**Effects of Water Salinity on Reproductive Performance of Female Hatchery-Reared Spotted Scat (Scatophagus argus Linnaeus, 1766) Broodstock**

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<td>Ruensirikul, Jirayuth; Coastal Aquaculture Research and Development Regional Center 6 (Songkhla), Department of Fisheries CHIAYVAREESAJJA, SOMMAI; Natural Resources, Aquatic Science</td>
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Original Article

Effects of Water Salinity on Reproductive Performance of Female Hatchery- Reared Spotted Scat (Scatophagus argus Linnaeus, 1766) Broodstock

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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-m³ 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 indicates that a holding salinity of 15 - 25 ppt is 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 advantage of this species is that it is both a valuable food fish and 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 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 its immune system as well as affecting its reproductive development (Campbell, Pottinger, & Sumpter, 1994; Castranova, King, & Woods, 2005; Davis, 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 for maintaining 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 has investigated the effects of salinity on the reproductive activity of spotted scat was that of Khanh et al. (2012). The aim of the study described herein was 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 fishes were reared together in a 28 m$^3$ cylindrical cement tank 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 °C, a 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, 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 fish selected were randomly put into the 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 comprised four treatments in which the broodstock fish were reared in varying 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 (6 females : 4 males). Each treatment was conducted in triplicate. Two females from each treatment were randomly tagged with passive-integrated transponder tags (PIT-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 °C, a 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

All the female broodfish in each tank were monitored monthly for 4 months after the experiment was initiated. The morphology of the fish’s abdomen was observed and females with 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 x 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 40x magnification and calculated as the number of post-vitellogenic oocytes x 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, depend 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 was first presented after massage. The ovulated oocytes were manually stripped and fertilized immediately with fresh pooled milt collected from 3 - 4 spermiating males obtained from the broodstock tank, with good motility. The ovulation rate (the number of ovulated females x 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 x100/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 minutes (Zakeri et al., 2009). Ovulated oocyte diameter and oil globule diameter was also measured as the post-vitellogenic diameter before hormone injection. The percentage of normal ovulated eggs (number of normal ovulated eggs x 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 centre, 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 x 100/total number of eggs), the hatching rate (number of larvae x 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 x 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/liter without aeration or feeding.

2.4 Sex Steroid Hormone Levels

At each time 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 °C and was then stored at -20 °C until the determination of the sex steroid hormone level (17β-estradiol: E2) by electrochemiluminescence immunoassay (ECLIA) 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, Massachusetts, USA) contemporary with steroid measurement. Distilled water (0 mOsmol/kg) was applied as a control solution.
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 were 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 difference in the monthly average serum osmolality among salinity treatments was also analyzed. The differences in serum and water osmolality were established using 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) (Table 1) showed no significant differences among the salinity treatments (\( P>0.05 \)).
After ovulation, no significant differences \((P>0.05)\) were detected in the latency time \((36.4 \pm 5.4 - 40.0 \pm 1.7\ h)\), buoyancy rate \((72.4 \pm 14.9 - 85.7 \pm 8.1\ %)\), ovulated oocyte diameter \((632.6 \pm 11.4 - 661.3 \pm 43.1\ \mu m)\), oil globule diameter \((237.0 \pm 21.7 - 244.5 \pm 23.1\ \mu m)\), or percentage of normal ovulated eggs \((61.1 \pm 21.6 - 81.4 \pm 11.4\ %)\) 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 \((61.0 \pm 22.7 - 80.7 \pm 13.0\ %)\), hatching rates \((40.5 \pm 10.0 - 62.9 \pm 16.7\ %)\), total length of larvae \((1.67 \pm 0.14 - 1.75 \pm 0.15\ mm)\), abnormality rates of larvae \((2.5 \pm 1.1 - 3.5 \pm 0.5\ %)\) or the survival of day-3 larvae \((65.9 \pm 3.9 - 68.1 \pm 8.8\ %)\) 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 \((65.9 \pm 16.7\ %)\) which was significantly different from all other salinities except the 15 ppt treatment \((48.9 \pm 1.9\ %)\). The females in 33 ppt salinity produced the lowest ovulation rate \((28.5 \pm 17.0\ %)\) which was significantly different from all other treatments apart from the 5 ppt treatment \((34.6 \pm 13.8\ %)\) (Table 1).

