Effects of Water Salinity on the Egg Hatching, Growth, and Survival of Larvae and Fingerlings of Climbing Perch, Anabas testudineus

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Effects of Water Salinity on the Egg Hatching, Growth, and Survival of Larvae and Fingerlings of Climbing Perch, *Anabas testudineus*

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A study on the effect of water salinity ranging from 0 ppt to 30 ppt on the hatching success of climbing perch, *Anabas testudineus*, was conducted in a 15–liter glass aquarium (water volume 10 liters) containing 100 eggs. Fertilization rates at 0, 5, 10, 15, 20, 25, and 30 ppt were 76.67, 61.33, 77.00, 47.33, 1.67, 0.33, and 0%, respectively. The fertilization rate at 0–10 ppt did not change. When the water salinity increased, the fertilization rate decreased. Hatching rates at 0, 5, 10, 15, 20, 25, and 30 ppt were 91.33, 87.90, 86.20, 77.49, 0.00, 0.00, and 0.00%, respectively. The hatching rate at 0–10 ppt was significantly ($P < 0.05$) higher than that at 15, 20, 25 and 30 ppt. The times (1,255–1,300 minutes) of hatching out among water salinity of 0–15 ppt did not change. The survival tolerance of climbing perch larvae in different water salinities (0–30 ppt) within 24 hours was then studied using a 50–liter glass aquarium (water volume 30 liters) containing 50 larvae at each level of water salinity. All treatments were done in triplicate. The survival rates of fish larvae in 0, 5, 10, 11, 12, 13, 14, 15, 20, 25, and 30 ppt were 100, 100, 100, 83.3, 83.3, 73.3, 70, 70, 0, 0, and 0%, respectively. The survival rates at water salinities of 0–12 ppt were not significantly different. The gain rate of total body length at 8 weeks at water salinities of 0, 5, 10, 11, and 12 ppt were 1.77, 1.41, 1.36, 1.36, and 1.63 cm, respectively. The gain rates of total body length at 0–12 ppt were not significantly different. We conclude that water salinity in the range of 0–12 ppt is suitable for climbing perch culture.

Key words: fertilization rate, hatching rate, hatching out, water salinity tolerance, climbing perch

INTRODUCTION

Anabas testudineus, commonly known as the climbing perch, is an indigenous species of Asia, i.e., Malaysia and Thailand (Talwar and Jhingran, 1992). It is also an important fish species due to its high demand, especially in Thailand, as it has notable nutritive benefits for consumers (Chotipuntu and Avakul, 2010). This species has been classified as vulnerable, as it is exposed to overfishing and pollution, and no culture approach has been established (Mijkherjee *et al*., 2002). Hence, most of the practiced cultures of climbing perch are semi–intensive, apparently depending on a diet prepared by the cultivator (Bungas *et al*., 2013). The optimal growth performance of climbing perch relies upon feeding them a diet with a protein level of around 20–40% (Hossain *et al*., 2012).

The climbing perch is a species that can grow naturally in various habitats, such as rice fields, irrigated canals, and ditches. Traditionally, the largest natural source of climbing perch production is from catching them in rice fields. However, the productivity of this fish had declined due to some problems in rice–producing areas and pesticide usage (Tan *et al*., 1973; Ali, 1990; Mohsin and Ambak, 1983).

In Southeast Asia, aquaculture plays an important role in the production of economically important aquatic organisms. However, suitable areas for the culturing of aquatic organisms are limited. Consequently, the coastal zone might be a potential alternative area for fish culture. Moreover, some shrimp ponds in the coastal zone have been abandoned, due to the outbreak of shrimp disease (Chotipuntu and Avakul, 2010). The possibility of using some abandoned shrimp ponds for the culture of freshwater fish such as the climbing perch should be studied. However, these ponds in the coastal zone might be in high–salinity water areas, which may affect the productivity of freshwater fish culture.

Water salinity is one critical factor that affects the survival rate, distribution, and metabolism of fish. Many studies on the climbing perch have focused on physiology, biology, nutrition, artificial breeding, and other subject, but few have focused on the effect of environmental parameters on the survival of this species (Amornsakun *et al*., 2005). Little published research on the effect of water salinity on this fish was found. Therefore, there is a need to study the effect of water salinity on the climbing perch. The objectives of this study were to evaluate the effect of water salinity on the hatching rate and the

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water salinity tolerance of the climbing perch by culturing them in various water salinities.

