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Original Article

Effects of water salinity on reproductive performance of the female hatchery-reared spotted scat, *Scatophagus argus* (Linnaeus, 1766) broodstock

Jirayuth Ruensirikul[1, 2*] and Sommai Chiayvareesajja[1]

[1] *Department of Aquatic Science, Faculty of Natural Resources*
Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand

[2] *Coastal Aquaculture Research and Development Regional Center 6 (Songkhla),*
Department of Fisheries, Mueang, Songkhla, 90000 Thailand

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Abstract

The effect of water salinity on the reproductive performance of female hatchery-reared spotted scat was investigated. Broodfish were reared in a hatchery for 14 months until maturation and selection. Selected broodfish were maintained in 1-m^3 tanks with holding water salinities of 5, 15, 25, and 33 ppt. The maturity of the female broodfish was monitored monthly for 4 months after the experiment was initiated. Each month, their reproductive parameters were monitored and their serum 17β-estradiol (E2) levels and osmolality were determined. The results showed that none of the reproductive parameters including the E2 profile and blood osmolality differed significantly between treatments except that the ovulation rate of the fish held in water salinities of 15 and 25 ppt were significantly higher than those of other salinities ($P<0.05$). This finding indicated that a holding salinity of 15–25 ppt was optimal for the culture of spotted scat broodstock in hatcheries.

Keywords: spotted scat, salinity, hatchery-reared broodstock, reproductive performance, estradiol

1. Introduction

The spotted scat, *Scatophagus argus* (Linnaeus, 1766) is a tropical finfish with the potential for coastal aquaculture. The two advantages of the spotted scat are 1) it is a valuable food fish and 2) it is a popular ornamental fish. Many countries have tried to breed this species (Cai, Wang, Hu, Zhang, & Lin, 2010; Chang & Hsieh, 1997; Ruensirikul, Assawaaree, Danayadol, & Chusrirat, 2008) but the results of mass seed production have been limited (Khanh, Hai, Huong, & Phuong, 2012). Nowadays, almost all the juvenile or mature spotted scat fish found in the market have been caught in the wild and the quantity is inadequate to support the market demand. One of the most important elements of the successful mass seed production of marine fish is the development of hatchery-reared broodstock because the availability of broodfish from wild sources is not consistent in either quantity or quality (Mair, 2002).

Before the establishment of a hatchery-reared broodstock, suitable environmental conditions for broodfish should be investigated to ensure appropriate fish rearing conditions that may affect the achievement of gonad development, such as water quality and nutrition (Mylonas, Fostier, & Zanuy, 2010). Water salinity is an important environmental factor which influences osmoregulation and ion-regulation in fish. These biological processes require energy, thus the broodfish has to make adjustments in order to maintain homeostasis balance with its surrounding environmental medium and to decrease osmoregulatory expenditure (Sampaio & Bianchini, 2002). Inappropriate external salinity levels can cause fish mortality and stress and long-term stress can impair the fish's growth and immune system as well as affecting its reproductive development (Campbell, Pottinger, & Sumpter, 1994; Castranova, King, & Woods, 2005; Davis,

*Corresponding author
Email address: jruensirikul@yahoo.com

Griffin, & Gray, 2002). The spotted scat is a euryhaline species (Chang, Hsieh, & Cheng, 2005) that can live in a wide range of salinities, from fresh to full marine water. However, Cai *et al.* (2010) stated that spotted scat broodfish require a high salinity level for spawning. The optimal level of salinity to maintain a normal reproductive rate in scat is still unclear. Haddy and Pankhurst (2000) found that gonadal maturation in black bream, *Acanthopagrus butcheri*, a euryhaline fish, was unaffected by salinity. However, the number of ovulations and egg volume of this species is low when reared in water with a salinity of 5 ppt.

Currently, there is no well-established hatchery-reared broodstock of spotted scat and little is known about the effect of water salinity on its gonadal development and reproductive performance. The only study that investigated the effects of salinity on the reproductive activity of spotted scat was by Khanh *et al.* (2012). This current study aimed to evaluate the reproductive performance of hatchery-reared spotted scat broodstock maintained under different water salinities ranging from 5 to 33 ppt. This study will provide important information for the establishment of domesticated broodstock in hatcheries, especially hatcheries located far from a seawater source since that will benefit mass seed production for this species in the future.

