



Neo-females production and all-male progeny of a cross between two Indian strains of prawn (*Macrobrachium rosenbergii*): Population structure and growth performance under different harvest strategies

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ABSTRACT

Culturing all-male giant freshwater prawn (*Macrobrachium rosenbergii*) presents a promising avenue for increasing yield and income. A sex reversal technology through androgenic gland (AG) manipulation was introduced, posing an increased risk of inbreeding. Thus, a scheme for Kerala (K) and West Bengal (WB) strains crossing using neo-females from one strain with males from the other strain was suggested.

Microsurgical sex reversal was applied in juvenile males of the Kerala strain at developmental stages of PL₁₅, PL₃₀, PL₄₅ and PL₆₀. Improved success rates of feminization were achieved when the intervention was performed at early developmental stages. Prawns operated at the PL₁₅ and PL₃₀ stages began developing ovaries as early as 105 days after metamorphosis and were able to produce offspring. A grow-out experiment in earthen ponds of all-male progeny originating from Kerala neo-female × West Bengal males was performed and the effect of selective harvest (SH) of <50 g prawns was compared to a final harvest (FH) strategy. The survival rate in the SH group was significantly higher than that in the FH group. Specific growth rate was significantly lower in the FH than in the SH group, while the feed conversion ratio was significantly lower in the SH than in the FH group. Distribution of fast-growing orange clawed (OC) males in the SH group was substantially narrower, peaking at 55–60 g, while in the FH group OC males were distributed over the size range of 30–150 g, suggesting further growth potential. Moreover, the terminally molted Blue Clawed (BC) males presented only small portion of the males; 7% and 0.7% in the FH and SH groups, respectively. The frequency of the large male (>100 g) marketable size group was significantly higher, and that of the medium-sized (5075 g) group was lower in the FH treatment in comparison with the SH.

The cross tested herein demonstrated substantially higher yield than that obtained in previous studies, however, no statistically significant difference in net productions was found between the FH and SH treatments (2207 ± 130 versus 2163 ± 137 kg ha⁻¹, respectively). Cost–benefit analysis after nine months of grow-out showed higher profit and higher benefit–cost ratio in the FH group. However, the SH treatment resulted in more uniform marketable prawns and suggested a continuous cash flow throughout the grow-out period.

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1. Introduction

The giant freshwater prawn *Macrobrachium rosenbergii* exhibits a bimodal growth pattern. Males grow larger and faster than females at maturity, and hence, there is an economical advantage in all-male cultures (Sagi and Aflalo, 2005). Experimental trials carried out in Israel (Cohen et al., 1988; Sagi et al., 1986), Vietnam (Aflalo et al., 2006), India (Nair

et al., 2006) and Bangladesh (Kunda et al., 2009; Rahman et al., 2010) revealed that an all-male freshwater prawn culture exhibits higher yields, higher average body weight at harvest and higher survival rates than either mixed-sex or all-female cultures under non-intensive conditions.

In decapod crustaceans, the order comprising crabs, crayfish, lobsters, prawns and shrimps, males possess a unique androgenic gland (AG) that regulates both male sex differentiation and male reproductive physiology. Unlike vertebrates, in crustaceans the endocrine and gametogenic functions are clearly separated into distinct organs, the AG and the testis respectively (Chang and Sagi, 2008). Thus, sex differentiation can be manipulated through the removal of the AG, without damaging

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the gonads, and such manipulations can play a key role in producing monosex cultures.

In previous studies carried out by our group, fully functional sex reversal of *M. rosenbergii* males, leading to the development of functional females capable of mating and producing progeny (neo-females) by AG ablation was achieved as late as 60 days post-larval stage (PL₆₀), but better success rates were achieved when the ablation was performed earlier: at 20–30 days post-larval stage (PL_{20–30}), prior to the development of external sex characters (Aflalo et al., 2006). These findings suggest that sex differentiation in *M. rosenbergii* is a dichotomous pathway, in which a 'decision' point for developing a male or a female reproductive system occurs. Before this point, the individual seems to be susceptible to changes, however, this option is lost when this point is passed in time ('point of no return'). Based on this, a feasible sex reversal procedure using two phases of microsurgical AG ablation and mass production of 'neo-females' capable of producing all-male progeny was established. Such neo-females could serve as broodstock for an all-male culture (Aflalo et al., 2006).

Even when stocked with the same cohort of PLs within a normal size distribution, the *M. rosenbergii* prawn population at harvest could be highly heterogeneous in size with three distinct male morphotypes (Kuris et al., 1987; Ra'anan and Cohen, 1985): the dominant, terminally molted blue-clawed males (BC); the fast growing, subordinate, orange-clawed males (OC); and the molting but not growing, small males (SM) (Karplus et al., 1989, 1992a; Peebles, 1979b). Ra'anan et al. (1991) reported approximately 50% SM, 40% OC and only 10% BC at harvest. The size variation in the *M. rosenbergii* male population is apparently not genetically-based; in the sense that it may be controlled by intra-population environmental social factors (Malecha et al., 1984). Conversely, according to Ranjeet and Kurup (2001), size disparity among male morphotypes could also have a genetic basis. As the market price of freshwater prawn heavily depends on size, such heterogeneous growth is one of the major bottlenecks for increasing profitability in its farming (Ranjeet and Kurup, 2002).

Strategies for farming freshwater prawns include variations in the rearing cycle period, and in the stocking and harvesting managements (Tidwell and D'Abramo, 2010; Valenti and Tidwell, 2006; Valenti et al., 2010). Prawns can be farmed for up to 6–9 month periods, when stocking newly-metamorphosed post-larvae or 15–60 day nursed juveniles (Karplus and Sagi, 2010; Tidwell and D'Abramo, 2010). Since *M. rosenbergii* prawns exhibit territorial and aggressive behavior (Peebles, 1979a, 1979b), dominant males inhibit growth of others and productivity cannot be managed only by increasing density and feeding input. Hence, strategies such as size grading, controlled densities and addition of substrates, for increasing final mean weight and productivity have been developed (Karplus et al., 1986, 1987; Tidwell et al., 2003, 2004a,b, 2005). Productivity seems to vary in different regions. Productivity above 2000 kg/ha has been obtained in temperate regions, with culture periods of 3–4 months preceded by a 60-day nursery period (Tidwell et al., 2003, 2004a,b). However, production at a commercial level of farming is approximately 50% of what has been reported in research trials (Tidwell et al., 2005). Productivity of 1280 kg/ha has been obtained in Saudi Arabia (1997) while in India Nair et al. (2006) reported 618 kg/ha after 5 months of grow-out.