MATERIALS AND METHODS

Animals

Fertilized eggs of climbing perch, *Anabas testudineus*, were produced by induced spawning using chemical injection with Suprefact (20 μg/kg) (CinnaGen, Tehran, Iran) and Motilium (5 mg/kg) (Janssen Pharmaceutica, Beerse, Belgium). The sexually mature fish were cultured in a fiberglass tank (water volume 300 liters) with a ratio of male to female brooders of 2:1.

Study on the fertilization rate, hatching out, and hatching rate of eggs of climbing perch in different water salinities

The fertilization rate and hatching rate experiments were carried out using 15–liter aquaria (water volume 10 liters), each containing 500 eggs, in different water salinities, which were 0, 5, 10, 15, 20, 25, and 30 ppt. The number of fertilized eggs was then observed four hours after incubation. In each aquarium, the fertilization rate was calculated by the (number of fertilized eggs/number of eggs) $\times$ 100. The time required for the appearance of the first newly hatched larvae, which would signal hatching out, was recorded. All newly hatched larvae were collected using a dropper. Hatching rates were calculated by the (number of newly hatched/number of eggs) $\times$ 100 (Tarnchalanukit, 1978).

Study of larvae's water salinity tolerance

Experiments were carried out using 50–liter aquaria (water volume 30 liters), each containing 50 fish (2.5 cm total length) in different water salinities, which were 0, 5, 10, 15, 20, 25, and 30 ppt. The mortality of the fish in each water salinity was recorded at 10–minute intervals for 24 h. The procedure was carried out with three replications.

Study of cultured larvae in different water salinities

Experiments were carried out using 50–liter aquaria (water volume 30 liters), each containing 50 fish (2.5 cm total length) in different water salinities (which in earlier experiments had shown a 100% survival rate). Throughout the rearing period with aeration, larvae were fed twice a day with normal commercial pellets. The water exchange rate was 10% of the tank volume every day. The total length of 20 specimens was measured every week until completion of the experiment after 8 weeks. The procedure was carried out with three replications.

Statistical analyses

The results of the analysis of variance of the fertilization rate, hatching out, and hatching rate in different water salinities are represented as means ± SD. The statistical significance among experimental groups was analyzed by Duncan's new multiple range test.

RESULTS

Study of the fertilization rate, hatching out, and hatching rate of the eggs of climbing perch in different water salinities

Fertilization rates in water salinities of 0, 5, 10, 15, 20, 25, and 30 ppt were 76.67, 61.33, 77.00, 47.33, 1.67, 0.33, and 0.00%, respectively (Table 1 and Figure 1). The fertilization rates at water salinities of 0–10 ppt were significantly ($P < 0.05$) higher than those at water salinities of 15–30 ppt. Fertilization rates among water

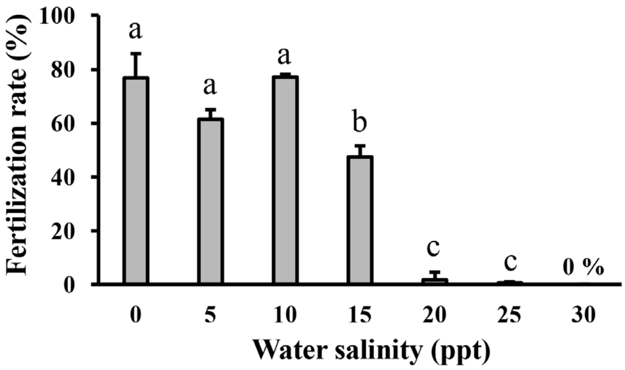

Fig. 1. Fertilization rate (%) of climbing perch in different water salinities.

Table 1. Fertilization rate (%), hatching rate (%) and hatching time (min) of climbing perch eggs incubating in different water salinities (n = 100)

Water salinity (ppt)	Fertilization rate(%)	Hatching rate(%)	Hatching time(min)
	Mean ± SD	Mean ± SD	Mean ± SD
0	76.67 ± 9.07[a]	91.33 ± 1.17[a]	1,276.66 ± 7.63[a]
5	61.33 ± 3.51[a]	87.90 ± 6.31[a]	1,255.00 ± 13.22[a]
10	77.00 ± 1.00[a]	86.20 ± 7.39[a]	1,283.33 ± 2.88[a]
15	47.33 ± 4.16[b]	77.49 ± 0.69[b]	1,300.00 ± 10.00[a]
20	1.67 ± 2.88[c]	0	0
25	0.33 ± 0.57[c]	0	0
30	0	0	0

Different superscript letters indicate significant differences ($P < 0.05$).

salinities of 0, 5, and 10 ppt were not significantly different ($P > 0.05$).

