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Toward a sustainable production of genetically improved all-male prawn (*Macrobrachium rosenbergii*): Evaluation of production traits and obtaining neo-females in three Indian strains

Eli D. Aflalo [a,b,*], Dandu V.S.N. Raju [c], Naidu A. Bommi [c], Johny T. Verghese [c], Thampi Y.C. Samraj [c], Gideon Hulata [d], Ofer Ovadia [a], Amir Sagi [a,b]

[a] Department of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel
[b] National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
[c] Scampi Broodstock Development Project, Rajiv Gandhi Centre for Aquaculture (RGCA), MPEDA, Ministry of Commerce & Industry, Government of India, AP, India
[d] Institute of Animal Science, Agricultural Research Organization, Bet Dagan, 50250, Israel

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ABSTRACT

Sex reversal technology, realized through androgenic gland (AG) manipulation, was recently introduced as a process for production of all-male producing broodstock. This technology exploits however, a relatively small number of sex-reversed broodstock. Thus, both genetic improvement via a breeding program and prevention of inbreeding are needed to ensure the sustainability of such technology. Three wild strains of prawns originating from geographically (though not necessarily genetically) isolated locations in India [Gujarat (G), Kerala (K) and West Bengal (WB)] were assessed for their suitability as breeders for all-male production. In addition, their potential for a selective breeding program was evaluated. A comparative evaluation of early sex segregation, sex reversal, growth performance, and population structure in the three selected strains was performed. Among the purebred strains, after eight months of grow out in earthen ponds, growth performance of the WB strain was the best (59.39 ± 1.08 g), while that of G was the poorest (26.50 ± 0.94 g). Strain-additive genetic effects for body weight at harvest were highest for the WB strain (+45.9%) and lowest for the G strain (−28.3%). Body masses of WB×K and WB×G crosses were 14.2% and 8.8% above the mean mass of the purebred strains, respectively, while that of the K×G cross was 23% below this value. In most crosses, males reached heavier mean body weights than did females with higher frequencies of the large male morphotypes being seen in the WB purebred strain and its respective crosses. Reciprocal effects for body mass ranged from 4% to 14.9% below the mean of the purebred strains. These negative signs mean that in the two crosses involving the WB strain, growth performance is higher when this stain was used as the sire strain. Similarly, the growth performance of the K×G cross was higher when the former was used as the sire strain. Average heterosis effect for body weight was minor (-0.51 ± 0.73) and did not differ significantly from zero. The high correlation between strain additive effects (the major source of variation in growth) and total performance for body weight ($r = 0.927$) indicate the existence of valuable genetic variation that could be exploited in a selective breeding program. For all-male production, males from the three strains were segregated at early post-larval stages and microsurgical AG removal was performed. In all the strains, similar low levels of complete sex reversal into functional neo-females (genetic males) were realized (0.17% −0.34%). These produced relatively small numbers of neo-female to be crossed with normal males to produce the desired all-male population, but raise the possibility that such a process could result in a genetic bottleneck. Thus, a genetic improvement scheme for each strain integrated with periodical crosses of the resulting neo-females from one strain with males from another strain is suggested to avoid inbreeding.

1. Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the most important crustacean species produced in inland aquaculture in many tropical and sub-tropical countries worldwide. In 2004, the total world farmed *M. rosenbergii* volume reached more than 194,000 tons, with an estimated market value that exceeded US $

* Corresponding author at: Department of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel. Tel.: +972 8 6479060; fax: +972 8 6479062.
E-mail address: aflaloe@bgu.ac.il (E.D. Aflalo).

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810 million, of which 99% was produced in Asia (FAO, 2009). India is the third largest producer of this species, after China and Thailand. Giant freshwater prawn production in India has increased from less than 178 tons in 1996 to 42,870 tons in 2005. However, reduction in productivity in major culture areas reduced total production to 12,806 tons in 2008 (FAO, 2009). During 2010, production reached an unprecedented low point only yielding 3721 tons (source: MPEDA, Min. of commerce and Industry, India). Productivity decline has also been reported in Taiwan and Thailand and was attributed to inbreeding depression in this species (Mather and de Bruyn, 2003). It was suggested that years of repeated culture of essentially unimproved stocks, caused declines in productivity in this species, that is of increasing concern to the industry (Mather and de Bruyn, 2003; Thanh et al., 2009). Thus, growth performance experiments and selective breeding programs were initiated to evaluate the genetic potential for selection of different strains of *M. rosenbergii* (Pillai et al., 2011; Thanh et al., 2010).

Significant productivity advances have been achieved via selective breeding programs in aquaculture in recent years, particularly in a number of fish species, including Atlantic salmon *Salmo salar* (Gjedrem, 2000; Quinton et al., 2005), Nile tilapia *Oreochromis niloticus* (Bentsen et al., 1998; Eknath et al., 2007; Ponzoni et al., 2005), and coho salmon *Oncorhynchus kisutch* (Hershberger et al., 1990; Neira et al., 2006), where improvements of up to 10–20%/generation have been achieved. In contrast, selective breeding programs have been initiated in only a few commercially important crustacean species (Jerry et al., 2005; Jones et al., 2000) and some marine penaeid prawn species (Argue et al., 2002; De Donato et al., 2005; Gitterle et al., 2005a; Gitterle et al., 2005b; Goyard et al., 2002; Hetzel et al., 2000; Preston et al., 2004). Essential to the successful development of any breeding program is reliable and species-specific information on phenotypic variation, non-additive genetic variation (e.g., heterosis) and additive genetic variation (i.e., heritability) (Gjerde, 1986). Selective breeding programs can provide significant economic benefit over the long term of operation. For example, the genetic improvement program for Nile tilapia has been highly beneficial with benefit:cost ratios ranging from 8.5 to 60 (Ponzoni et al., 2007).

In addition to selective breeding, monosex culture is a most promising avenue for increasing yields. Indeed, monosex culture has become a common practice in fish aquaculture (Beardmore et al., 2001; Devlin and Nagahama, 2002; Gomelsky, 2003). Attempts have been made to apply this aqua-technology to crustacean culture (Curtis and Jones, 1995; Lawrence, 2004; Lawrence et al., 2000; Sagi et al., 1986; Siddiqui et al., 1997) in species where male and females differ in terms of their growth rates, behavioral patterns and husbandry needs. Monosex all-male culture of *M. rosenbergii* has been carried out by hand segregation in normal populations, and results suggest possibility for increased yields (Sagi et al., 1986) up to a 60% increase in income (Nair et al., 2006). In recent years, a two-phase biotechnological approach to produce all-male populations via sex reversal of males was introduced (Aflalo et al., 2006). This biotechnology involves a two-generation manipulation of the sex differentiation process through androgenic gland (AG) ablation and creation of neo-females (genetic male with female phenotype) capable of producing all-male progeny. Due to relatively low rates of functional sex reversal however, this two-phase technology exploits a relatively small proportion of sex-reversed broodstock in the process of all-male production that could, in the long run, result in inbreeding depression.

In the current study, a combined approach was implemented in which three strains from wild populations of *M. rosenbergii* were collected from three discrete freshwater drainage systems in India and a diallel cross was set up. In parallel, an all-male producing scheme involving AG manipulation was applied to each of the three strains. We compared the relative performance of individuals from remote geographic localities, as well as the performance of all possible reciprocal crosses under earthen pond conditions over a long culture period to reflect real aquaculture conditions, with a focus on establishing sustainable all-male prawn culture.

