AACL BIOFLUX
Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Effects of salinity and water quality parameters on the breeding and larva rearing of black molly *Poecilia sphenops* in laboratory condition

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Abstract. A study was conducted to determine the effects of salinities (0, 3 and 6 PSU) and other water quality parameters on the breeding and larva rearing of black molly *Poecilia sphenops* (Valenciennes 1846) under laboratory condition. Each treatment was carried out in triplicates. Results showed that water salinity of 6 PSU represented the highest breeding success compared to salinities of 0 and 3 PSU. Nevertheless, no significant differences ($p > 0.05$) were observed at these three salinities for fry production in captivity. Moreover no significant differences were observed in weight increment when salinity raised from 3 to 6 PSU, however, these two treatments differed significantly when compared with 0 PSU. The survival rate was not significantly varied in comparison with 0 PSU. The highest total length increment was found at water salinity of 6 PSU followed by 0 and 3 PSU. Results on water quality parameters denoted no significant differences ($p > 0.05$) for all treatments except on ammonia (NH_3) rates. The highest ammonia level was found at 0 PSU followed by 3 and 6 PSU. The findings of the present study suggested to culture black molly (*P. sphenops*) in a slight saline condition from 3 to 6 PSU.
Key Words: *Poecilia sphenops*, aquarium trade, ornamental fish, fry production, Malaysia.

Introduction. Ornamental fish industry in Malaysia started in 1980 and progressively growing over time. This industry comprised of two sectors, freshwater and marine. In addition, Watson & Shiremen (1996) categorized ornamental fish into two types, live bearers and egg layers. Black molly (*Poecilia sphenops*) belongs to the family of Poeciliidae and is one of the top commercially important freshwater ornamental fish species which can withstand mass-cultured in captivity (Francis 1992; George & Pandian 1995; Beck et al 2003). Black molly is an euryhaline fish (Beck et al 2003) that live naturally in a brackish water ecosystem (Johnson 1981) and native from Mexico to Central America (Shipp 1986). According to Johnson (1981) and Beck et al (2003) black molly can evolve to cope with rapidly changing ionic concentrations in their environments. Black molly is a viviparous fish (eggs fertilized internally) (Kavumpurath & Pandian 1992, 1993; George & Pandian 1995, 1998). The gestation period for gravid female is between 28-35 days and their fry can swim after birth (Roy 1996). A matured female can produce around 20-60 fry/broodstock (Ramshorst 1995; Goodwin 2003). However, they do not display any parental care (Witte & Ryan 2002). The matured female may have more than one brood of fry from one fertilization process, but the production of fry will decrease in subsequent deliveries (Goodwin 2003). Black molly represents cannibalistic feeding behaviour although it is an omnivorous fish (consumes primarily algae, other plant materials and small invertebrates such as larvae of mosquitoes) (Rohde et al 1994).

At present, scientific documents pertaining to the breeding and culture of *P. sphenops* is lacking. The objective of this study was to investigate the effect of salinities and water quality parameters on (i) the breeding and fry production of black molly, and (ii) the body weight, total length increment and percentage of survival of black molly in laboratory condition.

Material and Method

Fish collection and conditioning. This study was conducted for ten months from May 2010 to February 2011. A total of 40 black molly broodstock (consisting of 20 males and 20 females) used in this experiment was procured from a local ornamental fish dealer and transported to the Endocrinology Laboratory of Department of Aquaculture, Universiti Putra Malaysia. Upon arrival to the laboratory, the samples were conditioned and acclimatize for 30 minutes and then released to the stocking aquaria (60 x 30 x 30 cm) separately according to their sexes. The samples were maintained under aerated re-circulating aquaria and fed frozen bloodworm *Chironomus* sp. for one month before being introduced for breeding process. The water temperature in the aquaria was maintained at 27°C.

Identification of broodstock. Matured black molly adults can be easily identified, of which male has a modified anal fin intromittent organ known as gonopodium while, female possessing much bigger and rounded bellies. The sizes of the matured broodstocks used were between 4.0 to 6.5 cm total length (TL, cm).

