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Hormone-induced spawning of cultured tropical finfishes

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Abstract — *Commercially important tropical freshwater and marine finfishes are commonly spawned with pituitary homogenate, human chorionic gonadotropin (HCG) and semi-purified fish gonadotropins. These preparations are often administered in two doses, a lower priming dose followed a few hours later by a higher resolving dose. Interval between the first and second injections may vary from 3 - 24 hours depending on the species. Variable doses are used even for the same species and may be due to variable potencies of the gonadotropin preparations.*

Synthetic analogues of luteinizing hormone-releasing hormone (LHRHa) are becoming widely used for inducing ovulation and spawning in a variety of teleosts. For marine species such as milkfish, mullet, sea bass, and rabbitfish, a single LHRHa injection or pellet implant appears to be effective. Multiple spawnings of sea bass have also been obtained following a single injection or pellet implant of a high dose of LHRHa. In a number of freshwater fishes such as the cyprinids, LHRHa alone however has limited efficacy. Standardized methods using LHRHa together with the dopamine antagonists pimozide, domperidone and reserpine have been developed for various species of carps. The technique may also be applicable for spawning marine teleosts that may not respond to LHRHa alone or where a high dose of the peptide is required.

Although natural spawning is the preferred method for breeding cultivated fish, induced spawning may be necessary to control timing and synchrony of egg production for practical reasons.

INTRODUCTION

There are more than 80 species of freshwater and marine fish cultured in Asia. Of these, about 50 species are cultured in tropical South and Southeast Asia (Rabanal, 1988). For most of the cultured species, wild fry from natural sources is insufficient to supply the requirements for culture. Moreover, the fry supply is dependent on the season, and fluctuates with environmental and climatic conditions.

Overfishing, pollution and various human activities have caused the destruction of natural spawning and fry grounds contributing largely to

the reduction in fry catch. Of the numerous species under cultivation only a few are bred in captivity. For those species that spontaneously breed under captive condition, the time of spawning is often not predictable. Problems such as viability of naturally spawned eggs and technical difficulties in egg and larvae collection are constraints to mass-scale fry production.

Most of the information on the physiological processes involved in the hormonal control of fish reproduction are derived from studies on a few species notably the salmonids, goldfish, and common carp. Although the basic biological principles arising from studies on these fish apply to tropical species, the diversity of fish cultured in the tropics present numerous problems. This paper summarizes the recent developments in induced breeding of important species cultured in Southeast Asia.

PRACTISES IN INDUCED BREEDING

Induced breeding of captive fish may be approached in two ways, hormonal and environmental. For most of the tropical cultured fish, the specific environmental cues that trigger ovulation and spawning have not been identified. Asian fish breeders however have succesfully developed methods that stimulate spawning conditions in a few freshwater species. Environmental manipulation to induce ovulation and spawning in fish has been reviewed by Lam (1983) and Lam and Munro (1987). Specific examples of tropical fish where environmental cues are known and used to stimulate breeding activities are cited in Lam (1985).

Induction of spawning using hormones provides a direct control over the final stages of the reproductive cycle in teleosts. Hormonal induction of spawning has been the subject of many recent reviews (Harvey and Hoar, 1979; Lam, 1982, 1985; Donaldson and Hunter, 1983; Crim et *al.*, 1987; Abraham, 1988). The physiological mechanisms involved in the final stages of oocyte maturation, ovulation and egg release have been thoroughly reviewed (Fostier and Jalabert, 1982; Goetz, 1983). Harvey and Hoar (1979) and Davy and Chouinard (1980) discuss traditional practises followed in induced spawning of tropical fish.

Ovulation and spawning in teleosts as in other vertebrates are controlled by several interacting factors. Environmental stimuli are translated by the brain into neural signals which result in the release of gonadotropin releasing hormone (GnRH) and/or inhibition of the release of gonadotropin release inhibiting factor (GnRIF) causing the pituitary to secrete gonadotropins (GtH) (Peter, 1982; 1983a; Peter et *al.*, 1986; Lin and Peter, 1986). When a certain GtH level is reached, vitellogenic oocytes undergo the process of final oocyte maturation : the germinal vesicle migrates to the periphery; theca and granulosa cells of the follicle are stimulated to secrete a maturation-inducing steroid (MIS); and the MIS induces germinal vesicle breakdown (GVBD) (Nagahama, 1983; Fostier and Jalabert, 1983; Goetz, 1983).

Evidence from studies on goldfish, salmonids and other species point to 17-alpha, 20-beta progesterone (17α, 20βP) as the MIS (Nagahama,1983; Scott and Canario, 1987) although other related progestogens have been

identified (Scott and Canario, 1987). Corticosteroids have also been implicated (Goetz, 1983; Fostier and Jalabert, 1983) but may play only a supportive role (Jalabert, 1976). An additional role for 17α, 20βP and other progestogens as reproductive pheromones particularly in those species where very high levels are found, has been suggested (Scott and Canario, 1987).

Hypophysation and Human chorionic gonadotropin (HCG) administration.

