# Impact of Salinity on the Incubation Rate and the Performance of Square Head Climbing Perch During the Nursery Phase (Anabas testudineus Bloch, 1792)

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## **Summary**

Square head climbing perch (Anabas testudineus Bloch, 1792) is a high quality fish without small bones. It grows in a wide range of temperatures (10-42 °C), pH (3.5-9.5) and salinity (up to 16%). As regards climate change, this species may become important for aquaculture in the Tam Giang-Cau Hai lagoon near Hue citv. To optimise the quality of fingerlings, we analyzed the effect of salinity levels on hatching and nursing in three subsequent experiments. Fertilization. hatching, survival and growth rates for 30 days were determined at salinity levels 0%, 5%, 7%, 13% and 15%. Water temperature and pH varied between 22-29.5 °C and 7.3-7.8, respectively. Between 0-5%, fertilization ratio was 77% to 83% and highest at 5%, but this dropped to 0% when salinity increased; hatching ratio was larger than 90%, but decreased to 0% at 13-15%. Between 0-11%, hatching time of fertilized eggs was not affected by salinity levels. The ratio of deformation gradually increased above 5%. After hatching at either 0 or 5%, survival ratios for square head climbing perch were above 13% up to 5%, but dropped to 0% at 9%. The growth after 30 days of nursing was higher at 5 and 7% compared with that of 0 and 3%.

## Résumé

Impact de la salinité sur l'éclosion et sur les performances de survie pendant l'élevage de la perche rampante à tête carré (*Anabas testudineus* Bloch, 1792)

La perche rampante à tête carré (Anabas testudineus Bloch, 1792) est un poisson de haute qualité sans petites arêtes. Cette perche évolue dans un vaste éventail de températures (10-42 °C), pH (3,5-9,5) et de salinité (au-delà de 16‰). Au regard des futurs changements climatiques, cette espèce pourrait s'avérer importante pour le secteur aquacole dans la lagune de Tam Giang-Cau Hai près de Hue city au Vietnam. Afin d'optimiser la qualité des alevins, nous avons analysé l'effet du degré de salinité de l'eau sur l'incubation et l'élevage des alevins en trois expérimentations successives. La fécondation, l'éclosion, la survie et la croissance pendant les 30 premiers jours ont été suivis. Les taux de salinité étudiés étaient de 0‰, 5‰, 7‰, 13‰ et 15‰. La température et le pH de l'eau variaient respectivement entre 22-29,5 °C et 7,3-7,8. Entre 0 à 5‰, la fécondation variait entre 77% à 83% mais était la plus élevée à 5‰. Entre 0 à 5‰, l'éclosion dépassait 90%. Avec l'augmentation du taux de salinité, la fécondation et l'éclosion ont été réduites à 0% pour des taux en sel entre 13 et 15‰. Entre 0 et 11‰, la durée d'éclosion des œufs fécondés n'était pas affectée. Néanmoins, le pourcentage de déformation auamentait progressivement pour des taux de salinités supérieures à 5‰. Après éclosion à des taux de salinité de 0 ou 5‰, le pourcentage de survie des perches était de près de 13%. Ce pourcentage était nul lorsque le taux de salinité était de 9‰. La croissance pendant les 30 premiers jours d'élevage était meilleure à un taux de salinité de 5 et 7‰ comparativement au taux de salinité de de 0 et 3‰.

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#### Introduction

Located in Thua Thien Hue province, Tam Giang Cau Hai is the biggest lagoon in Vietnam with an area of 22,000ha. The lagoon has a high value in terms of biodiversity and ecosystem services, and supports the livelihoods of thousands of households in the coastal areas. In recent years, global warming has strongly impacted on aquatic resources as well as aquaculture in this region. High temperature, saline intrusion and mud accretion are factors that impact the local aquaculture and the life of poor farmers (2, 6, 12, 13). In this context, the identification of fish species that deals with the influences of climate change is desirable (6).