The hatching rates at 0, 5, 10, 15, 20, 25, and 30 ppt water salinities were 91.33, 87.90, 86.20, 77.49, 0.00, 0.00, and 0.00%, respectively (Table 1 and Figure 2). The hatching rates at water salinities of 0, 5, and 10 ppt were significantly ($P < 0.05$) higher than those at 15, 20, 25, and 30 ppt. Hatching rates at water salinities of 0, 5, and 10 ppt did not change.

In order to further understand the effect of water salinity on the climbing perch, the time of hatching out was also recorded, and at 0, 5, 10, and 15 ppt water salinities, the times were 1,276.66, 1,255.00, 1,283.33, and 1,300.00 minutes, respectively (Table 1 and Figure 3). The time of hatching out was not influenced by water salinities of 0, 5, 10, and 15 ppt.

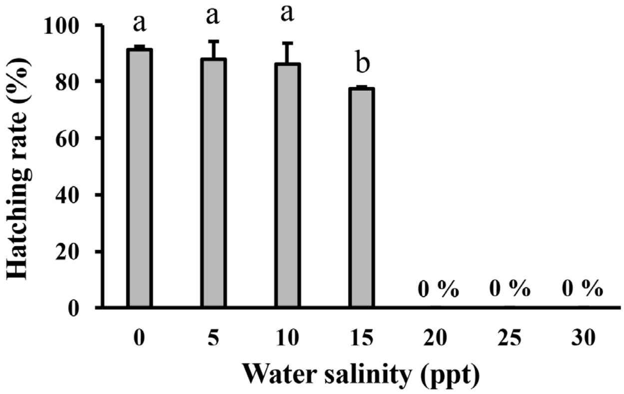

Fig. 2. Hatching rate (%) of climbing perch in different water salinities.

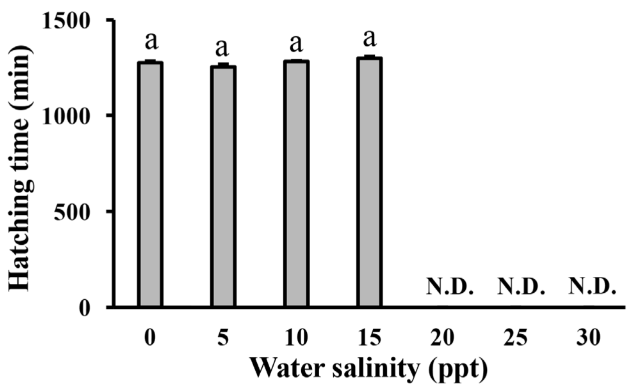

Fig. 3. Hatching out (min) of climbing perch in different water salinities.
N. D.: not detectable

Study of larvae's water salinity tolerance

The study on the tolerance of climbing perch larvae in different salinities within 24 hours was done in 50–liter glass aquaria (water volume 30 liters) containing 50 larvae each with fish 2.5 cm in total length. The survival rates of fish larvae at 0, 5, 10, 11, 12, 13, 14, 15, 20, 25, and 30 ppt were 100, 100, 100, 83.3, 83.3, 73.3, 70, 70, 0, 0, and 0%, respectively (Table 2). The water salinity tolerance was not significantly different ($P > 0.05$) at 0, 5, 10, 11, and 12 ppt salinities. As the water salinity increased, the survival rate decreased. At 20, 25, and 30 ppt, the fish larvae died.

Study of cultured larvae in different water salinities

The study of climbing larvae culture in different water salinities (in which 100% of the adult fish had survived) and experiments were completed in 8 weeks. The result showed total length gains at 0, 5, 10, 11, and 12 ppt salinities of 1.77, 1.41, 1.36, 1.38, and 1.63 cm, respectively (Table 3). Total length gains in water salinities of 0, 5, 10, 11, and 12 ppt were not significantly different ($P > 0.05$).

Table 3. Total length gain (cm) of climbing perch in different water salinities

Replication	Water salinity (ppt)				
	0	5	10	11	12
1	1.8	1.44	1.52	1.56	1.54
2	1.7	1.34	1.12	1.24	1.580
3	1.82	1.45	1.44	1.36	1.79
Average	1.77	1.41	1.36	1.38	1.63

The gain rates of total body length at 0, 5, 10, 11, and 12 ppt water salinity were not significantly different ($P > 0.05$).