Traditional methods of induced breeding involve the injection either intramuscularly or intraperitoneally of crude pituitary extracts. The pituitary extract is usually administered to the female in two doses, a stimulating dose followed after a variable time interval by a second resolving dose. Fresh or preserved pituitary glands from mature fish of the same species (homoplastic) or from other, usually related species (heteroplastic) are used. In some cases, glands from immature fish have been used but higher doses are required (Harvey and Hoar, 1979). Females that do not respond after the second injection are injected a third or even more doses. Injecting multiple doses however has seldom been successful and females regress probably as a result of stress from excessive handling. Males are injected the same or half the dose given to the female usually at the time the second or resolving dose is administered. Doses are given as fresh or dry weights of pituitary gland per unit body weight of the broodfish or in dose units, defined as the ratio of the body weight of the donor and the body weight of the recipient. Standardization of hypophysation is difficult since the potency of the pituitary extract depends on the age, sex and state of maturity of the donor. Method of collection and the technique used to preserve the pituitary also vary. Species specificity of gonadotropins has been demonstrated (Fontaine et *al*, 1972; Varikul and Sritongsook, 1981) and is an important factor to consider. The supply of pituitary glands is a problem and although crude or partially purified pituitary extracts with assayed gonadotropin potency is commercially available, the cost for the Asian fish breeder is prohibitive.

The problems of standardization and cost of hormone preparations are partly solved with the use of mammalian gonadotropin preparations. Two are available in purified form, human chorionic gonadotropin (HCG) and pregnant male serum (PMS). The dosage used varies widely between species and may be related to how closely HCG and PMS resemble the endogenous gonadotropin in each species (Lam, 1982). HCG has been successfully used in most species bred in Southeast Asia (examples are given in the succeeding sections). HCG is available and convenient to use although still expensive. The possibility that injecting HCG and pituitary extracts for several consecutive years to the same broodstock may result in the development of an immune response has been pointed out (Lam, 1982; Billard et *al* 1987).

Luteinizing hormone-releasing hormone (LHRH)

LHRH, a hypothalamic decapeptide and its synthetic analogues have been shown to stimulate gonadotropin secretion in teleosts (Crim et *al.*, 1987; Peter 1983a and b; Lin and Peter, 1986). The effectiveness of LHRH analogues in inducing ovulation and spawning of cultured fish was first

demonstrated in various species of carps by Chinese researchers (Anon 1977). The practise however was not widely adopted because consistent results were not obtained.

Recent studies have demonstrated the presence of a gonadotropin-release inhibiting factor in goldfish. Further evidences identify GRIF to be the catecholamine dopamine (for review, see Peter et *al.*, 1986). GRIF's inhibitory effect on GtH release is blocked by administration of dopamine receptor antagonists such as pimozide or metoclopramide (Chang et *al.*, 1984; Sokolowska et *al.*,1984, 1985; Peter et *al.*, 1985). Administration of dopamine antagonists potentiates LHRHa mediated GtH release in goldfish (Sokolowska et *al.*, 1984, 1985) and common carp (Billard et *al.*, 1983; Lin et *al.*, 1986) and to a lesser extent in coho salmon (Van Der Kraak et *al.*, 1986) and African catfish (de Leeuw et *al.*, 1985 a and b). LHRHa injected together with pimozide or other dopamine antagonists is highly effective in inducing ovulation in these species. The use of LHRHa alone or together with dopamine antagonists in spawning various species of cultured fish was recently reviewed by Crim et *al.*, (1987).

CATFISHES

Catfishes are a favorite food fish in Southeast Asia. They have high tolerance to crowding and adverse environmental conditions hence are easy to culture. Of the three species cultivated, *Clarias batrachus* and *C. macrocephalus* are extensively cultured in the region. Commercial culture of *Pangasius sutchi*, the riverine catfish is limited to Thailand. Only *C. batrachus* spawns in captivity.

Tab. 1. — Hormone induced spawning of *Clarias macrocephalus*

Hormone	Dose	% Spawned	Time to Ovulation/ Spawning (h)	Reference[b]
PG, dose unit[a] (Clarias sp.)	1.5-2.0	50-100	13-14	(1)
PG, dose unit (P. sutchi)	4-6	62.5-83	13-14	(1)
HCG, IU/kg	3000-4500	75-90	—	(2)
HCG, IU/fish	450-500	58	4	(1)
LHRHa, ug/kg	10-30	25-87.5	15	(1)
LHRHa, ug/kg	20	70	16-18	(3)

[a] dose unit is defined as the ratio : body weight of donor/body weight of recipient
b (1) Thalathiah et *al.* (1988); (2) Carreon et *al.* (1976); (3) Ngamvongchon et *al.* (1988).

