa administered in various ways (injection, cholesterol pellet,

osmotic pump, and silastic-based implants) were also successful in inducing single or consecutive spawnings at 24 h intervals over 4-5 days (Nacario and Sherwood 1986; Almendras et al. 1988).

Further improvement of the use of LHRHa was also achieved by defining the optimum dose required to induce single or consecutive spawnings. Implantation of pelleted LHRHa within the range 4.75-75 μg/kg stimulated a dose-dependent spawning response while higher dosages (150-300 μg/kg) resulted in significantly fewer spawnings (Toledo et al. 1991). Fertilization rates were low after implantation of the highest pelleted LHRHa dose (300 μg/kg), but hatching rates were not significantly different after induction of consecutive spawnings by various LHRHa dosages. Egg production peaked on the first day of consecutive spawnings, but declined on subsequent days. Similarly, single or consecutive spawnings by mature sea bass may be triggered by various amounts of injected LHRHa (Garcia 1989b). A single injection of 5 μg LHRHa/kg or less resulted in at least one spawning every 4 days. Higher dosages of LHRHa (10 μg/kg and above) stimulated more than one spawning in 4 days. Egg production, fertilization and hatching rates were not significantly influenced by various amounts (1-10 μg/kg) of the injected hormone.

To further refine the LHRHa spawning protocol, a technique was developed to obtain intra-ovarian oocytes for a more reliable means of determining the initial ovarian condition of fish prior to hormone treatment (Garcia 1989c). Eggs can be routinely sampled with a polyethylene tubing inserted into the genital pore of sea bass and then fixed in 5% buffered formalin for egg diameter measurement. The spawning response of sea bass to LHRHa can be better predicted if the initial egg size of recipient fish becomes known. Hence, mature sea bass with an initial egg diameter of 0.40-0.49 mm spawned after an injection of 100 μg LHRHa/kg, but fish with an initial egg size of less than 0.40 mm did not (Garcia 1989b). Fish having an initial egg diameter of 0.50-0.55 mm may spawn spontaneously with or without exogenous LHRHa. Also, the time of hormone injection can be manipulated to maximize the number of eggs spawned by LHRHa-treated sea bass (Garcia 1990a). Egg production was greater among LHRHa-injected fish which spawned at dawn than during the day. Sea bass spawn at dawn when LHRHa was administered during daytime.

One of the major objectives of fish breeding is to manipulate gonadal cycles to be independent of the annual or diurnal periodicities, so that gametes for seed production become available on demand. Chronic implantation of pelleted LHRHa and MT to cage-reared sea bass broodstock has partly achieved this objective. A hormonal therapy consisting of regular implantation of LHRHa alone or in combination with MT during the off-season months of February until March advanced gonadal maturation and spawning in May, which is earlier than the peak spawning months of July and August (Garcia 1990b). Attempts on off-season maturation of sea bass by hormonal and photoperiodic cues are in progress (SEAFDEC/AQD 1990). During the breeding season, spontaneous spawning of cage-reared sea bass coincide with the declining spring tides of quarter moons (Garcia 1992; Toledo et al. 1991). LHRHa treatment, however, can effectively override the lunar- and tide-synchronized spawning rhythm.

Although further studies are required, the milt response of sea bass to both LHRHa and MT suggests a potential role of this particular spawning protocol in the improvement of sperm production and milt dilution, and enhancement of spontaneous milt release (Garcia 1992). In addition, the maintenance of the biological potency of LHRHa can be improved by proper handling, preparation, and storage of the hormone. Hence, sea bass spawned after an injection of solubilized LHRHa stored for less than 90 days in a refrigerator (4-10°C) or 30 days at room temperature (28-30°C) (SEAFDEC/AQD 1990). Also, pelleted LHRHa stored for 120 days at room temperature retained its potency.

Rabbitfish (*Siganus guttatus*)

Earlier studies on breeding rabbitfish involved multiple injections of an anti-estrogenic compound (clomiphene citrate) in combination with HCG (SEAFDEC/AQD 1982). Later studies proved the effectiveness of HCG alone to induce spawning of mature rabbitfish (Juario et al. 1985) although fish with an initial egg diameter of at least 0.46 mm and greater did not require hormone treatment (Ayson 1991). Fish with an initial egg size of <0.45 mm require multiple HCG injections (2 IU/g) (Juario et al. 1985). Handling-associated stress also enhanced spawning of rabbitfish comparable with HCG-injected fish (Ayson 1989). In addition to HCG, silastic-based LHRHa implantation advanced spawning of female rabbitfish 1-2 days earlier than sham controls (Harvey et al. 1985). LHRHa also stimulated milt production and milt dilution a day after hormone injection (Garcia 1991).

Rabbitfish broodstock may spawn year-round without exogenous hormone intervention. Female rabbitfish fed a cod liver oil-rich diet spawned repeatedly for at least 4 consecutive months between the first quarter and full moon periods (Hara et al. 1986). Similarly, mature oocytes were present for 5 consecutive months among rabbitfish fed a lipid-enriched diet (SEAFDEC/AQD 1990).

Mullet (*Mugil* spp. and *Valmugil* spp.)

Mullet mature but do not spawn spontaneously in captivity. The hypophysation protocol of mullet has already been developed and established elsewhere, but trials using LHRHa require further work. Hypophysation using HCG and SPH induced ovulation in fish having an initial egg size of 0.6 mm (SEAFDEC/AQD 1980). However, mullet with an initial egg diameter of 0.89 mm were stimulated to spawn by a single injection of 1000 IU HCG/kg (SEAFDEC/AQD 1981). Ovulated eggs were normally stripped and artificially fertilized with fresh or cryopreserved milt (SEAFDEC/AQD 1982).

