A Review of SEAFDEC/AQD Finfish Breeding Research

Luis Maria B. Garcia
Aquaculture Department
Southeast Asian Fisheries Development Center
Tigbauan, Iloilo, Philippines

Abstract

Recent progress undertaken by SEAFDEC/AQD in the development of broodstock of a variety of cultured fish in the Philippines is reviewed. Spontaneous maturation and spawning has been achieved among captive breeders of grouper, milkfish, sea bass, rabbitfish, and tilapia. Hormonal intervention methods have been developed mainly to accelerated final gonadal maturation to synchronize release of mature gametes, and to control sex inversion among hermaphroditic fish such as grouper. These methods entailed the development of gonadal biopsy procedures and hormone administration protocols such as mode on introducing a variety of exogenous hormones to fish, administration intervals, and lately response times.

Enhancement of reproduction by improving the diet fed to Nile tilapia, rabbitfish, and milkfish breeders has also been achieved in recent years. Protein or lipid enrichment of the diet may enhance growth of broodstock to subsequently increase reproductive performance and fry survival.

Limited success has been achieved with photoperiod manipulation to effect year-round sexual maturation and spawning of milkfish and sea bass broodstock.

Introduction

The Southeast Asian region is endowed with a variety of marine and freshwater fish species, which have been cultured primarily as food fish for its growing population. But, like most cultured fishes elsewhere, seeds for nursery and grow-out are highly dependent on the natural productivity of fry grounds. For fish culture to be successful, the availability and reliability of seed supply must be ensured. Hence, since 1973, SEAFDEC/AQD has devoted efforts to establish broodstock for some of the economically important fish species in the region. This paper is an update on the research accomplishments on fish broodstock development undertaken by SEAFDEC/AQD since 1976. The list of species investigated follows the priority needs of the region for the next 3 years (1992-1994).

In: Marte CL, Quinitio GF, Emata AC (Eds.). 1996. Proceedings of the Seminar-Workshop on Breeding and Seed Production of Cultured Finfishes in the Philippines. 4-5 May 1993, Tigbauan, Iloilo, Philippines. SEAFDEC/AQD, Tigbauan, Iloilo, Philippines. p. 54-64.

Marine and Brackishwater Finfishes

1. Grouper (*Epinephelus* sp., *Cromileptes altivelis*)

Groupers are a highly priced fish commodity. Initial work in 1986 attempted to spawn mature *E. salmoides* by two injections of luteinizing hormone-releasing hormone analogue (LHRHa), human chorionic gonadotropin (hCG), and carp pituitary extract (Kungvankij et al. 1986). Although successful, the protocol was not further duplicated nor improved in subsequent years.

The problem of sex inversion (specifically, the lack of mature males) among groupers has only been recently addressed. Being protogynous hermaphrodites, grouper juveniles are most likely females, which invert to males at an unknown age. Female grouper, and very rarely males, are not therefore uncommon in wild catches. Synthetic androgens such as 17α-methyltestosterone (MT) and mibolerone generally failed to stimulate sex inversion among juvenile and adult *E. malabaricus* (SEAFDEC/ADQ 1989). Nonetheless, some histological signs in the gonads were observed, especially among fish weighing more than 1.2 kg, which underwent spermatogenesis after bi-weekly MT injections for 3-6 months (Tan-Fermin et al. 1989). Neither the largest fish in groupers held in a communal tank inverted sex (Tan-Fermin et al. 1989). Chronic release of MT and LHRHa from an oily vehicle, a cholesterol powder matrix or from silastic capsules to stimulate sex inversion is currently being investigated (SEAFDEC/AQD 1992).

Despite the lack of male fish, wild *E. suillus* reared in 50-ton concrete tanks and in sea cages have spawned spontaneously (SEAFDEC/AQD 1990). Tank-reared broodstock (6 females to 4 males) spawn year-round, from 6 to 14 times monthly. Caged broodstock spawn from July to October only, which apparently coincide with the natural breeding season of grouper in the Philippines. Preliminary results do not, however, appear to indicate that the quality of spawned eggs may be improved by cod liver oil or highly unsaturated fatty acid enrichment of their trash fish diet (SEAFDEC/AQD 1992).

In addition to *Epinephelus* sp., the collection of other species of grouper from fish traps has only recently been initiated (SEAFDEC/AQD 1992). Based on their gonadosomatic index (GSI), the natural spawning cycle of *C. altivelis* occurs from July until November. Mature males appear bigger (56 cm) and heavier (1.2 kg) than females (40 cm, 0.6 kg).