2. Materials and methods

2.1. Collection of wild *M. rosenbergii* populations and broodstock conditioning

Wild *M. rosenbergii* populations were collected at random from three isolated river basins in India (see Fig. 1) to form founder stocks for development of new culture strains. The populations also represent isolated agri-ecological regions suitable for normal culture practices. The degree of genetic differentiation among the three populations was, however, not estimated. The first population originated from Gujarat (hereafter termed G), north–west India, from the Narmada and Tapti river basins and their main tributaries (72°59′55.10″E and 20°41′45.36″N). The second population was collected from Kerala (K), south–west India, from the Vembanad lake area (76°23′23.03″E and 9°44′43.6″N), while the third population was collected from West Bengal (WB), north–east India, from the Ganges river basin and its tributaries (88°23′52.43″E and 22°53′13.06″N). Mature male and female prawns were collected from the above three sites to ensure that the samples represented broad genetic variation. Two hundred females and 100 males were originally collected for each strain and strains stocked separately in 500 m^2 in earthen ponds. Air supply systems were installed in each pond and operated during the early morning (2 am–6 am) to maintain optimal dissolved oxygen concentrations.

2.2. Broodstock maintenance and mating

The study was carried out at the Scampi Broodstock Development Project of RGCA (Vijayawada, AP). After three months of conditioning, healthy adult male and female prawns were selected for breeding and transferred to 7 m^3 cement tanks in the hatchery. The prawns were fed ad libitum with a 50% protein commercial maturation prawn pellet (Lucky Star Industrial Co. Taiwan). In addition, live feed, including squid, clam, beef liver and polychete worms, was supplemented. The design of the study comprised a complete 3×3 diallel cross, including three purebred strains ($G\times G$, $K\times K$ and $WB\times WB$) and six reciprocal crosses (the female parental strain is identified first in each cross). Female and male parents were chosen carefully based on the characteristics described by Sagi and Ra'anan (1985) to maximize mating success. Females with orange colored ovaries that occupied a large area of the cephalothorax were preferred. Males with a healthy appearance and long, thick, dark blue claws (BC males, (Kuris et al., 1987)) were obtained for mating with sexually receptive females. Breeding was carried out simultaneously in nine tanks equipped with shelters that were large enough to accommodate 12 females and six BC males (starting at the end of August, 2009). Females usually laid eggs within 1–3 days if they had mated successfully. Males were removed after the females became berried. Berried females were checked every 3 days for early embryonic development. Females carrying fertilized eggs were maintained in brood baskets for 15–18 days until the eggs had changed color to dust gray. At this stage, females were transferred to the hatchery for egg hatching in 100 l tanks. Hatching was carried out per single female (3–5 females were hatched synchronously from each cross). Larvae from individual females of each strain were stocked at a density of 50 larvae/l and placed in the same hatching containers. Containers were aerated vigorously using air stones laid down on the bottom. Larval rearing employed an open water system that required daily 20–40% water exchanges. Larvae were fed with newly hatched and frozen *Artemia* nauplii (O.S.I brand) for the first 4 days. Microencapsulated feeds (INVE, Belgium), and *Artemia* flakes (O.S.I brand, USA) were supplemented. Egg custard (egg, milk powder, shrimp or squid flesh, clam meat, beef liver, corn flour, soya

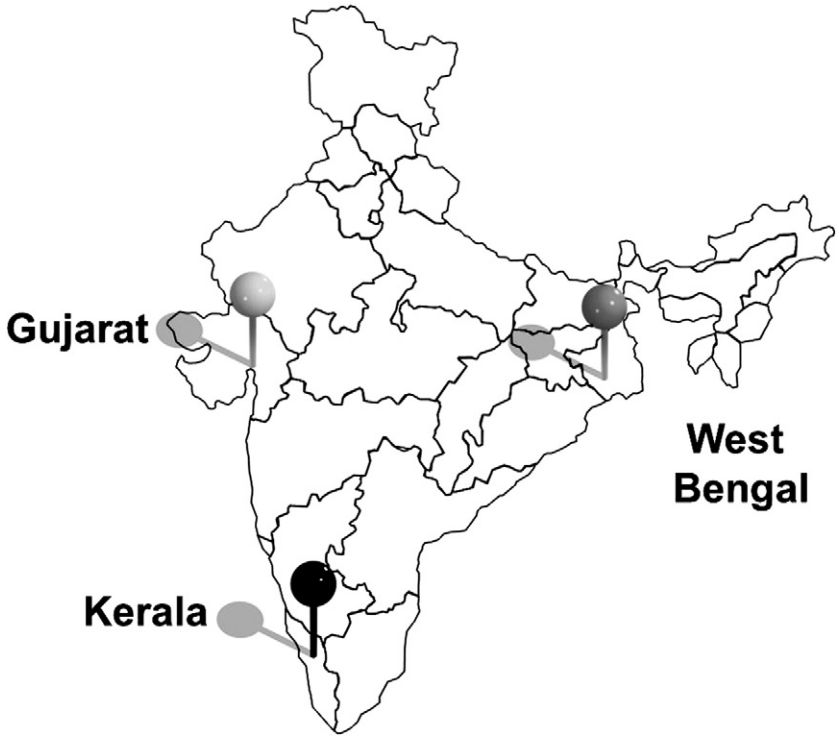

Fig. 1. Map of India, with circles representing the three different and distinct geographically agro-ecological regions from which *M. rosenbergii* strains were collected. Gujarat (North West), Kerala (South West) and West Bengal (North East) are indicated.

flour and shark liver oil) were provided starting from Day 11 and onwards. Synchronization of the experiment was achieved by allowing a maximum gap of only 13 days between hatching the first to the last females from all crosses raising 50,000–90,000 Post-larvae individuals (PLs) from each cross.

2.3. Juvenile production

PLs from each cross were mixed and two populations of 13,000 PLs (approx. 1850 PLs/m^3) were stocked at a density of 1.5−2 PL/l into 7 m^3 in cement tanks for a primary nursery stage of at least 30 days; maximal nursery duration was 35 days. Continuous aeration was provided and shelters covering 70% of the water volume of the tank were added. About 150% of the water was exchanged daily. A pelleted feed containing 35–42% protein (E-Larva500- Lucky Star Industrial Co., E-Pac XL- INVE, and farm Starter feeds, Waterbase, Nellore, India) was offered ad libitum. The first group of PLs started the nursery stage on November 2, 2009, and the last group began this stage on November 14, 2009. Following the nursery stage, juveniles were gradually transferred to the experimental ponds, one cross per day. The process of stocking for each cross began with a random sample of 2000 (+ some extra) juveniles from each of the two nursery tanks from any given cross. These juveniles, represented progeny from 3 to 5 females per cross, were mixed and transferred to the farm. The juveniles were then separated into four groups of exactly 1000 each and stocked at 4/m^2 into four replicated 250 m^2 earthen ponds designated and marked for each specific cross.