2. Materials and Methods

2.1 Broodstock rearing and conditions

Spotted scat broodstock were obtained from artificial propagation at the marine fish hatchery of the Coastal Aquaculture Research Institute, Songkhla, Thailand. About 200 fish were reared together in a cylindrical cement tank (28 m^3) for 14 months at a density of 10 fish/m^3 (around 1 kg/m^3) at a sex ratio of 1:1 before further selection. The water salinity was maintained at 15–30 ppt by diluting natural seawater with freshwater because spotted scat are commonly found in an estuarine habitat where the salinity levels fluctuate widely and it is an amphidromous fish that moves freely between waters with different salinities (Asha, Suson, Retina, & Nandan, 2014). The fish were fed to satiation twice a day with a commercially formulated marine fish diet containing 35% protein until selection. Half of the water was changed every two weeks and the water quality was maintained and measured by the standard methods of the American Public Health Association (APHA, 1998) at a temperature of 26–29 ^oC, pH of 7.4–8.2, dissolved oxygen at 5.4–6.7 mg/L, ammonia at 0.04–0.32 mg/L, and nitrite at 0.01–0.02 mg/L.

2.2 Broodstock selection and experimental design

The salinity of the broodstock tank was raised to 33 ppt for a week before selection and the broodfish were starved for a day before selection. Sex differentiation was determined by snout shape (Barry & Fast, 1992). Only post-spawning or resting stage females (non-swollen abdomen: abdominal width less than body width, gonadosomatic index [GSI] = 1.85±0.68, n=5) were selected for the experiment (106.4±7.1–124.2±23.3 g). Experimental males were selected with expressible milt present after gentle abdominal stripping, i.e. spermiating males (47.7±4.2–58.3±3.8 g). The selected fish were randomly put into experimental tanks and the salinity of each was adjusted by about 2 ppt/day by diluting it with freshwater until the targeted salinity was reached before the experiment was initiated.

The experiment consisted of four treatments in which the broodstock fish were reared in various water salinities of 5, 15, 25, and 33 ppt. The experimental fish were reared in 1,000 liter plastic tanks at a density 10 fish/tank at a ratio of 6 females:4 males. Each treatment was conducted in triplicate. Two females from each treatment were randomly tagged with passive-integrated transponder tags for broodstock identification, steroid hormone, and serum osmolality investigation. The broodstock fish were fed with the same diet and feeding procedure as in the broodstock tank but the water in the experimental tanks was changed at the rate of 100% daily. Sediment in the tanks was removed every day, and tank cleaning was performed weekly. The water salinity of each tank was monitored daily and other aspects of the water quality were maintained and measured according to standard methods (APHA, 1998) at a temperature of 26–29 ^oC, pH of 7.4–8.2, dissolved oxygen at 5.5–7.2 mg/L, ammonia at 0.04–0.41 mg/L, and nitrite at 0.01–0.02 mg/L.

2.3 Maturation monitoring and reproductive performance parameters

After initiation of the experiment, all of the female broodfish in each tank were monitored monthly for 4 months. The morphologies of the fish abdomens were observed and females with a swollen abdomen were classified as mature broodfish. Before hormone injection, the percentage of mature fish was calculated as the number of broodfish which had a swollen abdomen × 100/number of all females. The oocytes of each matured fish (>100 oocytes/fish) were sampled via cannulation using a polyethylene tube (0.5 mm in diameter) connected to a 0.1 mL syringe and inserted into the genital pore. Then, the percentage of post-vitellogenic oocytes was investigated with a compound microscope under 40× magnification and calculated as the number of post-vitellogenic oocytes × 100/total number of sampled oocytes. The post-vitellogenic oocyte diameter was monitored using an ocular and stage micrometer with a microscope. The 10 largest oocytes were measured for their mean diameter (Mylonas, Mitrizakis, Papadaki, & Sigelaki, 2013). Thereafter, each mature female that demonstrated a mean oocyte diameter of more than 350 µm (Barry & Fast, 1992) was artificially inseminated.