2. Milkfish (*Chanos chanos*)

Although milkfish has been extensively cultured in the Philippines, the source of seed has come mainly from the wild. Initial research to propagate milkfish in captivity began in 1975 when ecological studies were undertaken to determine

spawning sites, migration patterns, external sex markers, and sex of wild adult breeders or sabalo (Juario et al. 1983).

The effectiveness of known hypophysation methods to spawn sabalo was subsequently tested among sabalo. A semi-purified salmon gonadotropin preparation (SG-G100) induced ovulation of mature milkfish (SEAFDEC/AQD 1976). Other hypophysation agents such as salmon pituitary homogenate (SPH) and hCG were tested and its use standardized to determine the least effective dose, administration intervals, and initial egg size required for ovulation. About 20 mg SPH and 3,000 IU hCG per kg administered in 2 injections at 12-24 h intervals stimulated spawning of milkfish with an initial egg diameter of 0.66 mm (Juario et al. 1984). On the other hand, milt production can be prolonged for a week by a combination of hCG and a long-acting testosterone preparation (Juario et al. 1980). LHRHa injection or pellet implantation likewise stimulated spawning of mature milkfish 18-36 h after treatment (SEAFDEC/AQD 1980, Marte et al. 1987). Depending on its initial egg size, fish either undergo ovarian hydration or eggs are released completely after LHRHa treatment (Marte et al. 1987, 1988). LHRHa administered via an osmotic pump was less effective than either pellet implantation or injection (Marte et al. 1987).

The initiation of puberty, advancement of sexual maturation and rematuration have been attempted with some success. Since "gonadotropin" hormone (GTH) remained in circulation for only 1-2 days after hormone treatment (Marte and Crim 1983). SPH, thyroxine, and estradiol-17ß (E_2) injection or pellet implantations generally failed to induced maturation among sexually immature adults and spent sabalos (Lacanilao et al. 1985). Interestingly, these fish mature when left undisturbed in a cage (SEAFDEC/AQD 1980). But, recent information suggest that tank-reared adult female milkfish remature following regular implantation of 250 µg E_2 per fish. Males mature and remature when implanted several times with 17α-hydroxyprogesterone (SEAFDEC/AQD 1992). LHRHa implantation of 3- and 4-year old broodstock failed to stimulate sexual maturation, although LHRHa and testosterone induced precocious maturation among 4-year old milkfish reared in tanks (SEAFDEC/AQD 1986, Marte et al. 1988). The general ineffectiveness of these hormone protocols may somehow be related to the lack of information on endocrine changes occurring during sexual maturation of milkfish (Marte and Lam 1992).

Both cage- (Marte and Lacanilao 1986) and tank-reared (Emata and Marte 1990) milkfish broodstock undergo spontaneous maturation and spawning. Caged milkfish first attain sexual maturation at 5 years and continue to spawn yearly thereafter. The development of an egg sweeper collector (Garcia et al. 1988, Marte 1988) and the determination of the best time of day for collection (Toledo and Gaitan 1992) has markedly improved the number of milkfish eggs collected from sea cages. Likewise, initial results appear to suggest that a lipid-enriched broodstock diet may enhance egg production and improve larval quality (SEAFDEC/AQD 1987).

Cryopreservation of milkfish milt has also been characterized. Milkfish serum remains as the best extender for milt cryopreservation (Hara et al. 1982). Frozen milt in glycerine fertilized more eggs than milt cryopreserved in dimethyl sulphoxide.

In addition, morphological and physiological changes occurring during sexual maturation of milkfish have been described. Although changes in 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).

3. Snapper (*Lutjanus* sp.)

Domestication of wild-caught snapper broodstock have only recently started. Fish have spontaneously matured from May to October in sea cages and in concrete tanks, but do not spawn naturally in these holding structures (SEAFDEC/AQD 1992). A single injection of 50 µg LHRHa per kg or 1500 IU hCG per kg administered to mature females possessing oocytes with a diameter of 40-45 µm and to spermiating males induced spawning 27-36 h post-injection. A single female weighing 3-4.5 kg can spawn up to 1.2 million eggs. A spawning trial in August produced hatched larvae 16 h after spawning at 27-28 °C and 30-32 ppt salinity. Fish used for induced spawning trials rematured 4-5 weeks later.

