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Broodstock Rearing and Controlled Reproduction of Reeves Shad *Tenualosa reevesii*

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Abstract

Reeves shad *Tenualosa reevesii* is one of China's best known anadromous fish, but is presently on the verge of extinction. There is an urgent need for conservation of this species through artificial reproduction and culture. In this study, shad broodstock were held in captivity then were induced to spawn with serial injections of luteinizing hormone-releasing hormone analogue (LHRHa) following GtH priming by implantations of 17-methytestosterone (T) combined with environmental manipulations. Induced maturation of 4, 5, and 6-year-old females reached 83.3%, 87.5%, and 100%, respectively, while fish in control treatments remained immature. During the gonadal development priming period, mean serum 17-estradiol levels in the T treatment were significantly higher than the LHRHa treatment and control fish. Gonadal development of females was suppressed when their mesenteric fat index was below 1.5% by April. The effective accumulating temperatures for female and male maturation were 3,995 ± 31.4 C/d and 2,278 ± 8.9 C/d, respectively. Two injections of LHRHa in combination with domperidone (DOM) and environmental regulations were effective in inducing ovulation. About 800,000 fertilized ova were obtained (fertilization 75%) in 1995, and 5,000 fry were hatched in 1996. These results are an important step to develop shad culture and save this endangered species.

Reeves or Chinese shad *Tenualosa reevesii* is one of the largest species in the subfamily Alosinae and is one of the best known and valuable anadromous fish endemic to China. This species supported a lucrative commercial fishery before the early 60s, but is presently on the verge of extinction (Wang 1996). As a result, there is an urgent need to develop aquacultural and reproductive techniques for this species in order to meet the demand for markets and save this endangered species.

Traditional shad hatchery operations have largely depended on strip-spawning ripe fish on the spawning grounds (Leach 1925; Lu 1964; Malhotra et al. 1969; Anonymous 1978; Jia 1982; Hendricks 1986, 1996). Using this procedure, all adult fish were captured by gillnet and subsequently sacrificed during expression of eggs and milt. This process is limited by the difficulty in obtaining an ample supply of ripe fish, especially when shad resources are severely depleted. The sacrifice of valuable broodstock by strip-spawning in this manner is also damaging to natural spawning populations. As an alternative to strip-spawning, induced spawning of captured shad has been attempted, but initial trails have not been successful (Personal communication, Shunlin Qiu, Yangzte River Fisheries Institute, Jingzhou, China 1985) as the shad die easily during capture and handling. Therefore, for artificial spawning to be successful, domestication of the shad juvenile to sexual maturity must be achieved.

During the past few decades, there have been several attempts to produce domesticated broodstock of various shad species

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(Malhotra et al. 1969; Fleetwood 1978; Qju 1981; Jia 1982; De 1984; Sen et al. 1990). However, due to handling stress and low survival in captivity, no shad species to date has been successfully cultured to sexual maturity and spawned artificially.

The reproductive cycle of Reeves shad is characterized by a long delay before sexual maturation and, even at the early phase of the reproductive migration to rivers, the gonads remain immature. Furthermore, if the reproductive migration is blocked, such as by captivity, gonadal development is suppressed and sexual maturation will not occur (Wang et al. 1998). Therefore, after obtaining broodfish in captivity, it is important to find ways to trigger gonadal development.

Peter (1982) and Goos (1987) noted that positive feedback by testosterone on pituitary GtH level is important in triggering gonadal development of anadromous fish. Testosterone treatment has resulted in both pituitary synthesis and release of GtH in a number of salmonids, and stimulated gonadotropic hormone-releasing hormone (GnRH) activity in the hypothalamus (Goos et al. 1986). These and other studies have shown that above normal levels of testosterone influence the hypothalamus and pituitary, stimulating gonadal development via production and release of GtH by the pituitary.

Under natural conditions, Reeves shad do not feed during their anadromous period. Energy and nutrition for gonadal development depend on body fat stored at sea during winter. Under culture conditions, Reeves shad have to winter in holding ponds for about 4 mo. During that period, they do not feed much, due to low temperature, and fat does not accumulate. Therefore, fat reserves may be a key factor to gonadal development of cultured shad.