Ovulation was induced using a single intramuscular hormone injection of luteinizing hormone-releasing hormone analogue (LHRHa; Suprefact, Sanofi-Aventis Deutschland GmbH, Germany) at 20 µg/kg (Ruensirikul *et al.*, 2008). The induced broodfish were monitored for ovulation every 30 min to 2 h, that depended on the degree of external abdominal swelling, after 32 h post-injection, by gentle abdominal massage. The ovulation time was recorded as having occurred when the ovulated oocyte first presented after massage. The ovulated oocytes were manually stripped and fertilized immediately with fresh pooled milt with good motility collected from 3–4 spermiating males obtained from the broodstock tank. The ovulation rate (the number of ovulated females × 100/number of injected females) at each breeding time was monitored. The latency time (time from injection to ovulation) of each ovulated broodfish was recorded. The

buoyancy rate (mL of buoyant egg × 100/total mL of eggs) of 5 mL of stripped egg was measured as the percentage of viable buoyant eggs that were completely separated from dead eggs which sank in a 500-mL cylinder with 30 ppt seawater within 20 min (Zakeri *et al.*, 2009). Ovulated oocyte diameter and oil globule diameter were also measured as the post-vitellogenic diameter before hormone injection. The percentage of normal ovulated eggs (number of normal ovulated eggs × 100/total number of eggs sampled) was monitored under a light microscope for approximately 100 eggs. Ovulated eggs which were spherical in shape, had transparent cytoplasm and a single oil droplet located at the center, were defined as normal ovulated eggs. After fertilization, the fertilized eggs of each fish were incubated separately. The fertilization rate (number of eggs at the 4–8 cell stage × 100/total number of eggs), the hatching rate (number of larvae × 100/total number of fertilized eggs), the total length of the day 0 larvae (6–9 h post hatch, n=20), the larval abnormality rate (the abnormal larvae characterized by vertebral deformity, n=100) and the survival rate of day 3 larvae (number of surviving larvae × 100/total number of larvae initially) were determined by counting the surviving larvae reared indoors in 2-liter plastic containers after 3 days post hatching (experiment performed in triplicate) at a density of 15 larvae/L without aeration or feeding.

2.4 Sex steroid hormone levels

At each time point of investigating maturation, blood samples were taken from the caudal vein of four fish from each treatment. Serum was obtained from the blood by centrifugation at 6,000 rpm for 5 min at 4 ºC and then stored at −20 ºC until the determination of the sex steroid hormone level (17β-estradiol: E2) by electrochemiluminescence immunoassay using an Elecsys Estradiol III kit (Roche Diagnostics, Germany) with an immunoassay analyzer (Modular Analytics E170) according to the manufacturer's instructions. The sensitivity of the assay was 5.0 pg/mL.

2.5 Osmolality measurement

Sub-samples of the serum of the fish in each tank were measured monthly for osmolality using a freezing point depression osmometer (Fiske Micro-Osmometer Model 210, Fiske Associates, MA, USA) contemporary with steroid measurement. Distilled water (0 mOsmol/kg) was applied as a control solution. The osmolality of the ambient water in each treatment was also determined but only once, in order to determine the isosmotic point (Sampaio & Bianchini, 2002).

2.6 Statistical analysis

All data are expressed as mean±standard error of mean (SE). One-way analysis of variance (ANOVA) was used to compare the reproductive performance parameters, sex steroid level, and osmolality among the treatments followed by Duncan's multiple range test to determine significant differences among means with a significance level of $P<0.05$. The statistical differences in the monthly average sex steroid level for the different salinity treatments for the ovulated and non-ovulated fish were separately established and the differences among ovulated and non-ovulated fish within each treatment were also determined. Statistical differences in the monthly average serum osmolality among salinity treatments were also analyzed. The differences in serum and water osmolality were established using the t-test. Percentage data were subjected to logarithmic transformations and the oil globule diameter was square-root transformed prior to analysis.

3. Results

3.1 Reproductive parameters

The reproductive parameters before hormonal inducement of each treatment collected monthly representing the percentage of mature fish (35.4±17.2–45.6±24.4%), the percentage of post-vitellogenic oocytes (79.1±8.5–85.8±7.6%) and the post-vitellogenic oocyte diameter (391.1±38.6–415.1±9.6 μm) showed no significant differences among the salinity treatments ($P>0.05$) (Table 1).

