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All-female monosex culture in the freshwater prawn *Macrobrachium rosenbergii* – A comparative large-scale field study

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ABSTRACT

In crustacean aquaculture, size dimorphism between males and females is the main key factor determining the advantage of monosex aquaculture over that of mixed populations. This factor is particularly relevant for the freshwater prawn, *Macrobrachium rosenbergii*, for which intensification of cultures is complicated by a complex social structure in which large dominant males are territorial and inhibit the growth performance of smaller males and females. It has therefore been suggested that all-female mono-culture could be the practice of choice, since females are less aggressive and less territorial and are believed to exhibit a relatively homogenous growth pattern. Here we report the first large-scale comparative field study of all-female and mixed populations under extensive and intensive stocking conditions in earthen ponds. The study was facilitated by application of our novel biotechnology based on a single injection of suspended hypertrophied androgenic gland cells. Under both our intensive and extensive conditions, the all-female cultures showed better performance than the mixed cultures in most key aquaculture parameters including survival rate and yield per hectare. Also, the intensively stocked all-female ponds showed better feed conversion ratio than mixed ponds. Furthermore, while the mean size of the animals did not differ significantly between the two treatments, the all-female populations exhibited significantly higher size uniformity. Our study suggests that for *M. rosenbergii*, female monosex aquaculture is a sustainable method to yield a homogenous crop.

1. Introduction

In the commercial production of crustaceans – as in most animal husbandry sectors – monosex culture is highly desirable, particularly for economically important decapod species whose sexual dimorphism may give rise to products of different sizes (Sagi et al., 1986). In these species, the dimorphic growth pattern may be attributed to animal behavior, specific growth rates (Sagi and Aflalo, 2005) and even to the ability of the animals to utilize feed, i.e., the feed conversion ratio (Moss et al., 2002). For penaeid shrimp species, such as the whiteleg shrimp, *Litopenaeus vannamei* (Moss and Moss, 2006) and the giant tiger prawn, *Penaeus monodon* (Gopal et al., 2010; Hansford and Hewitt, 1994), it is readily apparent that all-female populations would constitute the preferable monosex culture, since females grow faster and reach a larger size at harvest than males. However, for the freshwater prawn, *Macrobrachium rosenbergii*, the choice of male vs female monosex culture is less facile: even though the males are larger at the end of the grow-out season (Nair et al., 2006) [suggesting that all-male culture would be the practice of choice (Nair et al., 2006; Sagi et al., 1986)], there are a number of considerations that call into question the overall advantage of culturing all-male populations. First, adult male prawns are differentiated into three main morphotypes, blue-claw males (BC), orange-claw males (OC) and small males (SM), which constitute different size classes with a wide size variation (Kuris et al., 1987). Second, these large-sized males comprise only a limited fraction of a mixed population (Karplus et al., 1986). Third, the dominant and aggressive BC males inhibit the growth of all other individuals (both males and females) (Ra'anan and Cohen, 1985). And, finally, over two decades ago, it was found that in all-male populations under intensive stocking densities, a strong hierarchy of male morphotypes is

Abbreviations: ADG, average daily growth; AG, androgenic gland; BC, blue-claw males; BWG, body weight gain; FBW, final body weight; FCR, feed conversion ratio; NC, no claw; OC, orange-claw males; SGR, specific growth rate; SM, small males

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established, leading to a wide size variation in the entire population; at harvest such a population will contain a large fraction of small-sized, and hence, unmarketable animals (Malecha, 2012). Malecha (2012), therefore, suggested a strategy of all-female monosex culture, since female prawns are less aggressive and less territorial and do not establish a morphotypic hierarchy. He also claimed that under conditions of intense aquaculture, the animals in an all-female population would direct most of their energy into somatic growth and the population would exhibit a homogenous size at the end of the grow-out season (Malecha et al., 2010), with the dual advantage of minimizing the need for frequent labor-intensive selective harvests during the grow-out season and for size grading at the end of the season, as is commonly practiced for crustacean mixed cultures (Gopal et al., 2010; Malecha, 2012; Otoshi et al., 2003; Sagi et al., 1986). It thus seems likely that the yields and profitability of all-female cultures would be superior (Levy et al., 2016; Malecha, 2012; Sandifer et al., 1991; Siddiqui et al., 1997), but testing this notion on a large scale in earthen ponds has not been possible due to the lack of an appropriate technology for the sustainable mass production of large all-female populations. Recently, however, such a technology has become available, and its principles and development are described below.

