Effects of stocking density on growth performance and survival of three male morphotypes in all-male culture of *Macrobrachium rosenbergii* (De Man)

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Abstract

A study was conducted to appraise the effect of different stocking densities on three male morphotypes named blue claw (BC), orange claw (OC) and small male (SM) at harvest in all-male culture and to assess the growth performance of BC, OC and SM in each isolated culture. Trials involving three stocking densities of all-male prawn viz., 20, 30 and 40 juvenile m^{-2} were carried out in replicates. After 4 months of culture, BC, OC and SM were sorted from all tanks and restocked at 5 m^{-2} in treatments BC, OC and SM respectively for 80 days. A difference in prawn density significantly ($p<0.05$) affected adversely on morphotypes. The highest survival rate combined with good yield performance was from 20 juvenile m^{-2} stocking density with 21% BC, 62.5% OC and 16.5% SM, respectively. In isolation culture, the average specific growth rate of the SM population (1.22) was significantly higher than that of the OC (1.01) and the BC (0.43) population. The survival of the SM population was 100% while for others it was 72%. Absolute weight of prawn was significantly greater in the male OC (23.87 g) than the SM (19.57 g) and the BC males (6.31 g). Impacts of isolated culture on population structure were much more pronounced in the SM population than others.

Keywords: Freshwater prawn, *Macrobrachium rosenbergii*, Heterogeneous individual growth, Male morphotypes, Stocking density, Isolation culture

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Introduction

All-male freshwater prawn *M. rosenbergii* (De Man, 1879) culture yields better outputs than mixed sex or all-female culture (Cohen *et al*., 1988; Nair *et al*., 2006; Kunda *et al*., 2009), and provides differential growth that contributes to a wide variation in size within male populations. Males *M. rosenbergii* however, exhibit heterogeneous individual growth (HIG), in some instances producing 50% undersized small males (SM), 40% orange-clawed males (OC) that are larger than SM, and only 10% large blue-clawed males (BC) at harvest (Ra'anan *et al*., 1991). Thus, HIG is one of the major problems affecting profitability in freshwater prawn culture (Karplus *et al*., 2000) as demand and market price of prawn depend largely on individual size.

Four social mechanisms are controlling factors of growth variation such as direct competition for food, appetite suppression in subordinate individuals, decreased food conversion efficiency and increased motor activity (Karplus, 2005) as well as genetic variation (Banu *et al*., 2015a). For management of size variation, early researchers have practiced several ways: supplementary shelters (Peebles, 1979; Smith and Sandifer, 1979; Cohen *et al*., 1983; Tidwell *et al*., 2002; Uddin *et al*., 2009), selective harvesting (Garcia-Perez, 1999; Jose *et al*., 2007; Rahman *et al*., 2010), claw ablation (Harpaz *et al*., 1987; Sagi and Aflalo, 2005; Rahman *et al*., 2010), size grading (D'Abramo *et al*., 1991; Daniels and D'Abramo, 1994; Daniels *et al*., 1995; Tidwell *et al*., 2003, 2004a,b) eyestalk ablation (Banu *et al*., 2014) and cold shock (Banu *et al*., 2015b). A practical management strategy, selective harvesting of BC, has been proposed to enhance the growth rate of SM, thereby minimizing the size variation of males and increasing the total productivity of ponds (Sagi and Aflalo, 2005; Rahman *et al*., 2010). According to Karplus *et al*. (1989) large second pair of claws of BC prawns is responsible for the stunting of growth of small males (SMs). However, stunting of SMs may be completely eliminated by removing the BC males from the culture systems (Karplus, 2005). Harvesting of BC males were conducted and SM individuals left in the pond to grow to larger size and hence to enhance overall biomass in polyculture system (Rahman *et al*., 2010).

Hence, the present investigation focused on male morphotypes of freshwater prawn *M. rosenbergii*. The aim of the current study was to determine the effect of different stocking densities on male morphotypes in all-male culture and to evaluate its influence on growth and survival of BC, OC and SM in isolation culture.