Several strategies have been suggested for overcoming the size heterogeneity of freshwater prawn. First, Sagi and Aflalo (2005) advocated that selective harvesting (SH) of the large BC and OC prawns at 2–3 week intervals may be a suitable strategy in warm regions, under long culture periods. Selective harvesting may consist of the removal of dominant males and mature females throughout the rearing cycle by seining the ponds (Valenti et al., 2010). The animals removed are those exhibiting slower growth rates, and these may be sold before total harvesting – prolonging the time during which the product is available for commercialization. In addition, following SH the density in the pond decreases and dominant BC males are removed, allowing the growth of small animals (Karplus and Sagi, 2010).

Practices have also been developed to support intensification of prawn production rates without compromising average animal size or negatively impacting water quality. These include adding substrate materials to the production ponds prior to pond stocking to disrupt negative social interactions (Tidwell and D'Abramo, 2010).

In our previous study on leading Indian strains of prawns, to prevent inbreeding, we recommended the use of crosses of sex reversed males (neo-females) from the Kerala (K) strain with West-Bengal (WB) strain selected males (Aflalo et al., 2012). The current study aims at: (a) checking the favorable conditions and age of AG ablation for scaling-up the production of neo-females (sex reversed males), (b) Comparing the social structure, growth performances and economic analysis of the K × WB cross all-male *M. rosenbergii*, and (c) determining the effects of monthly selective harvest on growth and production as a yield improvement practice.

2. Materials and methods

2.1. Broodstock maintenance and mating

The study was carried out at the Scampi Broodstock Development Project of RGCA (Vijayawada, AP). Kerala strain (K) and West-Bengal strain (WB) *M. rosenbergii* founder stocks were collected in the wild and used to raise pure bred progeny (Aflalo et al., 2012). Females and males from each strain were stocked separately in 500 m² earthen ponds.

2.2. Androgenic gland ablation

Juvenile males, progeny of K neo-females at developmental stages of PL₁₅, PL₃₀, PL₄₅ and PL₆₀ were used for the AG ablation experiment. At each time point, 416–680 juveniles were dissected. Juvenile males were mounted dorsally on molding clay under a dissecting stereo microscope. The bases of the 5th walking legs were removed, as were the terminal ampulae, sperm ducts and the adjacent androgenic glands, using fine scissors and forceps. The appearance of male gonophores at the base of the 5th walking legs served as an indication for the failure of AG ablation. Juveniles were inspected under a dissecting stereoscope for gonophore appearance 30, 60 and 90 days after AG ablation. Juveniles with developed gonophores were discarded, and the remainder prawns were monitored for ovary development. Prawns showing ovary development were placed with males from the same strain for mating, and were monitored frequently.

2.3. Mating

Neo-females (from K strain) were produced by AG ablation technique as described by Aflalo et al. (2006). Neo-females with developed ovaries which gave 100% male progeny (confirmed by a progeny test) were selected according to their readiness for mating and kept with males from the reciprocal strain (WB). Morphologically intact, healthy and active Orange Claw (OC) or Blue Claw (BC) male brooders at the weight of 80–120 g were selected from the WB pure strain broodstock ponds located at the project farm.

The selected neo-females and male brooders were transferred to the hatchery and placed in 7 m³ breeding tanks. The mating tanks were monitored once a week and egg-buried neo-females were transferred to hatching tanks, marked properly and the eggs were allowed to hatch and larvicate.

Larvae from individual neo-females were stocked at a density of a 50 larvae/L and placed in the same hatching tank. Containers were aerated vigorously using air stones laid down on their bottom. Larval rearing employed an open water system that required a 20–40% daily water exchange. Larvae were fed with newly hatched *Artemia* nauplii (O.S.I. brand) throughout the larvication period.

At the end of the larvication period, 20,000 post-larvae (PL₅) from the above hatching were stocked in two cement tanks (nursery tanks) of 7 m³ capacity (10,000 PLs each). Appropriate and equal conditions including feed, density, shelter and aeration were provided to the nursery tanks. Feed was supplied ad libitum 6 times a day at 6 am, 10 am, 2 pm, 6 pm, 10 pm and 2 am. Feed was composed of: 35–42% protein; 7% fat; 2.5% ash; and 10% carbohydrates, within the formulation of crumble feeds (XL e-pack 600 μ, e-larvae 500 μ, from Water Base, Nellore, INVE) and *Artemia* biomass. The prawns were nursed for 30 days and at the end of the nursery period, growth, average length (mm) and average weight (g) were recorded. Nine thousand PLs (PL₃₅) were selected from the two nursery tanks and packed in nine styrofoam boxes (1000 PLs each) with water from the nursery tanks. Artificial seaweeds were added to the boxes before closing them. The closed boxes supplied with aeration were transferred to the farm for stocking.

2.4. Stocking PLs in the farm

Five hundred-liter tanks, equipped with aeration and shelters, were placed near each of the designated ponds and filled with water from the ponds. Each styrofoam box was unloaded into a designated tank. From each tank 900 PLs were stocked directly in the designated pond and 100 PLs were stocked in a small hapa net (1 m × 1 m × 1 m in size) placed in the same pond (to monitor survival at stocking). Twenty four hours after stocking, the hapa net PLs were counted to approximate the survival rate in each stocked pond. Mean body weight at stocking was 0.14 g (range 0.09–0.2 g).

2.5. Experimental groups and design

This experiment was composed of two groups: 1) all-male population (progeny of neo-females) with a single final harvest in three pond replicates (termed FH); and 2) all-male population combined with selective harvest in six pond replicates (termed SH). Average prawn weight at harvest and yields (kg/ha) were compared between the two groups.

Ponds in the farm were randomly assigned and marked according to the experimental design. The following water parameters were monitored and recorded: temperature (°C), pH, alkalinity (ppm), hardness (ppm) and transparency (Secchi disk depth, in cm).

2.6. Grow-out

Water exchange and replenishment to compensate for evaporation/seepage losses were performed on alternate days. Water quality parameters were monitored regularly and ranged as follows: temperature 25–32 °C, pH 7.9–8.5, transparency (Secchi disk depth) 25–33 cm, dissolved oxygen 4.8–6.8 ppm, hardness 128 ppm and alkalinity 131 ppm. Ponds were supplied with air for 6 h each day; 4 h in the early morning (2–6 am) and 2 h during the day (if required, depending on the weather). As the age and size of prawns increased, aeration time was increased. Ponds were provided with additional artificial habitat in the form of vertical nets (2.5 cm mesh size) for prawns to hide in, to reduce cannibalism. Twelve nets (1 × 5 m size) were installed in each experimental pond using bamboo sticks.