M. rosenbergii, like most decapod species, is assumed to have the W/Z model of sex inheritance in which males are homogametic (ZZ genotype) and females are heterogametic (WZ genotype) (Benzie et al., 2001; Lecher et al., 1995; Parnes et al., 2003; Staelens et al., 2008). Thus, the first step towards achieving an all-female population requires a fully functional sex reversal of females into a 'neomales' with the WZ genotype. These 'neomales' are then crossed with normal females (WZ) to yield progeny containing 25% WW females. Finally, the WW females are crossed with normal males (ZZ) to yield an all WZ genotype female population (Levy et al., 2016; Malecha et al., 1992). To date, the major technological challenge in this procedure has been the production of the 'neomale' by implantation in a juvenile female (Nagamine et al., 1980) of an androgenic gland (AG) – a unique male crustacean endocrine organ – that has been surgically ablated from a male animal (Charniaux-Cotton, 1954; Manor et al., 2007; Sagi et al., 1997). The surgical procedure requires significant skill, with a reported very high mortality rate of ~90% (Malecha et al., 1992), which may explain why the procedure has not matured into a commercially viable protocol. Recently, however, our group has developed a novel biotechnology for the production of 'neomales' – by a single injection of hypertrophied AG cell suspension – which is suitable for the sustainable mass production of *M. rosenbergii* all-female populations (Levy et al., 2016). The ability to mass produce *M. rosenbergii* all-female populations has thus enabled us, for the first time, to compare true (not hand segregated) all-female populations with mixed populations in a large-scale study conducted in earthen ponds under extensive and intensive stocking densities, as reported here.

2. Materials and methods

2.1. Animals

2.1.1. Brood-stock preparation for all-female and mixed populations

To obtain the *M. rosenbergii* brood-stock producing an all-female population, WW genotype females were produced as previously described (Levy et al., 2016) and upon maturity were stocked with mature ZZ genotype males in communal tanks. The brood stock producing a mixed population was established by stocking mature WZ genotype females with ZZ genotype males in communal tanks. Animals were maintained as previously described (Levy et al., 2016). The exact genotypic profiles of the two brood stocks (i.e., WW × ZZ or WZ × ZZ) were validated using sex-specific DNA markers before the populations were stocked in the breeding tanks, as previously described (Levy et al., 2016; Ventura et al., 2011).

2.1.2. Larviculture and progeny testing

Gravid females were collected from the communal tanks and transferred into individual tanks, where the larvae were allowed to hatch. Larviculture was conducted according to common practice, as previously described (Aflalo et al., 2012). All-female and mixed population progenies were obtained from 25 and 24 successful matings, respectively. Progeny testing to validate the genotypic profile (i.e., all-female or mixed) of each batch of larvae was performed on groups of 20 representative individuals by using sex-specific DNA markers, as described above.

2.2. Comparative growth performance trial in earthen ponds

2.2.1. Stocking

Post larvae 14 days post metamorphosis (PL_{14}; weighing 30 mg on average) were stocked in 350-m^2 earthen ponds at the Aquaculture Research Station, Dor, Israel, as previously described (Sagi and Raanan, 1988). The two types of population (all-female and mixed) were stocked in triplicate at either an extensive stocking density of four individuals per square meter (1400 animals per pond) or an intensive stocking density of 34 individuals square meter (12,000 animals per pond). Although it seems reasonable to test another stocking density between the extensive and intensive stockings, due to limited number of ponds in the aquaculture research station we had to decide not to compromise on the number of replicates and to test only two densities.

