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Article

Aquaculture Potential of Climbing Perch, *Anabas Testudineus*, in Brackish Water

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Abstract

Climbing perch, *Anabas testudineus*, is a freshwater fish species commercially grown in Southeast Asian countries. This study investigated aspects of salinity on hatching viability and growth performance of climbing perch to use as a measure for aquaculture in brackish water. The experiments were carried out under controlled laboratory conditions. The hormonal induction spawned eggs, and farm reared fry fish of an average body weight of 1.51 - 1.54 g were used in this study. The tested media were prepared using fresh seawater diluted with dechlorinated supplied tap water. It was found that hatching rates for the artificially fertilized eggs were 77 - 92 % in salinities of 0 - 4.5 ppt. The LC50 (24 h) was found to be 5.1 ppt. Hatching was not observed in salinities higher than 7.5 ppt. When fry were reared in different salinities for 40 days, they were found to grow best in salinity of 6.9 ppt. Normal growth rates were found in salinities up to 10.2 ppt. The fry stopped growing and gradually died off in salinities higher than 12.4 ppt. This study suggests that brackish water can potentially be used for climbing perch aquaculture. Saline water up to 4 ppt is practical for propagation of the fish. The fry climbing perch may be nurtured in brackish water of 6 - 7 ppt to obtain the best growth performance.

Keywords: Climbing perch, *Anabas testudineus*, salinity, aquaculture

Introduction

In Southeast Asia, fishery productions play a major role in providing a protein source for local consumption. However, the world fuel crisis makes fishing unprofitable. A number of fishermen have abandoned their fishery activities and turned to grow freshwater fish for household consumption and as a substitute income. This demands land for aquaculture use along the coastline. A large number of shrimp ponds on the coastlines of Southeast Asian countries were abandoned due to an outbreak of shrimp diseases in the past decade. These ponds have potential for growing alternative species especially for the production of commercial freshwater fishes. However, water intake into the ponds is habitually contaminated with seawater, the culture species thus should tolerate certain levels of salinity. The climbing perch (*Anabas testudineus*) is economically important in Southeast Asia, and is in high demand especially in southern Thailand, Malaysia and the Philippines. Climbing perch are reported to be found in estuaries [1] but the effect of salinity on its biology is undetermined. Studies have demonstrated an important role of salinity on the growth performance of freshwater fishes [2-11]. However, salinity tolerances of freshwater fish vary among species and with their developmental stages. Researchers have shown that while most adult freshwater fish live comfortably in salinity between 7 - 13 ppt, eggs and larvae may not survive a salinity higher than 1 ppt [12,13]. This suggests that a comprehensive investigation throughout a fish's life cycle is essential in order to successfully grow freshwater fishes in saline water.

This study evaluated fertilization viability of climbing perch eggs in saline water. Salinity tolerance of larvae and fry, and growth performance of fry fish were measured.

Materials and methods

Preparations of the Tested Media

The tested media were prepared using seawater collected from a coastal area of the gulf of Thailand. The supplied tap water was dechlorinated using sodium thiosulfate (Na_2SO_3) and used as a diluent. Salinity was measured in terms of gram-salt in 1,000 g of the saline solution as part per thousand (ppt) using a pH Salinity Conductivity Temperature YSI 63 - 50 FT meter. The batches of saline water were kept in closed containers to prevent evaporation.

Effects of Salinity on Hatching Viability

Eggs and milt were obtained from an artificial spawning of a female and a male brood fish using Luteinizing Hormone Releasing Hormone analogue. Male milt was stripped, drawn and stored in a 2.5 ml dry syringe. At the same time the ripe eggs were stripped into a dry plastic container. The stripped eggs were taken from the egg batch using a small spoon and placed in containers filled with 300 ml of the tested media. The male milt was immediately dripped into the containers through an injection needle No.12, a single drop followed the eggs and was stirred to prompt fertilization. The fertilizations were done in a random order among the tested salinities.

The experiments were repeated twice using different pairs of brood fish. The first trial comprised 10 salinity levels: 0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 and 22.5 ppt, the second trial comprised 11 salinity levels: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ppt, 3 replications for each salinity. After mixing of eggs and milt, the containers were covered with lids and placed in a water bath vessel at a constant temperature of 28 °C. The incubation containers were continuously aerated through small slits on the lids. The numbers of viable hatchings were recorded. The median lethal concentration (LC50) was evaluated using the Probit analysis.