Feed was supplied 3 times daily and feed quantity was calculated per pond according to the prawn size in each pond as sampled biweekly using a cast net. Balanced feed pellets contained 35% protein, 4% fat, 7% fiber and 12% moisture (Waterbase Ltd., Nellore, India) were supplied during the grow-out period. Check trays were installed in each pond and were monitored regularly for feed consumption.

The grow-out experiment was conducted for nine months, with selective harvesting performed every month, from the fourth month onwards. The shelter nets were removed prior to the selective harvest and a dragging net (2.5 cm mesh size) was used to select the large sized prawns in the pond. The net was dragged along the pond length

on the bottom of the pond, four times in each pond and the harvested prawns were subjected to biometric measurements. In the FH ponds all the prawns were released back into their designated ponds while in the SH ponds, prawns weighing 50 g or more were removed from the experiment and the rest were released back into their designated ponds. Feed quantity was adjusted according to the estimated biomass of prawns remaining in each pond.

2.7. Data collection

The following variables were recorded or measured for each individual prawn at each selective harvest event and following the final harvest: body length (in mm), weight (in grams), and male morphotype [males were classified as BC, OC or SM as described by Kuris et al. (1987); large males without claws were classified as no-claw males (NC) and female reproductive stage (empty, with gonads, with eggs) was according to Hulata et al. (1990)]. Cumulative yield was calculated in every experimental pond and included the total biomass of harvested prawns (≥50 g). The total number of prawns harvested from each pond was recorded to test for differences in survival rates among treatments.

2.8. Economic analysis

A simple economic analysis was performed to estimate the profit (= total benefit – total cost) and benefit–cost ratio (BCR = total benefit ÷ total cost) in different treatments. The analysis takes in consideration the cost of: land rental, pond preparation, fertilizers and chemicals, electricity, fuel, shelters (vertical nets), seed, feed and labor. The prices of inputs and freshwater prawn correspond to the local Indian wholesale market prices in 2012. Prawns were graded and priced according to their weight (head count) as: >20, 14–20, 10–13 and <10 prawns per kg.

2.9. Data analysis

To test for the effect of AG ablation timing (PL₁₅, PL₃₀, PL₄₅ and PL₆₀) on failure rate (%/day) of sex reversal (appearance of male sexual characters) and rate of sex reversal expressed as ovary development (%/day), egg bearing and larval hatching (%/day) of AG ablated males, we used the Cox Proportional Hazards regression model. The model is given by the equation $\mu(t; z) = \mu_0(t) \exp(\beta z)$, where $\mu(t, z)$ represents the “hazard function” (i.e., male sex character development rate, ovary development rate, egg bearing or larval hatching rate); and $\mu_0(t)$ corresponds to the baseline hazard function rate that can change over time (t). The regression coefficient to be estimated, β , represents the independent effect of the timing of AG ablation (PL₁₅, PL₃₀, PL₄₅ and PL₆₀) on the hazard function appearance. The expected change in the different function rates following the AG ablation is represented by $\exp(\beta)$. For example, if $\exp(\beta)$ for sex reversal expressed as ovary development equals 5, a one day delay in AG ablation increases the overall rate of ovary development by a factor of 5. These analyses were performed using S-PLUS 2000 software (MathSoft, Inc., Cambridge, MA).

Differences in the proportion of surviving individuals per pond between the FH and SH groups in the grow-out experiment were tested using ANOVA. As accepted for proportions, an angular transformation ($\arcsin(\sqrt{p})$) was applied prior to the analysis, preventing the possible dependence between the variance and the mean (see Sokal and Rohlf, 1995). R × C test of independence – a contingency table test where R and C stand for the number of rows and columns in the frequency table respectively – was used to test for differences in the distribution of male morphotypes (BC, OC and SM) and male size class between the FH and the SH groups. The calculated statistics used were $G = 2 \times \ln(\text{likelihood ratio})$, which can be approximated by the χ^2 distribution (i.e. G-test of independence) (see Sokal and Rohlf, 1995). Specific growth rate (SGR) was calculated as $(\ln W_2 - \ln W_1) / \Delta T$ where ΔT is the number of days between times T₁ and T₂ and W is the measured

weight at T_1 and T_2 . Food conversion ratio (FCR) was calculated as: $FCR = G / R$, where R is the total food consumption between days T_1 and T_2 , and G is the observed increase in biomass during the same period (Brett, 1979). Differences in the FCR and SGR between the two experimental groups were tested using a two-samples t -test. Similar results were obtained using the non-parametric Mann–Whitney U test. Measurements within ponds such as size class yield or prawn weight among different size classes are dependent on each other, and thus cannot be considered as statistical replicates. To account for such dependency, split-plot ANOVAs were used to test for differences in the size class yield or in prawn weight among different size classes (i.e. within plot/pond treatment) between the two experimental groups (i.e. whole plot/pond treatment) using the following statistical model (Zar, 1999):

$$y_{ijlm} = \mu + C_i + p_l(c)_i + S_j + C_i S_j + p_l(c)_j S_j + e_{ijlm}$$

where y_{ijlm} is the observed weight of the m th individual or yield of the m th size class, μ is the overall mean, C_i is the fixed effect of the whole plot/pond treatment i th ($i = FH, SH$), $p_l(c)_i$ is the random effect of the l th pond ($l = 1, 2, \dots, 24$) nested within the i th treatment, S_j is the within plot/pond fixed treatment of j th size class ($j = 1, 2, \dots, 4$), $C_i S_j$ is the interaction effect of the i th treatment by the j th size class, $p_l(c)_j S_j$ is the interaction effect of l th pond ($l = 1, 2, \dots, 24$) nested within the i th treatment by the j th size class, and e_{ijlm} is the residual error of the m th individual. Pairwise differences in prawn weight among the different size classes of the two experimental groups (i.e. FH and SH) were evaluated using a Bonferroni post-hoc test. Differences in size class yield between the two experimental groups were tested using planned comparisons. $P < 0.05$ was defined as a statistically significant difference. All these analyses were performed using STATISTICA v9.0 (StatSoft, Ltd., Tulsa, OK).

3. Results

3.1. AG ablation and neo-female production

During the AG ablation experiment, 416–680 juvenile males were AG ablated at developmental stages of PL₁₅, PL₃₀, PL₄₅ and PL₆₀. Between 4 and 9% mortality was observed 24 h after AG ablation, attributed mainly to the microsurgical intervention. After 30 days of growth, the survival rate declined to 70–80%, reflecting natural mortality and cannibalism due to the experimental husbandry conditions such as provision of shelters and stoking density. During the 90 days after AG ablation the prawns were monitored for male gonopore appearance and proven males were discarded. From that time (90 days post-AG ablation) onwards, the remaining prawns were monitored twice a week for female sexual maturation, manifested by developed ovaries. These prawns were termed “neo-females” and were able to mate, and lay eggs which proceeded to larval hatching (see Fig. 1).