4. Sea Bass (*Lates calcarifer*)

Although spawning of mature sea bass broodstock has been earlier achieved by hypophysation (SEAFDEC/AQD 1983), subsequent studies also demonstrated the effectiveness of LHRHa when administered by injection, pellet and silastic implantations or osmotic pumps (Nacario 1987, Almendras et al. 1988). Following treatment, sea bass normally spawn at 24 h intervals for over 4-5 consecutive days (Almendras et al. 1988, Harvey et al. 1985).

Although caged sea bass broodstock spontaneously mature and spawn (Toledo et al. 1991, Garcia 1992), LHRHa may be employed to supplement natural spawnings and to obtain eggs on demand. The LHRHa protocol was therefore further improved to enhance the effectiveness as a spawning agent in sea bass. For instance, the least effective dose of both pellet (Garcia 1989a) and injection (Garcia 1989b) of LHRHa has been determined. Depending on the LHRHa dose injected, mature females sea bass may be induced to spawn singly or consecutively over several days. Fertilization but not hatching rates were low after implantation of a high dose of LHRHa (300 µg per kg). Egg production of LHRHa-implanted sea bass peaked on the first day of consecutive spawning before declining on subsequent days. Further, the administration of LHRHa injection may be timed during the day to produce a

maximum number of eggs available for hatchery rearing (Garcia 1990). The initial egg diameter required for successful spawning to occur by LHRHa injection has been determined to be at least 0.40 mm (Garcia 1989). Both LHRHa and MT can stimulate spawner production and milt dilution (Garcia 1992). As an alternative to the introduction of exogenous LHRHa, rectal but not oral intubation can trigger ovarian maturation among mature females (SEAFDEC/AQD 1992).

The natural spawning cycle of sea bass appear to follow a lunar- and tide-synchronized spawning rhythm (Toledo et al. 1991, Garcia 1992), which could be effectively overcome by manipulating hormonal and photoperiodic cues. Regular implantation of LHRHa alone or in combination with MT during the off-season months (February and March) advanced early onset of maturation and spawning in May (Garcia 1990). Subjecting sea bass to a short (8 h) or a normal (12 h daylength) period enhanced testicular and ovarian maturation in December (SEAFDEC/AQD 1992).

5. Rabbitfish (*Siganus guttatus*)

Together with hCG, anti-estrogenic compound, clomiphene citrate, has been used to spawn mature rabbitfish (SEAFDEC/AQD 1982), although hCG alone is effective as well (Juario et al. 1985, Ayson 1991). Depending on the initial egg size of mature rabbitfish, single or multiple injections of hCG was required to spawn fish. Hence, fish with an initial egg diameter of at least 0.46 mm and greater do not require hormone treatment, but those with an egg size of less than 0.45 mm require multiple injections of 2 IU hCG per (Juario et al. 1985, Ayson 1989). Handling-associated stress has also been demonstrated to enhance spawning of rabbitfish which is comparable with hCG-injected fish (Ayson 1989). Chronic release of LHRHa from silastic implants advanced spawning 1-2 days earlier than sham controls (Harvey et al. 1985). Similarly, milt dilution and production was enhanced a day after LHRHa injection (Garcia 1991) and can be sustained by 3 weekly injections of LHRHa (Garcia 1993). Recently, thyroxine injection to female spawners increased maternal circulation of thyroxine and tri-iodothyronine, which were eventually transferred into the ovaries and subsequently to the developing larvae (Ayson and Lam 1993). Larvae from thyroxine-injected spawners appeared longer and survived better than the controls.

Rabbitfish broodstock may spawn year-round even without exogenous hormonal intervention. Female rabbitfish fed a cod liver oil-enriched diet spawned repeatedly for at least 4 consecutive months, between the first quarter and full moon periods (Ayson and Lam 1993). Similarly, mature oocytes were present for 5 consecutive months among rabbitfish fed a lipid-enriched diet (Hara et al. 1986).

6. Mullet (*Mugil* sp. and *Valamugil*)

Under captive conditions, mullets attain sexual maturity but do not spawn spontaneously. Hypophysation has been utilized to spawn mature mullets. Hence, hCG and SPH induced ovulation in fish with an initial egg diameter of 0.60 mm (SEAFDEC/AQD 1980). Ovulated eggs have been stripped and artificially fertilized with fresh or cryopreserved milt (SEAFDEC/AQD 1982).