Catfishes are spawned mainly by hypophysation or HCG injection and recent trials using LHRHa have also proved to be successful (Table 1). For *C. macrocephalus*, a single injection of 0.0026 - 0.0039 mg/fish or 1.5 - 2 dose units pituitary gland extract (PG) is effective (Tongsanga et *al.*, 1963; Thalathiah et *al.*, 1986). Doses of HCG reported to be effective are 450 - 500 IU/fish (Carreon et *al.*, 1976) and 3 000 - 4 500 IU/kg (Thala-

thiah et *al.*, 1988). LHRHa at doses of 10, 20 and 30μg/kg was tried, the most effective being 20 μg/kg (Thalathiah et *al*, 1988). A similar result was obtained by Ngamvongchon et *al.* (1988). *C. macrocephalus* spawns from 13 - 16 hours after hypophysation or HCG injection and 15 - 18 hours when LHRHa is used.

Although *C. batrachus* breeds naturally in ponds, induced spawning of this species has been reported (Sundararaj and Goswami, 1969; Devaraj et *al.*, 1972; Zonneveld et *al.*, 1988; Wembiao et *al.*, 1988). Doses of HCG and PMS effective for spawning *C. batrachus* are 250 - 375 IU/fish (cited by Shehadeh, 1975). In Southern China, 500 - 800 IU/kg HCG and a combination of 500 IU/kg HCG + 1 mg/kg carp pituitary are commonly used (Wembiao et *al.*, 1988). Males are injected half the dose used for females. The broodfish are returned to the pond or tanks and allowed to spawn naturally. Of several criteria used by Zonneveld et *al.* (1988) to standardize methods for spawning *C. batrachus*, the stripping response (weight of stripped ovary/ weight of stripped ovary × number of larvae) and working fecundity (number of larvae per 100 g female body weight) were the most reliable. Based on these criteria, a single intramuscular injection of 6 mg/kg of carp pituitary extract and a stripping time of 17 hours at 25 °C gave the best result (Zonneveld et *al.*, 1988). There has been no report of induced spawning trials using LHRHa although Wembiao et *al.*(1988) indicated that LHRHa had no effect on *C. batrachus* even when a maximum dose was applied.

Of the catfishes, *Pangasius sutchi* is relatively difficult to spawn, requiring two to three injections of pituitary extract, HCG or LHRHa. If two injections of HCG+PG are given, doses of 300 IU/kg for the first (stimulating) injection and 500 IU/kg for the second (resolving) injection combined with 1 - 2 doses units of PG gave consistent results (83-100 % spawning). The time interval between stimulating and resolving doses is 8 hours (Thalathiah et *al.*,1988). Sometimes a priming dose of 100 IU/kg HCG or 1 dose unit PG is given followed 24 hours later by the stimulating injection. Acetone-dried pituitary extract (CPE) at doses of 3-4 mg/kg body weight for stimulating and 6-12 mg/kg body weight for resolving injection is also used in place of PG. When LHRHa is used alone, the most effective protocol is 20 μg/kg for the first injection followed 8 hours later by 50 μg/kg as a resolving dose. When combined with PG, highest spawning rate (83 %) is obtained using 20 μg/kg LHRHa + 1 - 1.5 dose units as the first injection followed by 30 μg/kg + 1 - 2 dose units PG as a resolving injection (Thalathiah and Abu-Fauzi, 1986). Ovulation occurs 10 - 12 hours after the last HCG or LHRHa injection.

CHINESE CARPS

The Chinese carps commonly cultured in Southeast Asia include the common carp (*Cyprinus carpio*), bighead carp (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), and grass carp (*Ctenopharyngodon idella*). These fishes with the exception of common carp generally have to be artificially spawned. Traditional practises for spawning Chinese carps involve the use of fresh or preserved pituitary glands from the same or

other species. HCG in combination with pituitary glands is also extensively used. Common practises in induced spawning carps in Southeast Asia are found in Harvey and Hoar (1979) and May et *al.* (1984).

Tab. 2. — Induced spawning of bighead carp (*A. nobilis*), silver carp (*H. molitrix*), and grass carp (*C. idella*) by hypophysation and HCG injection

Hormone[a]	Injection First	Second	Third	Interval (h)	Time to Ovulation/ Spawning (h)	% Spawned (No. of females)	Reference[b]
Bighead Carp (*Aristichthys nobilis*)							
PG	1	2		5-6	5-6	100 (4)	(1)
HCG	200	1500-1800		6	6	100 (4)	(1)
	100-200	700-1000		6-8	6	?	(2)
CPE	2-4	10-20		5-6	6-8	?	(2)
HCG +	0-50	250	—	24,6	?	80-90 (10)	(3)
CPE	—	—	4				
Silver Carp (*Hypophthalmichthys molitrix*)							
PG	1	2		5-6	6	100 (4)	(1)
PG, mg/kg	0.67	1.33		6	?	60-70(10)	(3)
CPE	5	10		6	?	70(10)	(3)
	2-4	16-30		4-5	4-6	?	(2)
HCG	200	1500-1800		6	6	10 (4)	(1)
	220	1350		12	?	?	(4)
	100-200	700-1000		5-6	6-8	?	(2)
Grass carp (*Ctenopharyngodon idella*)							
CPE, mg/fish	4	5		12	6	86.6 (15)	(5)
CPE	1-2	6-12		5-6	?	?	(2)
HCG +	220	1800	—	12, 24	6	?	(4)
PG, mg/fish	—	—	2.5				
HCG +	50	0-250	—		6		
CPE	—	1	4-6	24,6		60-80	(3)

[a] except where indicated hormone dosages are : PG, carp pituitary gland in dose unit; CPE, acetone-dried carp pituitary, mg/kg; HCG, human chorionic gonadotropin, IU/kg.