The objectives of this study were: 1) to determine if Reeves shad could be domesticated to sexual maturity by employing pond culture procedures, and 2) to evaluate the effects of hormonal and environmental manipulations on sexual maturation and the ability of captive broodfish to be spawned artificially.

Materials and Methods

Broodstock Rearing

Juvenile shad with total length from 17.0 to 37.5 mm and body weight from 0.18 to 0.51 g were captured near the mouth of Pearl River and transported to Dongguan Aquacultural Experiment Station (Guangdong Province, China) in September of 1989–1991. Shad were reared in 667-m^2 earthen ponds at ambient temperature (annual 14–34 C) and salinity (0.5–12 ppt) and under a natural photoperiod. Ponds were fertilized by applying 300 kg of grass manure weekly and 1 kg of soybean cake daily. Zooplankton in the ponds were maintained at about 1,500 ind./L. Shad age 1–6 yr were fed commercial eel feed (43% protein, 12% fat) supplemented with different kinds of marine fish trash gruel, 2–3 times daily, during the period of April to November each year. Feeding rates were 1–1.5% and 2–3% of body weight, respectively. From December to March, fish were kept in holding ponds for winter and fed commercial eel feed once a day, at 0.5% of body weight.

Environmental Manipulations

During brood fish culture, ponds approximated natural conditions for shad spawning in the rivers, salinity was maintained at above 10 ppt in winter, and then gradually decreased to 0.5 ppt from spring to summer. Temperature was controlled at above 15 C, mean 23.3–24.6 C, for different years. Fresh water was introduced into ponds for 4–6 h/d at a rate of about 36 m^3/h, and dissolved oxygen concentration was maintained at 5.9–9.7 mg/L. During induction of ovarian development, broodfish were fed eel feed three times daily.

Hormonal Manipulation

17-methytestosterone (T) was purchased from Sigma Chemical Co., USA; LHRHa,

TABLE 1. *Summary of experimental design for inducing maturation of cultured Reeves shad* Tenualosa reevesii *with luteinizing hormone-releasing hormone analog (LHRHa) and 17-methytestosterone (T) during 1992–1996. TL = T-Lacquer butter, TS = T-silastic pellet, Im = Implantation, Inj = Injection.*

Trial no.	*N*	Age (yr)	BW (kg)	Treatments	Dosage (/kg)	Times	Intervals (d)	Period
1	8	3	0.9–1.1	LHRHa-Inj	10–15 g	6–8	10–15	April–June
	4	3	0.9–1.1	Sham control				
2	8	4	0.9–1.2	LHRHa-Inj	10–15 g	6–8	10–15	April–June
	4	4	0.9–1.2	Sham control				
3	8	5	1.2–1.4	TL-Im	25 mg	2	14–15	March–April
	8	5	1.2–1.4	+LHRHa-Inj	10 g	4–6	10–15	April–June
	3	4–5	1.2–1.4	LHRHa-Inj	10 g	6	10–15	April–June
	7	4–5	1.2–1.4	TS-Im	50 mg	3	30	April–June
	3	5	1.2–1.4	Sham control				
4	9	4	0.9–1.2	TL-Im	25 mg	2	14–15	March–April
	9	4	0.9–1.2	+LHRHa-Inj	10 g	4–6	10–15	April–June
	7	6	1.4–1.6	TL-Im	25 mg	2	14–15	March–April
	7	6	1.4–1.6	+LHRHa-Inj	10 g	4–6	10–15	April–June
	2	6	1.4–1.6	Sham control				
5	3	5	1.2–1.4	TL-Im	25 mg	2	14–15	March–April
			1.2–1.4	+LHRHa-Inj	10 g	4–6	10–15	April–June

salmon gonadotropin-releasing hormone analog (sGnRHa), human chorionic gonadotropin (HCG), and domperidone (DOM) were purchased from the Ningbo Fish Hormone Factory (Zhejiang Province, China). T was dissolved in *molten lacquer butter* (a kind of butter made from seeds of lacquer tree) or incorporated into silastic implants as described by Lee et al. (1986a). Crystalline T was mixed with unpolymerized elastomer (Silastic 382 Medical Grade Elastomer, Dow Corning Co.) at 50 mg/g elastomer, 5 μl of accelerator added, and the mix spread into 2 × 2 × 30-mm molds to give 0.2-mg T/mm pellets. LHRHa was dissolved in freshwater teleost physiological saline (PS), whereas DOM was suspended in PS.