Freshwater Finfishes

7. Tilapia (*Oreochromis* sp.)

The acquisition in 1979 of several varieties of tilapia initiated SEAFDEC/AQD's research in broodstock development of this popular freshwater food fish. All possible crosses among *O. niloticus, O. mossambica, O. aurea,* and red tilapia revealed that progenies of red tilapia x *O. nilotica* exhibited high growth and survival rates (SEAFDEC/AQD 1979). Subsequent studies focused on improving production yields of fry and fingerlings reared in ponds and cages, and, more recently, on developing high yield, salinity-tolerant genetic strains (SEAFDEC/AQD 1979, Villegas 1990, Basiao and Doyle 1990). Although not statistically significant, spawning frequency and growth of female Nile tilapia tend to increase when the crude protein of dry pellets was increased to 50% (Santiago et al. 1983). Feeding Nile tilapia pelleted supplementary diets with 40% crude protein resulted in high body weight gains and high fry production, possibly due to more eggs produced by larger females (Santiago et al. 1985). Further manipulation of the Nile tilapia broodstock diet revealed that ipil-ipil (*Leucaena*) leaf meal as protein source should not contain more than 40% of the diet (Santiago et al. 1988). High concentrations of *Leucaena* invariably resulted in weight loss of both sexes on Nile tilapia breeders, including slow gonadal growth and low fry production.

Seed production of Nile tilapia in concrete tanks and hapa net enclosures is popular among tilapia farmers. Egg and fry production in these broodstock holding structures peaked at a 1:4 male-female ratio at 4 females per m^2 (Bautista et al. 1988).

8. Catfish (*Clarias macrocephalus*)

Initial efforts to propagate the native catfish in captivity at SEAFDEC/AQD dealt with the development of a technique of preserving eggs in suitable fixative (Tan-Fermin 1992). Such a technique is essential when egg diameter is used as an index of sexual maturity of catfish and other fishes.

Although catfish has been previously spawned by hypophysation, LHRHa together with a potent dopamine antagonist (pimozide, PIM, and domperidone, DOM) to block the endogenous gonadotropin release-inhibiting factor have been found effective in inducing ovulation among mature catfish. LHRHa doses ranging from 0.01 to 0.1 µg per g and 1 µg PIM per g stimulated ovarian maturation and ovulation 15-16 h after a simultaneous injection of these spawning agents (Tan-Fermin 1992). The least effective dose of LHRHa and PIM was demonstrated to be 0.05 µg and 1 µg per g, respectively (Tan-Fermin 1993). At this dose, complete ovulation occurred at 16-20 h post-treatment; fertilization (84-90%) and hatching (51-79%) rates were high.

Improvement of the dry fertilization protocol or catfish has also been recently improved by maximizing the use of very limited quantities of stripped milt to fertilize ovulated eggs (Tambasen 1993). Milt can be stripped 24 h after injection of 0.04 µg salmon GnRHa per g and 20 µg DOM per g (equivalent to 2 µl Ovaprim per g). A volume of 25-50 µl of stripped milt diluted 3.5 times in saline can adequately fertilize 10 g of ovulated eggs. Ovaprim-injected catfish can produce about 6 µl per g stripped milt, which can fertilize 290-570 g eggs.

9. Bighead Carp (*Aristhicthys nobilis*)

Gonadal maturation of bighead carp reared in net cages in Laguna Lake occurred year-round even without supplemental feeding (Fermin 1990). Peak maturation in March coincided with low natural food productivity during high inorganic turbidity, suggesting that broodstock may have largely fed on suspended food particles to augment the low natural supply of zooplankton, the major component of its natural diet.

In addition to the year-round availability of mature bighead carp broodstock, fish may be spawned by hCG and LHRHa (Fermin and Reyes 1989). Various dose combinations of these substances administered in 1 or 2 injections stimulated ovulation and spawning. As well, LHRHa and DOM were as effective as LHRHa and hCG in spawning mature bighead carp (Fermin 1991), but the LHRHa and DOM protocol cost less than the other.

Bighead carp broodstock fed a protein-rich (40%) artificial diet produced more eggs than those fed diets with lower protein content or those not fed artificial diet (Santiago et al. 1991). Fertilization and hatching rates among eggs spawned by broodstock fed various levels of protein in their diet were similar. More fry were also produced by broodstock fed a protein-rich diet. After 10 days of starvation, fry from such broodstock survived better than those fry whose parents were not fed an artificial diet.

