マーブルゴビOxyeleotris marmoratusの卵発生,孵化および仔魚の奇形に及ぼす塩分の影響

<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
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<tbody>
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Effects of Salinity on Egg Development, Hatching and Larval Deformation in the Marble Goby *Oxyeleotris marmoratus*

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**Abstract:** Marble goby *Oxyeleotris marmoratus* is an important aquaculture freshwater (FW) species in Southeast Asia. To improve hatching technique, eggs from a river population in Sabah, Malaysia were incubated in FW and seawater (SW) diluted to 5, 10, 15, 20 and 30 psu, comparing hatching rates and larval deformation. Egg development, hatching rates, hatching periods, larval deformation and survival at 10 days after fertilization (dAF) were then compared in FW and 10 psu SW. Salinities from FW to 15 psu SW were tolerated, with the highest hatching observed at 10 psu SW (60.0 ± 2.0%, mean ± SD). Significantly higher hatching rates and lower deformation rates were observed at 10 psu SW than in FW. In FW, embryonic developed at similar rate with 10 psu SW, but hatching was delayed and all larvae died by 10 dAF. Peak of hatching in 10 psu SW observed in 48-60 hours after fertilization (hAF) (33.1 ± 5.6%) while hatching in FW was delayed and peaked 72-84 hAF (10.6 ± 3.4%). Larvae that hatched later had higher deformation rates. The eggs incubated in 10 psu SW had a shorter hatching period, higher hatching rate and better larval survival than those in FW.

**Key words:** Marble Goby *Oxyeleotris marmoratus*; Salinity; Hatching; Deformation

The marble goby *Oxyeleotris marmoratus* (Bleeker 1852) (Eleotridae) is a freshwater (FW) fish widely distributed in Southeast Asia and is one of the most popular aquaculture species (Robert 1989; Cheah et al. 1994; Rainboth 1996; Inger and Chin 2002; Amornsakun et al. 2003; Luong et al. 2005). Typically retailing at US$50-60/kg, it is the highest priced freshwater aquaculture fish in Southeast Asian countries (Senoo 2003a; Lam et al. 2008). Over the last 30 years, the natural resource of marble goby has decreased drastically due to overfishing (Ikenoue 1991; Senoo et al. 1992; Senoo 2006) and this has also affected fish farmers who require a steady supply of the fish seed. To meet this demand and to protect the wild resource, artificial techniques for seed production have been developed. In West Malaysia (Peninsular Malaysia), Senoo et al. (1994a, 1994b) have described egg development, hatching, and larval development of marble goby in FW, and have reported behavioural changes of the larvae. However, in East Malaysia (the State of Sabah), high larval mortality presents a significant obstacle for the successful larval rearing of this species. Senoo et al. (2008) successfully produced marble goby seeds by increasing the salinity from FW to 10 psu diluted seawater (SW) after the larvae were hatched. They concluded that 10 psu SW was necessary for effective larval rearing during the first 10 days. However, it remained unknown

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whether incubation of the eggs in elevated salinities would lead to higher hatching success. Moreover, there is limited information concerning the effect of salinity on egg development and hatching in this species. Thus, in an attempt to improve seed production techniques, a series of experiments was carried out to determine the optimum salinity for incubation of marble goby eggs.

**Materials and Methods**

**Brood fish management**

The experiments were conducted at the Fish Hatchery, Borneo Marine Research Institute, Universiti Malaysia Sabah, Sabah, Malaysia during September to October, 2010. Twenty females and 30 males of brood fish with body weight (BW) 259.9 ± 52.7 g and 307.5 ± 77.9 g (mean ± SD) were collected from a river in the Penampang area (Fig. 1). Female and male fish were separately reared for five months in FW in 1,000 l high density polyethylene tanks (1.5 m diameter x 0.6 m deep). They were fed with fresh fish (*Sardinella* sp.) until satiation at 2 days intervals. Water temperature, dissolved oxygen (DO) and pH ranges of the brood fish tanks were 26.2–30.8 (28.4 ± 2.5) °C, 6.8–8.2 (7.7 ± 0.5) mg/l and 7.3–8.5 (8.0 ± 0.5), respectively (mean ± SD).