Time-to-event analysis (Cox proportional-hazards regression model) was used to test for differences in failure rate of sex reversal (male sexual development) and appearance rate of feminine reproductive stages (i.e., ovary development, egg laying and larval hatching) between the four AG ablated male groups (i.e. PL₁₅, PL₃₀, PL₄₅ and PL₆₀). The later the intervention was performed, the lower was the feminization success rate. In other words, every one-day delay in AG ablation resulted in a decrease of 1.8% in the rate of male sexual character appearance (data not shown), an overall reduction of: 3.3% in the rate of ovary development (Fig. 1A; $\exp(\beta) = 0.967$, $z = -6.2$, $P < 0.001$), 6.5% in the rate of egg carrying (Fig. 1B; $\exp(\beta) = 0.935$, $z = -6.72$, $P < 0.001$) and 9.8% in the rate of larval hatching (Fig. 1C; $\exp(\beta) = 0.902$, $z = -5.44$, $P < 0.001$). Prawns in the PL₁₅ and PL₃₀ AG ablated groups began developing ovaries as early as 105 days after metamorphosis and few of them even mated and produced offspring (larval hatching). Two hundred and thirty days after metamorphosis there were 60 prawns with developed ovaries in the PL₁₅ AG ablated group,

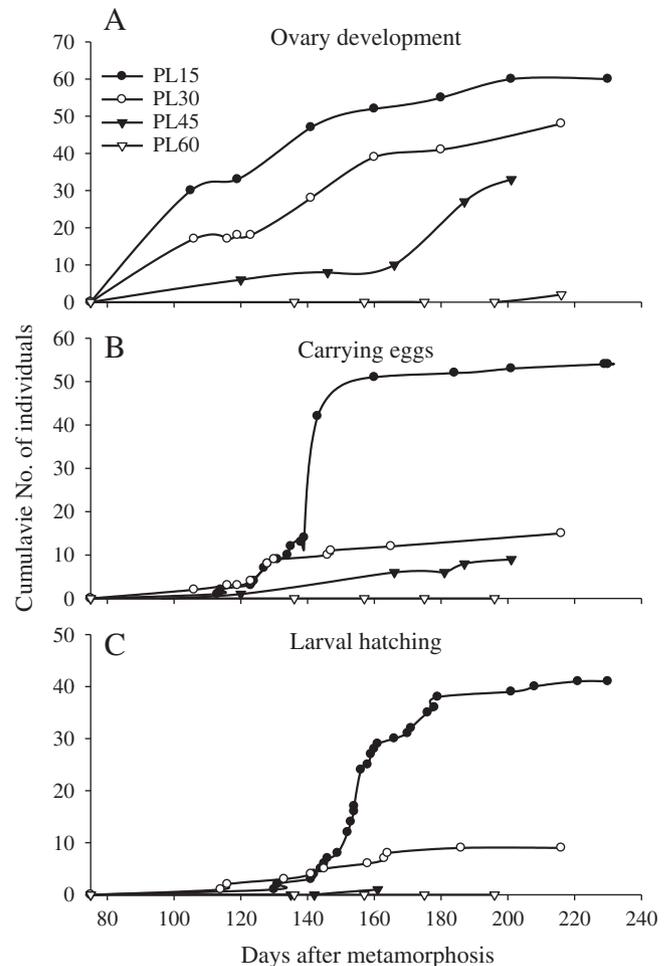


Fig. 1. Cumulative reproductive instances of neo-females of the Kerala strain, and rectomized at different post-larval stages (i.e. PL₁₅, PL₃₀, PL₄₅ and PL₆₀), showing ovary development (A), carrying eggs (B) and at larval hatching stage (C).

41 of them hatched. In the PL₃₀ AG ablated group 48 prawns developed ovaries, but only nine of them were able to produce offspring. By the same time only 2 prawns in the PL₆₀ AG ablated group exhibited ovarian development and both failed to produce offspring.

3.2. Population social structure

The distribution of male morphotypes in the FH (final harvest, Fig. 2A) and SH (cumulative selective harvest, Fig. 2B) groups was compared using the $R \times C$ test of independence. Male morphotype frequencies differed significantly between the FH and SH groups ($G = 377.57$; $Df = 2$, $P < 0.001$). The fast growing OC males presented a remarkable difference in distribution between the FH and SH groups. In the FH groups, OC were distributed over the size range of 30–150 g, while in the SH groups the size distribution of the OC males was substantially narrower, peaking at 55–60 g. BC males constituted only a small portion of the males; 7% and 0.7% in the FH and SH groups, respectively. In the FH group BC males reached the size of 200 g, while in the SH groups no BC males were found from the third selective harvest event.

3.3. Growth performances and yield

The frequency of the three different morphotypes differed significantly between the FH and SH groups: BC – 7.05% and 0.7% ($\chi^2 = 10.36$; $Df = 1$; $P < 0.001$), OC – 63.5% and 79.9% ($\chi^2 = 49.08$; $Df = 1$; $P < 0.001$), and SM – 19.2% and 11.2% ($\chi^2 = 38.4$; $Df = 1$; $P < 0.001$), in the FH and SH groups, respectively (Fig. 3A).