[b] (1) (Ngamvongchon et *al.*, 1988); (2) (Peter et *al.*, 1988); (3) (Thalathiah et *al.*, 1988) (4) (Ali and Hossain, 1984); (5) (Kumarasini and Seneviratne, 1988).

Similar protocols are used for spawning the different species of carps by hypophysation and HCG administration (Table 2). Carp pituitary gland (PG)is given at 1 dose unit as stimulating injection followed by 2 dose units as resolving injection for bighead and silver carp. When acetone dried carp pituitary (CPE) is used, a stimulating injection of 2-4 mg/kg is followed by 10-20 mg/kg or 16-30 mg/kg as resolving doses for bighead and silver carp respectively. For grass carp a stimulating dose of 4 doses units PG or 1-2 mg/kg CPE followed by 5 doses units PG or 6-12 mg/kg CPE as resolving dose is commonly used. The resolving injection is usually given six hours after the stimulating injection and stripping is carried out six hours after the second injection. Different combinations of HCG, CPE or PG were reported to give good results (Ali and Hossain, 1984; Thalathiah et *al.*, 1988).

LHRHa has been succesfully used for induction of ovulation and spawning of cultured carps in China (Anon., 1977). Information from studies on the neuroendocrine regulation of ovulation and spawning in goldfish and common carp, have been applied in field trials using LHRH analogues and dopamine antagonists to induce spawning of various species of Chinese carps. Standardized spawning methods have now been developed and are currently popularized as the LinPe method (Peter et *al.*, 1987, 1988). The LinPe method involves administering a single injection of [D-Ala^6-Pro^{9}NHEt-LHRH] (LHRHa)or [D-Arg^6-Pro^{9}NHEt-LHRH] (sGnRHa) together with one of the dopamine antagonists, pimozide, domperidone or reserpine. The required doses of LHRHa and dopamine antagonist differ for the different species and range between 10 - 100 µg/kg for LHRHa, 1 - 15 mg/kg for domperidone and 1 - 10 mg/kg for pimozide. The combination of domperidone and sGnRHa is more potent than

Tab. 3. — Induced spawning of bighead carp (*A. nobilis*), silver carp (*H. molitrix*), and grass carp (*C. idella*) using LHRHa alone or combined with HCG and domperidone (DOM)

Hormone[a]	Injection First	Second	Interval (h)	Time to Ovulation/ Spawning (h)	% Spawned (No. of females)	Reference[b]
Bighead carp (*Aristhichthys nobilis*)						
LHRHa	10			18-20	100 (4)	(1)
	5	15	18-20	4-8	100 (4)	(1)
LHRHa + DOM	50 + 5			8-12	?	(2)
LHRHa + DOM	7.5 + 1.5	67.5 + 13.5	12	6-14	75 (4)	(3)
LHRHa, ug/fish	15-20	—	12	?	100(3)	(4)
+ HCG	—	300-800				
LHRHa	—	10	5-6	6-8	?	(2)
+ HCG	100-200	400				
Silver carp (*Hypophthalmichthys molitrix*)						
LHRHa	5	15	18-20	4-8	100 (4)	(1)
LHRHa + DOM	50 + 5			8-12		(2)
sGnRHa + DOM	10 + 5			8-12		(2)
LHRHa	20	—	12	6-14	100 (3)	(3)
+ HCG	—	1500				
Grass carp (*Ctenopharyngodon idella*)						
LHRHa	20	50	12	?	100 (12)	(4)
+ CPE	—	0.5-1.5				
LHRHa	10	10	5-6	6-8	?	(2)
+ CPE	—	2-4				
LHRHa + DOM	10 + 5			8-12	?	(2)

[a] LHRHa, ug/kg (except where indicated); DOM, domperidone, mg/kg; CPE, carp pituitary extract, mg/kg; HCG, human chorionic gonadotropin, IU/kg.
[b] (1) (Ngamvongchon et *al.*, 1988); (2) (Peter et *al.*, 1988); (3) Fermin, A. (unpublished) (4) (Kumarasini and Seneviratne, 1988).

pimozide + LHRHa for spawning the same species of carp (Peter et *al.*, 1988). Ovulation in the three species occurs from 8 - 12 hours after the injection.