In an attempt to induce maturation, five trials were done from 1992 to 1996 (Table 1). Parent shad were subdivided into two groups and were randomly stocked into two ponds. The sex ratio was assumed to be 1:1 as shad do not exhibit sexual dimorphism prior to maturation. In 1992 and 1993 trials (experiments 1 and 2, respectively), one group received PS and served as a control, and another group received 6–8 intraperitoneal injections of LHRHa at a dose of 10–15 g/kg body weight at 10–15 d intervals from April to June. In experiment 3 the groups were treated as follows: 1) Fish received T-lacquer butter implants followed by injections of LHRHa. Fish were primed with two implantations of T at a dose of 25 mg/kg body weight at biweekly intervals from March to April to stimulate GtH accumulation in the pituitary. T, dissolved in molten lacquer butter, was injected intraperitoneally at the level of the pelvic fins at a temperature of 35 C. Following GtH priming, animals were treated with 4–6 injections of LHRHa at a dose of 10 g/kg body weight at 10–15 d intervals between April to June. LHRHa was injected intraperitoneally at the level of pectoral fin; 2) Fish received 6 injections of LHRHa at the same dose and intervals from March to June; 3) Fish received T-silastic pellets at a dose of 50 mg/kg body weight, using the procedure described by Lee et al. (1986a, 1986b), three times at monthly intervals between April and June; and 4) Control. In 1995 (experiment 4), above groups (1) and (4) were repeated. In 1996 (experiment 5), group (1) was repeated.

TABLE 2. *Sexual stages and their characteristics of Reeves shad* Tenualosa reevesii *according to the international (Hjort) scale (Bowers and Holliday 1961) as modified for pacific herring by Hay (1985).*

Stage	Characteristics
Stage I	With undeveloped or thread-like gonads
Stage II	With "starting" or ribbon-shaped gonads; some of the oocytes are developing yolk vesicles
Stage III	Early vitellogenesis stage; the developing gonads are in an early growth phase
Stage IV	Late vitellogenesis stage; maturing gonads are in a late growth phase
Stage V	Mature eggs become transparent and can be extruded with abdominal pressure although ovulation has probably not occurred; milt will flow under pressure from the testes
Stage VI	"Ripe" females have ovulated; sperm flows without pressure
Stage VII	"Spent" fish have spawned

Six trials were conducted to induce spawning, each having a control treatment. Four trials were carried out during July to August in 1994. Mature fish received two intraperitoneal injections of LHRHa plus HCG in the first three trials, and sGnRH and DOM in the fourth trial. In trials of 1995 and 1996, fish received LHRHa plus DOM by two injections with an interval of 12 h.

Evaluation of Sexual Maturity and Determination of Hormone Levels

Sexual maturity of the males and females was evaluated by the international (Hjort) scale (Bowers and Holliday 1961) as modified for pacific herring *Clupea harengus* pallasi by Hay (1985). In this scale, sexual maturity of the shad can be divided into seven stages (Table 2).

Fish were sampled monthly by seining. At the time of treatment, sexual maturity as defined (Table 2) was assessed by gentle squeezing of the abdomen and collection of ovarian samples using the cannulation technique. Samples for histological analysis were fixed in Bouin's fluid, hydrated, and embedded in paraffin. The 4–8 μm thick sections were stained with Regaud's hemotoxyline, orange G, aniline blue for males, and with Heindenhein's Azan for females. Diameters of over 1,000 eggs were measured with IBAS-2000 image analysis system for each developmental stage. Spawned eggs were collected and their diameters were measured similarly. Maturity was judged by the gonadosomatic index (GSI = 100 × gonad weight/whole body weight). Body fat stores were evaluated by mesenteric fat index (MFI = 100 × mesenteric fatty tissue/whole body weight).