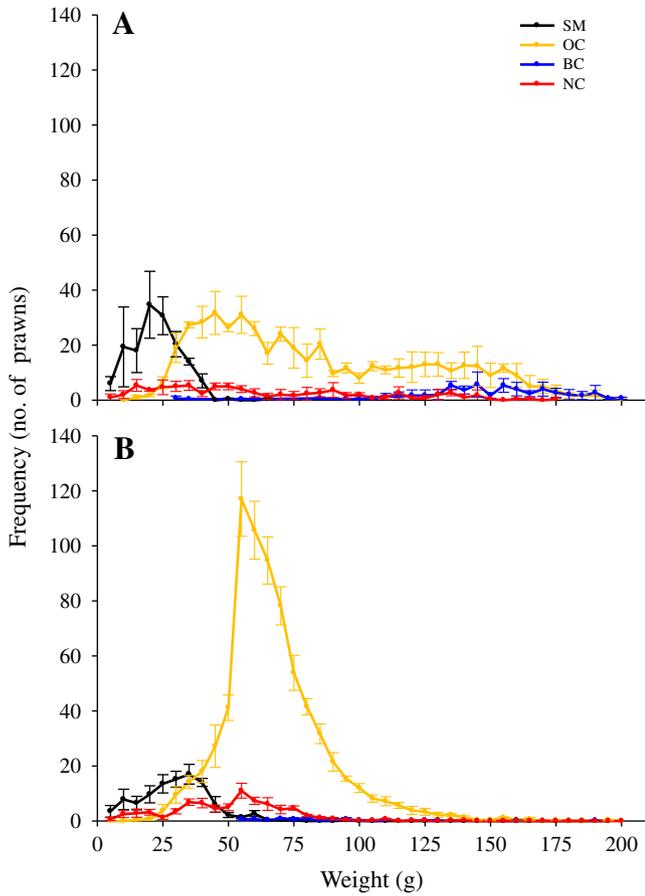


Fig. 2. Size distribution of the various male morphotypes showing the social structure of all-male populations of the Kerala neo-female \times West Bengal male cross under different harvest strategies: (A) a single final harvest at the end of the grow-out period (FH) and (B) cumulative consecutive selective harvests (SH). Morphotypes refer to: BC (Blue Claw), OC (Orange Claw), SM (Small Male), and NC (No Claw).

Mean weights of BC and of OC were significantly higher in the FH in comparison with the SH group (BC: 150.47 ± 14.42 g and 80.86 ± 4.6 g, OC: 82.04 ± 6.17 g and 64.99 ± 1.1 g, for FH and SH, respectively), while the opposite pattern was observed for the SM morphotype (21.16 ± 1.66 g and 28.76 ± 2.1 g, for FH and SH respectively) ($F_{2,14} = 17.97$, $P = 0.001$, a split plot ANOVA followed by a Bonferonni post-hoc test, Fig. 3B).

The survival rate of prawns in the SH group was calculated on the basis of the cumulative harvests (Fig. 4A) and was significantly higher compared to that of the intact FH group ($90.4 \pm 2.65\%$ and $76.3 \pm 5.74\%$, for SH and FH respectively; ANOVA, $F_{1,7} = 7.1417$, $P = 0.032$). SGR was significantly lower in the FH (1.57%) than in the SH (1.72%) group ($t = -11.33$; $Df = 7$; $P < 0.001$; Fig. 4B). Feed conversion ratio was significantly lower in the SH than in the FH group (0.65 ± 0.02 and 1.453 ± 0.04 , for SH and FH respectively; $t = -14.04$; $Df = 7$; $P < 0.001$; Fig. 4C). The frequency of the four different marketable size class prawns differed significantly between the FH and SH groups. However, these differences were not consistent between size classes: <10 (mainly BC males, above 100 g each) – 28% and 4%, ($\chi^2 = 128.24$; $Df = 1$; $P < 0.001$), <20 (less than 50 g each) – 41% and 25% ($\chi^2 = 12.47$; $Df = 1$; $P < 0.001$), and 14–20 (50–75 g, mostly OC males) – 16% and 55%, ($\chi^2 = 12.56$; $Df = 1$; $P < 0.001$), in the FH and SH groups, respectively (Fig. 5A). Mean weight of the >10 size-class was significantly higher in the FH than in the SH group (138.8 g and 118.3 g, respectively), while the opposite pattern was observed for the >20 size-class (28.42 g and 32.49 g, respectively) ($F_{3,21} = 17.46$, $P < 0.001$, a split plot ANOVA followed by a Bonferonni post-hoc test, Fig. 5B).

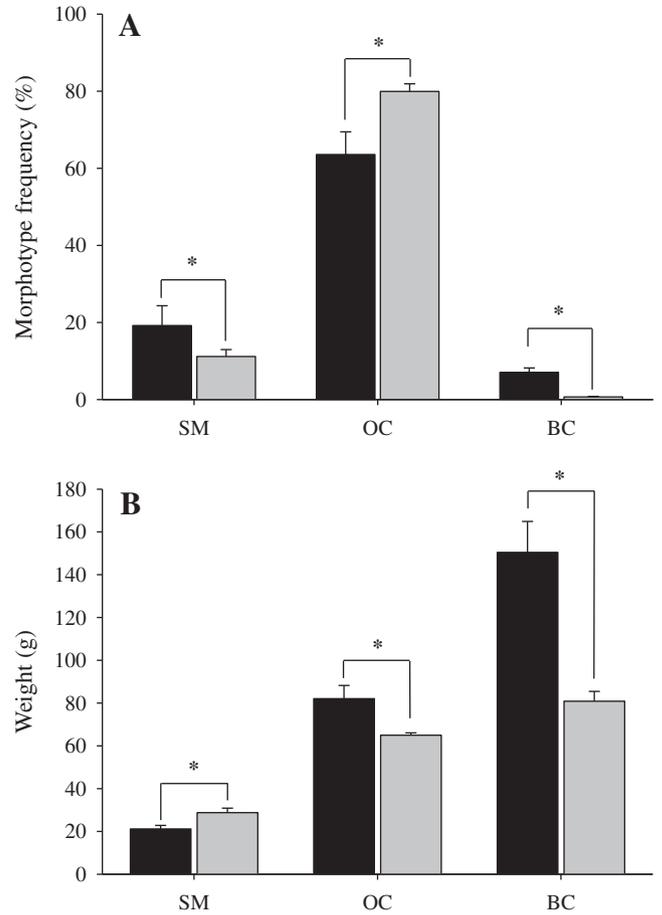


Fig. 3. Morphotype frequency (A) and average weight (B) in all-male populations of the Kerala neo-female \times West Bengal male cross under different harvest strategies: a single final harvest at the end of the grow-out period (FH) and cumulative consecutive selective harvests (SH). Morphotypes refer to: BC (Blue Claw), OC (Orange Claw), and SM (Small Male). Asterisk (*) on top of the column pairs represents significant differences ($P < 0.05$).

The yield per size-class and the cumulative yield are presented in Fig. 6. The largest prawns (mainly BC males, above 100 g each) were the main contributors to the total yield in the FH group, comprising 53% of the total yield (See Fig. 6A), while the main contributors to the cumulative yield of SH groups were individuals ranging in weight between 50 and 75 g (mostly OC males), comprising 56% of the total yield (see Fig. 6A).

The net production (cumulative yield) of the prawns was slightly higher in the FH treatment (2207 ± 130 kg ha $^{-1}$) compared to the SH (2163 ± 137 kg ha $^{-1}$), however, this trend was not statistically significant (Fig. 6B).