The use of dopamine antagonists for potentiating the effect of LHRHa on induction of ovulation and spawning in Chinese carps has not been applied in other Southeast Asian countries. A preliminary trial with bighead and silver carp reared to maturity in cages in a freshwater lake in the Philippines had promising results although the doses used were higher (75 μg/kg LHRHa + 15 mg/kg domperidone)(Fermin, A. personal communication) compared to those used in China (50 μg/kg LHRHa + 5mg/kg domperidone). LHRHa alone or in combination with HCG or PG however has been tried in several institutions (Table 3) with good results. A single injection of 10 μg/kg was reported to be as effective for spawning bighead carp as two injections (5 μg/kg for priming and 15 μg/kg for resolving injection) administered 18-20 hours apart Table 7, Ngamvongchon et *al.*, 1988). The time required from the first injection for ovulation or stripping appears to be longer (18-28 hours) when LHRHa is used alone compared to the combined treatment of LHRHa + domperidone (8-12 hours) or LHRHa + HCG (11-14 hours). Needless to say, the Linpe method is obviously a convenient and reliable technique for spawning Chinese carps and it should be validated in other countries to simplify and lower the cost of producing carp fry.

INDIAN MAJOR CARPS

The Indian major carps, rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigal*) and catla (*Catla catla*) have been introduced in many Asian countries. In their natural habitat, Indian carps spawn once a year during the monsoon season. Increased rainfall and lower water temperatures are believed to be the factors which trigger final gonadal maturation. In countries where these species are introduced, they are usually spawned by hypophysation (May et *al.*, 1984; Harvey and Hoar, 1979). Pituitary gland from common carp or from the same species is given as a single or two injections spaced 6 hours apart. Dosages used in different countries vary slightly : from 7 - 14 mg/kg PG injected once or as two injections, 1/3 the dose as a priming injection followed by 2/3 the dose as a resolving injection. A single injection of 250 IU/kg HCG together with 6 mg/kg CPE is also effective for spawning rohu, catla and mrigal (Thalathiah et *al.*, 1988). Males are injected the same or one-half the dose used for females given at the same time as the last injection. Results using HCG and PG are relatively consistent. Efforts to further optimize dosages or try new spawning agents such as LHRHa have not been done perhaps because Indian major carps are easy to spawn and the hormone dosages required are low.

OTHER CYPRINIDS

Other freshwater fishes that have been succesfully spawned by hormone induction are the barb, *Probarbus jullieni*, puntius carp, *Puntius*

gonionotus and the sultan fish, *Leptobarbus hoevenii.* A single injection of 3-12 mg/kg SG-G100 or 3-6 mg/kg CPE is effective for *P.goniomotus, P. jullieni* and *L. hoevenii* are spawned with a stimulating injection of 250 IU/kg HCG together with 1 mg/kg CPE followed 6 hours later by 4 mg/kg CPE (Thalathiah et *al.*, 1988).

Tab. 4. — Hormone protocols for induced spawning of grouper species

Hormone[a]	Injection First	Second	Third	Interval (h)	Time to Ovulation/ Spawning (h)	% Spawned (No. of females)
I. *Epinephelus akaara* (Tseng and Ho, 1979)						
HCG, IU/ fish	1000	500		24	14-23	100 (8)
II. *E. fario* (Kuo et *al.*, 1988)						
HCG, IU/Kg	1000	1000		24	24-48	80 (5)
	1000	1000	1000	24	24	50 (4)
III. *E. salmoides* (Kungvankij et *al.*, 1988)						
A. HCG, IU/fish	500	500-1000	0-500	12-24	12-15	100 (10)
+ CPG, ug/kg	3	0-3	0-3			
B. LHRHa, ug/kg	10	10	10	12	12-15	100 (2)
IV. *E. tauvina* (Chen et *al.*, 1977)						
A. HCG, IU/kg	500	500		12.5-15	?	?
B. HCG, IU/kg	500-1000	500-1000	0-500	10-13	?	66-100 (15)
+ SPE, mg/kg	0-10	0-10	0-15			
V. *E. salmonoides* (Huang et *al.*, 1986)						
HCG, IU/kg	1000	1000	0-1000	24	11-24	?
+ PG (*E. salmonoides*)	0-1	1				

GROUPERS

Several species of groupers are commercially cultured in Southeast Asia. Fry or juveniles are obtained from natural source but are scarce. At present, hatchery production of grouper fry is at the experimental stage and only few reports on induced breeding of groupers are available. At the Kuwait Institute for Scientific Research, *Epinephelus tauvina* broodstock reared from juveniles spawn naturally in concrete tanks between April and July (Hussain et *al.*, 1975). In Southeast Asia there has been no report

of natural spawnings of groupers although this may occur. The few reports on induced spawning of grouper species include those of Tseng and Ho (1979), Chen et *al.* (1977), Huang et *al.* (1986), Kungvankij et *al.* (1986a), and Kuo et *al.* (1988).

In species where induced spawning has been attempted, all were spawned with HCG alone (*E. akaara*, Tseng and Ho, 1979; *E. fario*, Kuo et *al.*, 1988), in combination with salmon pituitary extract (*E. tauvina*, Chen et *al.*, 1977) or pituitary gland from the same species (*E. salmonoides*, Huang et *al.*, 1986) (Table 4). For *E. salmoides*, 3 injections of 10 *u*g/kg LHRHa are as effective as HCG (500 IU/fish) combined with 3 *u*g/kg CPE (Kungvankij et *al.*, 1986a). Males were given one to two injections of half the dose given to females at the same time as the females. Females are usually stripped 10 - 24 hours after the last injection.