Blood samples (3 mL) were taken from fish in group 1, 2, and 4 of experiment 3 during the gonadal development priming period (March–April) by puncturing the caudal vasculature with a 25-gauge needle attached to 1-mL disposable syringe. Blood samples were allowed to clot on ice for several hours, and the serum was separated by centrifugation and stored at −25 C prior to determination of 17-estradiol (E_2) by RIA. Differences in mean responses among three groups were evaluated with one-way analysis of variance (ANOVA; $P < 0.05$) on ranked data followed by Duncan's test for group separation.

Results

Domestication of Shad Juvenile to Broodfish in Captivity

About 215 broodfish were domesticated from juveniles under pond conditions. The survival rate of domesticated shad ranged from 38.5–98.7% from 0⁺age juvenile to 6-age adult. The body weight of broodfish ranged from 805–1,590 g from age of 3 to 6 yr (Fig. 1).

Induced Gonadal Development and Maturation of Domesticated Shad

Experiments 1 and 2. By the end of July of each year, all LHRHa treated as well as control fish were immature. Ovaries of fish of two treatments were at stage II and III with average oocyte diameters of 58.7 μm and 202.1 μm, respectively. The sexual ma-

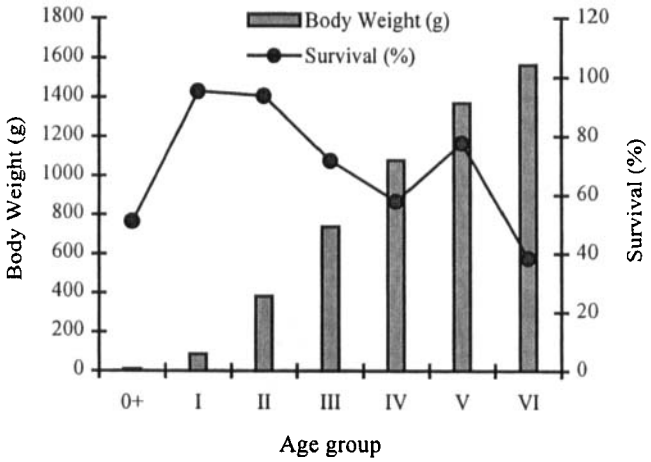

FIGURE 1. *Growth and survival of domesticated shad from juvenile to broodfish in captivity. Here age 0⁺ means the age when juvenile were captured from river, about 2-mo old; Age I means one full year of age; Age II means two full years of age, and so on and so forth.*

turity was at stage III–V for both treated and control male.

Experiment 3. On day 35, 7 d following the two implantations of T, the development of ovaries was primed and oocytes were at previtellogenesis to early vitellogenesis stage (stage III; Fig. 2A). At the end of May, after three injections of LHRHa, ovaries of T-treated fish were well developed, oocytes were at middle vitellogenesis stage (early stage IV; Fig. 2B), and average egg diameter was 508 μm. By the end of June after six injections of LHRHa, 87.5% of hormone-treated females were maturing (late stage IV) and possessed vitellogenic ova with an average egg diameter of 691.5 μm (Fig. 2C). In contrast, the maturity of fish receiving six injections of LHRHa or three implantations of T-silastic pellets alone were at early vitellogenesis stage (Fig. 2D, E), and control animals were only at previtellogenesis stage (Fig. 2F). Hormone-treated males matured earlier than treated females. At the end of May, the maturity of all treated fish reached stage V and sperm could be expelled from the urogenital pore with slight pressure exerted by stroking the abdomen. The histological examination of testes showed that a great number of spermatozoa were already present in the lobular lumen (Fig. 2G). On the other hand, the maturity of control animals was at stage IV–V and the germ cells of the testes were only spermatogonia (Fig. 2H).

Experiments 4 and 5. The same results as for experiment 3 were obtained for females in both experiments. For males, both treated fish and control 6-age shad were mature in experiment 4. The experimental results of hormone-induced maturation of pond-domesticated shad are summarized in Table 3.