Selection of broodstock. After one month of conditioning, broodstock was introduced in pairs into breeding tanks (40 x 25 x 25 cm). Broodstock selected was based on activeness, health, color and without deformities. Aeration was provided for each breeding tank. Prior to the introduction of broodstock, all breeding tanks was filled with 15 L water and aerated for three days to remove the chlorine. Then, 3 set of treatments with salinity of 0, 3 and 6 Practical Salinity Units (PSU) were prepared with the addition of seawater. Experiments were carried out in triplicates. Substrate such as PVC pipe or wire mesh was added into each tank to provide hiding place for newly born fry.

Breeding process. Stocking ratio of 1:1 (male and female) was used in this study. Fishes were left for two weeks to spawn at different salinity regimes (0, 3 and 6 PSU). After the completion of spawning, male and female broodstocks were separated. The gravid female remained in the aquarium tank, while male returned into conditioning tank.

Stocking fry. Newly hatched fry from the breeding experiment were placed in 10 L experimental aquaria (20 x 15 x 15 cm) with three different salinity regimes (0, 3 and 6 PSU) at stocking density rate of 2 fry L^{-1}.

Fry sampling. Ten fry were sampled randomly at every two weeks interval. Growth performance (total length) was measured at the nearest centimeter (±0.01 cm) by a measuring board and body wet weight to the nearest gram (±0.01 g) using digital balance (Simon et al 2008, 2009, 2010, 2013; Alam et al 2013). Percentage of survival was monitored daily and calculated at the end of the study period.

Feeding. The fries were fed to satiation with brine shrimp nauplii (*Artemia salina*), twice a day (10:00 and 17:00 hrs) throughout the study period.

Water quality parameters Water quality parameters were measured once a week. Parameters measured were pH, dissolved oxygen (DO), temperature, ammonia (NH_3), nitrite (NO_{2-}) and nitrate (NO_{3-}). Excess organic waste was removed by siphoning out the remnant of waste left at the bottom of the experimental aquaria. This is necessary to reduce organic loading in the culture system.

Data collection and analysis. Data collected throughout this study were breeding success, fry production, growth performance (body weight and total length) and percentage of survival (%). Data were initially analyzed for their normality test and normally distributed data were analyzed using one-way ANOVA (SAS system version 9.2 and Duncan's test). Data that are not normally distributed were analyzed by a non-parametric statistical test such as Kruskal Wallis. The levels of significant differences were compared between all treatments.

Results

Fry production. There were no significant differences ($p > 0.05$) in the number of fry production (within range of 33-56% of breeding success) at water salinity of 0, 3 and 6 PSU as shown in Table 1. The highest number of fry production was at 6 PSU followed by 0 and 3 PSU.

Table 1

Percentage of breeding success and number of fry production in all treatments

Treatments	*No. of female broodstock*	*Breeding success percentage (%)*	*Fry production (mean ± S.D)*
T1 (0 PSU)	9	44	24 ± 2.65
T2 (3 PSU)	9	33	22 ± 2.00
T3 (6 PSU)	9	56	26 ± 1.00

Growth performance and survival rate. Figure 1 shows the body weight increment for black molly fry at water salinity of 6 PSU was the highest (0.205 g) followed by 3 (0.203 g) and 0 (0.200 g) PSU. Results showed that there were significant differences ($p < 0.05$) in the weight increments for black molly fry when cultured at water salinity of 0, 3 and 6 PSU from 2 to 8 weeks. Figure 2 shows the length increment for black molly fry during the 8 weeks study period. Fry reared in 6 PSU recorded the highest (2.77 cm) total length increment as compared to 3 (2.70 cm) and 0 (2.57 cm) PSU. There were significant differences ($p < 0.05$) between all treatments in the total length increments.

