Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 6 (7): 834-839, 2011
ISSN 1816-4927 / DOI: 10.3923/jfas.2011.834.839

Breeding Performance and the Effect of Stocking Density on the Growth and Survival of Climbing Perch, *Anabas testudineus*

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ABSTRACT

Anabas testudineus was successfully induced to spawn using LHRHa. Egg production, hatching rate and GSI were 5126 eggs/fish, 62 and 10.41%, respectively. Results showed that the survival and growth of *A. testudineus* during the 30-day nursing period were stocking density dependent. The highest survival rate was recorded in T_1 (35/L; 75%), followed by T_2 (55/L; 53%) and lastly T_3 (75/L; 43%). The weekly growth performance in T_1 (35/L), in terms of total body length and body weight was significantly better than other treatments, especially after 21-day of nursing. Fish larvae were fed with a combination of live and prepared foods. Water quality parameters were stable and not influenced by the stocking densities tested.

Key words: *Anabas testudineus* (Bloch.), stocking densities, larval rearing

INTRODUCTION

Anabas testudineus, commonly known as climbing perch is a popular source of fish protein. It is suitable for cultivation due to its ability to withstand harsh environmental conditions (Amornsakun *et al*., 2005). In order to meet demands, the cultivation of this species must be optimized. However, survival during nursing is low due to many factors, such as their tiny size and cannibalistic behavior (Trieu and Long, 2001). Recent studies on this species have led to improved production, enabling enough seed to be supplied to fish farmers in South Asia (Dooligindachabaporn, 1994; Trieu and Long, 2001; Tuan *et al*., 2002). In Malaysia, grow out production is still low. Farmers prefer to culture this fish in earthen ponds and hapas. The use of homemade feed has resulted in high FCR and low survival rates (Dooligindachabaporn, 1994).

In larviculture, the stocking density determines the economic effectiveness of production (Turkowski *et al*., 2008). Stocking densities directly affect fish growth rates and survivals (Baras *et al*., 2003; Molnar *et al*., 2004; Kupren *et al*., 2011).

In order to obtain production of high quality fry, effort is needed to develop larval rearing protocols for this species. Considering the promising future prospect of this species, the present study was conducted to establish the optimal fry stocking density of *A. testudineus* nursed in aquaria and fed with natural and artificial feeds.

MATERIALS AND METHODS

Broodfish selection: Research was conducted at the Aquaculture Research Station, Puchong, Universiti Putra Malaysia. A pair of mature and healthy adult male and female *A. testudineus* broodfish was selected from stocks reared in 300 L fiberglass tanks. They were fed twice a day with commercial fish pellet containing 40% protein at 2% body weight. Ovarian maturity in the female was checked by inserting a cannula tube into her genital pore. Oocyte samples were taken (n = 10 oocytes) to observe the Germinal Vesicle (GV) stage. For the male fish, a gentle massage was applied to its abdomen. Free-flowing sperm indicated that the fish was ready to spawn.

Induced spawning: Luteinizing Hormone Releasing Hormone analogues (LHRHa), purchased from Syndel Laboratories Ltd. Canada was used to artificially induce the broodfish for breeding. Both male and female were injected intramuscularly with LHRHa using a dose of 2 μg kg^{-1} b. wt. The male weighed 30 g while the female weighed 41 g. After approximately 11 h, the broodfish were stripped for eggs and sperm which were mixed well and fertilized in-vitro. Fertilized eggs, which were pelagic and non-adhesive were incubated in a 10 L aquarium.

Egg production was determined by counting the number of eggs in a representative 1 g sample. The total number of eggs in the sample were then counted and multiplied with the total weight (g) of eggs released.

Samples of fertilized eggs were taken to determine the fertilization and hatching rates. The fertilization rate was observed and recorded after 1 h and hatching rate was monitored after 20 h. Fertilization rate was calculated by measuring the number of fertilised eggs divided by the total number of eggs released. The hatching rate was calculated from the number of fertilized eggs that actually hatched.