Conclusion

While much has been done after 20 years of research by SEAFDEC/AQD, much remains to be accomplished in addressing broodstock development of various finfishes important to the Philippines and to the entire Southeast Asian region. To be successful, fish culture requires an adequate source of fry produced from captive fish breeders. Broodstock must be able to produce mature gametes year-round, without compromising on seed quality. Such a scenario may be close to reality for some of the fish species reviewed, but for other species, this goal has remained elusive. Research at SEAFDEC/AQD shall continue to investigate most aspects of fish broodstock development until such time that fry and fingerlings produced in the hatchery will significantly decrease the dependence of fish farmers on wild seed catch.

References

Almendras JM, Dueñas C, Nacario J, Sherwood NM, Crim LW. 1988. Sustained hormone release. III. Use of gonadotropin-releasing hormone analogues to induced multiple spawnings in sea bass, *Lates calcarifer.* Aquaculture 74:97-111.

Ayson FG. 1989. The effect of stress on spawning of brood fish and survival of larvae of the rabbitfish, *Siganus guttatus* (Bloch). Aquaculture 80:241-246.

Ayson FG. 1991. Induced spawning of rabbitfish, *Siganus guttatus* (Bloch) using human chorionic gonadotropin (HCG). Aquaculture 95:133-137.

Ayson FG, Lam TJ. 1993. Thyroxine injection of female rabbitfish *Siganus guttatus* (Bloch) broodstock: Changes in thyroid hormone levels in plasma, eggs, and yolk-sac larvae, and its effect on larval growth and survival. Aquaculture 109:83-93.

Basiao ZU, Doyle RW. 1990. Interaction between test and reference populations when tilapia strains are compared by the "internal control" technique. Aquaculture 85:207-214.

Bautista AM, Carlos MH, San Antonio AI. 1988. Hatchery production of *Oreochromis niloticus* L. at different sex ratios and stocking densities. Aquaculture 73:85-95.

Emata AC, Marte CL. 1990. Natural and induced spawning of milkfish, *Chanos chanos* (Forsskal), in concrete tanks. SEAFDEC Asian Aquaculture 12:1-3.

Fermin AC, Reyes DM Jr. 1989. HCG and LHRHa-induced spawning in bighead carp *Aristichthys nobilis* Rich. reared in floating cages in Laguna de Bay. Philipp. Sci. 26:21-28.

Fermin AC. 1990. Year-round sexual maturation of bighead carp reared in floating cages in Laguna de Bay (Philippines). J. Appl. Ichthyol. 6:129-135.

Fermin AC. 1991. LHRH-a and domperidone-induced oocyte maturation and ovulation in bighead carp, *Aristichthys nobilis* (Richardson). Aquaculture 93:87-94.

Garcia GH. 1992. Milt production response of the sea bass *Lates calcarifer* (Bloch) to luteinizing hormone-releasisng hormone analogue and 17α-methyltestosterone. MSc Thesis. University of the Philippines in the Visayas. 51 pp.

Garcia LMaB. 1989a. Dose-dependent spawning response of mature female sea bass, *Lates calcarifer* (Bloch), to pelleted luteinizing hormone-releasing hormone analogue (LHRHa). Aquaculture 77:85-96.

Garcia LMaB. 1989b. Spawning response of mature female sea bass, *Lates calcarifer* (Bloch), to a single injection of luteinizing hormone-releasing hormone analogue: Effect of dose and initial oocyte size. J. Appl. Ichthyol. 5:177-184.

Garcia LMaB. 1990. Advancement of sexual maturation and spawning of sea bass, *Lates calcarifer* (Bloch), using pelleted luteinizing hormone-releasing hormone analogue and 17α-methyltestosterone. Aquaculture 86:333-345.

Garcia LMaB. 1990. Spawning response latency and egg production capacity to LHRHa-injected mature female sea bass, *Lates calcarifer* Bloch. J. Appl. Ichthyol. 6:167-172.

Garcia LMaB. 1991. Spermiation response of mature rabbitfish, *Siganus guttatus* Bloch, to luteinizing hormone-releasing hormone analogue (LHRHa) injection. Aquaculture 97:291-299.

