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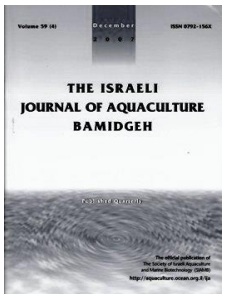

Embryonic and Larval Development and Effect of Salinity Levels on Egg and Ovary Performance in Rabbit Fish (*Siganus guttatus*)

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Keywords: *Siganus guttatus;* salinity; oocyte maturation; spawning; embryo, larvae

Abstract

In this study we investigated embryonic and larval development of rabbit fish (Siganus guttatus) in captivity. Embryonic development lasted 18-19 hours at 28-30°C, pH: 7.6-8.0, salinity: 30-32‰. Average total length of larvae at day 1 after hatching (DAH) was 1.58 mm, average yolk sac length was 0.35 mm containing one yolk ellipsoid (0.92±0.146 mm in length and 0.54±0.107 mm in width) in dorsal and lateral views. At day 2 (DAH) larval length increased to 2.16 mm, and pigmented eyes, mouth, and caudal-fin rays developed. Day 3 (DAH) larval length was 2.25 mm and the yolk was completely absorbed. Our findings provide fundamental information regarding seed larvae production of rabbit fish. In a further experiment we investigated the effect of salinity on gonad maturation, gonadosomatic index (GSI), egg biochemical composition and fecundities. Broodfish were cultured in three salinities, 25, 30, and 35 ‰ during reproductive season from February to July 2015. No statistically significant effects on gonadal development, fecundities, and egg quality in rabbit broodfish held in different salinities during the reproductive season were found.

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Introduction

Rabbit fish *Siganus guttatus* is a commercially valuable species in Vietnam however culture of this species has not developed due to dependence on larvae collected in the wild. Seed production is critical for industrial scale culture of rabbit fish. There has been limited research on this species, especially under conditions found in Vietnam (Le and Le, 2006). Knowledge regarding embryonic and larval development metamorphosis is important in developing techniques and procedures in seed production of fish. The aim of this study is to acquire a basic understanding of the early developmental stages of rabbit fish from fertilized egg to larval development. Our findings may provide fundamental knowledge and contribute to seed production of rabbit fish.

Teleost reproduction is basically regulated by external environmental factors that trigger internal signals into action. During the seasonal reproductive cycle the release of gametes are controlled by environmental stimuli or hormones (Zohar et al., 2009). Internal signals that regulate spawning are similar in most teleosts, however external environmental factors that control reproduction vary between different fish species (Zohar et al., 2009). Water temperature, photoperiod, salinity, water current, diurnal cycles, rainfall, available spawning substrates play an important role (Nguyen et al., 2003; Weltzien et al., 2004). However, little attention has been devoted to the role of ionic seasonal changes in the water. Salinity be either beneficial, or become a limiting factor during certain gametogenic stages. In some marine finfish species, ovulation may be inhibited and egg quality might be reduced when brood fish are exposed to low salinity (Kjørsvik et al., 1990). Processes associated with final oocyte maturation and ovulation (FOMO) may be particularly sensitive to environmental factors (Wen and Lin, 2001). In the present study, we investigated gonad maturation, GSI, fecundities, egg biochemistry, and reproductive performance of rabbit fish at different salinity levels. Our findings aim to find suitable salinity for broodfish management and conditioning of this species.

Materials and methods

Experimental design. Before the exposure to salinity experiment, one year old brooders were held in floating sea cages in which salinity was around 30‰. From the cages, fish 28-30 cm long, weighing 450-600 g/fish were selected. These were held in fiberglass tanks under conditions similar to the natural environment. Water temperature was 27-32 ^{0}C, pH: 7.8-8.4 and dissolved oxygen (DO): 4.5-6 mg/l. Brooders were fed daily at 2-3% of their body weight with a commercial barramundi pellet, proximate composition of protein: 43%, lipid: 7%, ash: 16%, fiber: 5% and moisture: 11%. The study was conducted between February and July 2015. This period is considered the main reproductive season for rabbit fish in Vietnam. Three groups comprising 30 females in each (90 in total) were randomly distributed into tanks at different salinities of 25, 30, and 35 ‰ with a load of 3 kg/m^3. It should be noted that 30‰ is the normal salinity for this species while 25 and 35‰ are assumed to be suboptimal salinities. The different salinities were achieved by dilution with freshwater.