The Gonadosomatic Index (GSI) was calculated as the percentage of gonad weight to body weight. Unfertilized eggs which float on the water surface were removed by siphoning. Hatching was completed after 20 to 24 h of incubation. Then, the larvae were collected and transferred to 5-liter aquaria for rearing.

Larval rearing: The experiments were run in aerated 5 L rectangular-shaped aquaria filled with 2 liters of water. Larvae were divided into three stocking density treatments (35, 55 and 75 L^{-1}), consisting of three replicates per treatment. Throughout the nursing period, larvae were fed progressively with freshwater rotifer (*Branchionus* sp.), followed by a mixture of finely strained boiled egg yolk, fish meal and *Artemia* nauplii. Leftover feed and waste were removed twice a day, once in the morning and once in the evening. Dead fish in each aquarium were recorded and removed. Water temperature, dissolved oxygen, ammonia, nitrate and pH were measured every three days. At weekly intervals, for each aquarium, 10 larvae were randomly sampled, weighed and the total length measured. After 30 days of rearing, all surviving larvae from each aquarium were collected, counted, weighed and their total body lengths measured.

Statistical analysis: Data collected in this study were analysed using one way analysis of variance (ANOVA), followed by Duncan's multiple comparison test.

RESULTS

Breeding performance: The diameter of fertilized *A. testudineus* eggs ranged from 830 to 850 μm. The eggs were transparent and pelagic. High rate of final oocyte maturation (100%)

Table 1: Breeding performance of *A. testudineus* female induced with 2 μg kg^{-1} LHRHa

Fish	Fertilization rate (%)	Egg production (no of eggs/fish)	Hatching rate (%)	Gonadosomatic Index (%)
Female	91	5126	62	10.41

Table 2: Survival rates, mean total length and mean body weight of *A. testudineus* larvae nursed in aquaria for 30 days at three stocking densities

Tank Density	Survival rate (%)	Mean total body length (mm)	Mean body weight (mg)
T_1 (35 L^{-1})	75.7±5.2^a	8.4±2.0^a	41.0±0.1^a
T_2 (55 L^{-1})	53.5±3.2^b	8.2±2.0^a	39.0±0.1^a
T_3 (75 L^{-1})	43.3±2.1^c	8.0±2.0^a	39.0±0.1^a

Different superscript in the same column indicate significant difference amongst treatments ($p<0.05$). Values are expressed as mean±SE

Table 3: Weekly mean length (mm) of *A. testudineus* nursed in aquaria for 30 days at three stocking densities

Treatment	At stocking	Day 7	Day 14	Day 21	Day 28	Day 30
T_1 (35 L^{-1})	1.2±0.0^a	4.7±0.3^a	6. 7±0.6^a	11.8±1.2^a	13.5±2.1^a	14.7±3.3^a
T_2 (55 L^{-1})	1.2±0.0^a	4.7±0.2^a	6.6±0.3^a	10.8±1.1^b	12.1±1.8^b	13.3±2.9^b
T_3 (75 L^{-1})	1.2±0.0^a	4.6±0.1^a	6.6±0.3^a	10.7±1.1^c	11.9±1.8^c	13.0±2.8^c

Different superscript in the same column indicate significant difference amongst treatments ($p<0.05$). Values are expressed as Mean±SE

Table 4: Weekly mean body weight (mg) of *A. testudineus* nursed in aquaria for 30 days at three stocking densities

Treatment	At stocking	Day 7	Day 14	Day 21	Day 28	Day 30
T_1 (35 L^{-1})	0.10±0.00^a	0.60±0.80^a	35.20±2.00^a	59.70±4.10^a	68.00±4.60^a	78.30±5.70^a
T_2 (55 L^{-1})	0.10±0.00^a	0.58±0.60^b	34.80±1.70^a	56.80±3.70^b	65.30±4.20^b	73.50±5.50^b
T_3 (75 L^{-1})	0.10±0.00^a	0.56±0.60^b	35.10±1.70^a	55.60±3.60^c	61.70±4.10^c	70.00±5.50^b

Different superscript in the same column indicate significant difference amongst treatments ($p<0.05$). Values are expressed as Mean±SE

during ovulation and spawning resulted in high fertilization rate (91%). Egg production, hatching rate and GSI were 5126 eggs/fish, 62 and 10.41% respectively (Table 1).

