Effect of stocking density and salinity on the growth and survival of golden Anabas fry

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Received: January 2017 Accepted: March 2017

Abstract

Stocking density and salinity influence on the growth and survival of golden *Anabas* fry were studied. Experiments were carried out at Hatchery Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang Selangor, Malaysia. Growth parameters were measured once a month. While survival was monitored daily throughout the experimental period. Water quality such as temperature, dissolved oxygen (DO), pH, ammonia and nitrite were measured once a week prior to water change. During the study period, fry were fed to apparent satiation with commercial crumble diet (Cargill 6103) containing 34% crude protein. After 6 weeks of culture, fry were weaned on Starfeed 9910 (1mm) containing 32% crude protein. Feedings were carried out two times daily at 0830and 1700. In the stocking density experiment, 3 densities tested were 2, 3 and 4 fry/L. All treatments were conducted in triplicates in 10L plastic aquaria. Golden *Anabas* fry with wet weight of 1.02g weight and total length of 3.60cm were used in this experiment. As for salinity experiment, golden *Anabas* fry were cultured in water with salinities of 0, 5, 10 and 15 ppt, at stocking of 3 fry/L, in 8L aquaria. Statistical analysis showed that there were significant differences (p<0.05) in the growth between the treatments. The best growth recorded was in 2 fry/L, followed by 3 and 4 fry/L. As for salinity experiment, 0 ppt showed significantly better (p<0.05) growth as compared to the rest of the treatments. However, the percentage of survival was not significantly different (p>0.05) among the treatments. In conclusion, golden *Anabas* fry showed better growth when cultured at low stocking density (2 fry/L) and can tolerate up to 15 ppt salinity.

Keywords: Stocking density, Salinity, Golden climbing perch, Growth, Survival

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Introduction

Anabas testudineus is a well known freshwater fish species, distributed widely in Asian countries like Malaysia, Thailand, India, Philippines and Bangladesh (Chotipuntu and Avakul, 2010; Rahman *et al.*, 2010; Singh *et al.*, 2011). This fish can survive out of water for a long period of time with the help of accessory respiratory organ, which allows it to swallow air therefore able to withstand poor water condition (Atack, 2006). It is commonly called climbing perch due to its ability to walk on land (Talwar and Jhingran, 1991). There are 3 varieties of *Anabas* in Malaysia. First is the small sized local *Anabas testudineus* found naturally in rice field areas? Second one is the Thai *Anabas* with size 2-3 times bigger, cultured by fish farmers in Thailand? This variety was produce through selective breeding of *Anabas testudineus*, producing bigger sized *Anabas*. Finally, the medium sized golden *Anabas*, similarly was produced through selective breeding with prominent golden body coloration devoid of black botches as seen in the wild type. Thus popular as ornamental fish. Golden *Anabas* has better economic value, sold at around USD1 per adult as compared to the other two varieties, sold at USD4 per kg as food fish.

Anabas testudineus is usually found in low lying swamps, marsh lands, lakes canals, pools, small pits and puddles (Jayaram, 1981; Talwar and Jhingran, 1991; Vidthayanon, 2002). According to Rainboth (1996), this fish is found in water body with compact vegetation. Report also showed that it inhabit brackish water body (Davenport and Martin, 1990) and in harsh environment with low oxygen (Graham, 1997). *Anabas testudineus* has the ability to migrate overland from one water body to another (Davenport and Martin, 1990; Pethiyagoda, 1991; Sakurai *et al.*, 1993). This migration usually occurs at night and after rain storms. This fish uses its tail and spiny opercula to move on land, often triggered by the lack of food and overcrowding population (Liem, 1987). Bhattachrjee *et al.* (2009) suggested its potential as biological control for mosquito in rice field and temporary pools.

Growth and development of most fishes are influenced by the environmental factors. Growth is a continuous process, fish become larger depending on their external environment (Brett, 1979; Boeuf *et al.*, 1999). Ecological factors like temperature, salinity and photoperiod act directly through receptors to increase or decrease the growth of the organism, and the limiting factors, like ammonia, oxygen, and specific threshold or tolerance range like pH. Boeuf (2001) stated that all these factors must be considered when establishing fish culture to produce fish of the best quality with most economical method. Thus this requires details and careful use of ecological factors to produce consistent quality of product for the market.

Among the ecological factors, salinity is specific to the aquatic environment. Studies by Fuentes and Eddy (1997), Usher *et al.* (1988), Shehadeh and Gordon (1969), Carroll *et al.* (1994 and 1995) and Perrott *et al.* (1992) demonstrated the influence of salinity on growth capacities in fish. Certain juvenile's fish preferred intermediary salinities like those in estuaries, tidal coast or lagoons.