Materials and methods

Tank preparation and stocking

The experiment was conducted in the Centre of Marine Science at Port Dickson, Negeri Sembilan, Malaysia for a period of 120 days from February to June 2012. Nine fiberglass tanks (volume 1 ton) were selected for three stocking densities of male freshwater prawn: 20, 30 and 40 juvenile m^{-2} (herein called treatments R_{20}, R_{30}, R_{40} , respectively) with three replications, using completely randomized design. Lime ($CaCO_3$) was applied before filling tank up with water from reservoir tank. Lime was liquefied into an earthen pot and then applied by spreading homogenously into the tanks. Water preparation was done one week before stocking juveniles. Each tank was aerated by an air stone supplied with air from a regenerative blower. The water depth was maintained around 0.5 m with keeping good water quality and water was exchanged at 50% every week. Water temperatures in all experimental tanks were maintained at (27.34±0.66)°C.

Total length of nursery period was 2 months. Males and females juveniles of *M. rosenbergii* larger than 3.5 cm total length can be easily identified (Janseen, 1987). The sexes can be manually segregated with accuracy of more than 95% by skilled persons (Nair and Salin, 2005). The segregation of male juvenile of *M. rosenbergii* was done manually on the basis of external morphological characteristics by visually examining the base of the last pereopods. The gap between the origins of the two walking legs is less in case of males and in addition they possess a shield-like covering that hides the genopore. The male juveniles of *M. rosenbergii* were collected from a commercial aquaculture hatchery farm, Jelubu, Negeri Sembilan. Juveniles were brought to the experimental site by plastic bag equipped with aerators. The bags were kept underwater in the experimental tanks for about 30 minute's acclimatization of temperature. Individual mean stocking weight (x ±S.D.) for each treatment was: 1) R_{20}, 5.80±0.09 g; 2) R_{30}, 5.80±0.07 g; and 3) R_{40}, 5.83±0.44 g. Tanks were randomly assigned to receive juveniles from one of the three treatments. Shelter was provided in each tank to reduce cannibalism of prawns. The length and weight of around 10% of prawn juveniles of each tank was measured and recorded for estimating initial stocking biomass and to adjust initial prawn feeding rates.

Feeds and feeding

Prawns were fed with commercial pelleted feed containing 34% protein (BLANCA, 7704, Star feed mills (M) SDN BHD) daily at a rate of 6 % of body weight for the 1st month, 4% of body weight for the 2nd month and 3% of body weight for rest of the culture period (D'Abramo *et al*., 1995). Half of the required feed for a day was supplied in the evening and the rest in the morning. Feed rate was calculated and adjusted after sampling of prawns twice in a month.

Samples

Freshwater prawns were sampled biweekly using scoop net to assess growth and health condition. At least 5 prawns from each tank were taken to make assessment of growth trends and to readjust feeding rate. Prawns were handled carefully to avoid stress during sampling. In the last two sampling attempts prior to harvest, prawns were also individually weighed and classified into one of three male morphotypes: blue claw (BC), orange claw (OC) and small (SM) as described by Cohen *et al.* (1981), Kuris *et al.* (1987) and modified by D'Abramo *et al*. (1989).

Harvest and restocking

On day 120 of stocking, water was drained out from each tank and all prawns were harvested. Then, individual body weight, and number of prawns from each tank were recorded. Percentage of BC, OC and SM male morphotypes were measured at three treatments. The BC, OC and SM males were segregated from all treatments into 3 tanks. After 1 week of harvesting, prawns were restocked at 5 prawn/m^2 per tanks with three treatments BC, OC and SM (also called treatments R_{BC}, R_{OC} and R_{SM}), respectively with three replicates per treatment. On day 80 of restocking, all prawns were harvested and weighed.

Water quality management

Water temperature and dissolved oxygen levels and pH (Dissolved Oxygen Meter, YSI Model 58, Yellow Springs, Ohio, USA) were measured daily. Nitrogen compounds (NH_3-N, NO_2-N, and NO_3-N) and orthophosphate (PO_4-P) analyses were performed bi-weekly using a digital HACH kit (model DR 2010, HACH Co., Loveland, USA).