Sampling reproductive parameters. In order to obtain measurements 5 females from each treatment were anesthetized in freshwater. Measurements taken were: weight, to the nearest 0.1 g, length to the nearest 0.1 cm, measurement of gonads and calculation of gonadosomatic index. To obtain gonad maturation status, females and males were checked for maturity with a catheter. For the assessment of fecundity, ovaries were removed from female cavity. Fragments of eggs from the posterior, middle and anterior parts (0.5-1 g) and all eggs at stage III and IV (yolk formation) were counted. Absolute fecundity (AF) defined as total number of eggs at stage III and IV in the ovary and relative fecundity (RF) was calculated as $RF=100 \times AF \times W - 1$ (where AF is absolute fecundity and W is total weight of the female fish).

Egg diameter and larval length were determined as the average of 50 eggs and larval measurements under a microscope equipped with a micrometer. Fish were considered to be mature when they had soft abdomens, swollen, protruding and reddish genitals, swollen papilla, and reddish anus.

Maturation status was determined as the ratio (%) between mature fish in relation to the number of all fish examined in each treatment group.

The gonadosomatic index (GSI) was calculated as 100 x gonad weight (g)/total body weight (g). Biochemical parameters of eggs were analyzed at the institute of biotechnology at Nha Trang University, Vietnam.

Statistical analysis. Statistical differences in reproductive variables including gonad maturation, egg and larval diameter, egg biochemical composition, and reproductive parameters were assessed using one-way analysis of variance (ANOVA). Least significant difference and Duncan's multiple range tests at 95% confidence level (Post Hoc Test) was used to compare mean values within individual exposure groups. All computations were performed with Statistical Package for Social Sciences software, Version 18 (SPSS 18). Values are expressed as mean ± standard error (SE) or standard deviation (SD).

Results

Embryonic and larval development. The fertilized eggs hatched within 18-19 hours when water temperature was 28-30^{0}C with salinity of 30-32 ‰. The embryonic stages of development of the rabbit fish eggs are given in Table 1. First cleavage occurred about 25 minutes after fertilization. Cell division continued every 20 to 25 minutes and the eggs developed to the multi-celled stage within 2 hours and 50 minutes. Embryonic heart started to function in about 16 hours and hatching occurred about 18.5 hours after fertilization (Table 1). Eggs were swollen about 15 minutes after fertilization.

Table 1: Embryonic development of rabbit fish (*Siganus guttatus*) at temperature: 28-30 ^{0}C, pH: 7.6-8.0, salinity: 30-32‰

Stages of embryonic development	*Time (h:min)*
Fertilization	0:15
2 - cell	0:25
4 - cell	0:45
8 - cell	0:55
16 - cell	1:20
32 - cell	1:50
Multi - cell	2:50
Blastula	4:10
Gastrula	5:20
Neural stage	7:05
Early embryo with eye vesicle	12:35
Heart function, free movement of tail	16:10
Hatch out	18:30

Cleavage occurs only in the blastodisc, defined as a thin region of yolk-free cytoplasm at the animal cap of the egg. Most of the egg cell is full of yolk. First cell movement of fish gastrulation is the epiboly of the blastoderm cells over the yolk. In the initial phase, the deep blastoderm cells move outwardly to intercalate with the more superficial cells. Later, these cells move over the surface of the yolk to envelop it completely.

The first day after hatching larvae have an average total length of 1.58 mm. Average yolk sac length is about 0.35 mm. One oil globule is located at the anterior part of the yolk sac which enables the hatchling to float almost vertically or about 45 degree from its usual horizontal position. The body is slender and pale in color with a distribution of pigments. The eyes, digestive tract, anus and caudal fins are distinctly seen but the mouth remains closed for about three days. Three days after hatching, most of the yolk sac is absorbed and the oil globule diminishes to a negligible size. At this stage, the mouth opens and the jaw begins to move as the larva starts to feed. The yolk sac is completely absorbed at day 4 after hatching. At day 31, average larval size was about 17.53 mm long and weight was 101.73 mg. For rate of absorption of the yolk sac under normal conditions, see Table 2.

