Songklanakarin J. Sci. Technol.
36 (4), 433-437, Jul. - Aug. 2014

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Short Communication

Suitability of water salinity for hatching and survival of newly hatched larvae of climbing perch, *Anabas testudineus*

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Received 24 June 2013; Accepted 7 May 2014

Abstract

Experiments were conducted between July and December 2012 at Universiti Malaysia Terengganu, Malaysia, to determine the effect of water salinity on the hatching rate and survival of newly hatched larvae of climbing perch *Anabas testudineus*. A total of 2,700 morula stage embryos of climbing perch were directly transferred to water salinities of 3, 6, 9, 12, and 15 ppt at 27-28°C. Control embryos were incubated alike in freshwater. Hatching rate (i.e. total and viable hatchings) and the survival of newly hatched larvae were recorded. Results showed the highest total hatching (97.3%) and viable hatching (95.3%) were both observed at 3 ppt. The survival of newly hatched larvae was highest at 3 ppt (90.0%), but was not significantly different ($p>0.05$) from the control (86.0%). Thus, our results suggest the feasibility to culture climbing perch in a slightly saline environment (i.e. 3 ppt) particularly during the endogenous feeding stage to increase the larval survival.

Keywords: *Anabas testudineus*, climbing perch, hatching rate, water salinity, newly hatched larvae

1. Introduction

Detailed knowledge on the reproductive biology and early life stages of individual fish species are important for successful cultivation of fish larvae. Higher rates of mortality normally occur during the early life stages in both freshwater and marine fish species (Chotipuntu and Avakul, 2010), although hatchery operators often tend to compensate the mortality by collecting as many eggs as possible during spawning. Therefore, high survival of good quality larvae in early life stages is particularly important for increasing the hatchery production.

In most fish species, fertilization occurs outside the body of the female. Prior to spawning, eggs retain their maternal osmotic conditions but rapidly adjust to the surrounding osmotic pressure by absorption of water into the perivitelline space upon fertilization (Finn, 2007). However, water permeability and passive transfer of osmolytes between the embryo and its environment are minimized by the chorion and perivitelline space after water hardening (Alderdice, 1988). The effects of salinity have been extensively examined in marine and estuarine fish embryos and larvae (Haddy and Pankhurst, 2000). However, only a few studies have been conducted on the tolerance of freshwater fish to increased water salinity. In general, many freshwater fish embryos can only tolerate low salinity, such as catfish *Clarias gariepinus* (Gbulubo and Erondu, 1998) and *Heterobranchus longifilis* (Fashina-Bombata and Busari, 2003), whereas embryos of some marine and euryhalineous fish can tolerate a wider range of salinity changes, such as black sea bass *Centropristis striata* (Berlinsky *et al.*, 2004), European sea bass (Conides and Glamuzina, 2001), and rabbitfish *Siganus guttatus* (Young and Duenas, 1993). Salinity has also been suggested to contribute to better hatching, and improve survival of some freshwater fish species such as tilapia, striped bass, and catfish (Molokwu and Okpokwasili, 2002).

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According to Chotipuntu and Avakul (2010), the hatching rate of artificially fertilized climbing perch eggs was 77.0-92.0% in salinities of 0-4.5 ppt. In addition, for most fish species, the egg fertilization and incubation, yolk sac absorption, early embryogenesis, swim bladder inflation, and larval growth depends on salinity (Cingi *et al.*, 2010). Salinities beyond optimal osmoregulatory ranges of embryos will have negative impacts during incubation, which usually manifest itself in a decreased growth and development (Bœuf and Payan, 2001; Sampaio and Bianchini, 2002). Smaller newly hatched fish are likely to have reduced chances of survival (Cingi *et al.*, 2010).

Climbing perch has been categorized as vulnerable (Vu) by the International Union for Conservation of Nature and Natural Resources (IUCN) (Sarkar *et al.*, 2005). At present, most of the studies on climbing perch have been focused on the fry and adult stages (Chotipuntu and Avakul, 2010). Reports on the effect of salinity on its early stages of embryogenesis are lacking. Salinity changes may affect fertilization, development and survival of embryos and larvae. Culture of freshwater fish in brackish water has the advantages in terms of metabolic cost and nutritional utilization efficiency (Woo and Kelly, 1999). In addition, there are many abandoned shrimp ponds in South Asian countries that can be used to culture potential freshwater fish (Chotipuntu and Avakul, 2010). However, there is also a gap of existing biological information on the effect of salinity during early life stages of climbing perch particularly during its endogenous feeding stage. Therefore, the purpose of this study is to determine the effect of water salinity on mortality, hatching rate and survival of newly hatched climbing perch larvae, in order to provide useful baseline information on the culture and management of this species in brackish environment.