Salinity Tolerance of Larvae

A total 300 of 3 day old larvae from a single female were used in this experiment. The tested media were divided into 10 salinity levels: 0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 and 22.5 ppt, each replicated 3 times. Ten larvae were immediately transferred to each replicated container filled with 300 ml of the tested media. The containers were covered with lids to prevent evaporation and continuously aerated. The containers were then placed in a water bath vessel at a constant temperature of 28 °C. The numbers of dead fish were recorded within 24 h. The lethal concentrations caused death to 1 % (LC1) and 50 % (LC50) of fish population were evaluated.

Salinity Tolerance of Fry

A total of 300 fry fish with an average weight of 1.51 ± 0.07 g were used in this experiment. The tested media were divided into 10 salinity levels of 0, 3, 6, 9, 12, 15, 18, 21, 24 and 27 ppt. The experiment was carried out using the same method for that of larval salinity tolerance. The lethal concentrations causing death to 1 % (LC1) and 50 % (LC50) of the fish population were evaluated. The LC1 was then used as a criterion for preparing the media used for the growth study of the fry fish.

Effects of Salinity on Growth Performances of Fry

The fry of climbing perch were obtained from a local hatchery and acclimatized to the laboratory conditions until they accepted food well. A total of 105 fish with an average weight of 1.54 ± 0.26 g were then transferred to rear in the tested salinities of 0, 2, 4, 6, 8, 10 and 12 ppt for a period of 40 days. Each salinity level comprised 3 replications each of 5 fish. The fish were reared in glass aquaria filled with 2 liters of the tested media. The rearing tanks were placed in a closet at a constant temperature of 28 °C. Fish were fed to satiation once a day with the live adult brine shrimp (*Artemia salina*). The leftover food and the excrement were removed daily. Water in the rearing tanks was replaced 25 % daily. Water qualities were measured every 5 days in terms of total ammonia, nitrite, hardness, pH and temperature. The final weights were measured at the end of the experiment. The growth rates were calculated in terms of the specific growth rate ($\ln Wt - \ln Wo \times 100/t$: %/day) where Wo is the initial weight, Wt is the final weight and t is the period of culture. The relationship between growth rate and salinity is to be determined.

Data of growth rates were subjected to one-way analysis of variance (ANOVA) and Duncan's new Multiple Range Test (MRT) procedures with SPSS version 10 software. A significance of 5 % was used.

Results

The viable fertilized eggs of climbing perch hatched within 24 h. In the first trial (0 - 22.5 ppt) hatchings were found in salinities up to 7.5 ppt. The estimated mortality of 50 % was obtained at 5.1 ppt. In the second trial (0 - 5.0 ppt) eggs found to hatch in all tested salinities. Hatching rates of 77 - 92 % and 57 % were observed at 0 - 4.5 ppt and 5.0 ppt (**Table 1**), respectively.

When larvae were exposed to salinities of 0 - 22.5 ppt (an interval of 2.5 ppt) for 24 h all fish died at 15.0 ppt. The estimated mortalities of 1 % and 50 % were obtained at 8.8 ppt and 12.2 ppt (**Table 2**), respectively.

When fry fish were exposed to salinities of 0 - 27 ppt (an interval of 3 ppt) for 24 h all fish died at 21.0 ppt. The estimated mortalities of 1 % and 50 % were obtained at 12.5 ppt and 17.7 ppt (**Table 2**), respectively.

When fry were reared in salinities of 0 - 12 ppt (an interval of 2 ppt), fish showed better growth in mild saline water than in freshwater. The growth rates at 4, 6 and 8 ppt were not different ($p > 0.05$) but significantly higher than those at 0, 2, 10 and 12 ppt ($p < 0.05$) (**Table 3**). The relationship between growth rates and salinity levels is drawn as the cubic polynomial function with a correlation coefficient (R^2) of 0.9816 ($p < 0.05$); $y = -0.0093\ x^3 + 0.0993\ x^2 - 0.0449\ x + 2.9691$, when x and y represent salinity levels (ppt) and relative weight gains (%/fish/day) (**Figure 1**), respectively. The relation equation was used to determine the optimal salinity (at which the maximal growth rate was obtained), threshold salinity (at which the growth rate was equivalent to the growth rate found in freshwater) and upper limited salinity (at which fish stop growing and started to die off). The calculation using the relation equation obtained optimal, threshold and upper limited salinities of 6.9, 10.2 and 12.4 ppt, respectively. Water qualities in the rearing tanks were apparently similar among the tested salinities accepted for the hardness that increased corresponding to the increasing salinity resulted from the $CaCO_3$ containing in the seawater (**Table 4**).