Table 2: Larval development (DAH: day after hatching) of rabbit fish (*Siganus guttatus*) at temperature: 28-30 ^{0}C, pH: 7.6-8.0, salinity: 30-32‰. Values are given as mean ± SD

Age	*Total length (mm)*	*Yolk sac diameter (mm)*	*Body weight (mg)*
01 DAH	1.58 ± 0.18	0.35 ± 0.02	-
02 DAH	2.16 ± 0.16	0.26 ± 0.02	-
03 DAH	2.25 ± 0.15	0.12 ± 0.04	-
10 DAH	6.65 ± 0.29	-	-
17 DAH	12.36 ± 0.60	-	-
24 DAH	15.20 ± 0.75	-	78.83 ± 10.96
31 DAH	17.53 ± 0.69	-	101.73 ± 12.32

Effect of salinity levels on egg and ovary performance.

No significant differences were observed in gonad maturation among treatment groups subjected to different salinity levels (P>0.05). Most broodfish reached maturity in July (Table 3). During the study period, the female fish GSI ranged from 2.8-7.6% for all salinity exposure groups and reached highest mean GSI value in April and July (Table 4).

Table 3: Gonad maturation status of the broodfish during experimental period

Sampling date	*Treatment groups*					
	25 ‰		*30 ‰*		*35 ‰*	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*
15/03/2015	(+)	(-)	(+)	(+)	(-)	(-)
10/04/2015	(-)	(-)	(-)	(-)	(-)	(-)
30/04/2015	(+)	(+)	(-)	(-)	(+)	(+)
09/05/2015	(+)	(-)	(+)	(-)	(+)	(-)
30/06/2015	(+)	(-)	(+)	(-)	(-)	(-)
20/07/2015	(-)	(+)	(+)	(+)	(+)	(+)

(+) indicates mature; while (–) indicates immature

For females: "Immature" was defined as eggs in stage II or III and not ready to spawn; while "mature" was defined as eggs in stage IV or V and ready to spawn.

For males: "Mature" was defined as fish with semen released when gently pressing along the abdomen; while "immature" fish do not release semen.

Table 4: Mean female GSI (%) during experimental period

Sampling date	*Treatment groups*		
	25 ‰	*30 ‰*	*35 ‰*
15/03/2015	4.0	5.5	2.8
10/04/2015	3.5	2.8	3.5
30/04/2015	6.7	6.2	7.0
09/05/2015	4.2	4.0	4.6
30/06/2015	3.0	4.2	4.2
20/07/2015	6.3	7.6	6.6

Protein and lipid in the eggs ranged between 18.32-21.21% and 7.35-8.15% respectively (Table 5). See Table 6 for fecundity and maturation rate at different salinity levels. No significant difference was observed in fecundity in the different salinity treatment groups (P>0.05).

Table 5: Biochemical composition of ovary at vitellogenin phase

Biochemistry	*Treatment groups*		
	25‰	*30‰*	*35‰*
Protein (%)	18.32	21.21	20.77
Lipid (%)	7.35	7.95	8.15
Ash (%)	1.63	1.46	1.34
Moisture (%)	68.23	66.54	69.06

Table 6: Mature rate, fecundities and egg size in female rabbit fish exposed to different salinities. Values given as mean ± standard deviation (SD)

Reproductive parameters	*Treatment groups*		
	25‰	*30‰*	*35‰*
Average matured rate (%)	20 ± 12	30 ± 18	22 ± 16
Absolute fecundity (egg/female)	570,625 ± 42,217	625,433 ± 86,673	620,182 ± 96,200
Relative fecundity (egg/ g female)	1358 ± 185	1250 ± 265	1360 ± 167
Egg size (μm)	550 ± 46	580 ± 55	570 ± 50