Statistical analyses

For statistical analysis, one-way analysis of variance (ANOVA) and Tukey's Test were performed using SAS version-9.2 (Statistical Analysis System: SAS Institute, 1990). Significance was assigned at the 0.05% level. Specific growth rate (SGR) was estimated as: SGR = [Ln (final weight) - Ln (initial weight) X 100] / culture period (days). Survival rate of prawn was calculated as Survival (%) = (No. of harvested individual ÷ No. of stocked individual) x 100.

Results

During the all-male culture and isolation culture of BC, OC and SM, parameters of water quality were not significantly different (p<0.05) among treatments. Data on mean values of water temperature, dissolved oxygen (DO), ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N) and orthophosphate (PO_4-P), and pH are presented in Table 1.

All-male culture

Growth and yields performance results for prawns are presented in Table 2. Initial stocking weight of prawn was not significantly different (p<0.05) among treatments whereas the final harvested weight was the highest in the treatment R_{20} as compared to the R_{30} and R_{40}. Although the survival rate of freshwater prawn was not significantly different among treatments, it reduced slightly at high stocking density.

Table 1: Range and mean (SD) of the water quality parameters were measured in all tanks.

Parameters	Range	Mean±SD
Dissolved Oxygen (mgL^{-1})	5.61- 8.32	7.5±0.23
Temperature (°C)	25.47- 29.93	27.34±0.66
pH	7.39-9.18	7.98±0.29
NH_3-N (mgL^{-1})	0.47-2.37	0.93±0.51
PO_3-P (mgL^{-1})	0.39-2.28	1.20±0.57
NO_3-N (mgL^{-1})	0.10-1.05	0.492±0.31
NO_2-N (mgL^{-1})	0.006-0.481	0.1570±0.16

Table 2: Growth and production parameters of prawn (Mean±SD).

Parameters	Stocking density (Juvenile/m^2)		
	R_{20}	R_{30}	R_{40}
Initial stocking weight(g)	$5.80±0.09^a$	$5.80±0.07^a$	$5.83±0.44^a$
Final harvesting weight(g)	$25.83±1.33^a$	$20.22±3.05^b$	$23.98±2.26^{ab}$
Survival rate (%)	$90.00±0^a$	$89.0±9.89^a$	$88.5±2.12^a$
Production (gm^{-2})	$386.15±30.21^a$	$379.78±82.28^a$	$464.74±65.49^a$
SGR(%/day)	$1.24±0.03^a$	$0.99±0.11^b$	$1.15±0.05^{ab}$

Mean values with different superscripts in the same row indicate significant difference (p<0.05).

Specific growth rate (SGR) of prawn was significantly greater (p<0.05) in the R_{20} than others.

Percentages of different male morphotypes within treatments are presented in Table 3. The proportion of the BC was significantly higher (p<0.05) in the R_{20} and R_{30} as compared to the R_{40}, representing 21, 23 and 10 % of total harvested male prawn. An opposite trend was evident for SM: 17% in R_{20}, 15% in R_{30} and 26% in R_{40}. Relative percentages of the OC males (62-65%) however, were not significantly different among stocking densities.

Isolation culture

Growth and survival rate of prawn were significantly (p<0.05) different among all the treatments (Table 4). Specific growth rate (g/day) trend was significant and thrice in the R_{SM} (1.22±0.28) and twice in the R_{OC} (1.01±0.11) compared to the R_{BC} (0.43±0.06). The survival rate of prawn was not significantly different (p>0.05) between the R_{BC} and R_{OC} whereas it was 100% in the R_{SM}. The absolute weight of prawn was significantly greater in the OC males (23.87g) than the SM (19.57 g) and the BC males (6.31g) (Fig. 1).

Discussion

Water parameters monitored in the current study remained within acceptable range for the cultivation of freshwater prawns (New, 2002). The stocking density of prawns had no significant effect on the water quality parameters, therefore differences observed in the productivity was related to the effect of the treatments.