Conclusion and prospects

Marine fish broodstock development continues to be a major research effort at SEAFDEC/AQD, although much has been achieved over the years. The breeding of captive milkfish, sea bass, and rabbitfish is already a fairly routine achievement which is ready for adoption by trained fish farmers. Off-season spawning of milkfish and sea bass broodstock has to be assured to produce seed year-round. Improvements on the present broodstock diet is necessary to produce quality seed stock.

The development of grouper and snapper broodstock, however, requires further research prior to being considered an established technology. Specifically, sex inversion among hermaphroditic fish species must be controlled to maintain an adequate sex ratio among broodstock.

The lack of a keen interest in Southeast Asia for producing mullet seed from broodstock is apparent due, in part, to its relatively low popularity as food fish in many areas of the region. Nonetheless, the improvement of hatchery techniques will continue until production will significantly augment supply from natural sources. The development of broodstock of other marine fish species with culture potential in Southeast Asia will also continue.

REFERENCES

Almendras, J.M., C.E. Dueñas, J. Nacario, N.M. Sherwood, and L.W. Crim. 1988. Aquaculture 74:97-111.

Aqua Dep't News (ADN). 1992. 7:1.

Ayson, F.G. 1989. Aquaculture 80:241-246.

Ayson, F.G. 1991. Aquaculture 95:133-137.

Department of Agriculture - Bureau of Fisheries and Aquatic Resources (DA-BFAR). 1989. Bangus Breeding (Philippines), Final Report. 36 pp.

Emata, A.C. and C.L. Marte. 1990. SEAFDEC Asian Aquaculture 12:1-3.

Garcia, G.H. 1992. MSc Thesis. University of the Philippines in the Visayas. 51 pp.

Garcia, L.Ma.B. 1991. Aquaculture 97:291-299.

Garcia, L.Ma.B. 1992. J. Fish Biol. 40:359-370.

Garcia, L.Ma.B. 1990a. J. Applied Ichthyol. 6:167-172.

Garcia, L.Ma.B. 1990b. Aquaculture 86:333-345.

Garcia, L.Ma.B. 1989a. Aquaculture 77:85-96.

Garcia, L.Ma.B. 1989b. J. Applied Ichthyol. 5:177-184.

Garcia, L.Ma.B. 1989c. Aquaculture 77:97-102.

Garcia, L.Ma.B., C.L. Marte, and V.3. Traviña. 1988. Aquaculture 68:83-86.

Hara, S., M.N. Duray, M. Parazo, and Y. Taki. 1986. Aquaculture 59:259-272.

Hara, S., J.T. Canto Jr., and J.M.E. Almendras . 1982. Aquaculture 28:339-346.

Harvey, B., J. Nacario, L.W. Crim, J.V. Juario, and C.L. Marte. 1985. Aquaculture 47:53-59.

Juario, J.V., M.N. Duray, V.M. Duray, J.F. Nacario, and J.M.E. Almendras. 1985. Aquaculture 44:91-101.

Juario, J.V., M.N. Duray, V.M. Duray, J.F. Nacario, and J.M.E. Almendras. 1984. Aquaculture 36:61-70.

Juario, J.V., R.P. Ferraris, and L.V. Benitez (eds.). 1983. Advances in Milkfish Biology and Culture. Island Publishing House, Inc., SEAFDEC/AQD and IDRC. 244 pp.

Juario, J.V., G.F. Quinitio, J.E. Banno, and M. Natividad. 1980. Kalikasan, Phil. J. Biol. 9:321-326.

Kungvankij, P., L.B. Tiro, B.P. Pudadera, and I.O. Potestas. 1986. In: Maclean J.L., L.B. Dizon, and L.V. Hosillos (eds.). The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines. pp. 663-666.

Liao, I.C., J.V. Juario, S. Kumagai, H. Nakajima, M. Natividad, and P. Buri. 1979. Aquaculture 18:75-93.

Marte, C.L. 1988. Aquaculture 71:387-392.

Marte, C.L., L.W. Crim, and N.M. Sherwood. 1988. Aquaculture 74:131-145.

Marte, C.L., N.M. Sherwood, L.W. Crim, and B. Harvey. 1987. Aquaculture 60:303-310.

Marte, C.L. and F. Lacanilao. 1986. Aquaculture 53:115-132.

Marte, C.L. and L.W. Crim. 1983. Kalikasan, Phil. J. Biol. 12:100-106.

Nacario, J.F. and N.M. Sherwood. 1986. In: Maclean J.L., L.B. Dizon, and L.V. Hosillos (eds.). The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philip pines; p. 13.

SEAFDEC/AQD Annual Report(s). 1976, 1980, 1981, 1982, 1983, 1984, 1986, 1987, 1989, 1990.

Tan-Fermin, J.D., L.Ma.B. Garcia, and A.R. Castillo Jr. 1989. In: Chan, D.K.O. (ed.). Hormones and the Environment. Society for the Study of Endocrinology, Metabolism and Reproduction, Department of Medicine, University of Hong Kong; pp. 19-20.

Tan, J.D. 1985. J. Fish Biol. 26:657-668.

Toledo, J.D, C.L. Marte, and A.R.Castillo Jr. 1991. J. Applied Ichthyol. 7:217-222.