The average individual weight of cumulative harvested prawns (≥ 50 g) in the SH groups ranged from 62.4 to 72.9 g in the different sampling events, while in the FH group the weight of the harvest size prawns started at 60.9 g in the first sampling event, and increased to 107.1 g in the final harvest. These differences were mainly due to the harvesting of the large marketable prawns in the monthly selective harvest, leaving behind the slow growing individuals for the rest of the culture period.

The percentage of monthly selective harvested prawns (≥ 50 g) in the SH groups at different sampling events was 4.5–13.8% of the total stocked prawns, which constituted 60% in weight of the total harvested prawns.

3.4. Economic analysis of the yield

Cost–benefit analysis of the grow-out of FH and SH groups is presented in Table 1. The profit (334,606 and 212,191 INR/ha) and

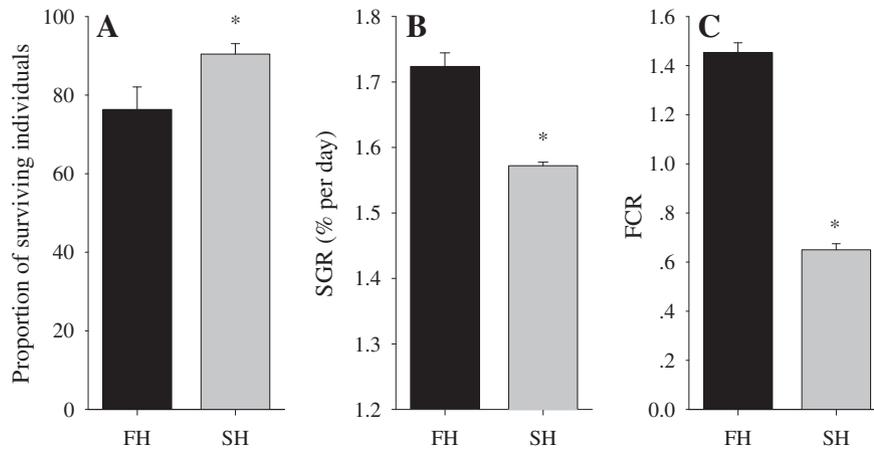


Fig. 4. Survival rate (A), specific growth rate (SGR) (B), and feed conversion ratio (FCR) (C) in all-male populations of the Kerala neo-female \times West Bengal male cross under different harvest strategies: a single final harvest at the end of the grow-out period (FH) and cumulative consecutive selective harvests (SH). The survival rate of prawns in treatment SH was calculated on the basis of the cumulative harvests. Asterisk (*) on top of the column pairs represents significant differences ($P < 0.05$).

benefit–cost ratio (BCR) (1.99 and 1.65) were higher in the FH than in the SH group. Prawn juveniles present an input of 11.5% of the total cost in both treatments. However, prawn feed cost was

markedly higher in the FH than in the SH group (32% and 14% of the total cost, respectively). Labor cost was higher in the SH group, reflecting the working hours needed in the monthly selective harvest events. Total income was significantly higher in FH than in the SH

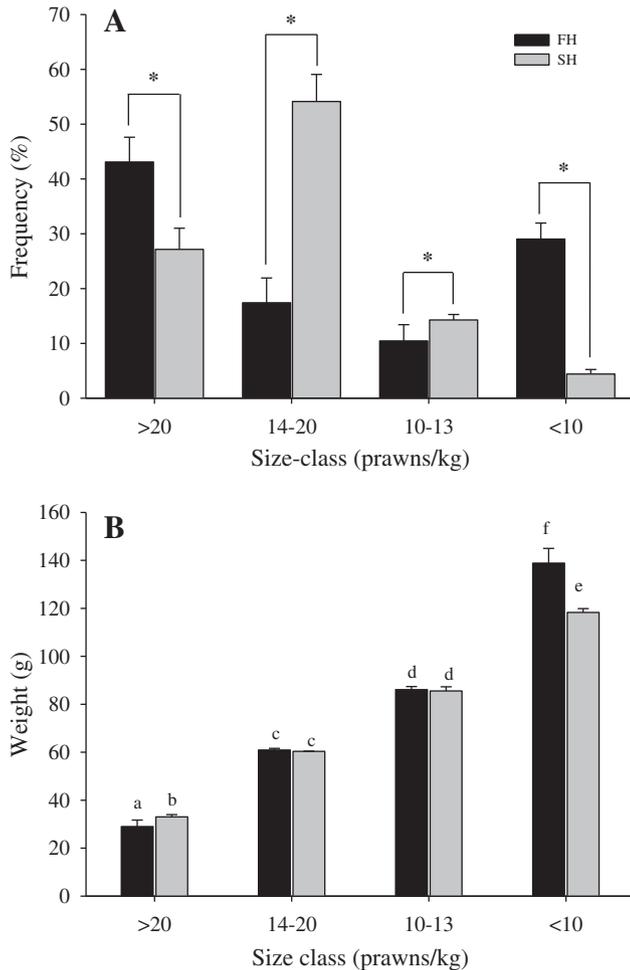


Fig. 5. Frequency (A) and average weight (B) of different size classes in all-male populations of the Kerala neo-female \times West Bengal male cross under different harvest strategies: a single final harvest at the end of the grow-out period (FH) and cumulative consecutive selective harvests (SH). Prawns were graded according to their weight (head count) as: >20 (more than 20 prawns per kg), 14–20 prawns per kg, 10–13 prawns per kg and <10 (less than 10 prawns per kg). Different letters on top of the columns represent significant differences ($P > 0.05$).