Survival rate and growth: After 30 days of nursing, the survival rates of *A. testudineus* were significantly different ($p<0.05$) between treatments. The highest survival rate was recorded in T_1 (75.7%), followed by T_2 (53.5%) and lastly T_3 (43.3%) (Table 2). Evidently, rearing *A. testudineus* larvae at lower density resulted in higher survival rate. The mean total length of larvae was significantly longer in T_1 (8.4±2.0 mm) where stocking density was the lowest, compared to the other treatments. Although mean larvae body weight registered the highest for T_1 (41.0±0.0 mg), statistically, there were no difference when compared to T_2 39.0±0.0 and T_3 39.0±0.0 mg.

The weekly mean body length (Table 3) and mean body weight (Table 4) of *A. testudineus* in all treatments increased as the nursing period progressed. The mean length of *A. testudineus* in all treatments were not significantly different ($p>0.05$) up to day 14. Beyond day 14 however, differences in mean lengths were significantly different. Clearly, *A. testudineus* nursed at the lowest stocking density (T_1; 35 L^{-1}) grew the fastest up to day 30, recording 14.7±3.3 mm in mean length, followed by T_2 (55 L^{-1}; 13.3±2.9 mm) and finally T_3 (75 L^{-1}; 13.0±2.8 mm).

Significant differences is mean body weight were clearly evident on day 21 and 28 between all treatments. On day 30, similar to the observation on mean length, T_1 recorded the highest mean

Table 5: Water quality parameters in nursing aquaria throughout the study period

Treatment	Temperature (°C)	pH	Dissolved oxygen (ppm)	Ammonia (ppm)	Nitrite (NO_2) (ppm)
T_1 (35 L^{-1})	28.3±0.3[a]	8.8±0.3[a]	5.8±0.4[a]	0.12±0.02[a]	0.16±0.02[a]
T_2 (55 L^{-1})	28.3±0.4[a]	8.7±0.1[a]	5.8±0.3[a]	0.18±0.01[a]	0.17±0.03[a]
T_3 (75 L^{-1})	28.3±0.1[a]	8.7±0.1[a]	5.7±0.6[a]	0.18±0.03[a]	0.17±0.04[a]

Different superscript in the same column indicate significant difference amongst treatments ($p<0.05$). Values are expressed as Mean±SE

body weight (78.30±5.70 mg). However, the mean body weight for T_2 (73.50±5.50 mg) was not significantly different ($p>0.05$) from T_3 (70.00±5.50 mg).

Water quality in nursing aquaria: Water quality parameters during the nursing period were relatively stable, as indicated in Table 5. There were no significant differences ($p>0.05$) in water quality parameters among all treatments. Water temperature kept around 28.3±0.4°C. pH ranged narrowly between 8.7±0.1 to 8.8±0.3 and DO always registered above 5.7±0.6 ppm. Ammonia concentration in T_1 (0.12±0.22 ppm), T_2 (0.18±0.1 ppm) and T_3 (0.18±0.3 ppm) showed an increasing trend as stocking density increased among treatments but variations among treatments were not significant ($p>0.05$). Nitrite (NO_2^-) concentration showed no significant differences ($p>0.05$) although T_3, with the highest stocking density (75 L^{-1}) also recorded the highest NO_2^- level (0.17±0.4 ppm).