In Malaysia there are many abandoned shrimp ponds. There is a potential to utilize these ponds for fish culture, provided that the fish can tolerate certain level of salinity. The ability of *A. testudineus* to tolerate harsh environment, made it a suitable candidate.

Stocking density is one of the most important factor in fish culture. Health, growth and survival of fish culture are depending on the stocking density (Backiel and LeCren, 1978). Higher stocking density may reduce the growth and survival of fish during culture (Shagunan, 1997). Better growth performance and survival of fishes when cultured in lower density has been demonstrated in *C. gariepinus* (Hecht *et al.*, 1996) sturgeon *Acipenser schrenckii* (Zhu *et al.*, 2011), in silver cat fish *Rhamdia quelen* (Poueyet *al.*, 2011), crayfish *Astacus leptodactylus* (Mazlum, 2007), *Oreochromis spp.* (Sorphea *et al.*, 2010) and *Macrobrachium rosenbergii* prawn (Cuvin-Aralar *et al.*, 2007).

High stocking density sometimes may not affect the survival of fishes, but it may increase the yield which lead to higher gross and net return at a lower production cost (Abou *et al.*, 2007). Therefore, determination of suitable stocking density of golden *Anabas* is the key to establish a proper culture technique.

Thus this study was conducted to determine the suitable stocking density and salinity tolerance for golden *Anabas* fry.

Materials and methods

Location of study

The present study was carried out for 3 months at Hatchery Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Stocking of fry

Golden *Anabas* fry with average total length of 3.6 cm and 3.3 cm used in stocking density and salinity experiment respectively were bought from local fish farmer in Rawang, Selangor.

Stocking density

In this study, 270 fry of golden *Anabas* were prepared into stocking density of 2, 3 and 4 fry/L in triplicate treatments. Rectangular-shape aquaria with 11L capacity were filled with 10L water. Sampling for wet weight and total length were carried out once a month for a period of 3 months.

Salinity tolerance

Prior to the initiation of salinity experiment, golden *Anabas* fry were acclimatized gradually to designated salinity. An increase of 2 to 3 ppt in two days, prepared by mixing seawater with seasoned tap water until the required salinity. Treatments were 0, 5, 10 and 15 ppt, with stocking density of 3 fry/L.

Survival and growth

Mortality of fry were observed daily and noted for the determination of survival (%). While, growth were recorded by measuring the wet weight and total length once a month, throughout the study period. Fry were weighed using an electronic balance (Sartorius BP310S), while total length measurement using an ordinary plastic ruler measured to the nearest gram and centimeter respectively.

Water quality measurements

Water quality parameters (temperature, dissolved oxygen, ammonia, nitrite and pH) were measured once a week, prior to water change. Temperature and dissolved oxygen of water were determined using DO meter (YSI 550A), ammonia and nitrite, API master test kit, and pH using pH pen (Ezodo, Taiwan). Salinity was measured using refractometer (Milwaukee MR32).

Feeding

Fry were fed to apparent satiation twice a day at 0830 and 1700, with commercial crumble diet (Cargill 6103) containing 34% crude protein, then 6 weeks later weaned on pellet sized 1mm (Starfeed 9910) with 32% crude protein until then end of the experimental period.

Statistical analysis

Data of weight and length were analysed statistically by using one way analysis of variance (ANOVA), followed by Duncan's multiple comparison test. All data in percentages were transformed using Arcsine before being used for ANOVA.

Results

Stocking density

Significant differences (p<0.05) were observed in the total length (TL), length gain, weight (Wt) and weight gain for golden *Anabas* fry cultured at 2, 3 and 4 fry/L (Table 1). At the end of first month culture, stocking density of 2 fry/L showed the highest (p<0.05) TL, length gain, Wt and Wt gain. However, at the end of second month, culture of 2 fry/L showed the highest (p<0.05) TL and Wt as compared to 3 and 4 fry/L. As for length gain, no significant different (p>0.05) was observed between all the treatments. Wt gains were higher in both 2 and 3 fry/L as compared to 4 fry/L. At the end of the third month culture, TL, length gain, Wt and Wt gain were again the highest (p<0.05) at 2 fry/L as compared to 3 and 4 fry/L, similar to the first month.

Survival of golden *Anabas* fry, ranging from 95-100% were not significantly different (p>0.05) between stocking density of 2, 3 and 4 fry/L for all the 3 months of culture.