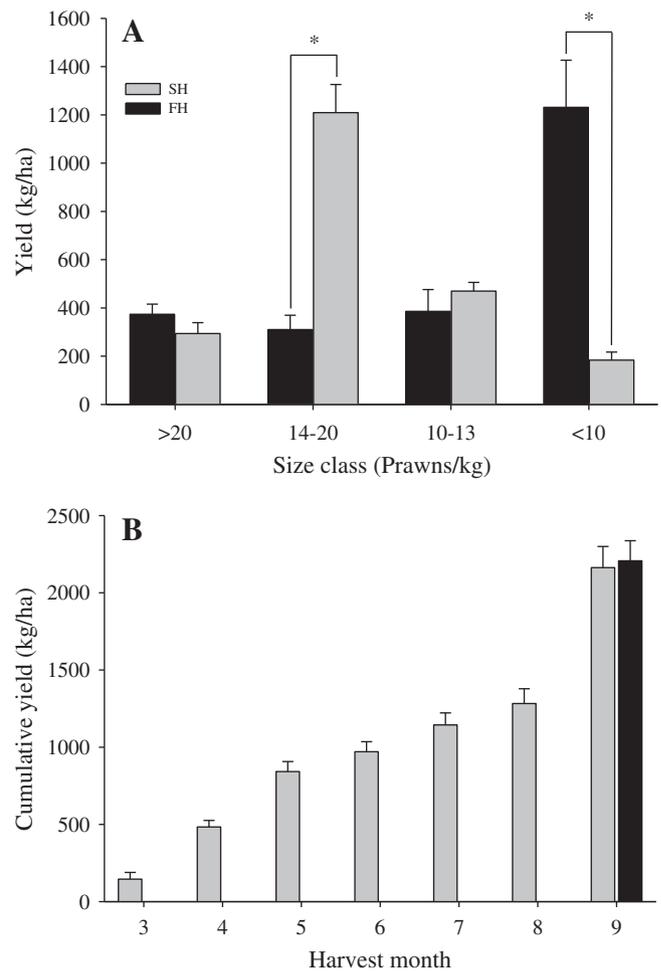


Fig. 6. Size class yield (A) and total cumulative yield (B) in all-male populations of the Kerala neo-female \times West Bengal male cross under different harvest strategies: a single final harvest at the end of the grow-out period (FH) and cumulative consecutive selective harvests (SH). Prawns were graded according to their weight (head count) as: >20 (more than 20 prawns per kg), 14–20 prawns per kg, 10–13 prawns per kg and <10 (less than 10 prawns per kg).

Table 1

Comparison of economics of single final harvest (FH) and cumulative selective harvest (SH) treatments calculated on the basis of a one-hectare pond.

Items	Quantity	Price rate (INR)	FH	SH
(A) Cost				
Land rent (9 months)	1 ha		30,000	30,000
Pond preparation	1 ha		10,000	10,000
Urea/superphosphate/manure	1 ha		2000	2000
Probiotics/other feed supplements	10 kg/ha		3000	3000
Liming	1 ha		3000	3000
Fuel/electricity	1 ha		50,000	70,000
Prawn juvenile	40,000	1 INR/PL	40,000	40,000
FCR			1.45	0.65
Feed			3204	1300
Feed cost		35 INR/kg	112,140	45,500
Vertical hideout nets	700 m/ha	50 INR/m	35,000	35,000
Labor	Worker/day	250 INR/worker/day	96,000	110,000
Harvesting	Worker/day	250 INR/worker/day	10,500	20,000
Production	kg/ha		2207	2114
Total cost (A)			391,640	338,500
(B) Benefit (return)				
Market price	<10/kg	350 INR	431,008	64,218
	10–13/kg	330 INR	127,230	155,056
	14–20/kg	220 INR	68,246	266,029
	>20/kg	180 INR	67,262	52,888
Total benefit (B)			693,746	538,191
(C) Profit (B – A)				
			302,106	199,691
(D) Benefit–cost ratio, BCR (B/A)				
			1.771	1.590

Figures in bold were included in the economic analysis.

group (693,746 and 538,191 INR/ha, respectively, Fig. 7A). Dividing the income according to the marketable size-class prawns showed that the major contribution to the total income in the FH group is attributed to the <10 size-class (431,007 INR/ha), while in the SH group the largest contribution came from the 14–20 size-class (266,028 INR/ha, Fig. 7B). The income of the SH group was distributed along the last 5 months of the grow-out period, as opposed to the income of the FH group that came only at the end of the grow-out period.

4. Discussion

Intervention in the sexual differentiation process in *M. rosenbergii* via manipulation of the AG according to the two-phase scheme introduced previously (Aflalo et al., 2006), results in full and functional sex-reversal and establishment of neo-females, capable of mating and producing all-male progeny. Higher rates of successful sex reversal were achieved when AG ablation was performed at early stages of development and sexual differentiation. These higher rates found in prawns that were ablated at PL₁₅ and PL₃₀ stage are in agreement with the “window of opportunity” for sexual differentiation as the earliest known point of sexual differentiation starts from PL₂₀ onwards, as observed with onset of the *M. rosenbergii* insulin-like androgenic gland hormone (*Mr-IAG*) expression in males (Ventura et al., 2011). Ablation of the AG closer to this starting point shifts the differentiation towards femaleness (Ventura et al., 2011). Rungsin et al. (2006) reported that the ability to identify male post-larvae at an early stage of development enhanced successful production of neo-females. Thus, we suggest that the most complete sex-reversal will occur when intervention is applied at early PL stages (PL_{15–30}). Moreover, the time required for mass production of all-male offspring was reduced by half when using the two-phase procedure under which operations were performed at an early stage without the need to identify the animal's gender (Aflalo et al., 2006). The results of the latter study paved the way to more focused and specific interventions in the sexual differentiation processes in crustaceans as recently demonstrated by temporal and transient *Mr-IAG* shutdown, using RNA interference, enabling complete and functional sex shift in *M. rosenbergii* (Ventura et al., 2009).

The use of the two phase technology (Aflalo et al., 2006) might create a genetic bottleneck and could lead to establishment of founder

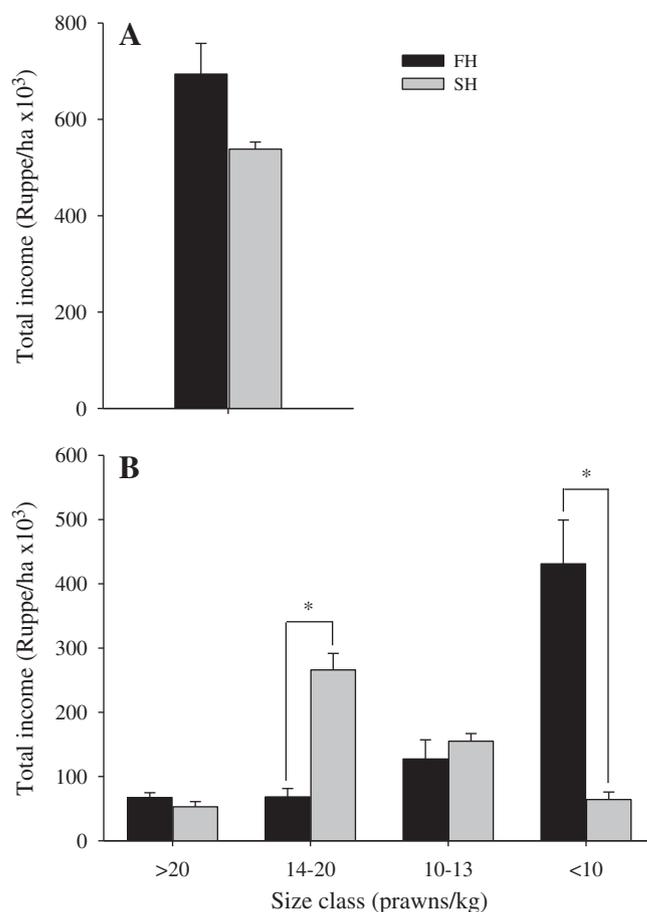


Fig. 7. Total income (A) and income by size class (B) in all-male populations of the Kerala neo-female × West Bengal male cross under different harvest strategies: a single final harvest at the end of the grow-out period (FH) and cumulative consecutive selective harvests (SH). Prawns were graded and priced according to their weight (head count) as: >20 (more than 20 prawns per kg), 14–20 prawns per kg, 10–13 prawns per kg and <10 (less than 10 prawns per kg).