2. Materials and Methods

2.1 Climbing perch

Healthy male and female climbing perch broodstocks (one each) weighing 60-80g were acquired from the freshwater hatchery of Universiti Malaysia Terengganu (UMT) for induced breeding. The male and female fish were given intramuscular injection of Ovaprim (Syndel, Canada) over the dorsal region at 0.25 ml/kg and 0.5 ml/kg, respectively. The injected broodstocks were paired and released into water tanks maintained at 27±l°C for natural spawning. The same broodstocks were used repeatedly for the experiments.

2.2 Salinity and hatching rate

The first experiment was performed to examine the effect of water salinity on embryo mortality and hatching rate. Saline water of 3, 6, 9, 12, and 15 ppt was prepared by diluting seawater with freshwater based on refractometer measurement. The highest salinity in this study (15 ppt) was based on the 24h-LC_{50} of climbing perch fry reported by Chotipuntu and Avakul (2010). Fifty morula stage embryos (formed 150 min after fertilization) were transferred directly from freshwater to saline water of 3, 6, 9, 12, and 15 ppt (500 ml each) in closed plastic containers with gentle aeration. The containers were set up in triplicates, and placed in water bath maintained at 27-28°C. Control embryos were incubated alike in freshwater. Dead embryos were removed and recorded. The embryos were allowed to develop until hatching. The hatching percentages (total and viable hatchings) were calculated. In addition, the time of first hatching (hatching of first egg) was determined and used as an index to compare the rate of embryonic development at different salinities (Yang and Chen, 2005). Water salinity in each container was recorded before and after treatment. Experiments were repeated three times using different batches of embryos from the same broodstocks.

2.3 Salinity and survival rate

Another experiment was conducted to determine the survival rate of newly hatched larvae in different salinities. Fifty healthy newly hatched larvae of similar size were sampled from hatching tanks and immediately transferred to water of 3, 6, 9, 12 and 15 ppt salinities (500 ml each) respectively in closed plastic containers with gentle aeration. The containers were set up in triplicate and placed in water bath maintained at 27-28°C. The control larvae were incubated alike in freshwater. Survival rate was determined at the end of the yolk sac absorption (i.e. 5 days after hatching, DAH). Experiments were repeated three times using different batches of newly hatched larvae from the same broodstocks.

2.4 Statistical analysis

All data were tested for normality. Percentage data were arcsine-transformed and subject to analysis of variance (ANOVA) at $p<0.05$. Tukey's test was used to determine significant differences between the mean of all groups.

3. Results and Discussion

3.1 Salinity and hatching rate

The fertilized eggs of climbing perch were epipelagic, round in shape, bright and clear in appearance. Table 1 summarizes the time of first hatching, hatching rate, and survival rate of yolk sac larvae at different salinities. The time of first hatching was shortest at 3 ppt i.e. 21 hrs compared to other salinities and control. In contrast, the longest hatching time was 24 hrs at 9 ppt. Meanwhile, there was no hatching at 12 and 15 ppt as all the embryos died. The highest hatching occurred at 3 ppt with 97.3% total hatching and 95.3% viable hatching, although it was not significant ($p>0.05$) when compared to the control. At 6 and 9 ppt, total hatching and viable hatching were significantly lower ($p<0.05$) when compared to control and 3 ppt. In addition, it was observed

Table 1. Time of first hatching, hatching and survival rates of *Anabas testudineus* at different salinities (n=50; mean±SD).

Salinity (ppt)	Time of first hatching (hour)	Hatching rate (%)		Survival rate (%)
		Total	Viable	
0	21.5	92.7±3.1[a]	91.3±5.0[a]	86.0±2.0[a]
3	21.0	97.3±2.3[a]	95.3±1.2[a]	90.0±2.0[a]
6	23.0	80.0±4.0[b]	78.7±3.1[b]	74.5±5.0[b]
9	24.0	70.7±3.1[b]	63.3±4.2[c]	54.0±7.2[c]
12	-	0	0	0
15	-	0	0	0

*Values with the same superscript in the same column are not significantly different ($p>0.05$).

that the number of yolk sac larvae showing deformity (i.e. stomach edema) was highest at 9 ppt (7.4%).

The development of early life stage in teleost fish generally follows the same pattern (Falk-Petersen, 2005). The eggs of freshwater fish are susceptible to water influx and continuous ionic loss due to different osmotic gradient (Fyhn et al., 1999). Embryonic water balance is essential for growth, thus the water salinity may affect yolk utilization and nutrient mobilization rate from the yolk to the tissues (Swanson, 1996). In addition, hatching success is principally affected by the level of chorionase activity and movement of the embryo in the chorion (Yamagami, 1988). However, the hatching time may vary among fish species. In climbing perch, the hatching time after a single injection of Ovaprim was reported to be approximately 19-23 hrs after spawning (Patowary and Dutta, 2012). In our study, the time of first hatching (hatching of first egg) was recorded for comparison of embryonic development. In contrast, based on the same method of observation, Amornsakun *et al.* (2005) reported hatching time of 20 hrs 30 min in climbing perch with 87.4% average hatching success at 27.0-30.5°C. Jalilah *et al.* (2011) reported a longer hatching time of 28 hrs in climbing perch embryo incubated at 25-27°C. However, the authors did not mention the mean hatching success acquired.