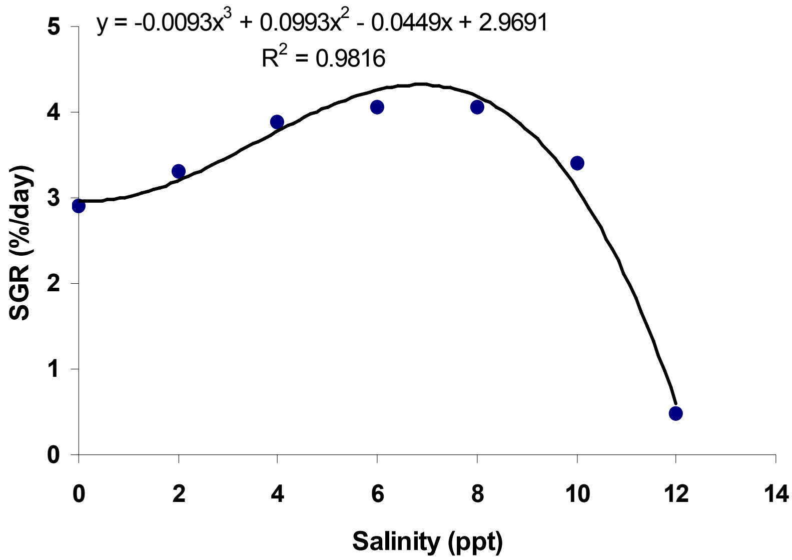

Figure 1 Specific growth rate (SGR) of climbing perch reared for 40 days in different salinities, the correlation drawn as the third polynomial regression.

Table 1 Hatching rates of climbing perch eggs fertilized and incubated in different salinities.

Salinity (ppt)	Number of Eggs	Hatching Rate (%)
0	209 ± 44	78 ± 10
0.5	198 ± 63	86 ± 7
1.0	164 ± 59	92 ± 3
1.5	203 ± 51	88 ± 3
2.0	197 ± 89	84 ± 12
2.5	205 ± 60	87 ± 10
3.0	167 ± 57	90 ± 8
3.5	201 ± 41	84 ± 8
4.0	212 ± 55	77 ± 11
4.5	203 ± 53	82 ± 6
5.0	172 ± 82	57 ± 18

Table 2 Lethal salinities of fertilized eggs and fry of climbing perch within 24 h.

Organism	LC1(24 h) (ppt)	LC50(24h) (ppt)
Fertilized eggs	Not determined	5.1
3-day old larvae	8.8	12.2
Fry (0.51 ± 0.07 g)	12.5	17.7

Table 3 Growth of climbing perch reared in different salinities for 40 days.

Salinity (ppt)	Initial Weight (g)	Final Weight (g)	SGR (%/day)	Survival (%)
0	1.54 ± 0.26	4.97 ± 0.80	2.91 ± 0.41 [a]	100
2	1.54 ± 0.26	5.83 ± 1.14	3.30 ± 0.47 [ab]	100
4	1.54 ± 0.26	7.33 ± 0.70	3.89 ± 0.24 [bc]	100
6	1.54 ± 0.26	7.81 ± 0.60	4.05 ± 0.19 [c]	100
8	1.54 ± 0.26	7.81 ± 0.69	4.06 ± 0.22 [bc]	100
10	1.54 ± 0.26	6.01 ± 0.60	3.39 ± 0.25 [ab]	100
12	1.54 ± 0.26	1.88 ± 0.29	0.48 ± 0.40 [d]	87

Table 4 Water qualities in fry rearing tanks (28.3 ± 0.5 °C).

Salinity (ppt)	Total Ammonia (mg/L NH_3-N)	Nitrite (mg/L NO_2-N)	Hardness (mg/L $CaCO_3$)	pH
0	0.22 ± 0.03	< 0.02	45 ± 13	7.34 ± 0.17
2	0.26 ± 0.06	< 0.02	49 ± 07	7.56 ± 0.13
4	0.21 ± 0.02	< 0.02	55 ± 12	7.39 ± 0.21
6	0.27 ± 0.09	< 0.02	67 ± 06	7.35 ± 0.12
8	0.31 ± 0.03	< 0.02	79 ± 11	7.44 ± 0.16
10	0.26 ± 0.11	< 0.02	91 ± 13	7.47 ± 0.10
12	0.21 ± 0.04	< 0.02	101 ± 09	7.38 ± 0.09

Discussion and conclusions

Results show that percentages of egg hatched following fertilization in the saline media varied among degrees of salt concentration. It was found that eggs successfully hatched in salinities up to 4.5 ppt. In higher salinities hatching dropped and met the lethal median concentration at 5.1 ppt. No hatch was observed in salinities beyond 7.5 ppt. This suggests that a salinity of 4.5 ppt is a threshold concentration for hatching viability of climbing perch eggs. The unsuccessful hatching of freshwater eggs in saline water may result from various factors such as a reduction in activity and viability of sperm [14] due to the inhibition of sperm-activating substances [15]. Salt contained in media may also cause chemical changes in the eggs that triggers releasing of a developmental block or generates irreversible changes that prevent fertilization [16].