After ovulation, no significant differences ($P>0.05$) were detected in the latency time (36.4±5.4–40.0±1.7 h), buoyancy rate (72.4±14.9–85.7±8.1%), ovulated oocyte diameter (632.6±11.4–661.3±43.1 μm), oil globule diameter (237.0±21.7–244.5±23.1 μm), or percentage of normal ovulated eggs (61.1±21.6–81.4±11.4%) between the female spotted scat broodstock in each treatment for all salinity levels. Similarly, after insemination, there were no significant differences ($P>0.05$) found between the fertilization rates (61.0±22.7–80.7±13.0%), hatching rates (40.5±10.0–62.9± 16.7%), total length of larvae (1.67±0.14–1.75±0.15 mm), abnormality rates of larvae (2.5±1.1–3.5±0.5%) or the survival rate of day 3 larvae (65.9±3.9–68.1±8.8%) obtained from each treatment. However, some significant differences ($P<0.05$) were found in the ovulation rate. The highest ovulation rate was obtained from broodfish in the 25 ppt treatment (65.9± 16.7%) which was significantly different from all other salinities except the 15 ppt treatment (48.9±1.9%). The females in 33 ppt salinity produced the lowest ovulation rate (28.5± 17.0%) which was significantly different from all other treatments apart from the 5 ppt treatment (34.6±13.8%) (Table 1).

3.2 Sex Steroid hormone levels

The average serum E2 level of the ovulated-female fish (797.6±95.0–1,546.0±448.8 pg/mL) was significantly higher than that of the non-ovulated fish (206.3±106.6–415.6±198.7 pg/mL) ($P<0.05$) in every treatment, except those in the 15 ppt treatment where data was collected only for non-ovulated fish since none of the tagged fish ovulated. However, the E2 level among the treatments was not significantly different ($P>0.05$) either in the ovulated fish or in the non-ovulated fish when they were separately analyzed (Figure 1).

3.3 Serum osmolality

There were no significant differences in serum osmolality of the fish among any of the treatments. The serum osmolality of the fish in all the treatments remained stable and ranged from 323.4±16.6 to 345.0±4.0 mOsmol/kg and thus did not vary according to the holding water osmolality which increased along with the salinity (Figure 2).

Table 1. Reproductive performance parameters of female spotted scat broodstock reared in different water salinities for 4 months in a hatchery.

Reproductive performance parameters	Water salinity (ppt)			
	5	15	25	33
Mature female (%)	45.6±24.4[a]	42.5±15.7[a]	43.6±23.2[a]	35.4±17.2[a]
Before injection				
Post-vitellogenic oocyte (%)	85.8±7.6[a]	82.1±6.8[a]	79.1±8.5[a]	79.2±5.8[a]
Post-vitellogenic oocyte diameter (µm)	400.1±15.3[a]	391.1±38.6[a]	400.1±5.2[a]	415.1±9.6[a]
After ovulation				
Latency time (h)	36.4±5.4[a]	38.9±1.2[a]	40.0±1.7[a]	38.4±2.9[a]
Ovulation rate (%)	34.6±13.8[a]	48.9±1.9[ab]	65.9±16.7[b]	28.5±17.0[a]
Buoyancy rate (%)	83.9±8.2[a]	85.0±9.0[a]	85.7±8.1[a]	72.4±14.9[a]
Ovulated oocyte diameter (µm)	657.9±23.8[a]	642.5±14.6[a]	632.6±11.4[a]	661.3±43.1[a]
Oil globule diameter (µm)	244.5±23.1[a]	237.8±20.4[a]	237.0±21.7[a]	243.0±18.8[a]
Normal ovulated egg (%)	75.8±4.6[a]	77.5±12.9[a]	81.4±11.4[a]	61.1±21.6[a]
Fertilization rate (%)	62.9±10.7[a]	80.7±13.0[a]	61.0±22.7[a]	68.8±30.4[a]
Hatching rate (%)	48.4±16.6[a]	61.7±12.7[a]	62.9±16.7[a]	40.5±10.0[a]
Total length of day-0 larvae (mm)	1.75±0.15[a]	1.73±0.03[a]	1.67±0.14[a]	1.74±0.07[a]
Larval abnormality rate (%)	3.0±0.3[a]	2.5±1.1[a]	3.5±0.5[a]	3.1±1.7[a]
Survival rate of day-3 larvae (%)	65.9±3.9[a]	66.1±1.9[a]	68.1±8.8[a]	67.7±6.6[a]

Note: Data were averaged from monthly investigations and expressed as mean±SE. Means sharing the same superscript indicate no significant differences (P>0.05) between salinity treatments.