Climbing perch is a popular fish and already farmed in aquaculture systems. Recent studies have discovered a climbing perch with slightly different morphology (14). This native species is identified with the local name square head climbing perch. This fish has high flesh quality and has no small bones (10,15,16). It grows fast, it is easy to culture and is well adapted to low oxygen content because of its auxiliary respiration organ. Previous research has determined their environmental comfort zone which ranges from 10-42oC and a pH of 3.5-9.5 (12, 13). These wide temperature and pH ranges allow the square head climbing perch to adapt well in brackish and marine water up to salinity levels of 16% (16). From the perspective of global warming and saline intrusion, the square head climbing perch is expected to become an effective and valuable species for both freshwater and brackishwater aquaculture in Thua Thien Hue province. Currently, there is no published research on the impact of salinity levels on the incubation and nursery phases of square head climbing perch in Vietnam. The identification of appropriate salinity and temperature for seed production may improve the quality of fingerlings, and therefore, enhance the local aquaculture and improve the farmers' livelihoods.

This study identified the optimal salinity level for square head climbing perch at incubation and nursery phases in order to optimise the quality of fingerlings for different environmental conditions. Thereto a three-staged experiment was carried out.

#### Materials and methods

#### **Materials**

The experiments were conducted at the Fish Breeding and Nursing Center, Thua Thien Hue province, Vietnam. After breeding, incubated eggs were taken and released into 6 L tanks with eight (8) different salinity levels: 0%, 3%, 5%, 7%, 9%, 11%, 13% and 15% designed by using the Pearson Square method. The different salinity levels were composed by mixing freshwater taken from a fish pond at the center with saltwater transferred from Thuan An beach near Hue city. During the experiments, all other factors were maintained at a similar level. The tanks were syphoned and water was changed daily.

Feed was added to the tanks four times per day. In both the hatchery and nursery phases, the feeds were: water-dissolved cooked chicken egg yolk, and industrial feed (protein>42%, lipid:6-8%, cellulose:<3%, maximum moister:11%).

Length was measured by using a panme ruler with a precision of 0.01 mm. For all three experiments, the water environmental parameters, such as temperature, DO and pH were checked daily at 8am and 2pm, and maintained within the optimal range for square head climbing perch. Temperature was measured by thermometer, while DO and pH were measured with a Serra test kit. Salinity was checked in a water sample taken from each tank with a refractometer.

### **Methods**

Three experiments were carried out: Impacts of salinity levels on three phases; namely: incubation, nursery and after hatching at 0% of square head climbing perch. The nursery was monitored for a period of 30 days.

# Impact of salinity on the incubation

The experiment involved 8 treatments with salinity levels of 0%, 3%, 5%, 7%, 9%, 11%, 13% and 15% in three replications withour air-blower. The treatments were distributed in the tanks (Table 1.1).

Impact of salinity on nursery after hatching at 0% The experiment involved 5 treatments with salinity levels of 0%, 3%, 5%, 7% and 9% in three replications and withour air-blower. The treatments were distributed in the tanks (Table 1.2).

**Table 1.1** Design of experimental treatments.

T1	Т3	T2	T5	T7	T4
T8	Т6	Т3	T1	T5	Т6
T2	T4	T7	T8	T1	Т3
T7	Т6	T5	T2	T4	Т8

Remark: T: treatment; T1: 0%; T2: 3%; T3: 5%; T4:

7%; T5: 9%; T6: 11%; T7: 13%; T8: 15%

**Table 1.2**Design of experimental treatments.

T1	Т3	T4	T5	T2
T4	T5	T2	T1	Т3
T2	T4	Т3	T5	T1

Remark: T: treatment; T1: 0%; T2: 3%; T3: 5%; T4: 7%;

T5: 9%

# Impact of salinity on nursery, after hatching at optimal salinity level

The experiment involved 5 treatments with salinity levels at 0%, 3%, 5%, 7% and 9% at three replications and withour air-blower. The optimal salinity level was determined from the experiment in nursery phase after fry was hatched at 0%. The treatments were distributed in the tanks (Table 1.3).