Fry of climbing perch were found to tolerate higher salinity than larvae and eggs. The LC50s within 24 h were obtained at 17.7, 12.2 and 5.1 ppt, respectively. Nielsen & Hillman [17] also stated that many fish species might be able to survive at elevated salt levels during the adult stages while juveniles, larvae and eggs were generally more susceptible.

Growth of fry fish reared in different salinities was shown to immediately increase with the increasing salinity. This suggests that growth is enhanced by salt in the rearing media. A number of studies also demonstrate a better growth of freshwater fish in mild saline water such as *Dicentrarchus labrax* [18], *Micropogonias furnieri* [19], *Bidyanus bidyanus* [20,3] and *Cyprinus carpio* [6]. The enhancement of salinity on growth results from a reduction in the osmoregulation metabolic rate and a re-organisation of metabolism which allow protein sparing in favor of a shift towards preferential utilization of carbohydrates and lipids [21]. In freshwater condition fishes expend a certain amount of energy to compensate the salt lost through passive diffusion, depending on the degrees of difference of osmolarity between the culture media and their body circulating fluid. Providing small amounts of salt in the culture media reduces energy expenditure and consequently promotes growth [21]. In this study the increase in weight gains of fry climbing perch continues elevating up to a maximal point at a salinity of 6.9 ppt and starts falling down to a salinity of 10.2 ppt at which growth rate is equivalent to the growth rate in freshwater. This point determines the threshold salinity or the point of no return. Beyond this point, increasing salinity results in adverse effects on the physiological mechanisms of fish. The chronic effects were observed in terms of the reduction in growth and skin infection due to immune deficiency [22]. When salinity continues increasing, fish stop growing and eventually die when salinity reaches the upper limit of 12.1 ppt. Ye *et al* [23] have stated that the reduction in growth may be explained partly by the decreased food intake of the stressed fish affected by salinity. De Boeck *et al* [24] have also stated that fish are likely to ingest less food in unsuitable salinities.

The growth characteristics of climbing perch fry in this study shows the point at which energy utilized for salt uptake attains a minimal level at a salinity of 6.8 ppt. The optimal salinity for the fry is comparable to those of some freshwater fish species such as 7.0 ppt in *Cyprinus carpio* [5] and in *Morone saxatilis* [25], 6.0 ppt in *Ctenopharyngodon idella* [4] and 6.2 to 6.8 ppt in *Maccullochella peelii peelii* depending on water temperature [12]. Febry & Lutz [26] and Potts [27] have stated that energy expenditure for water-salt balance mechanisms in fish is lowest in the iso-osmotic condition, which should result in optimal growth performance. However, studies of Kumaragura and Kamalam [28] in *Cyprinus carpio*, Morgan and Iwama [29] in *Oncorhynchus mykis* and *O. tshawytscha*, and Lambert *et al* [30] in *Gadus morhua* did not conform to this assumption. They have stated that the optimal salinities for growth of freshwater fish appear to vary according to individual species, life stage and seasonal depended cues. Chotipuntu [12] reported that optimal salinity for growth of *Maccullochella peelii peelii* improved when fish were acclimatized to low salinity. However Guo *et al* [31] found that the pre-acclimatizing of juvenile *Bidyanus bidyanus* did not improve the survival rates in higher salinity. Chervinski [32] has stated that there are two types of freshwater fish the so-called primary and secondary freshwater fish. The primary freshwater fish which migrate wholly in freshwater such as Claridae and Cyprinidae are not able to tolerate salinities higher than 9.8 ppt. The secondary freshwater fish which experience a high salinity as a part of their life history such as Cichlidae, Cyprinodontidae and Poecilidae are able to survive higher salinities through gradual adaptation due to a more developed osmoregulatory capability.

This study demonstrates a promising aquaculture potential of climbing perch in brackish water. It suggests growing this fish species in salinity of 6 - 7 ppt to obtain the best growth. Brackish water ponds of salinities up to proximately 11 ppt are utilizable for climbing perch culture. Brackish water ponds of salinities levels up to proximately 4 ppt are practical for spawning and nursing of climbing perch. Culture of freshwater fish in brackish water has advantages not only in reduction of energy expenditure and enhancing nutritional utilization efficiency of fish. Salt also effectively controls outbreak of the freshwater ectoparasites in the culture ponds.

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