Effect of T Implantation on Plasma E_2

Mean serum E_2 levels of fish receiving T administration exhibited a significant increase (ANOVA; $P < 0.05$) on day 1, to 2.7 ± 0.6 and 3.3 ± 0.8 ng/mL in March and April, respectively, while concentrations of E_2 of fish receiving LHRHa administration were 0.4 ± 0.1 and 0.5 ± 0.1 ng/mL, respectively; not significant higher (ANOVA; $P > 0.05$) than the E_2 levels of control fish which remained at the levels of 0.3–0.4 ng/mL (Fig. 3).

Effect of Fat Stores on Gonadal Development and Maturation

Studies on mesenteric fat indices of broodfish in the same treatment showed that only fish with 2.0% of mesenteric fat index

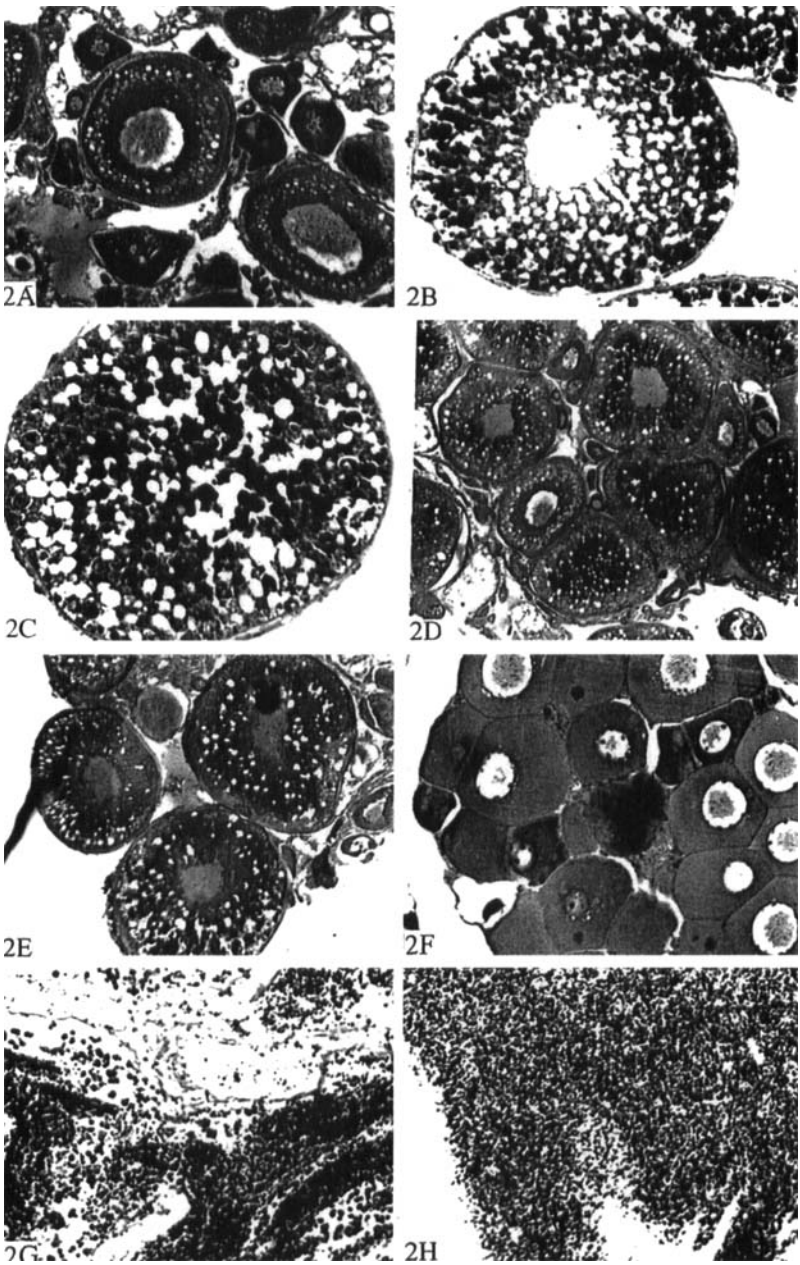

FIGURE 2. *Effect of injections of luteinizing hormone-releasing hormone analogue (LHRHa) following two implantations of methytestosterone (T), and LHRHa or T treatment alone, on gonadal development and maturation. Seven d following the two implantations of T, the development of ovaries was primed and the oocytes were at early stage III (Fig. 2A). At the end of May after three injections of LHRHa, the ovaries of T-treated fish were well developed, the oocytes were at early stage IV (Fig. 2B). By the end of June after six injections of LHRHa, 87.5% hormone treated females were at late stage IV (Fig. 2C). In contrast, the maturity of fish receiving six injections of LHRHa or three implantations of T-silastic pellets alone were at early vitellogenesis stage (Fig. 2D, E), and control animals were only at previtellogenesis stage (Fig. 2F). To the end of May, the maturity of all treated fish reached stage V (Fig. 2G), while the maturity of control animals were at stage IV–V (Fig. 2H).*

TABLE 3. *Summary of experimental results of induced maturation of domesticated shad* Tenualosa reevesii *with luteinizing hormone-releasing hormone analog (LHRHa) and 17-methytestosterone (T) in captivity during 1992–1996. TL = T-Lacquer butter, TS = T-silastic pellet, IM = Implantation, Inj = Injection.*

Trial no.	N	Age (yr)	Treatment	Maturity stage	
				Female	Male
1	8	3	LHRHa-Inj	II	III–IV