# **Data analysis**

Fertilization, hatching and heteromorphic rates are calculated by using the following formulas: Daily growth in length (DGL) was calculated by dividing the increase in length during a period of 15 days by this number of days.

Means of treatments for the three experiments were compared by using an ANOVA followed by a Tukey test with Minitab 16.0 software. Treatments were declared significant for the level p < 0.05.

**Table 1.3**Design of experimental treatments

De	sign of exp	perimental	treatmer	ıts.
T2	T1	Т3	T5	T4
Т3	T4	T2	T1	T5
T5	Т3	T1	T4	T2

Remark: T: treatment; T1: 0%; T2: 3%; T3: 5%; T4: 7%; T5:

9%

### **Results and discussion**

During the experiments, temperature, pH and DO remained in the optimal ranges for the growth of square head climbing perch (Table 2). Temperatures varied from 22-29 °C with means ranging between 26.4 and 26.6°C; all experiments lasted one month and therefore the temperatures varied within a wide range. DO varied between 4 and 5 mg l<sup>-1</sup>. The pH varied among the treatments (7.4 in 0% and 7.7 in 15%) due to mixing freshwater and brackishwater, but the pH of the tanks was adjusted to remain within a range smaller than 0.5. In the experiment conditions, this level of variation in these parameters was deemed appropriate for the incubation and hatching phases of square head climbing perch.

# Impact of salinity on the incubation

#### Fertilization ratio

The fertilization ratio was different (p<0.05) among the 5%, 7%, 9%, 11%, 13% and 15% treatments (Table 3). However, between 0% and 3%, these ratios were not significantly different. Fertilization and hatching ratios, 83% and 96%; respectively, were highest at 5%. Although the incubation of square head climbing perch eggs can be conducted up to a salinity of 11%, the resulting fertilization and hatching ratios were low: 8.4% and 5.8%, respectively. At these salinity levels, the embryo was damaged due to osmotic pressure.

### Hatching time and heteromorphic ratio

Salinity levels did not affect hatching time (Table 4). After 15.4 to 15.6 hours, all fertilized eggs were hatched. However, at 13% and 15%, the eggs were not fertilized, and therefore, hatching time and heteromorphic ratios were unavailable.

In contrast to hatching time, the salinity levels considerably affected the heteromorphic ratio (Table 4). The number of deformed larvae tended to increase at higher salinity; the ratio was 2.4 at 0% and to 4.8 at 11%. There were no statistical differences among the treatments of 0%, 3%, 5% and 7%, as well as among 5%, 7%, 9% and 11%. At 0% and 3%, the heteromorphic ratio was significantly lower than that at 9% and 11% (p<0.05).

**Table 2**Variation of Environmental parameters during the experiments.

Salinity	Tei	mpera	ture (0C)			рН		DO (	(mg/l)
•			Mean ± SE			Mean ± SE	Min	Max	Mean ± SE
0	22	29.5	$26.5 \pm 0.7$	7.3	7.5	$7.4 \pm 0.02$	4	5	$4.7 \pm 0.1$
3	22.5	29.5	$26.5 \pm 0.6$	7.4	7.6	$7.5 \pm 0.02$	4.5	5.5	$4.7 \pm 0.1$
5	22	29.5	$26.5 \pm 0.7$	7.4	7.7	$7.5 \pm 0.02$	4.5	5.5	$4.9 \pm 0.1$
7	22	29.5	$26.5 \pm 0.7$	7.5	7.7	$7.6 \pm 0.02$	4	5	$4.7 \pm 0.1$
9	22	29	$26.5 \pm 0.6$	7.5	7.8	$7.6 \pm 0.02$	4	5.5	$4.8 \pm 0.1$
11	22	29.5	$26.6 \pm 0.7$	7.6	7.8	$7.7 \pm 0.02$	4.5	5	$4.7 \pm 0.1$
13	22.5	29	$26.5 \pm 0.6$	7.6	7.8	$7.7 \pm 0.02$	4	5	$4.8 \pm 0.1$
15	22	29	$26.4 \pm 0.7$	7.6	7.8	$7.7 \pm 0.03$	4.5	5.5	$4.9 \pm 0.1$