2.2.2. Grow-out

Throughout the grow-out period, the prawns were fed daily with shrimp pellets (30% protein) according to the following protocol. In the first 60 days, 250 g of pellets were supplied to each pond. Thereafter, feeding rations were adjusted every four weeks according to prawns' average weight, as follows. Every four weeks, prawns from each pond were sampled and weighed, and feeding rations were adjusted to ~4% and ~5.5% of the prawns' total biomass in the extensively and intensively stocked ponds, respectively. For the calculation of the feeding rations, a mortality rate of 10% and 15% was assumed for the extensively and intensively stocked ponds, respectively. During the grow-out period the water temperature was maintained at 28 ± 2 °C. Ammonia, nitrite, oxygen and pH levels were monitored weekly and found to be within the normal range throughout the entire trial.

2.3. Harvest

At the end of the grow-out period (163 days post stocking), the earthen ponds were completely emptied, and the animals were harvested. The harvested all-female animals from each pond (both extensive and intensive stockings) were transferred into separate tanks. Thereafter, a sample group (as described below) was taken from each tank; animals were weighed, and their reproductive stage was determined (virgin, virgin with developed ovary, spent, spent with developed ovary and gravid). The data collection from the all-female ponds was performed on samples from each of the three extensive ponds ($n_1 = 310$, $n_2 = 309$, $n_3 = 300$) and from each of the three intensive ponds ($n_1 = 400$, $n_2 = 400$, $n_3 = 411$). All the remaining females from each pond were counted and weighed (in bulk), and the mean weight per animal was calculated. The harvested mixed population animals (from both extensive and intensive stockings) were also segregated into separate tanks, according to the following categories: females, SM, OC, BC and no-claw males (NC) (Kuris et al., 1987). Thereafter, the male morphotypes and a sample group of the small males and females were weighed individually and female's reproductive stage was determined. The data collection from the three mixed extensive ponds was performed on the following numbers of animals from each of the ponds: $males_1 = 287$, $females_1 = 152$, $males_2 = 330$, $females_2 = 207$, $males_3 = 264$, $females_3 = 241$. For the three intensive ponds, numbers of animals were: $males_1 = 360$, $females_1 = 200$,

$males_2 = 419$, $females_2 = 440$, $males_3 = 365$, $females_3 = 268$. The breakdown of the numbers of animals sampled according to male morphotypes and females from each mixed pond is presented in Table S1. The remaining females and the males of the different morphotypes in each pond were counted and weighed (in bulk), and the mean weight per animal was calculated.

2.4. Calculation of growth parameters

The following parameters were calculated at the end of the grow-out period:

$$\text{Specific growth rate (\%/day)} = \frac{ln(Wf - Wi)}{t} \times 100$$

where ln - natural log of the individual wet weight (g); *Wf* - final wet weight, *Wi* - initial wet weight, *t* - duration in days.

$$\text{Feed conversion ratio} = \frac{\text{Total feed supplied (kg)}}{\text{Total biomass harvested (kg)}}$$

2.5. Statistical analysis

The differences between the mixed and the all-female populations for both the extensive and intensive stocking densities were tested for each parameter by a two-tailed *t*-test ($p < 0.05$). The survival values were converted from percentages to proportions, followed by an arcsin transformation so as to facilitate a proper statistical analysis. The homogeneity of the variances was tested by Levene's test, and the residuals were tested for normality by the Shapiro-Wilk test. All statistical analyses were performed using Statistica v9.0 software (StatSoft Ltd., Tulsa, OK, USA).

3. Results

3.1. Post-harvest measurements

Upon harvest, the all-female cultures showed better survival rates, at both extensive and intensive stocking densities, with a 21% and 19% improvement, respectively ($p < 0.05$).The total crop harvested was also found to be larger for the all-female vs the mixed cultures at both stocking densities. Although under extensive stocking the improvement of ~11% was not significant ($p = 0.06$), in the intensive stocking there was an improvement of ~22% ($p < 0.05$). The all-female cultures were found to be better feed converters than the mixed populations. However, a significant improvement was found only under intensive stocking (~20%, $p < 0.05$). The differences between all-female and mixed cultures at each of the tested densities in terms of final body weight (FBW) at harvest, specific growth rate (SGR), body weight gain (BWG) and average daily growth (ADG) were not statistically significant (Tables 1 and 2).