**Experiment 1**

**Egg collection**

The aim of this experiment was to determine the optimum salinity for hatching of marble goby eggs. Fertilized eggs were obtained from a pair of sexually mature brood fish (female, 265 g; male, 280 g) using the method described by Senoo et al. (1993). The female was injected intramuscularly with human chorionic gonadotropin (Profasi, Laboratories Serono, Switzerland) with the dosage of 1,000 IU/kg BW (Senoo et al. 1993; Senoo 2001a, 2003a, 2006). At three days post-injection, approximately 20,000 eggs were collected from a natural spawning with a fertilization rate of 95%. The fertilized eggs measured 1.84 ± 0.03 and 0.64 ± 0.02 mm (mean ± SD, n=20) in the long and short axes, respectively, and weighed 1,668 eggs/g (n=3).

**Incubation and observation**

Eighteen 7 l transparent tanks (length, width, height; 18 × 26 × 17 cm) were prepared for incubation. They were divided into three groups of six tanks filled with either FW or SW diluted to a range of salinity 5, 10, 15, 20 or 30 psu. Salinity was adjusted by mixing filtered seawater with aged dechlorinated tap water and measured using a hand refractometer (H-50, ATAGO, Japan), precalibrated with distilled water. The prepared water was filtered through a 40 μm mesh plankton net.

One hundred eggs contained in a 9 cm diameter Petri dish were placed into each of the incubation tanks. Hatched larvae were removed and counted each day for 6 days. The removed larvae were observed with an optical microscope and the number of deformed individuals was recorded. Total hatching rate was defined as the percentage of stocked embryos that hatched. The deformation rate was the percentage of deformed larvae with a bent notochord among the hatched larvae.
Experiment 2

Egg collection

Based on the results of experiment 1, hatch­ing time, hatching rate and larval deformation rate of marble goby were compared in FW and 10 psu SW. To obtain eggs fertilized at a known time, eggs were collected by stripping and were fertilized artificially. Fertilized eggs were obtained from six pairs of brood fish (females, 287.5 ± 7.6 g BW, 24.2 ± 2.9 cm total length (TL); males, 307.5 ± 14.1 g BW, 28.8 ± 1.0 cm TL, mean ± SD) following hormone injection, as described above. At 3 days post-injection, ovu­lated eggs (approximately 15 g) were stripped from the female. Stripped eggs measured 0.89 ± 0.04 mm and 0.64 ± 0.03 mm (mean ± SD, n=6) in the long and short axes, respectively, and weighed 1,675 eggs/g (n=6). Milt was then stripped from the male and mixed with the eggs in FW in a 250 ml bottle previously coated with Vaseline™ to prevent the eggs from clumping (Senoo 2001a, 2003a, 2006).

Incubation and observation

In each trial, approximately 25,000 fertilized eggs were randomly deposited on two rectan­gular wooden-framed nets (30 x 38 cm) fitted with plankton gauze (250 µm mesh) and were incubated in FW or 10 psu SW. For the incubation, two aquarium tanks (60 x 39 x 36 cm, 70 l; Fig. 2) were prepared with a filtering system.

One of the wooden-framed nets was floated on the water surface of each tank and aerated at a rate of 500 ml/min. An electric heater was installed in each tank to maintain the temperature at 28–29 (28.5 ± 0.05°C (mean ± SD). Eggs were incubated under natural light conditions.

For determination of the hatching time and hatching rate, all hatched larvae were removed and counted every 12 hours from 24 to 144 hours after fertilization (hAF). Hatching rates and deformation rates at each observation time were determined as previously described. To compare larval survival in the two salinities, hatched larvae were transferred to 7 l transparent tanks (18 x 26 x 17 cm) and reared for a further 10 days (Senoo et al. 2008), recording mortality daily. The experiments were repeated six times under the same experimental condi­tions using different egg batches. During the observation period, water temperature, DO and pH were recorded at 06:00 and 18:00 h. In FW the ranges were 28.2–29.0 (28.3 ± 0.3)°C, 7.2–7.8 (7.5 ± 0.2) mg/l and 7.4–8.2 (7.8 ± 0.3); and in 10 psu SW they were 28.1–29.0 (28.3 ± 0.2)°C, 7.0–7.6 (7.2 ± 0.2) mg/l and 7.2–8.3 (7.8 ± 0.3), respectively (mean ± SD).