#### Table 3

The mean and standard errors of fertilization and hatching ratios (%) of square head climbing perch at different salinity levels.

Salinity (‰)	Fertilization ratio	Hatching ratio
0	$77.4 \pm 0.9^{a}$	$90.8 \pm 1.2^{a}$
3	$78.2 \pm 1.14^{a}$	$91.3 \pm 1.1^{a}$
5	$83.11 \pm 0.6^{b}$	$96.0 \pm 0.5^{b}$
7	$60.6 \pm 1.3^{c}$	$72.1 \pm 1.3^{c}$
9	$22.9 \pm 1.3^{d}$	$10.1 \pm 0.8^{d}$
11	$8.4 \pm 0.7^{e}$	$5.8 \pm 0.5^{e}$
13	0	0
15	0	0

Remark: Different superscripts in the same column indicate that the values are different (p< 0.05).

#### Table 5

The mean and standard errors of the length of square head climbing perch at different salinity levels and time after 15 and 30 days of nursery, after hatching in 0%.

Salinity	L	ength (mm/indiv	/idual)
(‰)	Initially	15 days	30 days
0	3.5	$10.39 \pm 0.08^{\circ}$	$25.44 \pm 0.32^{\circ}$
3	3.5	$10.36 \pm 0.07^{a}$	$26.06 \pm 0.29^{a}$
5	3.5	$10.38 \pm 0.07^{a}$	$27.53 \pm 0.3^{b}$
7	3.5	$10.43 \pm 0.07^{a}$	27.17 ± 0.23 <sup>b</sup>
9	3.5	$10.26 \pm 0.09^{\circ}$	=
11	3.5		

Remark: Different superscripts in the same column indicate that the values are different (p< 0.05).

#### Table 4

The mean and standard errors of hatching time and heteromorphic ratio of square head climbing perch at different salinity levels.

Salinity (‰)	Hatching time	Heteromorphic ratio
0	$15.4 \pm 0.2^{a}$	$2.4 \pm 0.3^{a}$
3	$15.5 \pm 0.2^{a}$	$2.8 \pm 0.4^{a}$
5	$15.5 \pm 0.2^{a}$	$3.3 \pm 0.4^{ab}$
7	$15.6 \pm 0.2^{a}$	$3.9 \pm 0.4^{ab}$
9	$15.4 \pm 0.2^{a}$	$4.4 \pm 0.3^{b}$
11	$15.4 \pm 0.2^{a}$	$4.8 \pm 0.3^{b}$
13	=	=
15	=	=

Remark: Different superscripts in the same column indicate that the values are different (p < 0.05).

#### Table 6

The mean and standard errors of the daily growth (DGL) of square head climbing perch at different salinity levels and time after 15 and 30 days of nursery, after hatching in 0%.

Salinity	DGRI	_ (mm/individual	/day)
(‰)	15 first days	15 last days	Average
0	$0.46 \pm 0.01^{a}$	$1.00 \pm 0.02^{a}$	$0.73 \pm 0.01^{a}$
3	$0.46 \pm 0.01^{a}$	$1.05 \pm 0.022^{ab}$	$0.75 \pm 0.01^{a}$
5	$0.46 \pm 0.01^{a}$	$1.14 \pm 0.02^{c}$	$0.80 \pm 0.01^{b}$
7	$0.46 \pm 0.01^{a}$	$1.12 \pm 0.02^{bc}$	$0.79 \pm 0.01^{b}$
9	$0.45 \pm 0.01^{a}$	-	-

Remark: Different superscripts in the same column indicate that the values are different (p< 0.05).