Table 1
Post-harvest parameters in all-female and mixed populations under extensive culture conditions (4/m^2).

	Mixed n = 3	All-female n = 3
Survival rate (%)	73.9 ± 1.3^a	89.9 ± 3.3^b
Total crop (kg/ha)	1269 ± 28^a	1405 ± 45^a
FBW (g)	42.9 ± 1.3^a	39.1 ± 1.4^a
SGR (%/day)	4.5^a	4.4^a
BWG (%)	143046 ± 4286^a	130334 ± 4552^a
ADG (g/day)	0.3^a	0.2^a
FCR (kg food/kg biomass)	2.4 ± 0.1^a	2.2 ± 0.1^a

Values are means or means ± SE of the experiments performed in triplicate. Different letters represent significant differences ($p < 0.05$).

Table 2
Post-harvest parameters in all-female and mixed populations under intensive culture conditions (34/m^2).

	Mixed n = 3	All-female n = 3
Survival rate (%)	62.0 ± 1.6^a	74.3 ± 3.5^b
Total crop (kg/ha)	2459 ± 58^a	3012 ± 94^b
FBW (g)	11.7 ± 0.5^a	11.8 ± 0.2^a
SGR (%/day)	3.7^a	3.7^a
BWG (%)	39,063 ± 1621^a	39,398 ± 784^a
ADG (g/day)	0.1^a	0.1^a
FCR (kg food/kg biomass)	3.0 ± 0.1^a	2.4 ± 0.1^b

Values are means or means ± SE of the experiments performed in triplicate. Different letters represent significant differences ($p < 0.05$).

3.2. Size distribution of harvested prawns

Prawns harvested from the mixed population ponds showed a marked size variation, with large animals (weighing ≥ 50 g) comprising 33% and 3% of the harvest at extensive and intensive stocking densities, respectively (Fig. 1A and C). The all-female cultures showed a much smaller variation in size, with 72% of the animals cultured at the extensive stocking density weighing 35 to 50 g; there was thus a 65% improvement in uniformity compared to the mixed population. For the all-female cultures that were intensively stocked, 75% of the animals weighed 15 g or less (Fig. 1B and D); the size uniformity was thus improved by 60% compared with the mixed cultures. The mean weights of the animals in the two types of culture did not differ significantly at either stocking density, being at 39.1 ± 1.4 g and 42.9 ± 1.3 g for all-female and mixed cultures under extensive stocking, respectively, and 11.8 ± 0.2 g and 11.7 ± 0.5 g under intensive stocking, respectively (Tables 1 and 2). Moreover, the same pattern of size variation was observed for each of the triplicates of the mixed population ponds at both stocking densities (Fig. S1), as well as for the all-female ponds at both densities (Fig. S2).

3.3. Distribution of male morphotypes in the mixed population ponds

Most males harvested from the mixed population extensively stocked ponds were classified as the OC morphotype (55%; 59.2 ± 2 g), while the rest of the animals were classified as BC (14%; 79.1 ± 2.3 g), SM (18%; 7.29 ± 0.17 g) or NC (13%; 58.2 ± 2 g) morphotypes. At the intensive stocking density, the vast majority of the harvested males were SM (64%; 7.2 ± 0.4 g), with the rest being classified as OC (26%; 26 ± 0.8 g), BC (2%; 39.1 ± 1 g) or NC (2%; 21.2 ± 1 g) morphotypes. The detailed distribution of the male morphotypes in two representative mixed population ponds (one extensive and one intensive) are shown in Fig. 2. A comparison of the males' final body weight under extensive and intensive stocking densities showed higher weights, with a greater size variation, under the extensive conditions (Fig. 3; 48 ± 1.8 g vs 21.6 ± 0.7 g). The marked size variation of the animals in the mixed ponds may be attributed mainly to male morphotypic differentiation. This notion is supported by the fact that even in the presence of males, the female fraction of the mixed population maintained a high size uniformity. Furthermore, 88% of animals in the female fraction of the mixed populations weighed 35 to 45 g under the extensive conditions, while 81% weighed 10 to 15 g in the intensive density. Overall, it seems there was a negative relationship between stocking density and size uniformity (Figs. 1 & 3). Finally, it should be noted that not even a single male was found in any of the all-female ponds.