Experiment 3

Morphological observation on egg development

Eggs from experiment 2 were used for observations on development. Approximately 20 eggs incubated in FW, and 20 eggs from 10 psu SW, were transferred to a Petri dish with some of their water and observed under an optical microscope (Eclipse E600, Nikon, Japan) at every minute to observe the divi­sion of cells up to morula stage, at 15 minutes intervals for 24 hours (morula stage to embryo ready to hatch) and then every 12 hours until 120 hAF. Each developmental stage was timed and photographed with a digital camera (Digital 600, Olympus). Development of the embryonic sensory organs was assessed using a scanning electron microscope (SEM) (JSM-5610, JEOL, Japan). For the SEM observations, 20 eggs each from the FW and the 10 psu SW tanks were sampled at 12 hours intervals from 24 to 144 hAF and preserved in 10% buffered formalin for one
month. The egg membranes were peeled off using micro-tweezers and the embryonic parts were processed by dehydration and gold sputtering for SEM observation (Senoo et al. 1994a; Kawamura et al. 2003).

Statistical analysis

Total hatching rates and deformation rates were compared between salinities by one-way analysis of variance (ANOVA) followed by a Tukey’s honest significant difference (HSD) test (experiment 1) or t test (experiment 2). To examine differences in the hatching period between two groups, repeated-measures ANOVA was used. Kaplan-Meier survival probabilities were computed for experiment 2 and the differences in larval survival between FW and 10 psu SW were tested using the log-rank test. All statistical analyses were carried out using JMP version 8 (SAS Institute, USA) and SPSS Statistics 17.0 software (IBM Corp., New York, USA).

Results

Experiment 1
Rates of hatching and deformation

Salinity significantly affected the hatching rate of marble goby (ANOVA, \( F_{4,10}=66.2 \), Tukey’s HSD test, \( P<0.05 \)). The highest hatching rate was observed in 10 psu SW (60.0 ± 2.0%), followed by FW (44.7 ± 5.0%), 5 psu SW (44.3 ± 5.5%) and 15 psu SW (37.0 ± 6.2%) with the lowest rate at 20 psu SW (3.0 ± 1.7%, mean ± SD). No hatching occurred in 30 psu SW (Fig. 3). Salinity also affected the occurrence of larval deformation (ANOVA, \( F_{4,10}=533.6 \), Tukey’s HSD test, \( P<0.05 \)). All larvae that hatched in 20 psu SW were deformed and died within three days. The deformation rate was lower in 15 psu SW (49.6 ± 1.9%), followed by FW (33.1 ± 3.9%) and 5 psu SW (29.1 ± 2.8%, mean ± SD). The deformation rate in 10 psu SW (11.1 ± 2.4%) was significantly lower than at all other salinities (Fig. 4).

Experiment 2
Rates of hatching, deformation, and larval survival

As shown in experiment 1, egg hatching and larval deformation rates were improved in 10 psu SW relative to FW. The mean hatching rate of eggs incubated in 10 psu SW (70.1 ± 13.2%) was significantly higher than in FW (52.3 ± 12.5%; \( t=-2.40, P=0.0376 \)) and the overall larval deformation rate was significantly lower in 10 psu SW (4.8 ± 4.4%) than in FW (26.8 ± 11.6%, mean ± SD; \( t=4.37, P=0.0014 \)).

The repeated-measures ANOVA showed a significant effect of the interaction between time and salinity on hatching time (\( F_{1,118}=0.568, P=0.037 \)), indicating a difference in the hatching period between the groups (Fig. 5). Hatching was commenced between 24–36 hAF in both groups and the eggs hatched in 10 psu SW were

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![Fig. 3](image1.png)

**Fig. 3.** Total hatching rates of marble goby eggs incubated in freshwater (FW), and in 5, 10, 15, 20 and 30 psu diluted seawater (means ± SD, \( n=3 \)). Different letters above each bar indicate significant differences between treatments (one-way ANOVA, \( F_{4,10}=66.2 \), Tukey’s HSD test, \( P<0.05 \)).