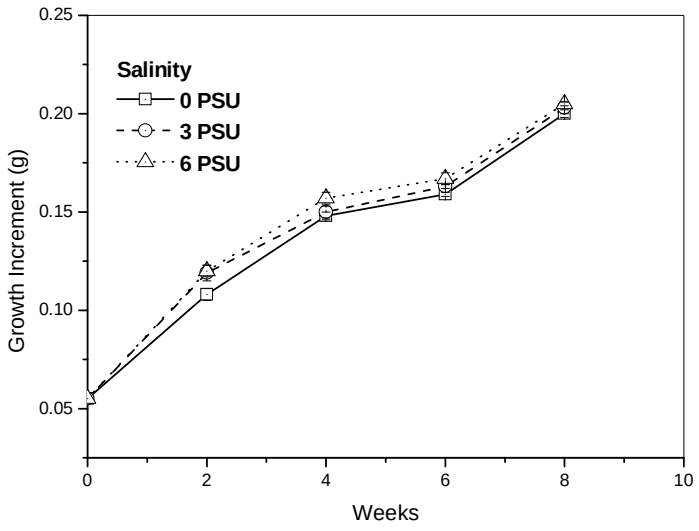

Figure 1. Body weight (g) increment of fry at fortnightly sampling during the 8 weeks study period at different salinities.

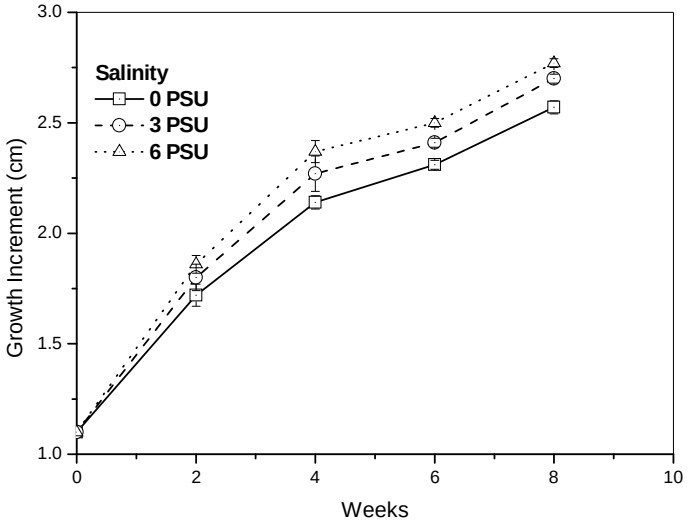

Figure 2. Total length (cm) increment of fry at fortnightly sampling during the 8 weeks study period at different salinities.

Percentages of survival for black molly fry cultured in different salinities (0, 3 and 6 PSU) ranged between 84 to 100% as shown in Table 2. Treatment 3 and 6 PSU showed significantly higher ($p < 0.05$) survival as compared to 0 PSU. However, percentage of survival was significantly ($p < 0.05$) lower in treatment with 0 PSU.

Table 2

Mean survival rates of black molly fry at the end of the 8 weeks culture period

Treatments	*Mean percentage of survival*	
	Initial (mean ± S.D)	*Final (mean ± S.D)*
T1 (0 PSU)	100 ± 0.0	84 ± 8.5[b]
T2 (3 PSU)	100 ± 0.0	97 ± 4.8[a]
T3 (6 PSU)	100 ± 0.0	100 ± 0.0

Mean values within the same column having the different superscript are significantly different ($p > 0.05$).

Water quality parameters. Table 3 shows the results for water quality parameters during the 8 weeks period of larval rearing. There were no significantly differences ($p > 0.05$) found in the water quality parameters among all treatments throughout the study period except for ammonia rates. The highest ammonia content was found at the salinity of 0 PSU followed by 3 and 6 PSU.

Table 3

Effects of different water quality parameters and salinity regimes on growth performance of black molly fry

Water quality parameters	*Treatments*		
	T1 (0 PSU) (mean ± S.D)	*T2 (3 PSU) (mean ± S.D)*	*T3 (6 PSU) (mean ± S.D)*
pH	7.33 ± 0.13[a]	7.30 ± 0.11[a]	7.29 ± 0.14[a]
Dissolved oxygen, DO (ppm)	3.09 ± 0.26[a]	3.19 ± 0.20[a]	2.98 ± 0.29[a]
Temperature (°C)	25.48 ± 0.69[a]	25.63 ± 0.74[a]	25.38 ± 0.73[a]
Ammonia, NH_3 (ppm)	1.12 ± 0.35[a]	0.81 ± 0.16[b]	0.54 ± 0.28[c]
Nitrite, NO_{2-} (ppm)	0.79 ± 0.32[a]	0.69 ± 0.30[a]	0.73 ± 0.47[a]
Nitrate, NO_{3-} (ppm)	10.42 ± 3.67[a]	8.96 ± 3.91[a]	9.79 ± 3.76[a]

Mean values within the same column having the different superscript are significantly different ($p > 0.05$).