3.4. Distribution of female reproductive stages

The vast majority of females harvested from all-female ponds

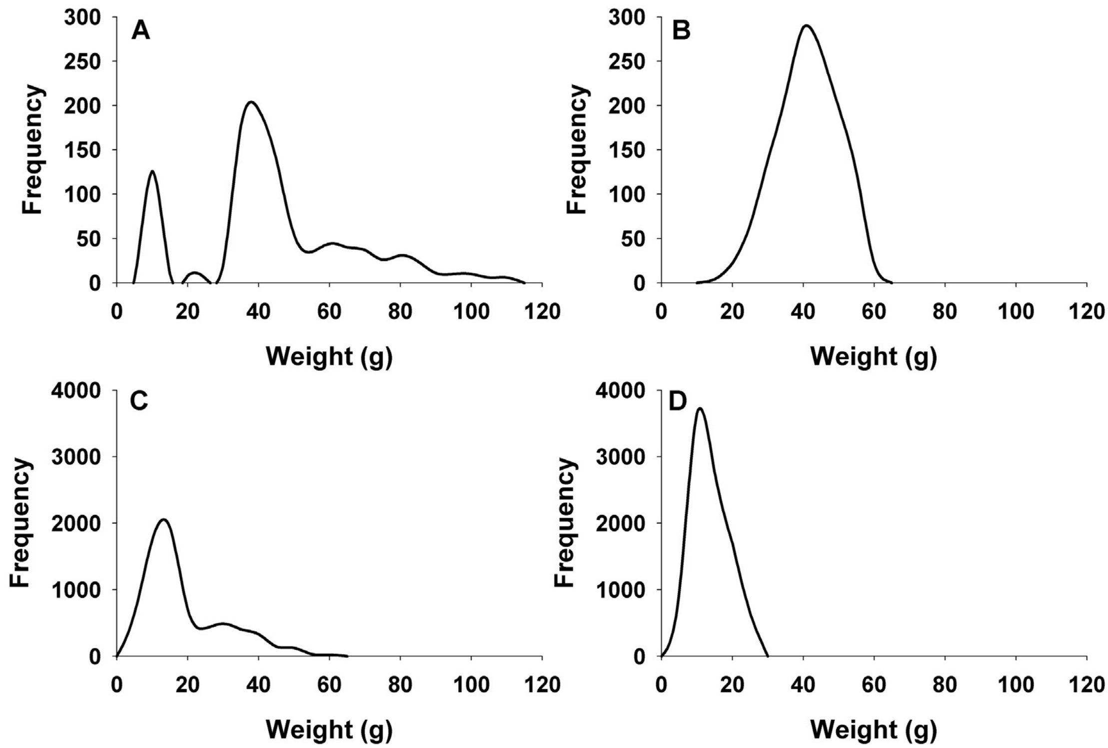

Fig. 1. Weight distribution of all-female and mixed populations under extensive and intensive stocking densities. (A) Mixed population at extensive stocking density (n = 439); (B) all-female population at extensive stocking density (n = 310); (C) mixed population at intensive stocking density (n = 560); and (D) all-female population at intensive stocking density (n = 400). Frequency was normalized to the total number of prawns harvested from each pond. Each of the plots represents one pond out of three.