![Fig. 4](image2.png)

**Fig. 4.** Deformation rates of marble goby larvae hatched in freshwater (FW), and in 5, 10, 15, 20 and 30 psu diluted seawater (means ± SD, \( n=3 \)). Different letters above each bar indicate significant difference between treatments (one-way ANOVA, \( F_{4,10}=533.6 \), Tukey’s HSD test, \( P<0.05 \)) and ND shows no data.
more rapidly than those in FW. In FW, hatching was occurred from 24 to 132 hAF (ANOVA, $F_{9,50}=19.5$, Tukey’s HSD test, $P<0.05$). The total hatching rates were significant higher during 60–72 and 72–84 hAF ($9.7 \pm 3.8\%$ and $10.6 \pm 3.4\%$), followed by a relatively constant rate of hatching during 36–48, 48–60, 84–96 and 96–108 hAF ($4.8 \pm 2.4\%, 8.1 \pm 2.6\%, 8.8 \pm 2.0\%$ and $7.0 \pm 2.6\%$, respectively), and then significantly lower during 24–36, 108–120, 120–132 hAF ($0.7 \pm 0.4\%, 2.0 \pm 0.9\%$ and $0.7 \pm 0.8\%$, mean $\pm$ SD, respectively). Of the eggs that hatched in 10 psu SW, significantly higher hatched in the period during 48–60 and 60–72 hAF (ANOVA, $F_{9,50}=98.5$, Tukey’s HSD test, $P<0.05$, $33.1 \pm 5.6\%$ and $18.2 \pm 4.5\%$), followed by 36–48, 72–84 and 84–96 hAF ($7.6 \pm 2.4\%, 7.3 \pm 3.3\%$ and $3.0 \pm 1.8\%$, mean $\pm$ SD, respectively). The hatching rate gradually declined, significantly lower during 24–36 hAF ($0.8 \pm 0.3\%$) and no future hatching was observed beyond 96 hAF in 10 psu SW.

Deformed larvae were clearly observed after 72 hAF in both waters. In FW, all of the larvae that hatched during 108–132 hAF were deformed and eventually died on the tank bottom (ANOVA, $F_{8,45}=46.5$, Tukey’s HSD test, $P<0.05$), followed by 84–96 and 96–108 hAF ($44.7 \pm 33.2\%$ and $72.8 \pm 20.7\%$ of total hatching rates at each period; Fig. 6), then 72–84 hAF ($43.7 \pm 6.9\%$ of total hatching rates), significantly lower at 60–72 hAF ($2.2 \pm 5.4\%$ of total hatching rates, mean $\pm$ SD) and no deformation were observed from 24 to 60 hAF. The highest deformation rate for the larvae in 10 psu SW was 48.4 $\pm$ 32.2% of total hatching rates, which was observed in the interval 84–96 hAF (ANOVA, $F_{5,30}=4.0$, Tukey’s HSD test, $P<0.05$), followed by 72–84 hAF ($23.8 \pm 38.2\%$ of total hatching rates), significantly lower during 60–72 hAF ($1.8 \pm 4.5\%$ of total hatching rates, mean $\pm$ SD), and no deformation were observed from 24 to 60 hAF (Fig. 7). In both groups, the larvae that

![Fig. 5. Total hatching rates of marble goby eggs incubated in freshwater (FW, white bar; ANOVA, $F_{9,50}=19.5$, Tukey’s HSD test, $P<0.05$) and in 10 psu diluted seawater (SW, black bar; ANOVA, $F_{9,50}=98.5$, Tukey’s HSD test, $P<0.05$) in different time intervals in hours after fertilization (hAF). Results are expressed as the means $\pm$ SD ($n=6$). The repeated-measures ANOVA also showed a significant effect of the interaction between time and salinity on hatching time ($F_{1,118}=0.568$, $P=0.037$).](image1)