DISCUSSION

Study of the fertilization rate, hatching rate, and hatching out of eggs of climbing perch in different water salinities

The results showed that water salinities of 0–10 ppt did not significantly affect climbing perch propagation. The fertilization rate of fish was related to the activation of spermatozoids (Sampaio *et al*., 2007). The effect of

Table 2. Survival rate (%) of climbing perch in different water salinities

Replication	Water salinity (ppt)										
	0	5	10	11	12	13	14	15	20	25	30
1	100	100	100	80	90	70	80	70	0	0	0
2	100	100	100	90	80	80	70	80	0	0	0
3	100	100	100	80	80	70	60	60	0	0	0
Average	100^a	100^a	100^a	83.3^a	83.3^a	73.3^b	70^b	70^b	0^c	0^c	0^c

Different superscript letters indicate significant differences ($P < 0.05$).

water salinity on the fertilization rate was also found in black bream, *Acanthopagrus butcheri*, in which the fertilization rate was reduced at 5 ppt (Haddy and Pankhurst, 2000). Some researchers considered that fish sperm were mostly activated in water with salinity below 10 ppt (Bush and Weis, 1983; Pissetti *et al.*, 2003). Furthermore, increases in the fertilization rate and hatching rate were produced by chemical injection. In this study, climbing perch injected with Suprefact (20 μg/kg) and Motilium (5 mg/kg) had an effective rate of fertilization and hatching. In addition, the chemical injection was the same as that used by Amornsakun *et al.* (2005), who reported that climbing perch injected with Suprefact (20 μg/kg) and Motilium (5 mg/kg) had a fertilization rate of 92.7%. Amornsakun *et al.* (2011) reported that snakehead fish injected with Suprefact (20 μg/kg) and Motilium (5 mg/kg) had a fertilization rate of 76.5%. In 2004, Amornsakun *et al.* reported that Siam gouramis injected with Suprefact (15 μg/kg) and Motilium (5 mg/kg) had a fertilization rate of 91.12%.

The number of hatched eggs is related to the hatchery technique, which can increase the number of larvae to support further culturing. Experiments in the present study found that hatching rate was not significantly affected by water salinities of 0–10 ppt. Yang and Chen (2006) mentioned that the puffer, *Takifugu obscurus*, hatched in water with salinities ranging from 0–20 ppt. Some freshwater fish such as silver carp, *Hypophthalmichthys molitrix* (Gao, 1965), and catfish, *Heterobranchus longifilis* (Fashina–Bombata and Busari, 2003), could only hatch in low–salinity water (6 ppt). In addition, hatching out took 1,255–1,300 minutes, showing that eggs quickly hatched in water with salinities of 0–10 ppt. Amornsakun *et al.* (2017) reported that snakehead fish hatched out in 1,192–1,442 minutes, showing that eggs quickly hatched in water with a salinity of 5 ppt. Moreover, it was also reported that the hatching time of green catfish, climbing perch, and Siamese gouramis were 1,080, 1,420, and 1,330 minutes, respectively (Amornsakun *et al.*, 1997; Amornsakun, 1999a; Amornsakun *et al.*, 2005; Amornsakun *et al.*, 2004).

Effect of larvae's water salinity tolerance

Although many studies have examined the water salinity tolerance of freshwater fish (Brett, 1979), only some studies could be applied to culturing those fish in the coastal zone (Partridge and Jenkins, 2002). Data in the present study show that climbing perch can survive in water with salinities below 12 ppt, and these data can be applied to culture in brackish water as well. Today, fish production for human consumption is necessary. However, freshwater fish culture is insufficient for catering to increasing demands. Some fish are starting to be cultured in brackish water to provide food. The common carp, *Cyrpinus carpio*, can survive in 15 ppt (Paye, 1983). Grass carp, *Ctenopharyngodon idella*, showed a water salinity tolerance of up to 14 ppt (Maceina and Shireman, 1979; Kilambi and Zdinak, 1980). The growth and survival rates of common carp larvae were actually better in water salinities of up to at least 3 ppt than in fresh water (Lam and Sharma, 1985). Amornsakun *et al.* (2017) mentioned that the maximum water salinities for snakehead fish to survive in brackish water were 0–14ppt, and the optimum water salinities for the culture of snakehead fish were 0–10 ppt. It is important to identify a salinity threshold for freshwater fish species. Nile tilapia, *O. niloticus*, showed the potential of freshwater fish to be cultured in the coastal zone (Lewbart *et al.*, 1999). Channel catfish, *Ictalurus punctatus*, and blue catfish, *Ictalurus furcatus*, have been captured in water with salinity of 11 ppt (Perry, 1968).