Table 2 showed the mean for water quality parameters. Ranges of water temperature pH, DO, ammonia and nitrite monitored during the experimental period were 27.5-27.7°C, 7.32-7.52 and 5.05-5.14ppm respectively. As for ammonia and nitrite, the levels remained constant at 0.25ppm. No significant differences (p>0.05) were observed in all the treatments for temperature, DO, ammonia and nitrite. Values of pH were significantly higher (p<0.05) in tanks with stocking density of 4fry/L. However, all these pH, DO, ammonia and nitrite were still within the acceptable limits for fish culture as described by Boyd (1982). Interestingly, even at stocking density 4 fry did not affect the water quality, evident with the high survival of golden *Anabas* during the 3 months culture period.

Salinity tolerance

Table 3 showed the significant differences (p<0.05) observed in the TL, length gain, Wt and weight gain for golden *Anabas* fry cultured in 0, 5, 10 and 15 ppt. At the end of the first month culture, fry cultured in 0 and 5ppt showed comparatively better TL and length gain as compared to 10 and 15 ppt. As for Wt and Wt gain, 0 ppt showed the best results compared to 5, 10 and 15 ppt. At the end of second month, fry cultured in 0 and 5ppt showed comparatively better TL but no significant different was detected in length gain when compared to 10 and 15 ppt. While Wt and Wt gain were highest (p<0.05) in 0ppt. At the end of the third month culture, 0, 5 and 10 ppt showed higher (p<0.05) TL compared to 15 ppt. There was no significant different in length gain for all the treatments, similar to those observed in the second month. Wt and Wt gain were highest (p<0.05) at 0 ppt when compared to 5, 10 and 15 ppt. Overall, the best TL, Wt and Wt gain were observed in fry cultured in 0 ppt, followed by 5 and 10 ppt, then the lowest in 15 ppt.

Survival of golden *Anabas* fry was quite high, ranging from 93-100%, with no significant different (p>0.05) between 0, 5, 10 and 15 ppt for the 3 months of culture period. Basically, golden *Anabas* showed high tolerance to salinity and able to strive in brackish condition of 15 ppt, without affecting the survival.

Ranges of water temperature, pH and DO monitored during this study were 27.6-27.8°C, 7.09-7.30 and 4.84-4.95ppm respectively (Table 4). While the levels of ammonia and nitrite remained constant at 0.25ppm and 0.25ppm respectively in all treatments. There were no significant differences (p>0.05) observed for temperature, DO, ammonia and nitrite in all the treatments. However, pH was noticeably higher in 0 ppt as compared to 5, 10 and 15 ppt.

Table 1: Mean of total length (TL), length gain, wet weight (Wt), weight gain and survival (%) of golden *Anabas* fry cultured for 3 months at stocking densities of 2, 3 and 4 fry/L.

Month of Culture	Parameter	Stocking (fry/L)		
		2	3	4
Initial	TL (cm)	3.60 ± 0.10^{a}	3.60 ± 0.05^{a}	3.60 ± 0.04^{a}
	Wt (g)	1.02 ± 0.03^{a}	1.02 ± 0.03^{a}	1.02 ± 0.03^{a}
	TL (cm)	5.84 ± 0.04^{c}	5.58 ± 0.09^{b}	5.33 ± 0.07^{a}
	Length gain (cm)	2.24 ± 0.04^{c}	1.98 ± 0.09^{b}	1.72 ± 0.05^{a}
1	Wt (g)	4.14 ± 0.09^{c}	3.71 ± 0.10^{b}	3.18 ± 0.07^{a}
	Wt gain (g)	3.09 ± 0.09^{c}	2.69 ± 0.10^{b}	2.16 ± 0.07^{a}
	Survival (%)	96.67 ± 1.67^{a}	97.78 ± 1.11^{a}	98.33 ± 0.83^{a}
	TL (cm)	7.70 ± 0.04^{b}	7.44 ± 0.10^{a}	7.21 ± 0.04^{a}
	Length gain (cm)	1.86 ± 0.01^{a}	1.86 ± 0.18^{a}	1.89 ± 0.01^{a}
2	Wt (g)	10.15 ± 0.09^{c}	9.40 ± 0.08^{b}	7.02 ± 0.08^{a}
	Wt gain (g)	6.04 ± 0.01^{b}	5.69 ± 0.18^{b}	3.84 ± 0.14^{a}
	Survival (%)	100 ± 0.00^{a}	95.40 ± 4.60^{a}	97.44 ± 2.56^{a}
	TL (cm)	9.32 ± 0.07^{c}	8.82 ± 0.06^{b}	8.40 ± 0.07^{a}
	Length gain (cm)	1.62 ± 0.03^{c}	1.39 ± 0.04^{b}	1.18 ± 0.03^{a}
3	Wt (g)	15.64 ± 0.08^{c}	13.62 ± 0.07^{b}	11.29 ± 0.05^{a}
	Wt gain (g)	5.49 ± 0.09^{b}	4.22 ± 0.07^{a}	4.26 ± 0.12^{a}
	Survival (%)	100 ± 0.00^{a}	98.67 ± 1.33^{a}	100 ± 0.00^{a}

Value in the same row with the same superscripts are not significantly different (p>0.05); ± Standard error.