![Fig. 6. Deformed larva of marble goby immediately after hatching at 96 hours after fertilization (hAF) in freshwater (FW). Bar = 0.5 mm.](image2)

![Fig. 7. Deformation rates of marble goby larvae hatched in freshwater (FW, white bar; ANOVA, $F_{8,45}=46.5$, Tukey’s HSD test, $P<0.05$) and in 10 psu diluted seawater (SW, black bar; ANOVA, $F_{5,30}=4.0$, Tukey’s HSD test, $P<0.05$) in different time intervals in hours after fertilization (hAF). Results are expressed as the means $\pm$ SD ($n=6$). ND shows no data.](image3)
hatched in later periods tended to have higher deformation rates. At 10 dAF, the larval survival rate in 10 psu SW was 52.7 ± 20.1% (mean ± SD), while almost all larvae were dead by 9 dAF in FW (Fig. 8). Kaplan-Meier survival analysis indicated that there was a significant difference in survival between the two groups (P < 0.0001).

**Experiment 3**

**Morphological observation on egg development**

Egg morphology was developed similarly in FW and 10 psu SW, as shown in Table 1. This result showed there are no salinity effects towards the egg development in both waters. Soon after fertilization, the eggs elongated along their vertical axis and were suspended below the plankton nets. At 1 hAF, the mean dimensions of the eggs in FW were 1.85 ± 0.04 and 0.65 ± 0.03 mm in long and short axes, respectively and, in 10 psu SW the dimensions were 1.87 ± 0.06 by 0.66 ± 0.03 mm (mean ± SD). In both waters, embryos began to form at around 9 hAF with the agrippa condition (Senoo et al. 1994a). Hatching was commenced at 24 hAF. Hatched larvae at 24–36 hAF showed unpigmented eyes, two clearly auditory vesicles with otolith, not open mouth and anus, and floated in water column. Hatched larvae were developed coincide as the embryo developed in both waters (Table 1). Those early hatched larvae had developed an air bladder and had commenced active swimming by 96 hAF. At 120 hAF, larvae hatched in 10 psu SW commenced exogenous feeding (Fig. 9). At this time, sensory organs in the eggs and hatched larvae were at similar stages.

**Table 1.** The time course of egg development of marble goby incubated in freshwater (FW) and in seawater diluted to 10 psu diluted seawater (SW)

<table>
<thead>
<tr>
<th>Egg Developmental Stages</th>
<th>FW</th>
<th>10 psu SW</th>
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<tbody>
<tr>
<td>Two-celled stage</td>
<td>0:00:24</td>
<td>0:00:22</td>
</tr>
<tr>
<td>Four-celled stage</td>
<td>0:00:42</td>
<td>0:00:40</td>
</tr>
<tr>
<td>Sixteen-celled stage</td>
<td>0:00:51</td>
<td>0:00:49</td>
</tr>
<tr>
<td>Morula stage</td>
<td>0:02:30</td>
<td>0:02:30</td>
</tr>
<tr>
<td>Blastula stage</td>
<td>0:04:15</td>
<td>0:04:15</td>
</tr>
<tr>
<td>Gastrula stage</td>
<td>0:05:00</td>
<td>0:05:00</td>
</tr>
<tr>
<td>Blastopore nearly closed</td>
<td>0:08:00</td>
<td>0:08:00</td>
</tr>
<tr>
<td>Embryo formed</td>
<td>0:09:00</td>
<td>0:09:00</td>
</tr>
<tr>
<td>Five-myomeres formed</td>
<td>10:15</td>
<td>10:15</td>
</tr>
<tr>
<td>Kupffer's vesicle appeared</td>
<td>11:30</td>
<td>11:30</td>
</tr>
<tr>
<td>Optic vesicle appeared</td>
<td>12:30</td>
<td>12:30</td>
</tr>
<tr>
<td>Tail separated from the yolk sac</td>
<td>13:00</td>
<td>13:00</td>
</tr>
<tr>
<td>Ototocyst vesicle appeared</td>
<td>14:30</td>
<td>14:30</td>
</tr>
<tr>
<td>Head formed</td>
<td>15:00</td>
<td>15:00</td>
</tr>
<tr>
<td>Lens and heart formed</td>
<td>17:15</td>
<td>17:15</td>
</tr>
<tr>
<td>Embryo commenced moving</td>
<td>20:15</td>
<td>20:15</td>
</tr>
<tr>
<td>First hatching commenced</td>
<td>24:00</td>
<td>24:00</td>
</tr>
<tr>
<td>Embryonic tail elongated</td>
<td>36:00</td>
<td>36:00</td>
</tr>
<tr>
<td>Embryonic mouth formed</td>
<td>48:00</td>
<td>48:00</td>
</tr>
<tr>
<td>Eyes commenced pigmentation, vesicle of air bladder formed, olfactory pit opened, free neuromast with cupulae observed</td>
<td>60:00</td>
<td>60:00</td>
</tr>
<tr>
<td>Eyes deeply pigmented</td>
<td>72:00</td>
<td>72:00</td>
</tr>
<tr>
<td>Embryonic pectoral fin formed, tail elongated to the head in FW</td>
<td>96:00</td>
<td>96:00</td>
</tr>
<tr>
<td>Egg membrane transformed by developed embryonic head in FW</td>
<td>120:00 (End of hatching)</td>
<td></td>
</tr>
</tbody>
</table>