Although the various combinations and doses of the hormones used have been effective, methods for spawning the different species of groupers have not been standardized mainly because of the limited number of broodstock. Fertilization rates reported for the various species, whether eggs were artificially fertilized or naturally spawned were generally low and may be related to the scarcity of ripe males or infertile males. Groupers are protogynous fishes and male broodstock are difficult to collect. Sex-inversed male *E. fario* obtained after six months of oral administration of methyl testosterone to mature females were not functional (Kuo et *al.*, 1988). Further work to determine the appropriate stage of egg development, time interval between injections and appropriate time for stripping ovulated eggs need to be done. For example, a low response rate was obtained even after three injections, if female *E. fario* had oocyte diameters less than 0.50 mm (Kuo et *al.*, 1988). The low fertilization rates reported for *E. salmonoides* (Huang et *al.*, 1986) may also be a result of inappropriate time employed for stripping the females.

MULLET

Mullet is widely distributed and is a good source of cheap animal protein but its culture is not as extensively practised in Southeast Asia as that of other species. This may be due to difficulty in identifying the fry of fast growing species like *Mugil cephalus* from other less desirable mullet species. Most of the information on mullet breeding are results of research done in Taiwan and at the Oceanic Institute in Hawaii. These studies have been reviewed (Liao, 1975; Nash and Shehadeh, 1980). Lee and Tamaru (1988) also provide an update of their recent work at Oceanic Institute.

The mullet, *Mugil cephalus* matures in captivity but has never been reported to spawn naturally. Among the marine species being bred, *M. cephalus* is probably one of the most difficult to induce spawning, requiring at least two injections of high doses of hormones. Various hormone protocols have been tried and these are summarized in Table 5. Gravid females will respond to hormone induction when oocyte diameter is at least 0.60 mm but preferably larger than 0.65 mm. The amount of gonadotropin required to complete final maturation is ontaneous proportional to the initial egg size (Nash and Shehadeh, 1975; Kuo, 1982).

Two injections are given at 24 hours interval and spawning occurs around 12 - 15 hours after the second injection but may be delayed until 45 or 50 hours (Table 5). The different protocols tried using sGTH, CPH and HCG were reported to give high ovulation rates but SGTH gave more predictable results (Nash and Shehadeh, 1980).

Tab. 5. — Hormone protocols for spawning mullet

Hormone[a]	Injection		Time to Ovulation/ Spawning (h)	% Ovulation	% Fertilization	Reference[b]
	First	Second				
PG +	1-2	1.5-30	16-45	15.8-53	62-100	(1)
Synahorin	10-20	20-30				
Vitamin E	100-200	0-200				
SGtH	3.1-9.1	4.6-16.8	12-25	100	53-98	(2)
	5.1-7.0	10.2-14	10-12.5		83-98	(3)
CPH (mg/ fish)	20	40	10-15.3	75	24-73	(4)
HCG	12000-19600	16400 50000	11.8-21	100	45-98	(2)
SGtH + HCG	6.7-9	33300-44900	12-13		94-96	(2)
CPH + HCG	50-66.7	30000-33000	9.5-11.7		77-95	(2)
SGtH + DOC	4.8-7.8	50-110	11.5-23		76-91	(2)
CPH + DOC	50-71	95-157	10.2-20.3		68-92	(2)
LHRHa	101-278	202-484	13-24	68.4	0-95	(5)
CPH + LHRHa	16.53-51.86	165-536	11.5-50.5	94.1	27.5-99.7	(5)

[a] PG - Pituitary gland/fish; Synahorin - RU (Rabbit Units)/fish; Vitamin E - mg/fish SGtH - SG-G100, mg/kg; CPH - carp pituitary extract, mg/kg (except where indicated); HCG- human chorionic gonadotropin, IU/kg; DOC - deoxycorticosterone, mg/kg; LHRHa, ug/kg

[b] (1) (Liao, 1975); (2) (Nash and Shehadeh, 1980); (3) (Kuo, 1982); (4) (Lee et al., 1988); (5) (Lee et al., 1987).

In efforts to optimize spawning procedures and reduce the cost of larvae produced in the hatchery, Lee et *al.* (1987, 1988) compared various strategies using CPE, HCG and LHRHa. Based on the price of the hormones and assuming : 1)that the males were in the same maturation condition, 2) that average fecundity is one million eggs/kg, and 3) that hatch rate is 50 %, the most cost effective protocol for spawning mullet is CPH (20 mg/fish) as a priming injection and 200 *u*g LHRHa as a resolving injection. The estimated cost for producing mullet larvae in the hatchery using this strategy was $0.97 - 1.17 per 100 000 larvae. This is a small fraction of the cost when HCG is used alone ($78.60) or even when HCG is replaced by CPH as a priming dose ($57.20/kg fish) (Lee et *al.*, 1987).