Garcia LMaB. 1992. Lunar synchronization of spawning in sea bass, *Lates calcarifer* (Bloch): Effect of luteinizing hormone-releasing hormone analogue (LHRHa) treatment. J. Fish Biol. 43:359-370.

Garcia LMaB. 1993. Sustained production of milt of the rabbitfish, *Siganus guttatus* Bloch, by weekly injection of luteinizing hormone-releasing hormone analogue (LHRHa). Aquaculture. 113:261-267.

Garcia LMaB, Marte CL, Traviña V. 1988. A collecting gear for naturally-spawned milkfish (*Chanos chanos* Forsskal) eggs in circular floating net cages. Aquaculture 68:83-86.

Hara S, Canto JT Jr, Almendras JME. 1982. A comparative study of various extenders for milkfish, *Chanos chanos* (Forsskal), sperm preservation. Aquaculture 28:339-346.

Hara S, Duray MN, Parazo M, Taki Y. 1986. Year-round spawning and seed production of the rabbitfish, *Siganus guttatus.* Aquaculture 59:259-272.

Harvey B, Nacario JF, Crim LW, Juario JV, Marte CL. 1985. Induced spawning of sea bass, *Lates calcarifer,* and rabbitfish, *Siganus guttatus,* after implantation of pelleted LHRH analogue. Aquaculture 47:53-59.

Juario JV, Duray MN, Duray VM, Nacario JF, Almendras JME. 1984. Induced breeding and larval rearing experiments with milkfish *Chanos chanos* (Forsskal) in the Philippines. Aquaculture 36:61-70.

Juario JV, Ferraris RP, Benitez LV (Eds.). 1983. Advances in Milkfish Biology and Culture. Proceedings of the Second International Milkfish Aquaculture Conference. 4-8 October 1983, Iloilo City, Philippines. 243 pp.

Juario JV, Quinitio GF, Banno JE, Natividad M. 1980. Effects of exogenous hormone injections on milt consistency in newly caught, wildmilkfish. Kalikasan, Philipp. J. Biol. 9:321-326.

Juario JV, Duray MN, Duray VM, Nacario JF, Almendras JME. 1985. Breeding and larval rearing of the rabbitfish, *Siganus guttatus* (Bloch). Aquaculture 44:91-101.

Kungvankij P, Tiro LB Jr, Pudadera BP, Potestas IO. 1986. Induced spawning and larval rearing of grouper (*Epinephelus salmoides* Maxwell). p. 663-666. In: The First Asian Fisheries Forum. Maclean JL, Dizon LB, Hosillos LV (Eds.). Asian Fisheries Society, Manila, Philippines.

Lacanilao F, Marte CL, Lam TJ. 1985. Problems associated with hormonal induction of gonad development in milkfish (*Chanos chanos*). p. 1247-1253. In: Current Trends in Comparative Endocrinology. Lofts B, Holmes WN (Eds.). Proceedings of the 9th International Symposium in Comparative Endocrinology, 7-11 December 1981, Hong Kong. Hong Kong University Press, Hong Kong.

Marte CL and Crim LW. 1983. Gonadotropin profiles in serum of milkfish treated with salmon pituitary homogenate. Kalikasan, Philipp. J. Biol. 12:100-106.

Marte CL, Crim, LW, Sherwood NM. 1989. Induced gonadal maturation and rematuration in milkfish: limited success with chronic administration of testosterone and gonadotropin releasing-hormone analogues (GnRHa). Aquaculture 74:131-145.

Marte CL, Lacanilao F. 1986. Spontaneous maturation and spawning of milkfish in floating net cages. Aquaculture 53:115-132.

Marte CL, Lam TJ. 1992. Hormonal changes accompanying sexual maturation in captive milkfish (*Chanos chanos* Forsskal). Fish Physiol. Biochem. 10:267-276.

Marte CL, Sherwood NM, Crim L, Tan J. 1988. Induced spawning of maturing milkfish (*Chanos chanos*) using human chorionic gonadotropin and mammalian and salmon gonadotropin releasing hormone analogues. Aquaculture 73:333-340.

Marte CL, Sherwood NM, Crim LW, Harvey B. 1987. Induced spawning of maturing milkfish (*Chanos chanos* Forsskal) with gonadotropin-releasing hormone (GnRH) analogues administered in various ways. Aquaculture 60:303-310.

Marte CL. 1988. An improved method for collecting naturally spawned milkfish eggs from floating cages. Aquaculture 71:387-392.