2.4. Grow out experiment

Water filling and replenishment of 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) — 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 hours (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 coconut leaves for prawns to hide in and under to reduce cannibalism. A nutritious balanced commercial pellet feed (Waterbase) containing: 35% protein, 4% fat, 7% fiber and 12% moisture was used. Feed was provided three times daily and rationed according to the recommendations of the feed manufacturer. Check-tray monitoring was done regularly to adjust the feeding regime as per the feed manufacturer's suggestion. Grow-out rearing lasted for 8 months in a total of 36 ponds (nine crosses within and among three strains using four replicates). Mean body weight at stocking was 0.14 g (range 0.09–0.2 g). Three sampling events of 60 individuals per pond captured randomly using a cast net were done at 2, 4 and 6 months (according to the stocking order) from stocking, respectively. At the end of the rearing period, all prawns were collected, one strain each day in the same order as for stocking so that the number of culture days was equal in all ponds (total of 243 culture days).

2.5. Data collection

The following variables were recorded or measured for each individual prawn at each sampling and at the end of the rearing period: gender (male or female), body length (in mm), weight (in g), male morphotype [males were classified as either blue claw (BC), orange claw (OC) or small claw males (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 classified according to Hulata et al. (1990)]. The total number of prawns harvested from each pond was recorded to calculate relative survival rates among crosses. Growth performance here is presented only as body mass since this trait was found to correlate with increment in body length [the correlation between body mass and body length was significant ($P>0.05$), and ranged from r = 0.79 to r = 0.93].

2.6. Segregation of juvenile males for AG ablation

Juvenile males at PL_{25-35} were segregated from the above three purebred strain populations (prepared according to Sections 2.2 and 2.3). Male juveniles were selected following the identification of the genital papillae or *appendix masculina* after examination under a stereo microscope (Aflalo et al., 2006). Weight range of the segregated males ranged from 0.1 to 0.45 g.

2.7. Androgenic gland ablation

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. As part of the quality control process, one of the second pleopods bearing the *appendix masculina* was also ablated. Monitoring the regeneration of the *appendix masculina* served as an indicator for the success/failure of AG ablation. Juveniles were inspected under a dissecting stereoscope for regeneration of *appendix masculina* 30, 60 and 90 days after AG ablation. Juveniles with regenerated *appendix masculina* were discarded. The non-regenerated *appendix masculina* prawns were monitored continuously for ovary development.

2.8. Data analysis

The following statistical mixed model was used to analyze growth traits (body weight and body length at harvest):

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

where y_{ijlm} is the observed growth performance of the mth individual, μ is the overall mean, C_i is the fixed effect of the i^{th} cross (strain) combination (i = 1, 2, …, 9), S_j is the fixed effect of j^{th} sex ($j = 1, 2$), $(CS)_{ij}$ is the interaction effect of the i^{th} cross combination by the j^{th} sex, $p_l(c)_i$ is the random effect of the l^{th} pond (l = 1, 2, …, 36) nested within the i^{th} cross, e_{ijlm} is the residual error of the m^{th} individual.

Least squares means were calculated for the two growth traits from each cross and significant mean differences were examined using a Tukey post-hoc test. Survival rates, proportions of males and females and proportions of morphotypes in males/reproductive status in females were analyzed using a generalized linear model (GLM) after angular transformation.

A second model was used to evaluate each of the genetic effects (strain additive, general reciprocal and total heterosis) of growth performance:

$$y_{lijm} = \mu + S_l + \sum_i a_i t_i + \sum_i r_i w_i + \sum_{ij} h_{ij} t_{ij} + e_{lijm}$$

where y_{lijm} is the recorded growth performance of the m^{th} individual of the i^{th} and j^{th} strain combination ($i = 1, 2, 3$; $j = 1, 2, 3$), μ is a constant, S_l is the fixed effect of the l^{th} sex ($l = 1, 2$), a_i is the regression coefficient of the additive genetic effect of the i^{th} strain on the proportion of genes of the strain ($i = 1, 2, 3$), t_i is the proportion of genes in the m^{th} individual originating from the i^{th} strain ($t_i = 0.0$, 0.5 or 1.0 and $\sum t_i = 1.0$), r_i is the regression coefficient of the reciprocal effect of the i^{th} strain on the proportion of genes of the strain, w_i is the proportion of genes of the m^{th} individual of the i^{th} strain ($w_i = 0.0$ for purebreds, $w_i = -0.5$ for sire strain origin, $w_i = 0.5$ for dam strain origin and $\Sigma w_i = 0.0$). h_{ij} is the regression coefficient of the total heterosis effect for the i^{th} and j^{th} strain combination on the proportion of genes originating from both reciprocals of the i^{th} and j^{th} strains ($i \neq j$ and $ij = ji$; thus $ij = 1, 2, 3$; $\sum h_{ij} = 0.0$), t_{ij} is the proportion of genes in the m^{th} offspring of the i^{th} and j^{th} strain combination ($t_{ij} = 0.0$ for $i = j$, $t_{ij} = t_{ji} = 1.0$ for $i \neq j$, $\sum t_{ij} = 0.0$ for purebreds and $\sum t_{ij} = 1.0$ for crossbreds), and e_{lijm} is a random error for the m^{th} individual. The strain additive and strain reciprocal effects were restricted so that $\sum_i a_i = \sum_i r_i = 0$. Similarly, S_l was restricted so that $\sum S_l = 0$. Under these restrictions, μ is the mean growth performance of the three purebred strains, meaning that strain additive (a_i), reciprocal (r_i) and total heterosis (h_{ij}) effects are expressed as a deviation from this value.

Total heterosis $h_{ij} = h + h_i + h_j + s_{ij}$ (Gardner and Eberhart, 1966) of a particular strain cross was partitioned into average heterosis (h), general strain heterosis (h_i and h_j) and specific heterosis (s_{ij}). Total heterosis (h_{ij}) pools records of both reciprocals, therefore, average heterosis was calculated as the mean of total heterosis estimates ($h = \frac{1}{3}\sum_1^3 h_{ij}$). The general heterosis effect of i^{th} strain was calculated as the mean of the total heterosis estimates from all crosses involving the i^{th} strain, expressed as a deviation from the average heterosis, i.e., $h_i = \frac{1}{2}\sum_{j=1}^{2} h_{ij} - h$ and with $\sum_i h_i = 0$ and $\sum_i s_{ij} = \sum_i s_{ji} = 0$ as required restrictions (Gardner and Eberhart, 1966). Specific strain heterosis was calculated as $s_{ij} = h_{ij} - (h + h_i + h_j)$.

3. Results

Total harvest biomass of the crosses at the end of the culture period ranged from 3.3 ± 2.2 kg/pond (K×G) to 31 ± 1.2 kg/pond (K×WB) (see Fig. 2A). These differences are mainly attributed to the contribution of specific strains (e.g. all the highest yielding ponds involved at least one WB parent) rather than survival rates among the ponds (Fig. 2B).

3.1. Relative growth performance of purebred strains and crosses

Least squares means (LSM) of body weight at harvest for purebred strains and crosses are presented in Fig. 3. The WB strain grew significantly faster than did the G and K strains (Fig. 3A) and was ranked 1st in performance. The purebred G strain and the K×G cross grew slower than did most cross combinations and were ranked 8th and 9th in performance, respectively (Fig. 3A). All crosses that included the WB strain as either sire or dam showed better growth performance (Fig. 3A), as compared with reciprocal purebred strains. In the K purebred strain and in all cross combinations involving K as either a sire or dam (except K×G), the growth of males was significantly better than that of females (Fig. 3B). The largest differences in the growth performance between genders were found in the K purebred strain and its sire crosses (Fig. 3B). Since body weight and body length were highly correlated (ranging from r = 0.79 to r = 0.93, $P > 0.05$), only growth performance in term of body mass was presented.