RABBITFISH

Herbivorous fishes like the rabbitfish are good candidate species for aquaculture especially in developing countries where cheap sources of protein are needed. Of the different species of rabbitfishes, *Siganus guttatus* and *S. canaliculatus* are especialy suitable for culture because of their high tolerance to environmental factors, handling and crowding (Carumbana and Luchavez, 1979). *Siganus canaliculatus, S. rivulatus* and *S. argenteus* have been spawned with single or multiple injections of HCG (Lam, 1982). The doses used for these species are similar and range from 250 to 300 IU/kg body weight. Multiple injections are given at 24 hours interval. Female *S. canaliculatus* are stripped 5-10 hours after the last injection (Soh and Lam, 1973) while *S. rivulatus* and *S. argenteus* spawn 36 hours after a single injection (Popper et *al.*, 1979). *S. guttatus* females having eggs with diameters of 0.46 mm will spawn after one injection of 2000 IU/kg HCG while those with smaller eggs (0.43 mm or less) require multiple injections or will not spawn (Juario et *al.*, 1985; Duray and Juario, 1988).

Captive *Siganus guttatus* spawn every month a few days after the first quarter moon throughout the year (Hara et *al.*,1986). However, only a few females appear to spawn each time. Harvey et *al.* (1986) however, reports that when implanted with LHRHa silastic pellets (D-Nal(2)6 LHRH, 6.7 mg/pellet) ten days before the expected spawning, eight of ten pellet-implanted females spawned on the ninth day, one day earlier than control females and all spawned within two days. Sham-operated females spawned over a period of four days beginning on the tenth day after treatment. Spawning on the succeeding month was also advanced in the LHRHa-implanted group and was relatively synchronized.

MILKFISH

Of the finfishes cultured in Southeast Asia, milkfish (*Chanos chanos*) accounts for over half of total fish production from aquaculture and is the single most important species being cultured in the region (Rabanal, 1988). Research on the artificial propagation of this fish has been recently reviewed (Lam, 1984; Kuo, 1985; Kelley and Lee, 1986; Marte, 1987). Table 6 gives a summary of successfull attempts at spawning wild and captive milkfish using salmon or carp pituitary homogenate (SPH or CPH) together with HCG. The hormone dosages, number of injections and time interval between injections varied. Females that were induced to spawn had oocyte diameters greater than 0.66 mm (Lam, 1984). Females were stripped from 4-24 hours after the last injection. Mullet pituitary together with HCG has also been used to spawn milkfish (Liao and Chen, 1984; Lin, 1984). In these early experiments, only one female spawned « slow » pellets following a single injection of 1000 IU/kg HCG.

Synthetic analogues of LHRH have been succesfully used to spawn captive milkfish broodstock (Table 7). LHRHa and D-Ala6-sGnRHa were equally effective when administered as a single injection, cholesterol-pellet

Tab. 6. — Summary of succesfull spawning attempts in wild and captive milkfish using Salmon pituitary homogenate (SPH), carp pituitary homogenate (CPH) and human chorionic gonadotropin (HCG)

Hormone	Dose-range SPH/CPH, mg/kg HCG, IU/kg	No. of Injections	Interval (h)	No. of Fish Spawned
SPH + HCG	42-100* 2,800-10,000*	2-4	8.2-24	8
CPH + HCG	5.6-14.4 430-5,714	2-5	9	4
HCG	1,000-1,429	1		7

* Total dose
Source : (Lam, 1984); (Liao and Chen, 1984); (Kuo, 1985).

implant or osmotic pump implant (Marte et *al.*, 1987). The pellets contained 100 *ug* of the analogue in a matrix of 100 % cholesterol (specific dose = 20.6-35.7 *ug*/kg body weight), the osmotic pump released 10-16 *ug* LHRHa /day (estimated actual dose = 2.8-4.4 *ug*/kg) and injection was given at 10 *ug*/kg. Lee et *al.* (1986 a,b) also succesfully spawned tank-reared broodstock with a single pellet implant or injection of 200-250 ug LHRHa. D-Arg6-sGnRHa was as effective as D-Ala6-sGnRH and D-Ala6LHRH, and HCG was comparable if not more effective than the analogues for spawning milkfish (Marte et *al.*, 1988). Spontaneous spaw-

Tab. 7. — Induced spawning of milkfish with analogous of luteinizing hormone - releasing hormone

Analogous	Mode of Administration	Dose ug/kg	Total dose ug/fish	No. of fish spawned	References**
LHRHa	Pellet implant	20.6-30.8	100	4/10	(1,2)
	Injection	10	34.5-62.5	5/7	
	Osmotic pump implant	58-92*	330	2/3	
	Pellet implant	41.7 ± 3.3	200-250	9/17	(3)
	Injection	58.7 ± 9.3	250	10/33	
D-Ala6-sGmRHa	Pellet implant	19.2-26.3	100	3/3	(1)
	Osmotic pump implant	65-69*	330	2/3	
D-Arg6-sGnRHa	Pellet implant	21.5-35.7	100	1/7	(2)
	Injection	24-34	100	4/4	