effects, that could, in turn, lead to higher rate of inbreeding and/or genetic drift, as pointed out by Tave (1999). Thus, a combined scheme was suggested (Aflalo et al., 2012). The scheme suggests a selective breeding process on each of the leading Indian strains, producing new neo-females every generation to be mated with males of another selectively bred strain.

Based on the above, in the present study we demonstrated the use of crosses of well-performing Indian strains. We used neo-females of the Kerala strain crossed with West Bengal strain males to produce all-male progeny for the grow-out experiment. This cross combination was chosen due to its significantly higher growth performance as shown in a complete diallel cross experiment (Aflalo et al., 2012). The long grow-out period employed in this study was used in order to allow the development of complete social interaction and hierarchy between the male morphotypes and perform a more relevant demonstration suitable for warm and tropical environments of aquaculture conduct (Valenti et al., 2010).

The prawn survival rate in the present study was similar to that reported elsewhere (Tidwell et al., 2004a,b). The common reasons for mortality of prawn are intra-specific interactions and possible cannibalism among the prawns (Zimmerman and New, 2000). In the present study, we used vertical nets as shelters for the prawns, which were found to be efficient in reducing the mortality rate (Coyle et al., 2010). We found significantly higher survival rate in the SH treatment, probably since the periodic harvest of large male individuals (<50 g) reduced prawn density compared to FH treatment, minimizing the intra-specific competition for food, space and shelter as suggested by Fujimura and Okamoto (1972). The significantly higher numbers of SM at harvest found in the FH treatment indicate that large numbers of SM were not able to transform into BC through OC, and their growth was stunted by the suppression by BC males (Karplus et al., 1992a,b).

In the present study, we found that male morphotype distribution was significantly different between the FH and SH treatments, showing lower final calculated frequencies of SM and BC, and higher frequency of OC in the SH treatment. Karplus et al. (1989) suggested that the periodic selective harvests of BC in SH freed the SM of this treatment from the growth suppression phenomenon by BC. The significant higher average weight of the SM morphotype in the SH treatment in our study supports this idea. Conversely, Rahman et al. (2010) reported higher frequencies of BC in selective harvest treatment compared to control, and significantly reduced frequencies of SM.

The difference between the FH and the SH in regard to male morphotype frequency may be a result of differences in density after the initiation of selective harvests, as prawns from the SH were harvested along the culture period, reducing the density compared to FH treatment. It is known that increased density leads to early maturation and therefore, early appearance of BC males with a smaller body weight (Cohen et al., 1981). A significantly lower frequency of BC males in the FH in comparison with that in the SH treatments found in the present study indicates the growth potential of this cross (K × WB). In both treatments high frequencies of fast growing OC males were observed, suggesting that the density of four prawns/m² employed in this experiment could be increased, increasing yield and profitability with little or no effect on the growth and survival of the prawns, and should be further investigated under all-male selective harvest conditions. In addition, the high frequency of OC males obtained in the selective harvest events leads us to consider the possibility of increasing the size threshold set for the selective harvest since the OC males that were harvested have the potential to grow more.

Regarding the FCR of prawns, the SH treatment performed better, reflecting efficient utilization of feed by the prawns with the reduced densities following the selective harvests. In addition, in the SH treatment increased frequency of the fast growing OC males along with the reduced frequencies of BC males also contributed to efficient feed utilization (Karplus, 2005). Higher FCR values were reported when grow-out was studied under higher densities, indicating underutilization of

the feed by the prawns (Siddiqui et al., 1997) or feed utilization by other species when fish-prawn polyculture was employed (Rahman et al., 2010).

Cumulative yield did not significantly differ between the FH and SH treatments, reaching more than 2200 kg/ha, representing 72% increase in yield found by Siddiqui et al. (1997) under similar stocking density (1280 kg/ha) and 360% higher yield compared to Nair et al. (2006), who reported 618 kg/ha for all-male culture after 5 months of grow-out. The high yield found in the present study (2207 kg/ha) can be attributed to the combination of several factors: (a) improved growth performance of the K × WB cross as previously reported (Aflalo et al., 2012); (b) longer grow-out period that was employed in this experiment; and (c) the advantage of the male monosex culture practice of freshwater prawns, as previously suggested (Aflalo et al., 2006; Nair et al., 2006) and supported by comparing the total yield of the all-male culture in the present study with that obtained from a normal mixed population of the same cross combination (Aflalo et al., 2012), showing more than two-fold higher yield. Segregating the prawns into four marketable size classes showed that the larger prawns comprising the <10 prawn/kg size-class were the main contributors to the total yield (weight and frequency) and income in the FH treatment, while the main contributors (frequency) to the yield and income of the SH treatment were the size-class of 14–20 prawn/kg. This significant increase in the frequency of the 14–20 prawn/kg size class (comprised mostly of OC males) adds additional advantage by creating a more uniform marketable product.

Jose et al. (2003), in their comparative study, recorded BCR values of 1.71 and 1.35 in the selective harvest and batch harvest, respectively, which is similar to the findings of Rahman et al. (2010) who attained BCR of 1.71 in selective harvest and 1.24 in single final harvest. In the present study the FH treatment had a higher BCR, indicating that the labor costs for periodic selective harvest on the one hand, and the higher prices gained by the large prawns in the FH treatment on the other hand, were the main reasons for this difference. Despite the lower BCR found in the SH treatment, it presents an economically secure and a better management option in an all-male prawn culture for small and medium growers in India, suggesting continuous income along the season and allowing to cover the running expenses.

5. Conclusions

The present study demonstrates the advantage of all-male culture, progeny of *M. rosenbergii* neo-females. The contribution of the use of a cross between the Kerala and West Bengal strains is emphasized. The all-male culture in itself, regardless of harvest strategy, led to an increase in yield. An increase in stocking density should be tested since it seems to bear potential to further improve the yield.

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