3.2. Strain additive genetic effects

Among the three purebred strains, the growth performance of the WB strain was the highest, while that of the G strain was the lowest. Body mass and body length of the WB strain were 45.9% and 17.7% higher than were the means of the purebred strains for these traits, respectively (Table 1). The G and K strains showed 28.3% and 17.6% lighter mean body mass, as compared with the purebred strains, respectively (Table 1). Similarly, their body lengths were 14.1% and 3.6% below the mean of the purebred strains, respectively (Table 1). Among the three crossbred strains, the growth performance of the two crosses involving WB was higher than that of the K×G cross. Body masses of the WB×G and the WB×K crosses were 8.8% and 14.2% above the mean mass of the purebred strains, respectively (Table 1). Similarly, their body lengths were 1.8% and 7.1% above the mean length of the purebred strains, respectively (Table 1). In contrast, body mass and body length of the K×G cross were 23% and 8.8% below the means of the purebred strains for these growth traits, respectively (Table 1).

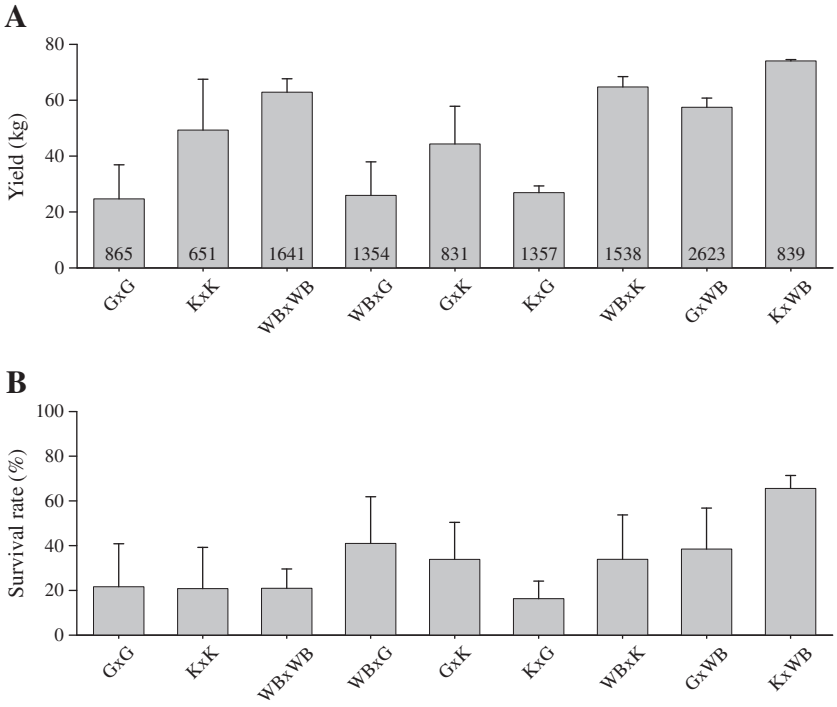

Fig. 2. Means with standard errors of the total harvest biomass per pond (A), and survival rate (B) of each of the purebreds and crosses at the end of the rearing period. G × G (Gujarat × Gujarat), K × G (Kerala × Gujarat), WB × G (West Bengal × Gujarat), G × K (Gujarat × Kerala), K × K (Kerala × Kerala), WB × K (West Bengal × Kerala), G × WB (Gujarat × West Bengal), K × WB (Kerala × West Bengal), WB × WB (West Bengal × West Bengal). Numbers on columns = *n*.

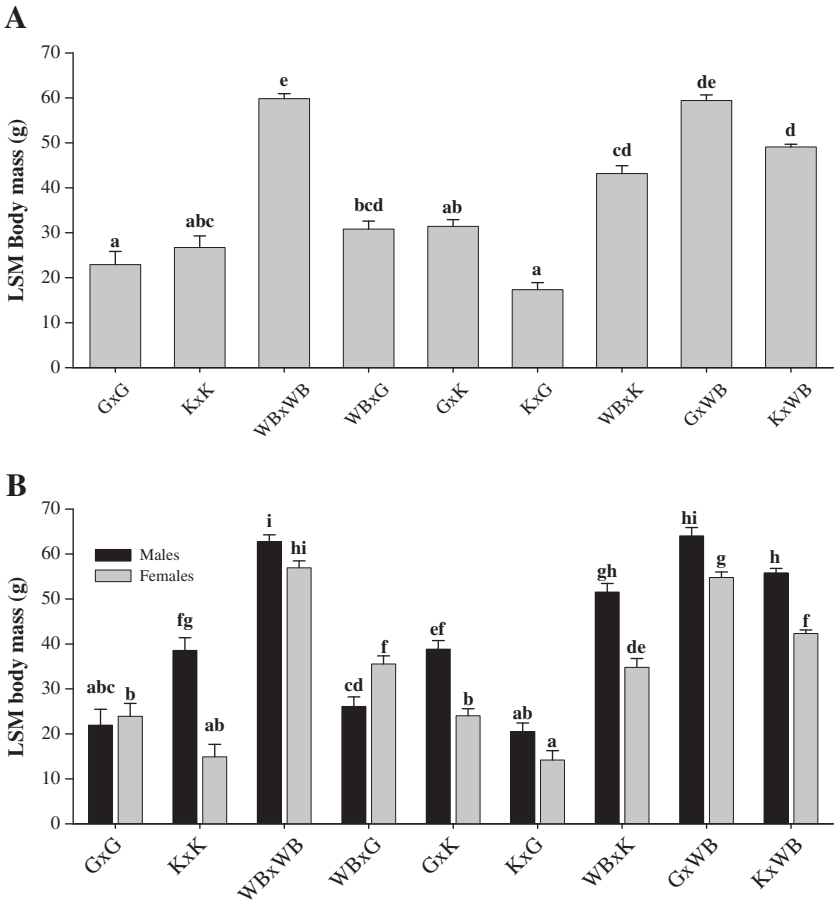

Fig. 3. Least squares means of body weight and standard errors of mixed population (A), and male/female individuals (B) of purebred strains and their reciprocal cross combinations estimated using the mixed model (Model 1). Males — dark columns; females — white columns. G × G (Gujarat × Gujarat), K × K (Kerala × Kerala), WB × WB (West Bengal × West Bengal), K × G (Kerala × Gujarat), WB × G (West Bengal × Gujarat), G × K (Gujarat × Kerala), WB × K (West Bengal × Kerala), G × WB (Gujarat × West Bengal), K × WB (Kerala × West Bengal). Different letters on top of the columns represent statistical differences ($P > 0.05$).

3.3. Strain reciprocal effects

Following Thanh et al. (2010), we considered the cause of the reciprocal effects to be unknown and of the same magnitude but with reverse signs for sires and dams of the same strain. Specifically, the coding of the reciprocal effects was set at 0 for purebreds, −0.5 for sire strain origin, and 0.5 for dam strain origin. All reciprocal effects for body mass were negative, ranging from 4% to 14.9% below the mean of the purebred strains (Table 2). Similarly, all reciprocal effects for body length were negative, ranging from 0.5% to 6.7% below the mean of the purebred strains, although the effect of the WB × K cross did not vary significantly from zero (Table 2). These negative signs mean that in the two crosses involving the WB strain, growth performance was higher when this strain was used as the sire strain. Similarly, the growth performance of the K × G cross was higher when the former was used as the sire strain.