The most common morphological abnormalities in many fish species after hatching are undeveloped head, deformed trunk, enlarged yolk sac, and curved tails (Teji and Thomas, 2006). However, we only observed stomach edema at 9 ppt salinity in the present study. From the results of our study, it is possible that water salinity stimulates the movements of the embryo due to higher oxygen availability through the perivitelline space when exposed to different osmotic gradients. In addition, it has been reported that both freshwater and brackish water fish species possess hatching enzymes with salt-dependent characteristics (Kawaguchi *et al.*, 2013). Osmotic gradient may also induce the chorionase secretion and activity, thus shorten the hatching time.

Compared to embryonic stage, fish larvae at the later developmental stages may be able to tolerate exposure to altered salinity by the development of osmoregulatory mechanisms. For instance, chloride cell also called mitochondria-rich cell (MRCs) or ionocyte typically found in the gill epithelium, is a major osmoregulatory site for teleosts (Hiroi *et al.*, 1999). In our study, the higher water salinities of 12 and 15 ppt resulted in highest mortality during the stage of embryonic development. Similarly, osmotic gradient has been reported to prevent embryonic development in some freshwater fish species (Yang and Chen, 2006). Others reported that fertilization and hatching rates of freshwater teleost decrease in saline water (Gbulubo and Erondu, 1998). Our finding agrees with Boeuf and Payan (2001) that freshwater fish show higher tolerance to a lower range of water salinities. Other experiments examining the abrupt transfer of black sea bass *Centropristis striata* to low salinities have helped identify the salinity threshold for successful culture of the species (Young *et al.*, 2006). Similarly, gradual acclimation experiments with Nile tilapia, *Oreochromis niloticus* and blackchin tilapia, *Sarotherodon melanotheron* (Lemarie *et al.*, 2004), and larval salinity tolerance experiments with cobia *Rachycentron canadum* (Faulk and Holt, 2006) have provided valuable information on osmoregulatory ability of the species. The ability of early stages of fish to withstand osmotic gradient range is possibly species-dependent.

3.2 Salinity and yolk sac larval survival

The yolk sac of newly hatched climbing perch larva appeared to be bilobed (data not shown). The yolk sac was fully absorbed in five days after hatching (5 DAH) at 0, 3, 6, and 9 ppt salinities. The survival rate in 5 DAH was significantly lower ($p<0.05$) at 6 ppt (74.5%) and 9 ppt (54.0%). The highest survival rate was observed at 3 ppt (90.0%), although it was not significantly different ($p>0.05$) compared to the control. Survival rate were significantly lower ($p<0.05$) at 6 and 9 ppt with 74.5% and 54.0%, respectively.

Our findings are in agreement with Jalilah *et al.* (2011) that yolk sac was fully absorbed in 5 DAH. However, Amornsakun *et al.* (2005) reported a complete yolk sac

absorption in climbing perch within 92 hrs after hatching at 27.0-30.5°C. In other species, for example, green catfish *Mystus nemurus*, yolk sac absorption was completed in 3 DAH at 25-30°C (Amornsakun *et al.*, 1997). In our study, salinity of 3 ppt did not affect the yolk sac absorption in climbing perch when compared to other salinities. It is possible that the salinity conditions allow the larvae to make efficient use of the ionic constituents available for other physiological developments. In addition, ameliorating effect of moderate salinity has been well documented in many fish species and has also been proposed as a stress reduction mechanism in fish husbandry (Pickering, 1993). For instance, Tsuzuki *et al.* (2001) showed reduction in cortisol level in *Odontesthes bonariensis* exposed to moderate salinities.

4. Conclusions

This study has demonstrated that water salinity of 3 ppt produced the highest percentage of hatching success and yolk sac larval survival. Thus, our study suggests that a slightly saline environment may increase hatching success and yolk sac larval survival. Further studies on the effect of salinity on oxygen consumption, growth rate, hatching enzyme activity during embryonic, and yolk sac larval development may provide essential information on the feasibility of introducing climbing perch into brackish water culture system.

Acknowledgements

This work was financially supported by Research Promotion Grant (Geran Galakan Penyelidikan: Grant No. 68007) awarded by Universiti Malaysia Terengganu to the first author. Authors are grateful to the School of Fisheries Science and Aquaculture, Universiti Malaysia Terengganu for providing necessary facilities.

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