Discussion

Fry production. Through this study, results denoted that fry production was not influenced by the different water salinities. This study showed that fry production ranged between 20-27 fry/broodstock which is close to the finding by Ramshorst (1995) and Goodwin (2003) which ranged from 20-60 fry/broodstock. Chong et al (2004) stated that feeding the female broodstock with higher protein diets would increase the number of fry produced.

Growth performance and survival rate. Results proved that 3 and 6 PSU produced the better weight increment and percentage of survival for black molly as compared to 0 PSU. However 6 PSU treatments produced the better length increment of black molly as compared to 0 and 3 PSU. This study signifies that salinity affected weight and length increments, and percentage of survival of black molly. These findings are similar to those recorded by Yang et al (2009). Furthermore, Schelkle et al (2011) stated that culture water of slight saline condition is the most effective against disease for tropical ornamental fish. This study indicated fry fed with live food by using *Artemia* nauplii increased the weekly growth performance. Similar observation was reported by Harpaz et al (1999) and Lim et al (2003).

Water quality parameters. Tropical ornamental fish are generally sensitive to poor water quality (Watson & Shiremen 1996). Water quality is very crucial in the breeding and rearing of fry as reported by Roy (1996). According to Ng et al (1992), pH requirements for tropical ornamental fish culture should be from 7.2-7.8. In this study pH range was 7-7.58 which is within close range to that the above report. However, values for DO ranged from 2.46-3.48 ppm were considerably lower than 4.9-7.4 ppm as stated by Ng et al (1992). This finding indicated that black molly fry were quite tolerant to low dissolved oxygen in the culture water. Hernandez & Buckle (2002) stated black molly is a eurythermal species and it can adapt to higher water temperature. This ability is clearly demonstrated in this study. Whereby, black molly were able to tolerate temperature ranged between 24-26.7°C. High ammonia in the aquarium can cause the mortality of black molly fry (Ng et al 1992; Roy 1996). Ammonia is the primary metabolic waste produced by fish. Therefore, mortalities of fry in the 0 and 3 PSU were most likely due to the high ammonia concentration. In this study, the ammonia ranged between 0-1.5 ppm. While, nitrite and nitrate ranged from 0-1.5 ppm and 2.5-15.0 ppm respectively were considerably higher than 0.025-0.058 ppm and 0.5-1.50 ppm as reported by Ng et al (1992). Nitrite is also toxic to fish but toxicity level is lesser than that of ammonia (Tomasso et al 1979).

Conclusions. It can be concluded in the present study that black molly can breed successfully in salinity 6 PSU. However, salinity has no significant influences on the number of fry production. The growth performance (weight and length increments) and percentage of survival of black molly fry are much better when cultured at 3 and 6 PSU. Black molly fry can tolerate a wide range of pH (7-7.58), dissolved oxygen (2.46-3.48 mg L^{-1}), temperature (24-26.7°C), ammonia (0-1.5 ppm), nitrite (0-1.5 ppm) and nitrate (2.5-15.0 ppm). The information obtained from the present study would be useful for better management and commercial production of this fish species in captivity.

Acknowledgements. We would like to thank Claudiu Gavriloaie (Editor-in-Chief, AACL Bioflux journal) and the reviewers for their very useful comments. We would like to thank the laboratory technicians of Aquaculture Department, University Putra Malaysia. We also would like to thank the Public Service Department of Malaysia (JPA) for the scholarship provided to the first author. Thanks to University Kebangsaan Malaysia for the financial support through the research grant # ERGS/1/2013/STG/UKM/01/2.

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Received: 09 December 2013. Accepted: 20 January 2014. Published online: 29 January 2014.
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How to cite this article:
Husna W. N. W. H., Christianus A., Cob Z. C., Mazlan A. G., Simon K. D., 2014 Effects of salinity and water quality parameters on the breeding and larva rearing of black molly *Poecilia sphenops* in laboratory condition. AACL Bioflux 7(1):8-14.