### 3.2 Sex Steroid Hormone Levels

The average serum E2 level of the ovulated-female fish \((797.6 \pm 95.0 - 1,546.0 \pm 448.8\ pg/mL)\) was significantly higher than that of the non-ovulated fish \((206.3 \pm 106.6 - 415.6 \pm 198.7\ pg/mL)\) \((P<0.05)\) in every treatment, except those in the 15 ppt treatment where data was only collected 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 nor in the non-ovulated fish when they were separately analyzed (Figure 1).
3.3 Serum Osmolality

There was no significant difference in serum osmolality of the fish among any 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).

4. Discussion

Typically, salinity can suppress the reproductive performance of fish but euryhaline fish are better adapted to environmental fluctuations. Although, this study clearly shows that the salinity tested ranging from 5 to 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 tested at 15 and 25 ppt. Reproductive impairment when broodfish are subjected to low salinity has been reported in previous studies. In a study of black bream (*Acanthopagrus butcheri*), a wide range of holding salinity (5 - 35 ppt) did not affect gonadal development or plasma steroid levels. However, the number of ovulations and egg volumes was lowest in fish held at 5 ppt (Haddy & Pankhurst, 2000). In other euryhaline marine fish such as Waigieu seaperch (*Psammoperca waigiensis*), holding broodfish in different salinities (5, 10, 20 and 30 ppt) during the breeding season affected gonadal development and spawning performance. Fish held at 5 ppt had 100% mortality and at 10 ppt, oocyte development or ovulation was diminished (Pham, Kjørsvik, Nguyen, Nguyen, & Arukwe, 2010) and similar results were found for mullet (*Mugil cephalus*) (Assem, Abdel Rahman, Al Absawey, & Mourad, 2015) in which different water salinities affected gonadosomatic index values.
Thus, based on the present study, spotted scat broodstock can be reared for aquaculture in any salinity (5 - 33 ppt) depending on available water sources and investment budget. However, to achieve higher ovulation rates in broodfish, salinities of 15-25 ppt are suggested as being 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 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 female scat was similar to that found in 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 has also been found in other fish, such as common snook, Centropomus undecimalis (Cruz-Botto, Roca-Lanao, Gaitán-Ibarra, Chaparro-Muñoz,
& Villamizar, 2018) for which the E2 level was not affected by different salinity environments (estuarine and seawater), being high in the spawning season (300 - 400 pg/mL) and decreasing 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 ranging 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). The serum osmolality of the scat in this study was close to that reported by Mu et al. (2015) and was slightly lower than that of 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 to 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 that there is a good opportunity for the establishment of broodstock of this species in hatcheries or in natural water for the purpose of breeding and mass seed production.

References


FIGURE CAPTION LIST

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 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, expressed as mean ± SE. No significant differences were observed among serum osmolality of any salinity treatment but at the same salinity serum and water osmolality are significantly different ($P > 0.05$).
Figure 1

Figure 2
### LIST OF TABLE

**Table 1** Reproductive performance parameters of female spotted scat broodstock reared in different water salinities for 4 months in a hatchery. Data was averaged from monthly investigation, expressed as mean ± SE. Means sharing the same superscript indicate no significant differences \((P>0.05)\) between salinity treatments.

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<td><strong>Mature female (%)</strong></td>
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<td>Before injection</td>
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<td><strong>Post-vitellogenic oocyte (%)</strong></td>
<td>85.8±7.6a</td>
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<td><strong>Post-vitellogenic oocyte diameter (µm)</strong></td>
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<td>After ovulation</td>
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<td>Latency time (h)</td>
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<td>Ovulation rate (%)</td>
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<td>Buoyancy rate (%)</td>
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<td>Ovulated oocyte diameter (µm)</td>
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<td>Oil globule diameter (µm)</td>
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<td>Normal ovulated egg (%)</td>
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<td>Fertilization rate (%)</td>
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<td>Hatching rate (%)</td>
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<td>Total length of day-0 larvae (mm)</td>
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<td>Larval abnormality rate (%)</td>
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<td>Survival rate of day-3 larvae (%)</td>
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