#### Table 7

The mean and standard errors of the length of square head climbing perch at different salinity levels and time after 15 and 30 days of nursery, after hatching in 5%.

Salinity	Length (mm/con)				
(‰)	Initially	15 days	30 days		
0	3.5	$10.21 \pm 0.12^{a}$	25.05 ± 0.29 <sup>a</sup>		
3	3.5	$10.32 \pm 0.10^{a}$	$26.43 \pm 0.27^{\circ}$		
5	3.5	$10.48 \pm 0.09^{a}$	$28.06 \pm 0.24^{\circ}$		
7	3.5	$10.46 \pm 0.08^{a}$	$27.67 \pm 0.25^{\circ}$		
9	3.5	$10.37 \pm 0.09^{a}$	-		

Remark: Different superscripts in the same column indicate that the values are different (p< 0.05).

# Impact of salinity during nursery after hatching at 0% and 5%

The results during hatching for square head climbing perch were best at 5%. Therefore the performance of the fish hatched in this salinity level was comparable with that hatched at 0%. Impact of salinity on length and growth after hatching at 0%

At 15 days after nursery, the fish of the treatments was the same (Table 5). The average maximum length reached 27.5 mm per individual after 30 days of nursery at 5%; the value was statistically the same as that at 7%, but higher than that at 0% and 3%. However, the p value indicated a statistical difference between 0% and 3%, and on the other hand, between 5% and 7% (p<0.05). At higher salinity levels, the survival was close to zero and the length could not be measured.

In the first 15 days the DGLs among the treatments were equal (Table 6). During the last 15 days, the DGL was different between the salinity levels and was highest at 5%: 1.1 mm/individual/day. The DGL at 5% was significantly different from that of the 0% and 3% treatments, but not significantly different from that of the 7% treatment Impact of salinity on length and growth after hatching at 5%.

After 15 days of nursery, the length was the same for all treatments (Table 7). After 30 days, the measured lengths of fish raised at 5% and 7% were longer (28 mm/individual) than those raised at 0% and 3 %.

#### Table 8

The mean and standard errors of the daily growth rate (DGL) of square head climbing perch at different salinity and time after 15 and 30 days of nursery, after hatching in 5%.

Salinity (‰)	DGRL	DGRL (mm/individual/day)				
(900)	Day: 1-15	Day: 16-30	Average			
0	$0.45 \pm 0.01^{\circ}$	0.99 ± 0.02°	$0.72 \pm 0.01^{a}$			
3	$0.46 \pm 0.01^{a}$	$1.07 \pm 0.02^{b}$	$0.76 \pm 0.01^{b}$			
5	$0.47 \pm 0.01^{a}$	$1.17 \pm 0.02^{\circ}$	$0.82 \pm 0.01^{\circ}$			
7	$0.46 \pm 0.01^{a}$	$1.15 \pm 0.02^{\circ}$	$0.81 \pm 0.01^{c}$			
9	$0.46 \pm 0.01^{a}$	=	=			

Remark: Different superscripts in the same column indicate that the values are different (p< 0.05).

During the last 15 days, the DGL at 5% and 7% salinity was higher than that at 0% and 3 %; about 0.8 and 0.7 mm/individual/day, respectively (Table 8).

# Impact of salinity on survival and growth

After hatching at 5%, the square head climbing perch reached a maximum length and DGL of 28 mm/individual and 1.2 mm/individual per day, respectively; while after hatching at 0%, these values were about 27 mm/individual and 1.1 mm/individual per day, respectively.

For the two levels of salinity (0% and 5%) at hatchery, the survival rates of square head climbing perch nursed at four different salinity levels were not different (Table 9). At 9%, no fish survived, and at 7%, the survival rate was only half (<7%) of those which reached at 0%, 3% and 5%. In the range of 0% - 5%, survival rate of the fish (>13%) was not significantly affected by salinity level at p>0.05.