Table 2: Water quality parameters in the 3 months culture of golden *Anabas* fry at stocking densities of 2, 3 and 4 fry/L.

Stocking density (fry/L)	Temperature (°C)	pH	Dissolved oxygen (ppm)	Ammonia (ppm)	Nitrite (NO_2^-) (ppm)
2	27.7 ± 0.06^{a}	7.32 ± 0.05^{a}	5.05 ± 0.13^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}
3	27.6 ± 0.09^{a}	7.44 ± 0.03^{ab}	5.05 ± 0.03^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}
4	27.5 ± 0.02^{a}	7.52 ± 0.05^{b}	5.14 ± 0.08^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}

Value in the same column with the same superscripts are not significantly different (p>0.05); ± Standard error.

Table 3: Mean of total length (TL), length gain, wet weight (Wt), weight gain and survival (%) of golden *Anabas* fry cultured for 3 months at salinities of 0, 5, 10 and 15ppt.

Month of Culture	Parameter	Salinity (ppt)			
		0	5	10	15
Initial	TL (cm)	$3.30 \pm 0.03^{a\backslash}$	3.30 ± 0.02^{a}	3.30 ± 0.02^{a}	3.30 ± 0.04^{a}
	Wt (g)	0.87 ± 0.01^{a}	0.87 ± 0.01^{a}	0.87 ± 0.02^{a}	0.87 ± 0.02^{a}
	TL (cm)	5.50 ± 0.07^{c}	5.44 ± 0.05^{bc}	5.28 ± 0.04^{b}	5.01 ± 0.05^{a}
	Length gain (cm)	2.20 ± 0.07^{c}	2.14 ± 0.05^{bc}	1.98 ± 0.04^{b}	1.71 ± 0.05^{a}
1	Wt (g)	3.37 ± 0.07^{c}	3.08 ± 0.05^{b}	2.96 ± 0.04^{b}	2.43 ± 0.06^{a}
	Wt gain (g)	2.50 ± 0.07^{c}	2.21 ± 0.05^{b}	2.09 ± 0.04^{b}	1.56 ± 0.06^{a}
	Survival (%)	100 ± 0.00^{a}	100 ± 0.00^{a}	97.78 ± 2.22^{a}	100 ± 0.00^{a}
	TL (cm)	7.32 ± 0.06^{c}	7.23 ± 0.07^{bc}	7.07 ± 0.06^{b}	6.87 ± 0.07^{a}
	Length gain (cm)	1.83 ± 0.05^{a}	1.79 ± 0.09^{a}	1.79 ± 0.06^{a}	1.86 ± 0.10^{a}
2	Wt (g)	7.43 ± 0.06^{c}	6.84 ± 0.07^{b}	6.69 ± 0.10^{b}	5.50 ± 0.09^{a}
	Wt gain (g)	4.07 ± 0.02^{c}	3.76 ± 0.06^{b}	3.72 ± 0.06^{b}	3.06 ± 0.08^{a}
	Survival (%)	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}
	TL (cm)	8.55 ± 0.08^{b}	8.41 ± 0.05^{b}	8.31 ± 0.08^{b}	7.85 ± 0.08^{a}
	Length gain (cm)	1.23 ± 0.07^{a}	1.19 ± 0.08^{a}	1.24 ± 0.13^{a}	0.98 ± 0.11^{a}
3	Wt (g)	12.68 ± 0.09^{c}	11.67 ± 0.12^{b}	11.49 ± 0.02^{b}	8.77 ± 0.04^{a}
	Wt gain (g)	5.25 ± 0.05^{c}	4.83 ± 0.06^{b}	4.80 ± 0.08^{b}	3.27 ± 0.14^{a}
	Survival (%)	95.56 ± 2.22^{a}	100 ± 0.00^{a}	97.62 ± 2.38^{a}	93.33 ± 3.85^{a}

Values in the same row with the same superscripts are not significantly different (P> 0.05); ± Standard error.