Table 3: Percentages of different male morphotypes of prawn by count (Mean±SD) within treatments.

Morphotype	Stocking density (Juvenile/m^2)		
	R_{20}	R_{30}	R_{40}
Blue claw male (%)	21.0±2.83[a]	23.265±0.37[a]	9.5±0.71[b]
Orange claw male (%)	62.5±0.71[a]	61.855±4.04[a]	65.0±0[a]
Small male (%)	17.0±2.83[ab]	14.880±4.41[b]	25.5±0.71[a]

Mean values with different superscripts in the same row indicate significant difference (p<0.05).

Table 4: Growth and survival rate of different male morphotypes by count (Mean±SD) within treatments in isolation culture.

Parameters	Male morphotype of prawn		
	BC	OC	SM
Initial stocking weight(g)	23.34±1.26	18.39±1.37	9.06±0.53
Final harvesting weight(g)	29.65±0.28	42.24±0.37	28.63±0.73
SGR(%bdwt/day)	0.43±0.06[b]	1.01±0.11[a]	1.22±0.28[a]
Survival (%)	72.33±9.24[b]	72.33±9.24[b]	100±0[a]

Mean values with different superscripts in the same row indicate significant difference (p<0.05).

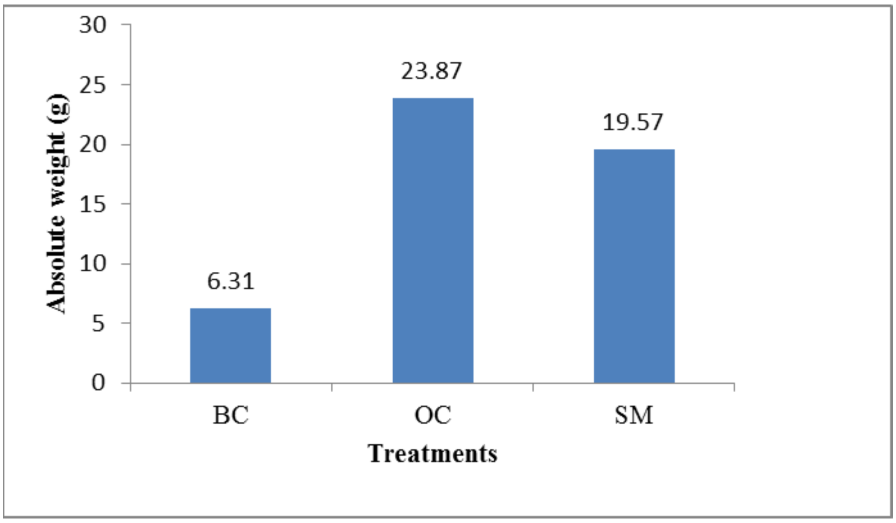

Figure 1: Absolute weight of each male (BC, OC, SM) morphotype in isolation culture.

Density had a major effect on growth and a minor effect on survival of prawns (Valenti *et al*., 2010). Stocking density significantly but slightly affected the final harvesting weight whereas no significant effects on final yield was detected. It has been reported that stocking density has dependent effects on growth pattern in *M. rosenbergii* (Karplus 2005; Pillai *et al*., 2007; Rahman *et al*., 2012). The mean size at harvest, specific growth rate and size class distribution can be significantly influenced by stocking density, with those at the lowest stocking density showing significantly better growth and overall proportion of larger prawns (Cuvin-Aralar *et al*., 2007; Kunda *et al*., 2008). In the present study, the significant effect of increasing stocking density from 20 to 40 juvenile m^{-2} was achieved on the mean percentage of three male morphotypes. The impact of low stocking of prawn was increased proportion of the BC and reduced SM as well while the impact of high stocking rate was vice versa (Ranjeet and Kurup, 2002; Rahman *et al*., 2012). The proportions of BC was significantly similar in the R_{20} (21%) and R_{30} (23%) and the OC was significantly high in all the treatments. On the other hand, the percentage of the SM was 15-25% in the treatments. The number of SM at final harvest reported by Ra'anan *et al.* (1991) was up to 50% in some instances. The percentage of the SM prawn was related to the stocking density and the inverse effect on the BC males as reported by Rahman *et al.* (2012) in their earlier study. Karplus *et al.* (1986) found a dynamic change in the mean percentages of male morphtypes with the increase in density from 1 to 4 prawn m^{-2}. Moreover, Rahman *et al.* (2012) achieved significant variation in the mean proportions of three male morphotypes. Contrary to these reports, no significant effect of increasing stocking density from 4 to 8 prawn m^{-2} (Daniels *et al.*, 1995) and from 5 to 20 prawn m^{-2} (Siddiqui *et al*., 1997) was found on the mean percentages of male morphotypes of freshwater prawn. Cohen *et al*. (1981) and Cohen and Ra'anan (1983) have also reported stable proportions of male morphotypes in mature populations.