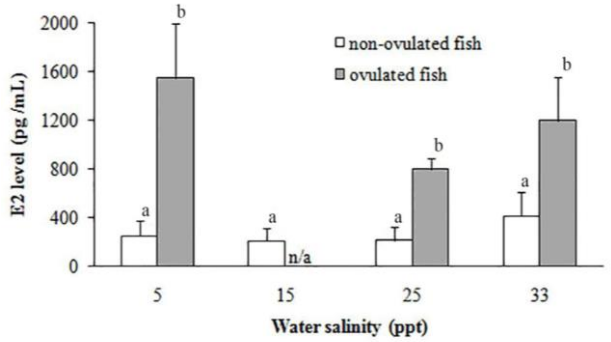

Figure 1. Sex steroid hormone level (17β-estradiol: E2) of female spotted scat broodstock reared in different water salinities in a hatchery for 4 months shown as overall average of each salinity treatment. In the 15 ppt treatment, no ovulated fish were observed in tagged females. Means with the same superscript are not significantly different (P>0.05); n/a=not available; expressed as mean±SE.

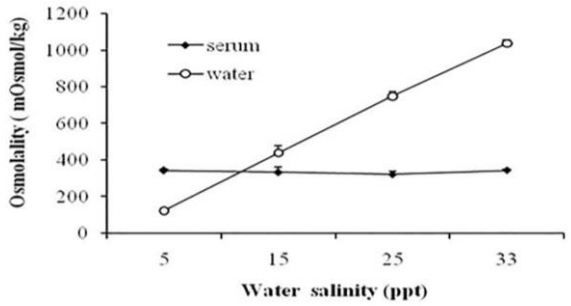

Figure 2. Serum osmolality of female spotted scat broodstock reared in different water salinities in a hatchery. Data were averaged from monthly investigation and are expressed as mean±SE. No significant differences were observed among serum osmolality of any salinity treatment but at the same salinity, the serum and water osmolality were significantly different (P<0.05).

4. Discussion

Typically, salinity can suppress the reproductive performance of fish but euryhaline fish are better adapted to environmental fluctuations. Although this study clearly showed that the tested salinity (range 5–33 ppt) did not affect the reproductive performance of the female scat fish, a higher ovulation rate was obtained within the middle range of salinities (15 and 25 ppt). Reproductive impairment was reported in previous studies when broodfish were subjected to low salinity. In a study of black bream (*Acanthopagrus butcheri*), a wide range of holding salinities (5–35 ppt) did not affect gonadal development or plasma steroid levels. However, the numbers of ovulations and egg volumes were lowest in fish held at 5 ppt (Haddy & Pankhurst, 2000). In other euryhaline marine fish, such as the Waigieu seaperch (*Psammoperca waigiensis*), the gonadal development and spawning performance were affected by holding broodfish in different salinities (5, 10, 20, and 30 ppt) during the breeding season. Fish held at 5 ppt had 100% mortality and at 10 ppt, oocyte development or ovulation was diminished (Pham, Kjørsvik, Nguyen, Nguyen, & Arukwe, 2010). Similar results were found for mullet (*Mugil cephalus*) (Assem, Abdel Rahman, Al Absawey, & Mourad, 2015) in which different water salinities affected the GSI values.

Thus, based on the present study, spotted scat broodstock can be reared for aquaculture in any salinity (5–33 ppt) depending on the availability of water sources and investment budget. However, to achieve higher ovulation rates in broodfish, salinities of 15–25 ppt are suggested as the optimum range for hatchery-reared spotted scat especially in hatcheries where the number of broodfish available is limited. In Vietnam, Pham *et al.* (2010) noted that the production cost of Waigieu seaperch could be reduced by decreasing the salinity levels to 20 ppt through increased use of freshwater instead of the exclusive use of seawater. However, a certain degree of water salinity was still necessary during the process of artificial insemination because success in propagating this species was achieved only in high salinity (>28 ppt)

(Ruensirikul *et al.*, 2008) which corresponds to the natural spawning behavior of spotted scat, which prefer to migrate to areas of high water salinity for spawning (Cai *et al.*, 2010).