	4	3	Sham control	II	III
2	8	4	LHRHa-Inj	II	IV–V
	4	4	Sham control	II	IV–V
3	8	5	TL-Im+LHRHa-Inj	IV	V
	3	4–5	LHRHa-Inj	III	V
	7	4–5	TS-Im	III	V
	3	5	Sham control	III	IV–V
4	9	4	TL-Im+LHRHa-Inj	IV	V
	7	6	TL-Im+LHRHa-Inj	IV	V
	2	6	Sham control	III	V
5	3	5	T-Im+LHRHa-Inj	IV	V

by April could reach final maturity. Maturation was suppressed when the mesenteric fat index failed to exceed 1.5% by April, although it could reach 2.1% by June.

Effective Accumulating Temperature of Gonadal Development

Cultured shad began to accumulate fat when the water temperature was above 20 C after wintering. Therefore, the effective accumulating temperature is cumulative total of temperature days above 20 C (Table 4). Table 4 shows that gonadal maturation of female shad is more sensitive to temperature than for males. Females need about 4,000 C/d of accumulating temperature and males only need about 2,280 C/d.

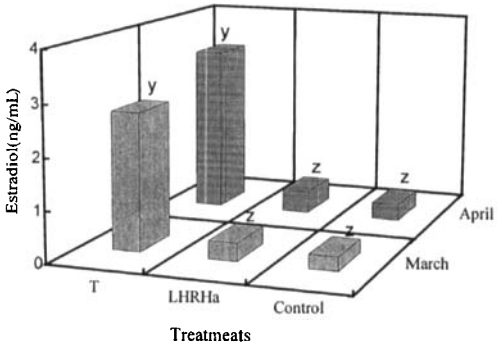

FIGURE 3. *Change in concentration of serum estradiol (E_2) in Reeves shad after treatment with methytestosterone (T) or luteinizing hormone-releasing hormone analogue (LHRHa) during the gonadal development priming period (March–April). Means among treatments not sharing similar superscripts differ significantly (P < 0.05).*

Relationship between Gonadal Development and Season and Water Temperature

Ovaries of cultured shad were at stage II during winter and GSI ranged from 0.21–0.95%. In March and April, the hormone-treated shad began to store fat and oocytes began to accumulate yolk material, but the ovaries were still at stage II–III and GSI ranged from 0.50–1.20%. In summer the ovaries developed rapidly and reached stage IV with an average GSI of 6.20% by the end of May and stage V with GSI of 13.50% at the middle of July (Fig. 4).

The development of testes of males was coincident with egg development in females during winter to spring. Development accelerated in summer and by June all fish reached a mature stage (V). The monthly changes of testis development of hormone-treated male are presented in Table 5.