3.4. Strain total, average and general heterosis

General heterosis for body mass for both the WB and G strains were positive (4.5% and 0.2% above the mean of the purebred strains, respectively), with only the former differing significantly from zero (Table 3). The respective K general heterosis values were 4.7% below the mean of the purebred strains (Table 3). All general heterosis effects for body length differed significantly from zero, with the WB strain being 4.6% above, and G and K strains being 1% and 3.6% below the mean of the purebred strains, respectively (Table 3).

Total heterosis for body mass of the WB × G cross was 8.1% above the mean of the purebred strains (Table 3). In contrast, the respective effects of both K × G and WB × K crosses were negative, being 10.3% and 1.6% below the mean of the purebred strains, with only the former effect differing significantly from zero (Table 3). Total heterosis effects for body length of both the WB × G and the WB × K crosses were 7.9% and 2.6% above the mean of the purebred strains, respectively (Table 3). Again, the respective effect of the K × G cross was negative, being 8.4% below the mean of the purebred strains (Table 3). Consequently, the average heterosis effects of both traits (−0.514 ± 0.726 body mass; 0.773 ± 0.779 body length) did not differ significantly from zero (Table 3).

3.5. Total performance

The correlation between additive effect and total performance was high for both growth traits (body mass: r = 0.927, $P<0.001$; body length: r = 0.875, $P=0.002$), indicating that additive genetic effects can largely explain total growth performance.

Table 1
Estimates of additive genetic effects for body weight and body length, in measurement units and as a percentage of the means of the purebred strains, for these growth traits.

Trait	Body mass (g)		Body length (mm)	
	Estimate ± SE	%	Estimate ± SE	%
Mean of pure strains	40.713 ± 0.639***		106.557 ± 0.686***	
Pure strain				
G	−11.523 ± 0.901***	−28.30	−15.019 ± 0.967***	−14.09
K	−7.166 ± 0.904***	−17.60	−3.796 ± 0.969***	−3.56
WB	18.689 ± 0.904***	45.90	18.815 ± 0.970***	17.66
Crosses				
K × G	−9.345 ± 0.452***	−22.95	−9.408 ± 0.485***	−8.83
WB × G	3.583 ± 0.452***	8.80*	1.898 ± 0.485***	1.78
WB × K	5.762 ± 0.451***	14.15**	7.510 ± 0.484***	7.05

G = Gujarat, K = Kerala, WB = West Bengal. Crosses = female × male.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

Table 2
Estimates of reciprocal effects for body weight and body length, in measurement units and as a percentage of the means of the purebred strains, for these growth traits.

Trait	Body mass (g)		Body length (mm)	
	Estimate ± SE	%	Estimate ± SE	%
Mean of pure strains	40.713 ± 0.639***		106.557 ± 0.686***	
Reciprocal crosses				
K × G	−4.456 ± 0.546***	−10.94	−6.631 ± 0.585***	−6.22
WB × G	−6.077 ± 0.486***	−14.93*	−7.178 ± 0.522***	−6.74
WB × K	−1.621 ± 0.466***	−3.98**	−0.547 ± 0.500	−0.51

G = Gujarat, K = Kerala, WB = West Bengal. Crosses = female × male.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

3.6. Morphotypic differentiation and reproductive development

A calculation of the proportion of different male morphotypes (among males only) showed that in the purebred WB strain and in its reciprocal crosses with the K strain, larger proportions of the large morphotypes (BC and OC) were present along with the smallest proportion of the SM morphotype (Fig. 4). In addition, we calculated the proportion of different reproductive stages in females (among females only), a higher proportion of the females in progressive reproductive stages (bearing developed gonads, berried or spent) were found when the purebred WB strain and its respective crosses served as sires (Fig. 5).

3.7. Sex reversal via AG ablation

The percentages of males clearly identified using the appearance of *appendix masculina* and/or genital papillae at different developmental stages are presented in Table 4. At PL_{30} and below, only a small fraction of the segregated prawns were found to be males (1.26% and 1.70% in the WB and K strains, respectively), while at PL_{90} and above, up to 7.87% males were detected in the G strain (Table 4). These segregated males were AG-ablated and a strict quality control process was performed to ensure the success of AG removal and sex reversal in the treated prawns. Survival rate of prawns 24 h after microsurgical AG ablation varied between 37.5% and 93.7%, with the highest survival rate being found in the $PL_{46–50}$ stage for all strains

Table 3
Estimates of general, specific, total and average heterosis for body weight and body length, in measurement units and as a percentage of the means of the purebred strains, for these growth traits.

Trait	Body mass (g)		Body length (mm)	
	Estimate ± SE	%	Estimate ± SE	%
Mean of pure strains	40.713 ± 0.639***		106.557 ± 0.686***	
General heterosis				
G	0.070 ± 0.327	0.17	−1.019 ± 0.351**	−0.96
K	−1.899 ± 0.331***	−4.67	−3.828 ± 0.355***	−3.59
WB	1.830 ± 0.362***	4.49	4.847 ± 0.389***	4.55
Specific heterosis				
K × G	−1.830 ± 0.362***	−4.49	−4.847 ± 0.389***	−4.55
WB × G	1.899 ± 0.331***	4.67	3.828 ± 0.355***	3.59
WB × K	−0.070 ± 0.327	−0.17	1.019 ± 0.351**	0.96
Total heterosis				
K × G	−4.173 ± 1.074***	−10.25	−8.920 ± 1.152***	−8.37
WB × G	3.285 ± 0.963***	8.07	8.428 ± 1.033***	7.91
WB × K	−0.653 ± 0.945	−1.60	2.811 ± 1.014**	2.64
Average heterosis	−0.514 ± 0.726	−1.26*	0.773 ± 0.779	0.73

G = Gujarat, K = Kerala, WB = West Bengal. Crosses = female × male.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

(e.g. 83.8% and 93.7% in strains K and G, respectively, Table 5). Data on success rate of sex reversal up to mating and progeny testing of the sex-reversed males (neo-females) are presented in Table 6.

There was a massive reduction in the number of sex-reversed prawns. Twenty-two months after AG ablation, the percent of neo-females capable of mating and raising progeny were only 0.17% and 0.34% in the K and WB strains, respectively (Table 6). The progeny of proven neo-females (with 100% male progeny) could be used for a second phase microsurgical AG ablation on a large scale so as to produce commercial quantities of PLs needed for grow out.

4. Discussion

We present the first comparative growth performance experiment undertaken on freshwater prawns conducted under grow-out pond conditions during an eight-month long growout period. The long duration of growout allowed exhibition of adult population structure and dimorphic growth patterns, properties that are of particular importance in such a social organism. It could be clearly seen that growth performance of the purebred strains and their reciprocal crosses examined here varied significantly. Although the three strains are geographically remote from each other, we cannot relate these differences to distinct differences in the genetic background of individual strains. Translocations of *M. rosenbergii* stocks for culture are known to have occurred among sites in India, and some mixing cannot be ruled out since we have not verified that the three populations are in fact genetically differentiated. Such differences could be used in a selective breeding program to improve growth performance of *M. rosenbergii* stocks in India. Variation in growth performance of different stocks of aquatic species, including rohu carp (*Labeo rohita*) (Gjerde et al., 2002), Nile tilapia (Eknath et al., 1993), red claw crayfish (*Cherax quadricarinatus*) (Gu et al., 1995) and recently, *M. rosenbergii* (Pillai et al., 2011; Thanh et al., 2009), has been reported. As in our study, none of the latter two studies presented evidence for the genetic differentiation of the stocks used either. Among the pure bred stocks tested in the present study, the West Bengal strain was found to show the best performance, while the performance of the Gujarat strain was poorest.