Survival rate of freshwater prawn in the treatments of the current study ranged from 88 to 90% that is similar with the finding of Tidwell *et al*. (2004a). A survival rate above 50% is considered acceptable by New and Singholka (1985). Malecha (1983) reported about 50 to 60% of prawn survival in continuous culture systems while Kunda *et al.* (2009) recorded a survival rate of 64% in their all-male freshwater prawn polyculture trials.

Periodic selective harvesting of the BC during the culture phase can improve relative biomass yields (Sagi and Aflalo, 2005; Rahman *et al.,* 2010). Similarly, sorting of BC, OC and SM from all tanks improved HIG effects of all-male by removing BC and OC, which was reared separately for sustainable production, and by reducing the stocking density. Isolation culture of BC, OC and SM freshwater prawn has also potential for increasing growth performance among OC and SM because of them having equal ability to compete for food and space among same group of males as well as increasing survival rate as a result of reduced stocking density.

After partial harvesting of the BC, cannibalism could still be present among the OC and SM in the rearing system. In the present study, SM population showed rapid growth and a 100% survival due to no cannibalism.

After isolation of BC and OC males, the stunted SM grew rapidly and developed into large BC males (Ra’anan and Cohen, 1985). It was found that the OC males surpass the BC males in absolute weight when reared in isolation culture. Isolated males transformed into the BC morphotype at a frequency three times higher than those in the communally cultured males (Karplus *et al*., 1991). The reason for this could be that the OC escaped from the BC and the SM evaded the BC & OC, hence, more space etc. This growth pattern, in isolation culture, was achieved mainly by delayed transition from the fast growing OC morphotype into the slow growing BC one, causing the male prawns attain a larger size upon metamorphosis (Karplus, 2005). This delay probably results from interaction among the male prawns. Male prawns studied under semi-natural conditions were found to be organized in a stable dominance hierarchy, sharing the same area with few low intensity aggressive interactions (Karplus and Harpaz, 1990).

As the absolute weight of the BC was significantly low in isolated culture, it would be better to sell it instead of wasting time and resources. In addition, the proportion of the OC from all male culture and the absolute weight of the OC males from the isolation culture were significantly high in this study; hence prawn farming through following this finding would be most profitable. After separating the BC and OC, the growth of the SM increased three times more than that of the BC with no mortality observed in our study. Therefore, after sorting of the BC, OC and SM from all-male culture, the OC and SM could be reared in isolated culture system until marketable size to make it a profitable and sustainable all-male prawn farming.

Increasing stocking density of all-male freshwater prawn had virtually no effect on water quality parameters; however it influenced the percentage of the BC, OC and SM in all-male culture. Although it is apparent that the percentage of the BC male prawn is significantly high with 21- 23% from stocking densities up to 30 juvenile m^{-2}, the highest SGR and final individual body weight was from 20 juvenile m^{-2} stocking density. In isolation culture, the SM prawn apparently displayed a compensatory growth response, through which the lower growth rates registered in the BC male population, showed three times more growth rate with no mortality during culture period in fiberglass tanks. Thus, the finding of present study can be used to improve the size variation management in all-male prawn farming.

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