Induced Spawning in Maturing Shad

All treated females were induced to a mature stage (V) by two intraperitoneal injections of LHRHa plus HCG or DOM in

TABLE 4. *The effective accumulating temperature (EAT) of hormone-induced development of gonads in Reeves shad* Tenualosa reevesii *during 1994–1996. EAT is cumulative total of above 20 C. MWT = Mean water temperature.*

	Male			Female		
Year	MWT (C)	Culturing period (d)	EAT (C/d)	MWT (C)	Culturing period (d)	EAT (C/d)
1994	27.9 ± 2.6	87	2,287.8	28.4 ± 2.5	141	4,004.4
1995	25.8 ± 2.5	92	2,270.4	27.0 ± 2.4	153	4,021.5
1996	28.1 ± 2.6	81	2,276.1	28.7 ± 2.5	138	3,960.6
Mean	27.3 ± 2.6	85	2,278.1 ± 8.9	28.0 ± 2.5	144	3,995.5 ± 31.4

1994. However, the treated shad were extremely sensitive to handling stress when they were in spawning condition. Only fish in the LHRHa+DOM group spawned; females in the other groups failed to ovulate after handling. On the basis of initial experiments, obstacles about ovulation mechanism and handling-induced stresses were overcome in the process of induced spawning by increasing water circulation and improving spawning condition, and about 800,000 fertilized ova were obtained (fertilization 75%) in 1995, and 5,000 fry were hatched in 1996 (Table 6).

Discussion

The culture and induced reproduction of shad has been a worldwide problem and studied for many years. The most important findings from the present experiment are: 1) shad juveniles can be cultured to broodfish size in captivity by improving environmental conditions and culturing techniques though they are extremely sensitive to handling stress and environmental changes; and 2) serial injections of LHRHa following GtH priming by implantations of T, combined with environmental manipulations, can stimulate the brain-pituitary-ovary axis of domesticated Reeves shad to induce gonadal development to maturation; furthermore, injection of LHRHa plus DOM can induce spawning. To our knowledge this is the first successful domestication of shad juvenile to broodfish and artificial induction of sexual maturation and spawning.

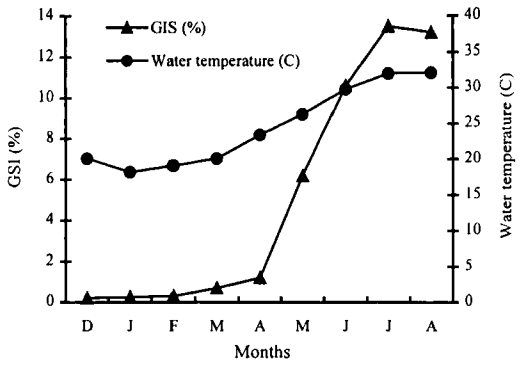

FIGURE 4. *Relationship between ovary development, judged by gonadosomatic index (GSI), of females and season and water temperature in 1996. All females were treated by implantations of methytestosterone (T) and injections of luteinizing hormone-releasing hormone analogue (LHRHa) from March to June.*

These results demonstrate that pelleted T is effective in the onset of gonadal development of cultured Reeves shad. T has some stimulatory effects on the brain-pituitary axis leading to an accumulation of GtH in the pituitary. This reflects a positive feedback effect of T on the brain-pituitary axis, and stimulation of GtH synthesis in the pituitary, similar to the positive feedback effects of sex steroids previously demonstrated in immature salmonids (Crim and Evans 1979, 1982, 1983). In those studies, T implants in Atlantic salmon parr stimulated precocious development.

Several studies (Peter 1982; Goos et al. 1986; Goos 1987) have shown that above normal levels of testosterone influence the hypothalamus and pituitary, stimulating go-

TABLE 5. *Monthly change of testes development of male Reeves shad* Tenualosa reevesii *treated with luteinizing hormone-releasing hormone analog and 17-methyltestosterone during 1994–1996. MWT = mean water temperature.*

	Developmental stage of testes[c]					Sperm index				
Year	Jan–Feb	March	April	May	MWT (C)	May	June	July	Aug	MWT (C)
1994	II	III	IV	IV–V	21.65	+	+++	++	+	30.06
1995	II	III	IV	IV–V	20.95	–	+++	+++	++	29.93
1996				IV–V	21.73	+	+++	++	+	30.01

nadal development via production and release of GtH by the pituitary. However, testosterone may not have a direct effect on the hypothalamus or pituitary. It may only provide the substrate for aromatisation to estrogen. Crim et al. (1981) demonstrated that only estrogens and aromatizable testosterone stimulated GtH production in trout. More significantly, they found that an aromatase inhibitor reduced GtH production in testosterone-treated fish. The aromatisation of testosterone may be a significant source of estrogen production in female and male fish (Greeley et al. 1988).