Mean body weights of crosses employing the West Bengal strain as dam or sire were significantly greater than those employing the purebred Gujarat or Kerala strains. This result suggests heterotic effects for certain cross combinations that could be trialed for heterotic outcomes among genetically divergent populations of *M. rosenbergii*. These results are, moreover, in agreement with an earlier study that reported that growth rates of crosses between Thai and Malaysian *M. rosenbergii* strains performed better than did a pure Malaysian strain (Dobkin and Bailey, 1979), a finding that influenced our final conclusions (see Section 4.5).

The relative performances of reciprocal crosses in the present study were influenced, in part, by the direction of the cross combinations. For

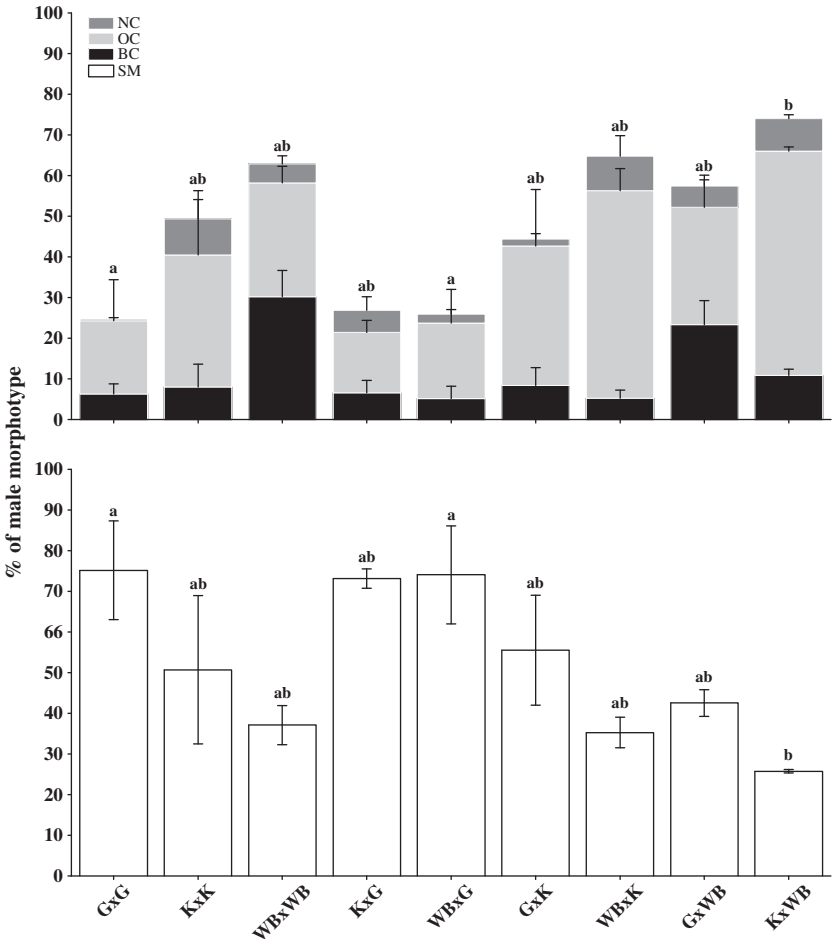

Fig. 4. The proportion of the male morphotypes from the male fraction in each of the purebred strains and cross combinations. The lower graph shows the proportion of SM (Small Male) and the upper graph the proportion of BC (Blue Claw), OC (Orange Claw), and NC (No Claws). G × G (Gujarat × Gujarat), K × K (Kerala × Kerala), WB × WB (West Bengal × West Bengal), K × G (Kerala × Gujarat), WB × G (West Bengal × Gujarat), G × K (Gujarat × Kerala), WB × K (West Bengal × Kerala), G × WB (Gujarat × West Bengal), K × WB (Kerala × West Bengal). Different letters on top of the columns represent statistical differences ($P > 0.05$, based on the generalized linear model after angular transformation, followed by a Tukey post-hoc test).

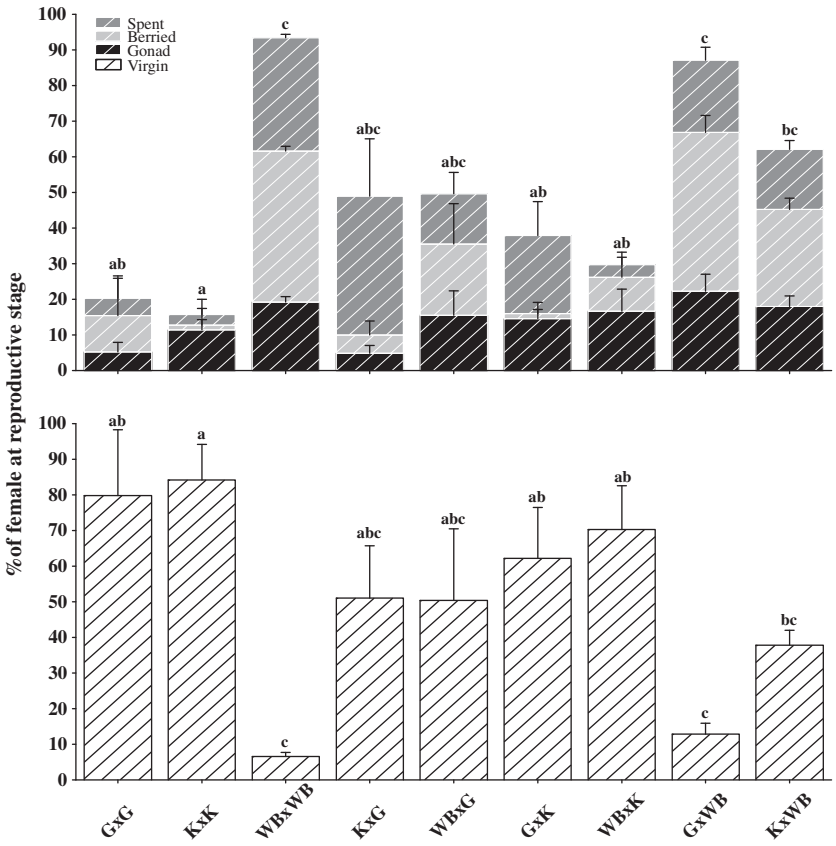

Fig. 5. The proportion of the female reproductive-staged females from the female fraction in each of the purebred strains and cross combinations. The lower graph shows the proportion of Virgin (immature female) and the upper graph the proportion of Gonad (developed ovary), Berried (female carrying eggs) and Spent (mature female which are not carrying eggs). G×G (Gujarat×Gujarat), K×K (Kerala×Kerala), WB×WB (West Bengal×West Bengal), K×G (Kerala×Gujarat), WB×G (West Bengal×Gujarat), G×K (Gujarat×Kerala), WB×K (West Bengal×Kerala), G×WB (Gujarat×West Bengal), K×WB (Kerala×West Bengal). Different letters on top of the columns represent statistical differences ($P>0.05$, based on the generalized linear model after angular transformation followed by a Tukey post-hoc test).

example, reciprocal crosses employing the WB with G strains performed significantly differently according to the direction of the cross (WB×G vs. G×WB). This suggests potential maternal or paternal effects on relative strain performance. Since the above results could dictate that appropriate crosses will be required for future mono-sex producing crosses (as discussed later), the potential for maternal and/or paternal effects should be investigated further so as to confirm that such effects are repeatable in independent trials. In such cases, maternal or paternal effects should be considered in future breeding *M. rosenbergii* programs.