Investigation of the water salinity tolerance of freshwater fish has been predominantly motivated by ecological concerns (Dhaneesh *et al.*, 2012). Flathead catfish, *Pylodictis olivaris*, studied by Bringolf *et al.* (2005), were shown to survive at water salinities ranging from 0–11 ppt after 48 hours of experiment; the study also included goldfish, *Carassius auratus* (Schofield *et al.*, 2006). These studies showed that freshwater fish could survive—and showed a high potential for being cultured—in brackish water. Yolk–sac larvae of African catfish *H. longifilis* survived 100% in water salinities ranging from 0–7.5 ppt (Fashina–Bombata and Busari, 2003).

There have been many studies on the effect of water salinity on the growth and survival rate of fish but few on the acclimation of freshwater species to sea water (Dhaneesh *et al.*, 2012). Brett (1979) reported that most freshwater fish showed high survival rates in approximately 10 ± 2 ppt. However, climbing perch can survive at 0–12 ppt water salinity. Therefore, this species can survive in brackish water better than other freshwater fish. Boeuf and Payan (2001) concluded that freshwater fish showed high growth rates and survival rates in high water salinity, and marine species could have high growth rates and survival rates in low–salinity water.

Effect of water salinity on cultured larvae

Climbing perch showed the best growth rate in water with 0–12 ppt salinities. Amornsakun *et al.* (2017) reported that snakehead fish showed their best growth rates in 0–10 ppt. Jomori *et al.* (2012) reported that pacu, *Piaractus mesopotamicus*, showed fast growth at 2–4 ppt. In some studies, these authors mentioned that suitable saline water could reduce physiological stress (Jomori *et al.*, 2012) as well as ionic and osmotic differences between external and internal fluids (Lam and Sharma, 1985; Wurts, 1995; Riley *et al.*, 2003). Osmoregulation was also an important factor affecting the growth rates of fish. Boeuf and Payan (2001) reported that freshwater fish transferred to high water salinity needed more energy for breathing and osmoregulation. In addition, Wang *et al.* (1997) mentioned that water salinity affected the digestive system, food conversion ratio, and growth rate of common carp and showed that suitable water salinity for this species was 2.5 ppt.

Britz and Hecht (1989) mentioned that the growth rate and survival rate can be affected by water salinity. A study of the growth rate and survival rate of catfish, *Clarias gariepinus*, in different water salinities, including 0, 2.5, 5, 7.5, and 10 ppt, showed that high mortality and low growth rates of fish occurred in water with a salinity of 7.5 ppt, but that fish fry would die after 48 hours at 10 ppt, and a water salinity of 0–2 ppt was best for fry of this fish to grow. The effect of water salinity on the survival rate of fish was probably related to water salinity (Jomori *et al*., 2012). The relationship between water salinity and the survival rate was studied in some freshwater species, such as Siam gouramis and giant gouramis, at 12 ppt (Amornsakun *et al*., 2004; Amornsakun *et al*., 2014); and in redtail catfish, green catfish, and snakehead fish, at 10 ppt (Amornsakun, 1999b; Amornsakun *et al*., 1997; Amornsakun *et al*., 2017).

It was concluded that fertilization rates, hatching rates, and hatching out times of climbing perch were high with water salinities ranging from 0–10 ppt, and hatching out times were in the range of 1,276–1,300 minutes. When climbing perch were directly transferred to water with salinities ranging from 0–30 ppt, the fish could grow in water salinities of 0–12 ppt. Water salinities in the range of 0–12 ppt were shown to allow growth. Further research should focus on this fish species' physiological adaptability to changing water salinity.

AUTHOR CONTRIBUTIONS

T. Amornsakun, A. B. Hassan, and N. Suzuki designed the study. S. Srithongthum, P. Promkaew, and T. Amornsakun performed experiments. T. Amornsakun, N. Suzuki, H. Matsubara, Y. Takeuchi, K. Mukai, Y. Shimazaki, and Y. Oshima analyzed the data and wrote the paper. Y. Oshima and Y. Shimasaki supervised the work. All authors assisted in editing the manuscript and approved the final version.

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