The time showed at each egg developmental stage was first observed.

![Fig. 8](image-url) Survival rates of marble goby reared in freshwater (FW) and in 10 psu diluted seawater (SW) for 10 days after fertilization (dAF). Results are expressed as the means ± SD (n=6). Kaplan-Meier survival analysis showed significant different of survival rates in 10 dAF (P < 0.0001).

![Fig. 9](image-url) Micrographs of an un-hatched egg (upper) in freshwater (FW) and a larva (lower) in 10 psu diluted seawater (SW) of marble goby at 120 hours after fertilization (hAF). The larva commenced feeding at this time and a rotifer provided as food is visible in the intestine (white arrow). A, anus; Ab, air bladdar; Ey, eye; Fn, free neuromast; Ie, inner ear; In, intestine; Lj, lower jaw; Og, oil globule; Op, olfactory pits; Ys, yolk sac. Bar = 0.5 mm.
Discussion

The present study showed clearly that salinity did not effect to egg development but had significant effects on egg hatching and larval deformation of marble goby. Egg hatching occurred within a wide salinity range, from FW to 20 psu SW. However, the eggs died in 30 psu SW and all larvae that hatched in 20 psu SW were deformed and died within three days. Previously, high salinity has been shown to delay hatching or in some cases, to cause precocious hatching, and also to cause malformations and death of fish eggs (Vetemaa and Saat 1996; Albert et al. 2004). For marble goby, eggs hatched into viable larvae at salinities of 15 psu SW or lower. Generally, their natural habitat ranges from freshwater to brackish environments including canals, rivers, reservoirs and swamps (Robert 1993; Rainboth 1996). However, there is lack information of spawning and distributional environments of larval stages in natural water body. Interestingly, despite the inhabited in FW and brackish environment, incubation of the eggs in 10 psu SW provided the best outcome, i.e., significantly higher hatching rate (60.0 ± 2.0% and 70.1 ± 13.2%) and lower deformation rate (11.1 ± 2.4% and 4.8 ± 4.4%, mean ± SD) than those in FW in experiment 1 and 2.

On closer comparison of the FW and 10 psu SW incubation it appeared that the hatching period is a key factor affecting embryo deformation. The deformation rate increased when egg hatching was delayed beyond 72 hAF, which coincides with the time of eye pigmentation. The majority of the eggs in 10 psu SW provided the best outcome, i.e., significantly higher hatching rate (60.0 ± 2.0% and 70.1 ± 13.2%) and lower deformation rate (11.1 ± 2.4% and 4.8 ± 4.4%, mean ± SD) than those in FW in experiment 1 and 2.