The present study showed a significant sex effect on body weight. In most crosses, males reached heavier mean weights than did females. These results reflect the sexual dimorphic growth patterns of *M. rosenbergii* in which males are generally larger than females (Ling, 1969; New, 1995). The dimorphic growth rate becomes apparent when females attain sexual maturation and start to divert much of their energy intake to ovarian maturation and less into growth, whereas males continue to grow at the same rate (Ling, 1969). Differential growth effect among sexes has been reported in many aquatic species, including tilapia, *Oreochromis shiranus* (Maluwa and Gjerde, 2006), *O. niloticus* (Bentsen et al., 1998; Nguyen et al., 2007), freshwater crayfish *Cherax destructor* (Jerry et al., 2005) and in red claw crayfish *C. quadricarinatus* (Manor et al., 2004). The

Table 4
Percentage of clearly identified males found in the different Indian strains by segregating post-larvae (PL) at different times (days) after metamorphosis. Juvenile prawns were distinguished as males through the identification of genital papillae (GP) and/or *appendix masculina* (AM).

	$PL_{<30}$ (GP)	PL_{30-50} (GP and AM)	PL_{50-70} (AM)	PL_{70-90} (AM)	$PL_{>90}$ (AM)
Gujarat	1.48	1.93	2.63	4.40	7.87
Kerala	1.70	1.98	2.69	4.34	7.86
West Bengal	1.26	2.41	2.38	3.92	5.21

Table 5
Success rates of sex-reversal in the different Indian strains through AG ablation performed at different times (days) post-metamorphosis. Data are presented as number of individuals, with the percent of AG-ablated individuals in parentheses. Regeneration of the male secondary sex character, the *appendix masculina*, was used as a bioassay for the failure of the sex reversal process as described in the Materials and methods section.

Strain	PL stage at AG ablation	AG-ablated prawns	Survival rate 24 h post-AG ablation	With *appendix masculina* (discarded)	Without *appendix masculina*
Gujarat	PL_{25-30}	296	253 (85.4%)	198 (66.9%)	55 (18.6%)
	PL_{31-35}	177	156 (88.1%)	68 (38.4%)	88 (49.7%)
	PL_{36-40}	224	175 (78.1%)	9 (4%)	166 (74.1%)
	PL_{41-45}	160	137 (85.6%)	61 (38.1%)	76 (47.5%)
	PL_{46-50}	112	105 (93.7%)	24 (21.4%)	81 (72.3%)
	PL_{50-up}	1138	657 (57.3%)	458 (40.2%)	199 (17.5%)
Kerala	PL_{25-30}	51	38 (74.5%)	10 (19.6%)	28 (54.9%)
	PL_{31-35}	32	12 (37.5%)	12 (37.5%)	0 (%)
	PL_{36-40}	167	105 (62.9%)	34 (20.4%)	71 (42.5%)
	PL_{41-45}	519	360 (69.4%)	157 (30.2%)	203 (39.11%)
	PL_{46-50}	142	119 (83.8%)	65 (45.8%)	54 (38.03%)
	PL_{50-up}	4787	2760 (57.7%)	2240 (46.8%)	520 (10.9%)
West Bengal	PL_{25-30}	–	–		–
	PL_{31-35}	25	18 (72%)	9 (36%)	9 (36%)
	PL_{36-40}	61	24 (39.3%)	24 (39.3%)	0 (0%)
	PL_{41-45}	16	11 (68.7%)	3 (18.7%)	8 (50%)
	PL_{46-50}	249	204 (81.9%)	128 (51.4%)	76 (30.5%)
	PL_{50-up}	2291	1740 (76%)	1113 (48.6%)	627 (27.3%)

Table 6
Success rates of sex reversal, up to progeny testing, in the different Indian strains. Data presented show the number of individuals, with the percent of AG-ablated individuals in parentheses.

Strain	AG-ablated prawns	Survival rate 24 h post-AG ablation	Without *appendix masculina*	Developed ovary	Mated	Hatched	Progeny test
Gujarat	2107	1483 (70.3%)	665 (31.5%)	300 (14.2%)	300 (14.2%)	32 (1.5%)	6 (0.3%)
Kerala	5698	3394 (59.5%)	876 (15.4%)	220 (3.8%)	165 (2.9%)	40 (0.7%)	10 (0.17%)
West Bengal	2642	2033 (77%)	720 (27.2%)	380 (14.4%)	300 (11.3%)	14 (0.52%)	9 (0.34%)

long grow out period employed in the present study allowed social hierarchy and male morphotypes to develop, including the appearance of three distinct male morphotypes (i.e. blue claw, orange claw, and small male) (Karplus and Sagi, 2010). Mean body weights of males separated into morphotypes in the current study were 125.55 g for BC, 68.81 g for OC, 9.00 g for SM and 48.95 g for NC. The least squares means of body weight for males and females at harvest differed significantly among the nine crosses although differences in the relative frequencies of male morphotypes among crosses were not significantly different. This suggests that there may be advantages to using the WB strain and K males. Current dogma states that male size may be a sex-limited secondary sexual characteristic and thus would be a non-genetic trait in the sense that it may be controlled by intra-population environmental-social factors (Malecha et al., 1984). Our study challenges this dogma, showing, based on reciprocal crosses, that the differences in growth rate of both sexes are derived mostly from their genetic background rather than from social factors (Fig. 3B).

4.1. Additive genetic effects

The significant additive genetic variance observed here indicates the potential for improving growth rates via application of artificial selection in *M. rosenbergii*. The WB strain ranked highest in terms of additive effect while the G strain ranked lowest. The K strain population was intermediate. Strain additive genetic effects in crossbred strains showed that the WB × K cross performed best.

Since 1999, the government of Kerala, India, has been implementing a large scale program to replenish natural stocks of *M. rosenbergii* in Vembanad Lake with seed from several local hatcheries (Kumar and Velayudhan, 2003). This practice would suggests that the K strain collected from this region may not truly be 'wild', but instead bearing some degree of domestication, as compared with the other strains examined here. Domesticated strains have sometimes been shown to perform better in culture environments than 'wild' strains due to better adaptation to the captive environment developed over time (Nguenga et al., 2000) as reported for *O. niloticus* (Brummett et al., 2004). This latter report suggested that poor genetic management resulted in a deterioration of genetic quality. Alternatively, growth performance of some wild *O. niloticus* strains was as good as or better than established farmed strains (Eknath et al., 1993). Thus, there are no consistent trends regarding differences between domesticated stocks and their wild counterparts in culture aquatic animal species.