## Table 9

The mean and standard errors of survival rate (%) of square head climbing perch in the nursery phase at different salinity levels, after hatching at 0% and 5%.

Salinity (‰)	(0‰)	(5‰)	
	Survival rate	Survival rate	
0	$13.7 \pm 0.4^{a}$	$12.9 \pm 0.4^{a}$	
3	$13.2 \pm 0.4^{a}$	$13.3 \pm 0.6^{a}$	
5	$13.4 \pm 0.5^{a}$	$14.0 \pm 0.7^{a}$	
7	$6.1 \pm 0.4^{b}$	$7.0 \pm 0.5^{b}$	
9	0	0	

Remark: Different superscripts in the same column indicate that the values are different (p<0.05).

## **Discussion**

The fertilization ratio of square head climbing perch was significantly higher in brackish water with 5% salinity than that in freshwater with other salinity levels. The fertilization ratios at 5% were slightly higher (83%) than those found by Dan (2) when testing the effects of LH-RHa on Anabas testudineus in freshwater (70 - 81%). In another study, Oanh (8) tested the effect of salinity levels at 0%, 3%, 5%, 7%, 9% and 11% for snakeskin gouramy (Trichogaster pectoralis Regan) with the highest fertilization rate at 5% (68.3%) and lowest at 11% (34.2%). The data show a better adaptation of square head climbing perch to salinity in the early stage at low salinity (from 0% to below 7%). At higher or lower salinity, fish loses energy to adjust to the osmotic pressure (9,18); fish also consumes more oxygen (17), which impacts on both hatching and growth of the fry. At 5%, osmotic pressure of the fry is equal to the pressure of external environment, whilst it is higher at 0% and lower at 7% or 9%. When salinity was higher than 9%, hatching rate dropped to 5.78% due to low function of osmoregulation (4). Energy was consumed considerably in this case; therefore, fish has no ability to grow (5).

Survival rate, as a result, is considerably lower at the higher salinity levels. Consequently, 5% is advised as an optimal salinity to retrieve a higher ratio of fertilization and hatching. In spite of being an euryhaline species, square head climbing perch is sensitive to salinity in the early stage in comparison with other fish. Sarma (15) studied the effect of salinity on the fingerling stage of *Clarias batrachus*.

In the first 30 days, there was no difference in survival rate among the treatments of 0%, 4% and 8%. Enayati (3) indicated 8% as the point of death for *Ctenopharyngodon idella* (fish was up to 120 g in body weight), while *Channa striata* tolerated salinity up to 10% (7). Similar result was obtained with *Pseudophoxinus stymphalicus* in the ranges of 6-13.5% (1).

Additionally, maintaining salinity levels at 5% and lower reduced the heteromorphic ratio and improved survival ratio of the fry. For best results, fry should be nursed in the brackishwater (3 to 5%).

For salinity intrusion, square head climbing perch is a good choice for aquaculture in the Tam Giang Cau Hai lagoon.

#### **Conclusion**

The optimal salinity level for hatching square head climbing perch is at 5% when fertilisation rate is highest. The fertilization ratios of 78 to 83% in the range of 0% - 5% dropped to 0% when salinity levels increased to 13% - 15%. In the range of 0% - 5%, the hatching ratio was larger than 90%, but decreased to 0% at 13% - 15% salinity. The ratio of deformation increased gradually from 2.4% at 0% to 4.8% at 11% salinity.

Salinity levels affected the growth and survival of square head climbing perch fry between day 15 and 30 of the nursery phase. After hatching at either 0 or 5%, survival ratios for square head climbing perch were above 13% up to 5% salinity, but dropped to 0% at 9% salinity. The growth after 30 days of nursery was higher in salinity levels of 5 and 7%, compared to that at 0 and 3%.

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