The pattern of the E2 level found in the female scat was similar to that found in the female black bream (*A. butcheri*) maintained at 5, 20, or 35 ppt salinity after being injected with 50 μg/kg LHRHa (Haddy & Pankhurst, 2000). The salinity did not appear to affect the E2 level in this species although it was significantly elevated after induced ovulation and in ovulated broodfish at all three salinities. In contrast, the plasma E2 levels were observed to be significantly affected by different levels of salinity during the spawning season of Waigieu seaperch (*P. waigiensis*). However, in that species, the E2 levels were high (308–629 pg/mL) during the spawning period and decreased in the off-season (6–54.8 pg/mL) (Pham *et al.*, 2010). This coincided with the E2 levels in spotted scat which were high in ovulated broodfish and low in non-ovulated fish. This pattern of E2 level profile was also found in other fish, such as the common snook, *Centropomus undecimalis* (Cruz-Botto, Roca-Lanao, Gaitán-Ibarra, Chaparro-Muñoz, & Villamizar, 2018) in which the E2 level was not affected by different salinity environments (estuarine and seawater), which was high in the spawning season (300–400 pg/mL) and low in the off-season (100–200 pg/mL). The similarity of the pattern of E2 profile of the spotted scat in each treatment indicated that the oocyte development of the broodfish held at each salinity was similar. The level of E2 tends to increase before maturation when the oocyte size increases because estradiol regulates ovarian development through vitellogenin synthesis (Heidari, Roozati, & Yavari, 2010; Kagawa, 2013).

The isosmotic point of spotted scat was 337.3 mOsmol/kg which corresponded to a water salinity of 11.8 ppt. The serum osmolality of this species seems to be constant in any water salinity. Similarly, Urbina and Chris (2015) found that the plasma osmolality of inanga (*Galaxius maculatus*) reared in salinities that ranged from freshwater to 43 ppt showed only minor changes and there were no significant changes in physiology such as in metabolic rate or energy expenditure. However, the period during which fish adapt following a change in salinity may differ and for Urbina and Chris's study of inanga, the period was only 16 days which was much less than in this study. Constant or almost constant variability of blood osmolality in fish has been commonly noted when they are maintained in different levels of water salinity, through ion and water regulation, mainly in the gills, kidneys, and intestines (Varsamos, Nebel, & Charmantier, 2005). In pure marine or pure freshwater fish that inhabit waters with a stable salinity, steady-state osmoregulatory mechanisms are adequate to sustain homeostasis. However, little is known about the mechanisms by which euryhaline fish adjust their osmoregulation to balance homeostasis in variable salinity surroundings (Kultz, 2015). Shui *et al.* (2018) stated that the main mechanism for maintaining serum osmolality involves gill Na^+/K^+-ATPase. Both inanga (Urbina & Chris, 2015) and scat (Asha *et al.*, 2014) are classified as amphidromous fish that can move freely in any salinity gradient. Typically, the isosmotic salinity and blood osmolality of adult teleost fish are about 10–12 ppt (Boeuf & Payan, 2001; Sampaio & Bianchini, 2002; Wada, Aritaki, & Tanaka, 2004) and 280–360 mOsmol/kg (Varsamos *et al.*, 2005), respectively. The serum osmolality of the scat in this study was close to that reported by Mu *et al.* (2015) and was slightly lower than common estuarine fish such as Asian seabass (*Lates calcarifer*) (Sarwono, 2004) and rabbit fish (*S. rivulatus*) (Saoud, Kreydiyyeh, Chalfoun, & Fakih, 2007) when they were reared in seawater ranging from 0–30 ppt and 10–40 ppt, respectively. Stable serum osmolality allows scat to exist in a wide range of salinities throughout their life cycle. The present study offers new insight into the range of holding salinity for successful broodstock rearing for breeding purposes.

5. Conclusions

The spotted scat is a versatile euryhaline fish that can be cultured in a wide range (5–33 ppt) of salinities in hatcheries with no effects on growth, survival, sex steroid (E2) level or in its overall reproductive performance. However, water salinities of between 15 and 25 ppt are recommended to maximize its ovulation rate. This finding suggests good opportunities for the establishment of broodstock of this species in hatcheries or in natural water for the purpose of breeding and mass seed production.

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