Fat stores play an important role in regulation of maturation in Reeves shad. Fat levels must increase during March to April for maturation to occur. Early feeding is needed for this, but the fats in food must also be stored. Under natural conditions, Reeve shad feed frequently and store abundant fat in the sea during winter. When they enter the mouth of the Yangtze River in April, the total lipid content reaches 20.43% and 21.82%, respectively, for females and males. Lipid content declines to 9.60% and 5.89% at the stage of ripening for females and males, respectively, when fish return to spawning grounds (Sun 1985).

Johnston et al. (1987) found that the timing of fat deposition was important for maturation to proceed in another anadromous species. They noted that oogenesis and vitellogenesis proceeded in reconditioned, previously spawned Atlantic salmon kelts *Salmo salar,* which restored fat and protein reserves early in spring. It did not occur in the fish that were slow to restore energy reserves. Rowe et al. (1991) provided evidence that in Atlantic salmon the size of the mesenteric fat store was important for the decision to proceed with maturation or to suppress it. When mesenteric fat failed to exceed an underlined level by May, maturation was suppressed.

Serial injection of LHRHa to T-primed shad cultured in ponds can induce gonadal development to the maturation stage.

TABLE 6. *Result of induced spawning in induced-maturing shad* Tenualosa reevesii *with luteinizing hormone-releasing hormone analogue (LHRHa), gonadotropin-releasing hormone analogue (sGnRHa), human chorionic gonadotropin (HCG), and domperidone (DOM) by two intraperitoneal injections with an interval of 12 h during 1994–1996.*

		Treated group			Control group		
Trial no.	Treatment	*N*	*N* of fish spawned	*N* of eye eggs	*N* of hatched fry	*N*	*N* of fish spawned
1	LHRH-a+HCG	2	0			2	0
2	LHRH-a+HCG	2	0			2	0
3	LHRH-a+HCG	2	1			2	0
4	SGnRH+DOM	1	0			2	0
5	LHRH+DOM	2	2	800,000		2	0
6	LHRH+DOM	2	2	30,000	5,000	2	0

Mclean (1991) reported that oral delivery of LHRHa to estradiol-primed coho salmon *Oncorhynchus kisutch* results in a significant release of GtH when compared to control, saline-treated fish. A single injection of LHRHa alone stimulated GtH release in female Japanese eel *Anguilla japonica* pretreated by estradiol (Lin et al. 1991). These results confirm that GtH release can be induced in T or E_2-treated fish. Evidence that LHRHa stimulates GtH secretion in teleost fish has been presented previously for common carp *Cyprinus carpio* (Weil et al. 1980), trout (Crim et al. 1981) and goldfish *Carassius auratus* (Peter and Paulencu 1980).

Two injections of LHRHa alone have a limited effect on inducing spawning in female Reeves shad. However, LHRHa in combination with DOM was more effective in inducing spawning. These results suggested that the neuroendocrine regulation of GtH secretion in shad also involves a dual control, with GtH releases stimulated by GnRH and inhibition by dopamine which acting as a gonadotropin release inhibition factor (GRIF). Kreibery et al. (1987) noted that dopamine inhibition is less important in the pacific herring *Clupes harengus* than in other species. In that study, herring were treated at a time shortly before natural spawning when dopamine inhibition of gonadotropin release may have been minimal.

The present findings have important significance to save valuable shad resources worldwide. These techniques make it possible to develop large-scale shad culture and release, which may provide a fundamental means for stock recovery. The current efforts to rebuild stocks of shad, such as Reeves shad, American shad, and Indian shad *Tenualosa ilisha*, depend on dry stripping of gillnet-caught naturally-ripened adults. The results reported in this study will promote the development of commercial culture of shad in a pond environment, which can help to meet market demands for these high-value fish while reducing dependence on commercial fisheries.

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