The high correlation between additive genetic effects and total performance (body mass: $r = 0.927$; body length: $r = 0.875$) reported here suggests that additive genetic effects are largely responsible for relative strain performance. As such, a selective breeding program that is based on additive genetic variation could improve total performance of *M. rosenbergii* strain combinations.

4.2. Reciprocal effects

Variation between the relative performances of different reciprocal strain crosses or species can largely be attributed to maternal effects (Lutz, 2001). In our study there was a significant negative effect on growth when WB × G and K × G crosses were compared with their reciprocal crosses G × WB and G × K ($P<0.001$), pointing to low maternal effects in these crosses. Maternal effects were also found to be a minor contributor to the performance of juvenile *M. rosenbergii* (Malecha et al., 1984).

Differential performance of reciprocal crosses has been reported in aquatic species, including tilapia (Bentsen et al., 1998), salmon (*Oncorhynchus tshawytscha*) (Bryden et al., 2004) and crustaceans (Bosworth et al., 1994). Thanh et al. (2010) reported that a strain reciprocal effect was an important source of variation affecting strain performance of *M. rosenbergii* in Vietnam. Reciprocal effects can be partitioned into maternal and non-maternal components. A significant non-maternal contribution to reciprocal variance would indicate strong interactions between extra-nuclear and nuclear factors (i.e. interactions between mitochondrial and nuclear genes) (Hedgecock and Davis, 2007). The present study has shown that additive genetic and reciprocal effects provided significant sources of variation for the sampled growth traits. This indicates that crossbreeding among existing culture strains of giant freshwater prawn in India is likely to produce only marginal genetic gains in practice because heterotic outcomes were several folds lower than was the additive genetic effect. Thus, we recommend selective breeding programs rather than crossbreeding (see Section 4.5).

4.3. Strain heterosis effects

In the present study, heterosis effects were significantly different from the mean of pure strains. A study of heterosis effects among three strains of *M. rosenbergii* in Vietnam yielded similar results (Thanh et al., 2010). The relative performance of crossbred offspring in some instances may be either intermediate or inferior to their parental lines (Lutz, 2001). Our observation of low heterosis in *M. rosenbergii* in India is consistent with what has been observed in some fin fishes (Bentsen et al., 1998; Bryden et al., 2004; Gjerde and Refstie, 1984; Gjerde et al., 2002) and shrimp (Benzie et al., 1995). In general, the heterotic effect is strain- or population-specific and depends on the test environments to which the animals are subjected (Bentsen et al., 1998) and could mask results obtained.

Based on the present study and considering the low heterosis effect, a selective breeding program could utilize both non-additive (heterosis) and additive genetic effects to generate much-needed genetic improvement of *M. rosenbergii* in India.

4.4. Sex reversal and all-male population production

Intervention in the sexual differentiation process via manipulation of the endocrine controlling gland, the AG, resulted in some degree of sex reversal and in several cases full and functional sex reversal and production of neo-females capable of mating and producing progeny (Aflalo et al., 2006). Mating sex-reversed individuals (neo-females) with normal individuals has been used in the past to confirm sex differentiation mechanisms (Ginsburger-Vogel and Charniaux-Cotton, 1982; Lécher et al., 1995). Here we used the sex ratio of the out-coming population to validate successful sex reversal in *M. rosenbergii* males. In only a few cases, complete functional sex reversal from male to functional neo-female was recorded (0.17–0.34% in the present study), values similar to what had been reported in previous studies (Aflalo et al., 2006). We

suggest that neo-females generated here can be used repeatedly as all-male-producing broodstock for the industry. Such a production scheme for a broodstock, however, poses a risk of reducing genetic diversity levels and could lead to inbreeding. Moreover, our use of second phase technology (Aflalo et al., 2006), in which the second AG ablation is performed on an all-male population (progeny of a single neo-female) on a large scale will, add to the bottleneck and could lead to establishment of founder 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 will be required in which both selective breeding of each strain are used, from which fresh neo-females are created every few generations. We propose that periodical crosses of neo-females be made from one cross with selectively-bred males from other distinct crosses.

4.5. Conclusions

The results of our study suggest that selective breeding programs to improve growth rates of *M. rosenbergii* strains in India have significant potential for the development of a sustainable giant fresh water prawn aquaculture in India, with an emphasis on the West Bengal and Kerala strains. Based on the above, a combined approach comprising independent selective breeding programs on the above two strains, in parallel with sex reversal of the selected strains, is suggested (Fig. 6). According to this scheme, sex-reversed males (neo-females) of one of the selectively bred strains should be crossed with selected males from the reciprocal strain and the all-male progeny will be used for grow out. Such a combined approach, which should still be tested for several generations, aims at genetic improvement of the selected strains while preventing inbreeding that could result from using neo-females and males from the same strain.

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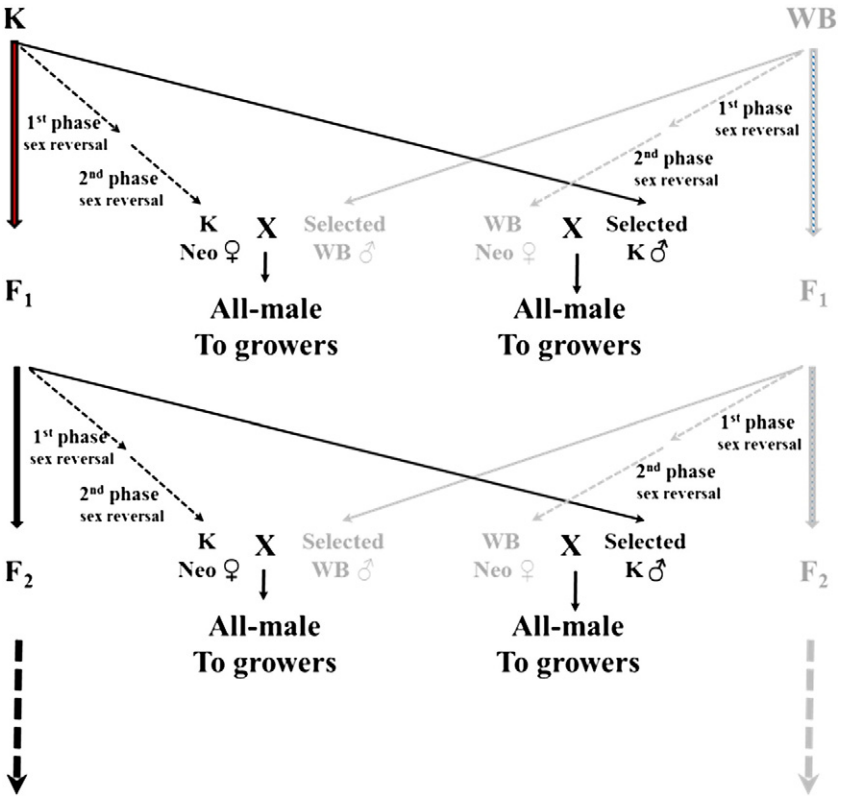

Fig. 6. A proposed combined all-male production and selective breeding scheme for genetic improvement and prevention of inbreeding in two leading Indian strains. The wide arrows represent selective breeding programs for a generation of each strain. The dashed narrow arrows represent the 1st and 2nd phases of sex reversal for mass production of neo-females. Narrow arrows represent selected males crossed with neo-females, black arrows represent the west Bengal strain (WB) and gray arrows represent the Kerala (K) strain.

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