In the present study, hatching period was obviously affected by salinity. Intraspecific variation in the hatching time of fish eggs depends on several other environment variables, (e.g., oxygen availability, temperature and light) but salinity has not been demonstrated to be among the main factors (Yamagami 1988). The delayed hatching was believed to be caused indirectly by the morphological and physiological deformation of eggs resulting from the salinity difference, rather than by a direct effect on the hatching glands.

In the present study, the salinities tolerated by marble goby eggs ranged from FW to 15 psu SW. In this and a previous study, larval survival was better in 10 psu SW than in FW (Senoo et al. 2008). This suggests that marble goby may be capable of reproducing in low salinity water and perhaps indicates that it should be classified as a euryhaline fish. The salinity tolerance pattern displayed by marble goby is similar to that of other euryhaline fish. For example, eggs of killifish Fundulus heteroclitus hatched...
from FW to 35 psu (Grosell et al. 2007). Eggs of the obscure puffer hatched at salinities from FW to 20 psu (Yang and Chen 2006) and hatching rates of the tawny puffer *T. flavidus* were above 70% from 5 to 40 psu (Zhang et al. 2010). Similarly, eggs of the Iceland capelin *Mallotus villosus* are able to hatch between 1.5 and 34.0 psu (Davenport 1989). The reproductive biology of marble goby in its natural environment is still unknown. Because marble goby has a wide distribution and diverse habitats, it is believed that there are several genetically distinct populations of this fish. Ha et al. (2011) revealed highly significant differences in the mtDNA control region between fish sampled in East Malaysia and West Malaysia. The marble goby brood fish used in the present study were captured from a “river” population and may possess different reproductive characteristics from “landlocked” fish inhabiting enclosed ponds and lakes, as used in other studies (Tavarutmaneeul and Lin 1988; Senoo et al. 1994a; Luong et al. 2005). Understanding the differences in biological and reproductive characteristics of river and landlocked marble goby needs further investigation.

The eggs used in the present study can be incubated in both FW and 10 psu SW. However, the eggs incubated at 10 psu SW had a shorter hatching period, higher hatching rate, and better larval survival than eggs incubated in FW. The present study showed that egg incubation at 10 psu is recommended for marble goby, at least for the brood fish used in the study. The most favourable incubation conditions for egg survival and hatching are considered to be those that result in the greatest numbers of normal larvae. Incubation in 10 psu SW, which resulted in a high hatching rate and a low deformation rate may produce better success in hatcheries. Hatched larvae should be reared for a further 10 days in 10 psu SW as this period is considered to be the most important period for larval survival. Larvae then can be reared in FW (Senoo et al. 2008). This study presents the first observations on the optimal salinity for egg incubation and hatching of marble goby, information which will be useful for its aquaculture and seed production. However, further studies are required to understand the mechanisms underlying the observed phenomena and to develop practical rearing techniques for this species, e.g., elucidation of the role of chloride cells in the yolk-sac membrane and body surface of embryos and newly-hatched larvae.

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### References


II (Fish culture in Southeast Asia).


マーブルゴビ *Oxyeleotris marmoratus* の卵発生、孵化および仔魚の奇形に及ぼす塩分の影響

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東南アジアにおいて重要な淡水養殖魚であるマーブルゴビ *Oxyeleotris marmoratus* の孵化率向上のため、マレーシア・サバ州の河川で捕捉されたマーブルゴビから得た卵を、淡水（FW）、5, 10, 15, 20および30 psu に希釈した海水（SW）でインキュベートし、孵化率および仔魚の奇形率を調べた。その結果、淡水から15 psu SW の間で正常孵化がみられ、10 psu SW において最も孵化率が高く奇形率が低かった。そこで FW と10 psu SW との間で孵化時期および10日齢の仔魚の生残率を比較したところ、FW では10 psu SW よりも孵化が遅れ、孵化した仔魚の奇形率は高く、さらに FW では全ての仔魚が10日齢までに飼死した。本実験の結果から、本実験で用いた卵を10 psu SW でインキュベートすると FW よりも孵化時期が早く、孵化率および仔魚の生残率が高くなることがわかった。