Table 4: Water quality parameters in the 3 months culture of golden *Anabas* fry at salinities of 0, 5, 10 and 15ppt.

Salinity (ppt)	Temperature (°C)	pH	Dissolved oxygen (ppm)	Ammonia (ppm)	Nitrite (ppm)
0	27.6 ± 0.08^{a}	7.30 ± 0.04^{b}	4.88 ± 0.03^{ab}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}
5	27.7 ± 0.04^{a}	7.09 ± 0.01^{a}	4.86 ± 0.02^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}
10	27.8 ± 0.06^{a}	7.12 ± 0.02^{a}	4.95 ± 0.01^{b}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}
15	27.8 ± 0.02^{a}	7.09 ± 0.03^{a}	4.84 ± 0.01^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}

Value in the same column with the same superscripts are not significantly different (p> 0.05); ± Standard error.

Discussion

In this study, stocking of golden *Anabas* at 2 fry/L was observed to produce the best growth by the end of the experimental period. A negative trend was observed, as stocking density increases, growth slows down. Sugunan and Katiha (2004) stated that it is chronically stressful for fish to be cultured at high density. Various studies showed that low stocking densities provide more space, food and less competition (Ahmed, 1982; Hasan, 1982; Haque *et al.*, 1984). Increase in growth and survival was observed in *Clarias gariepineus* fingerlings cultured at low density (Jamabo and Keremah, 2009). According to Stickney (1994), some species may be able to tolerate extreme crowding, however competition for food will affect the growth, leading to poor weight gain.

In the salinity experiment, the best growth was observed when golden *Anabas* fry were cultured in 0 ppt, while the lowest in 15 ppt. The increased of salinity to 5 ppt may have minimal effect on the growth of golden *Anabas*. However, further increase of salinity to 10 ppt and above, will cause detrimental effects on the growth, as observed in this study. Stress resulted from high salinity exposure reduces the feed intake by fish (Ye *et al.*, 1990; De Boeck *et al.*, 2000). However, a small amount of salt in the culture system may reduce energy usage and therefore promote growth (Woo and Kelly, 1995). Different freshwater fish species may have different tolerance to salinity. *Labeo rohita* was reported to be more tolerant towards salinity compared to *Cirrhinus mrigala* (Tarer, 2000). Fish ability to adapt to any medium depends on its ability to maintain body osmoregulation. In freshwater fish, the body fluids are hypertonic compared to the external medium therefore they are able to osmoregulate through the production of urine or increase uptake of ion through gills (Sahoo *et al.*, 2003).

In this study water quality parameters measured were within acceptable range for fish culture as described by Boyd (1982). Water temperature measured in the stocking density and salinity experiment were between 27.5 to 27.7°C and 27.6-27.8°C respectively. Range between 25 to 30°C is preferable for fish (Aminul, 1996; Akhteruzzaman, 1988; Adhikary *et al.*, 2009; Kohinoor *et al.*, 1998; Khan, 2008) cultured in the tropical regions. As for pH of water, it is the most important factor in fish culture. Rapid fluctuation may affect the physiological function in fish. The pH measured for both experiment in this study was between 7.09 to 7.52. This pH falls within the 6.5 to 8.5 recommended by Boyd (1982) as acceptable range for fish culture. In the wild, *Anabas testudineus* is known to withstand low oxygen (Graham, 1997).

In this study, DO ranges were 5.05-5.14 ppm and 4.84-4.95ppm for stocking density and salinity experiment respectively. Therefore close to the favorable level of 5 ppm as recommended by Rahman *et al.* (1982).

Ammonia and nitrite levels for both experiments in this study were constantly at 0.25 ppm. High ammonia and nitrite in water were due to the breakdown of excess of food, faeces and dead plankton (Bhatnagar and Devi, 2013; Zailie, 2009). Therefore, Bhatnagar and Singh (2010) recommended the level of ammonia for aquaculture system should be less than 0.2 ppm. Being a hardy fish is probably the reason why golden *Anabas* showed higher tolerance for ammonia and nitrite in this study.

Conclusion

Based on the findings of this study, it can be concluded that best stocking density for optimal growth of golden *Anabas* is at 2 fry/L. Eventhough this species is able to tolerate salinity up to 15 ppt, the recommended salinity for the culture of golden *Anabas* should not exceed 10 ppt for better growth. Most importantly, gradual acclimatization of golden *Anabas* fry should be carried out prior to the introduction into higher salinity water.

Acknowledgement

Authors would like to thank the Institute of Bioscience, Universiti Putra Malaysia for providing resources and facility for this study.

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