Nacario JF. 1987. Releasing hormones as an effective agent in the induction of spawning in captivity of sea bass (*Lates calcarifer*). p. 126-128. In: Management of Wild and Cultured Sea Bass/Barramundi (*Lates calcarifer*). Copland JW, Grey DL (Eds.). Australian Centre for International Agricultural Research, Canberra, Australia.

Santiago CB, Aldaba MB, Abuan EF, Laron MA. 1985. The effects of artificial diets on fry production and growth of *Oreochromis niloticus* breeders. Aquaculture 47:193-203.

Santiago CB, Aldaba MB, Laron MA, Reyes OS. 1988. Reproductive performance and growth of the Nile tilapia (*Oreochromis niloticus*) broodstock fed diets containing *Leucaena leucocephala* leaf meal. Aquaculture 70:53-61.

Santiago CB, Aldaba MB, Laron MA. 1983. Effect of varying dietary crude protein levels of spawning frequency and growth of *Sarotherodon niloticus* breeders. Fish. Res. J. Phil. 8:9-18.

Santiago CB, Camacho AS, Laron MA. 1991. Growth and reproductive performance of bighead carp (*Aristichthys nobilis*) reared with or without feeding in floating cages. Aquaculture 96:109-117.

SEAFDEC/AQD. 1976. Annual Report. Aquaculture Department, southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

SEAFDEC/AQD. 1979. Annual Report. Aquaculture Department, Southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

SEAFDEC/AQD. 1980. Annual Report. Aquaculture Department, southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

SEAFDEC/AQD. 1982. Annual Report. Aquaculture Department, Southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

SEAFDEC/AQD. 1983. Annual Report. Aquaculture Department, southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

SEAFDEC/AQD. 1986. Annual Report. Aquaculture Department, Southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

SEAFDEC/AQD. 1987. Annual Report. Aquaculture Department, southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

SEAFDEC/AQD. 1989. Annual Report. Aquaculture Department, Southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

SEAFDEC/AQD. 1990. Annual Report. Aquaculture Department, southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

SEAFDEC/AQD. 1992. Annual Report. Aquaculture Department, southeast Asian Fisheries Development Center. Tigbauan, Iloilo, Philippines.

Tambasen MVP. 1993. Milt-to-egg ratio in artificial fertilization of *Clarias macrocephalus* (Gunther) using salmon gonadotropin-releasing hormone analogue and domperidone. MSc Thesis. University of the Philippines in the Visayas. 46 pp.

Tan JD. 1985. A histological study of the hypophysial-gonadal system during maturation and spawning in the milkfish *Chanos chanos* (Forsskal). J. Fish Biol. 26:657-668.

Tan-Fermin JD, Garcia LMaB, Castillo AR Jr. 1989. Induction of sex inversion in juvenile grouper, *Epinephelus malabaricus* (Bloch and Schneider), by bi-weekly injection of 17α-methyltestosterone. p. 19-20. In: Hormones and the Environment. Chan DKO (Ed.).

Proceedings of an International Symposium, 18-20 December 1983, University of Hong Kong. University of Hong Kong Press, H.K.

Tan-Fermin JD. 1991. Suitability of different formalin-containing fixatives for the eggs of freshwater Asian catfish *Clarias macrocephalus* (Gunther). Isr. J. Aquacult-Bamidgeh 43:57-61.

Tan-Fermin JD. 1992. Induction of oocyte maturation and ovulation in the freshwater Asian catfish, *Clarias macrocephalus* by LHRHa and pimozide. J. Appl. Ichthyol. 8:90-98.

Tan-Fermin JD and Emata AC. 1993. Induced spawning by LHRHa and pimozide in the Asian catfish *Clarias macrocephalus* (Gunther). J. Appl. Ichthyol. 9:89-96.

Toledo JD, Gaitan AG. 1992. Egg cannibalism by milkfish (*Chanos chanos* Forsskal) spawners in circular floating net cages. J. Appl. Ichthyol. 8:257-262.

Toledo JD, Marte CL, Castillo AR. 1991. Spontaneous maturation and spawning of sea bass *Lates calcarifer* in floating net cages. J. Appl. Ichthyol. 7:217-222.

Villegas CT. 1990. Evaluation of the salinity tolerance of *Oreochromis mossambicus, O. niloticus* and their F_1 hybrids. Aquaculture 85:281-292.