DISCUSSION

In the present study, of *A. testudineus* larvae reared under low density had better growth and survival. Better growth obtained under low density might be associated with the production of stronger fish of the same age (Kentouri *et al.*, 1994; Hernandez-Cruz *et al.*, 1999; Gimenez and Estevez, 2005; Faulk *et al.*, 2007). High larval density lowers growth rates, mainly due to appetite reduction, higher aggressiveness behaviour and increased food competition (Wendelaar-Bonga, 1997).

Survival rates of *A. testudineus* were 75, 53 and 43% in T_1, T_2 and T_3, respectively. This indicates that the survival rate of larvae was affected by stocking density. Mortality of larvae was higher at the high stocking densities. According to Trieu and Long, 2001, the survival rates of *A. testudineus* reared in concrete tanks using artificial food at 500, 1000 and 1500 fry/m^2 stocking densities were 16.5, 14.3 and 4.90%, respectively. Morioka *et al.* (2009) reported that only 16.7% of *A. testudineus* survived from a total of 14,000 larvae nursed for 35 days.

Due to its cannibalistic nature, Devaraj (1975) found that less than 4% of *A. testudineus* survived after 7 month of rearing. Morioka *et al.* (2009) also reported that *A. testudineus* is cannibalistic, where up to 30% fry mortality was observed when nursed under normal day light conditions. Survival rate of Sutchi catfish *Pangasianodon hypophthalmus* and African catfish *Clarias gariepinus* fry was reported low under normal day light that exposed them to predators (Mukai, 2011; Mukai and Lim, 2011). Cannibalism is started by larger individual biting the caudal portion of smaller individuals (tail-first cannibalism) (Morioka *et al.*, 2009). In the present study, most of the dead individuals had ragged tail, indicating that they were bitten.

According to Van and Hoan (2009), the survival rate of *A. testudineus* fry in hapas and given different protein level diets was high, ranging from 89.27 to 91.4%. The results of Long *et al.* (2006) on survival rate of *A. testudineus* nursed in earthen ponds ranged from 3.7 to 15.6%. Apparently, the survival rate of *A. testudineus* nursed in hapas was higher than that in ponds (Tuan *et al.*, 2002).

In the present study, there were differences in growth of *A. testudineus* where larvae in higher density recorded significantly less values in length and weight after the nursing period. This suggested that there is improper acquisition of food by the larvae at higher densities, resulting in poor growth. Differences in the growth rate at various densities can be attributed to stress from interaction between fish, cannibalism as well as from physicochemical changes in the water (King *et al*., 2000; Alvarez-Gonzalez *et al*., 2001; Baras *et al*., 2003; Costas *et al*., 2008).

During the experiment, water quality parameters such as pH, DO, ammonia and nitrite were not influenced by the stocking density. However, much higher stocking density would eventually affect water quality, such as lowered dissolved oxygen. Decreasing dissolved oxygen in aquaria stocked with fish at high density could be attributed to the gradual increase in biomass (Wendelaar-Bonga, 1997). The effect of density is directly linked to the fish size and their ontogeny level, where a significantly negative effect of density seemed to increase along with their increasing size and level of ontogeny (Kupren *et al*., 2011).

CONCLUSION

Anabas testudineus was successfully induced to spawn using LHRHa. Since only a pair of broodfish was used in the present study, further studies need to be done for a more thorough research and analysis on the breeding performance of this species.Based on the results of this study, it can be concluded that stocking density had a significant effect on survival and growth of *A. testudineus*. The optimum stocking density for *A. testudineus* larvae reared in aquaria, according to the present study is 35 L^{-1}. More study on larval rearing of A. *testudineus* is required in order to verify our finding and to improve survival rates during larval stages.

ACKNOWLEDGMENT

We express our sincere gratitude to Head of the Department of Aquaculture, staff of Research Aquaculture centre, Puchong and staff of Aquaculture laboratory, Universiti Putra Malaysia for providing infrastructural facilities. The valuable assistance of everyone who contributed to the research is greatly appreciated.

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