* Release rate from the osmotic pump is 10-16 ug/day hence actual dose is estimated at 2.8 and 4.4 ug/kg.
** (1) (Marte et *al.*, 1987); (2) (Marte et *al.*, 1988); (3) (Lee et *al.*, 1986); (Kelley and Lee, 1986).

ning occurred 16-32 hours after treatment with the analogues or HCG. LHRHa is cheaper to use as a spawning agent than HCG, however, cost-effectiveness of this hormone will have to be assesed in terms of viable spawns and number of fry produced from induced-spawned females. Reported fertilization rates after LHRHa treatment were highly variable and ranged from 20-88 % (Marte et *al.*, 1987) and 14-99 % (Kelley and Lee, 1986).

SEABASS

The seabass *Lates calcarifer* is a popular foodfish in Southeast Asia. Techniques for culture of sea bass were first developed in Thailand in the early 1970's (Wongsomnuk and Manevonk, 1973). Seabass culture has since been popularized in Thailand and is now bred routinely in many government and private hatcheries by either environmental or hormonal means (Maneewong, 1986).

Natural spawning of seabass appears to be periodic. Ripe spawners are usually collected at around the full moon and from 18 :00 to 22 :00 hr at the time of the rising tide (Kungvankij et *al*, 1986b). Natural spawning of cage-reared broodstock also occurs at this time (personal observation). Induced spawning is thus normally done during this period and injections timed such that spawning occurs between 18 :00 - 22 :00 hr.

The widely practised method for induced spawning of seabass involves injecting wild spawners or mature captive broodstock with either Puberogen,Pregnyl, HCG or HCG + pituitary gland of Chinese carps or sea bass (Kungvankij, 1986; Kungvankij et *al.*, 1986b; Maneewong, 1986). Mature females are injected once or twice with 50 - 200 IU/kg body weight Puberogen. A double dose is given as a second injection if two injections are administered. Males receive 20 - 50 IU/kg body weight. Spawning occurs 36 hours after a single injection or within 12-15 hours after the second injection. The doses used for HCG and carp pituitary extract are 150 - 1000 IU/kg body weight and 2-3 mg/kg respectively following the same injection protocol as for Puberogen.

Various methods of LHRHa administration for spawning seabass have been tried with interesting results. One or two injections of from 10 to 75 *u*g LHRHa/kg body weight induced two successive spawnings at 24 hours interval (Lim et *al.*, 1986; Nacario, 1987; Nacario and Sherwood, 1986). Pelleted LHRHa in a cholesterol matrix at doses ranging from 9-23 *u*g/kg induced spawning 72 hours after implantation (Harvey et *al.*, 1985). Implantation of an osmotic pump designed to deliver a continous release of 9 *u*g of the analogue per day at 26 °C induced up to five consecutive spawnings in individual females (Nacario, 1986; Nacario and Sherwood, 1986; Almendras et *al.*, 1988). Up to four consecutive spawnings were also obtained after daily injections of 60-100 *u*g LHRHa (Almendras et *al.*, 1988). Similarly, multiple spawnings were induced after a single implantation of « quick-release » or « slow-release » LHRHa cholesterol pellets. The pellets contained 100 ug LHRHa in a matrix consisting of different proportions of cholesterol and cellulose. « Quick » pellets had 80 % cholesterol whereas « slow » pellets had either 95 % or 100 % cholesterol.

Garcia (in press) has determined the minimum dose of LHRHa in cholesterol pellet that will induce the maximum spawning rate (total number of spawnings per fish over four days multiplied by 100) to be between 37.5-75.0 *u*g/kg body weight. Total number of eggs produced by females implanted with the graded doses of LHRHa did not differ. The most number of eggs was spawned on the first day progressively decreasing during succeeding spawning days. Fertilization rate was significantly lower in fish implanted with the high LHRHa dose (300 *u*g/kg). Hatching rate although not significantly different in fish implanted with increasing doses was also lower in females implanted with the high LHRHa dose. A graded spawning response to LHRHa injection was also demonstrated (Garcia, submitted). At similar doses (50-100 *u*g/kg) up to three consecutive spawnings were obtained following a single LHRHa injection, the lower doses (1-10 *u*g/kg) induced at most 2 spawnings.

Seabass will spawn from 30-36 hours after hypophysation (Kungvankij et *al.* 1986), LHRHa injection (Nacario, 1987) or LHRHa implantation (Almendras et *al.*, 1988; Garcia, in press and submitted) but may be as long as 72 hours after a single pellet implantation (Harvey et *al.*, 1985) In these experiments, oocyte diameter of mature females was usually less than 0.50 mm. The response time however was shortened to 8-9 hours in females with oocyte diameter greater than 0.50 mm (Garcia submitted).

The results of these experiments demonstrate that administering LHRHa via pellet implants is an alternative method for spawning fish. The method may prove to be more cost-effective and convenient especially for batch or sequential spawners like seabass.

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