Discussion

Embryonic and larval development. In some countries in the Pacific region rabbit fish have been considered a major food fish species (Lam, 1974). It is also farmed in the Philippines (Pillai, 1962). There is no cultured propagation of this species. An attempt was made to produce and culture rabbit fish larvae at the Southeast Asia Fisheries Development Centre in the Philippine (SEAFDEC) (Juario et al., 1985) and they have been cultured in Indonesia but survival rates of early larval stages were low (<2%) (Rachmansyah et al., 2007). Lack of understanding of the diverse natural environmental conditions has been a major challenge to the development of fish farming and artificial propagation and cultivation of rabbit fish. Under natural conditions, the spawning of rabbit fish is synchronously linked to tides and specific lunar phases (Duray, 1990). *Siganus guttatus* species in the Philippines can lay eggs all year around (Soletchnik, 1984); Hara et al., 1986; Duray, 1998). This is a spawning strategy that has not been verified in natural populations in Vietnam. However, rabbit fish fry from 1.5-2.0 cm are often observed in the wild in April-May of the Vietnamese lunar calendar, in at least two regions of Tam Giang - Cau Hai Lagoon (Thua Thien – Hue: 16°19'22"N 107°51'2"E) and Thi Nai Lagoon (Binh Dinh province: 11° 37' 00"N 109° 02' 00"E) (Le and Le, 2006).

Rabbit fish is a relatively new candidate for marine aquaculture compared to other tropical species, such as grouper (*Epinephelus spp*), cobia (*Rachycentron canadum*), mangrove red snapper (*Lutjanus erythropterus*), red drum (*Scyaenops ocellatus*) and Asian seabass (*Lates calcarifer*) which all have a relatively long history of culture.

In the present study conducted in Vietnam, the development of embryonic and larval stages of rabbit fish is described for the first time from fertilized egg to 31 day old larvae after hatching. Like other marine finfish species, their embryonic development follows the usual developmental stages such as cleavage, blastula, gastrula, neurola and embryonic development. Our results showed that embryonic development lasted about 18-19 hours at water temperature of 28-30^{0}C and salinity of 30-32‰, and larvae survived until day 31 after hatching. A complete metamorphosis from larva to juvenile stage was described. Egg size and cleavage are important indicators for egg and larval quality, viability during incubation, and rearing in aquaculture (Kjørsvik et al., 1990; Warga and Kimmel 1990). The average diameter of rabbit fish eggs is around 0.5-0.6 mm. In this study, no relationship between egg size and larval performance was observed. Our findings indicate that rabbit fish eggs are adhesive and demersal, and the cleavage pattern is the same as that of other tropical marine finfish. The results of this study might provide a basis for further studies to determine the complete early life history of the rabbit fish. They can be used to explain some aspects of the early life cycle under culture conditions and can help develop better larval culture techniques in marine finfish hatcheries in developing countries such as Vietnam.

Effects of salinity levels. One of the most serious bottlenecks in the development of sustainable aquaculture in developing countries is the control of reproductive processes of fish in captivity (Bromage et al., 1992). Many fish species exhibit reproductive dysfunction when reared under unfavorable salinity conditions. Most commonly, females fail to undergo oocyte maturation, ovulation, and subsequent spawning (Zohar and Mylonas, 2001). Once reproduction can be controlled under cultural conditions, we can advance or delay broodstock spawning during annual cycles and maximize productivity.

In regard to the influence of salinity on fish reproduction, information is limited and varies depending on species, population, and life history strategies (Ishimatsu et al., 2007). Some studies have indicated that different species with similar salinity habitats showed different physiological responses to ranges of ambient salinity (Haney and Nordlie, 1997). In our study no significant difference in fecundity, GSI, egg quality, and maturation rate were observed ($P>0.05$) when female rabbit fish were held at different salinity levels from 25-35‰. The results of this study indicate that it is advisable to hold brooders in lower salinity to reduce costs and seawater use. Further study is needed to understand the physiological mechanisms involved in these findings.

Under culture conditions, egg size may not be very important although in a study with rainbow trout, it appeared that larger eggs produced larger fry (Bromage et al.,

1992) and the effect of size differences are often masked by other environmental factors. On the other hand, egg size may be important in the wild for subsequent larval growth and survival (Brooks et al., 1997). Proteins and lipids are important for embryonic and larval development. In our study salinity did not influence egg biochemical composition. Although brooders held at 25‰ salinity level showed lower protein and lipid percentages, no significant differences were observed between the treatment groups ($P>0.05$). In fish, the major components of the egg are yolk proteins (vitellogenin) and eggshell protein. They are important aspects of oogenesis and are associated with egg size (Arukwe and Goksoyr, 2003). This was reconfirmed when no significant difference in egg size was found among treatment groups ($P>0.05$). The observations from this study suggest that salinity between 25-35‰ does not affect some aspects of reproductive performance of rabbit fish. Further study should be conducted with lower and higher salinity levels to further explore this issue.

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