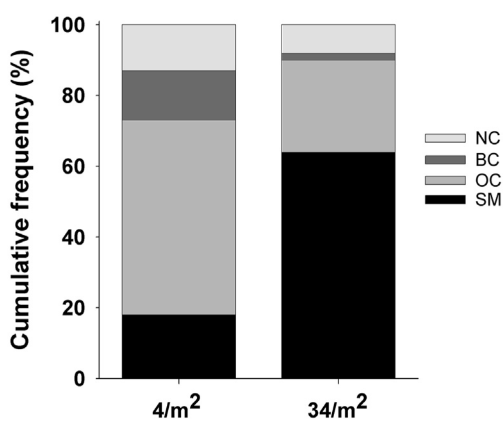

Fig. 2. Cumulative frequency of male morphotypes in mixed populations under extensive (n = 463, left) and intensive (n = 3725, right) stocking densities. Each bar represents one pond out of three. The frequency of each morphotype was divided by the total number of males so as convert the frequency to percentage. SM - small-male, OC - orange-claw, BC - blue-claw, and NC - no-claw.

stocked under intensive conditions were found to be either virgins (66%; 9.1 ± 0.2 g) or virgins undergoing ovarian development (21%; 13.9 ± 0.4 g), while the rest were classified as either spent (2%; 16.8 ± 1.3 g) or spent undergoing ovarian development (12%; 16.6 ± 0.6 g). For the intensively stocked mixed ponds, similarly to above-mentioned all-female cultures, most of the females were either virgins (50%; 8 ± 0.4 g) or virgins undergoing ovarian development (17%; 12.8 ± 0.4 g), while the rest were spent with developed ovaries (22%; 13 ± 0.4 g) or spent (5%; 11.1 ± 0.8 g), but some were gravid (7%; 12.8 ± 0.7 g) (Fig. 4). Under the extensive stocking conditions, most animals in the all-female ponds were found to be spent undergoing ovarian development (42%; 42.6 ± 0.6 g), while the rest were virgins (29%; 33.3 ± 1 g), virgins undergoing ovarian development (18%; 37.2 ± 1 g), or spent (11%; 41.8 ± 1.2 g). No egg-carrying females were found in the all-female ponds at harvest, regardless of the stocking density, suggesting that the spent females had probably dropped their unfertilized eggs prior to harvest. However, 20% of the females harvested from the mixed cultures under the same density were found to be gravid (35.1 ± 0.6 g) (Fig. 4). The other females in the extensively stocked mixed cultures were classified as spent undergoing ovarian development (56%; 34.7 ± 0.5 g), virgin undergoing ovarian development (12%; 34.7 ± 1.5 g), virgin (10%; 32.7 ± 1.6 g) or spent (1%; 28.5 ± 3.6 g). Detailed distributions of female reproductive stages in representative ponds of mixed and all-female populations under extensive and intensive stocking densities are shown in Fig. 4. The final body weights at harvest of females under extensive conditions (34.5 ± 0.4 g and 38.9 ± 0.5 g for mixed and all-female ponds, respectively; Figs. S1–S3) were found to be higher, but with a wider variation, than the weights under intensive conditions (10.4 ± 0.3 g and 11.1 ± 0.2 g for mixed and all-female ponds, respectively; Figs. S1–S3).

4. Discussion

In this study, we report the first large-scale comparative experiment under aquaculture conditions of true all-female prawn cultures produced through the novel single cell injection biotechnology (Levy et al., 2016) vs mixed cultures. The *M. rosenbergii* all-female cultures in the current large-scale study outperformed the mixed cultures in most major aquaculture criteria tested, namely, survival rate, total crop harvested, size uniformity and FCR. Based on this improvement in performance, we believe that – in light of the following considerations – all-female cultures will indeed be more profitable than mixed cultures. The survival rates in the all-female cultures were significantly higher than those in the mixed cultures, in both extensively and intensively stocked ponds, probably as a result of the less aggressive behavioral pattern of the females, as previously reported (Sagi and Aflalo, 2005). Moreover, while premium-sized individuals were found only in the mixed ponds among the male morphotypes, these animals constituted

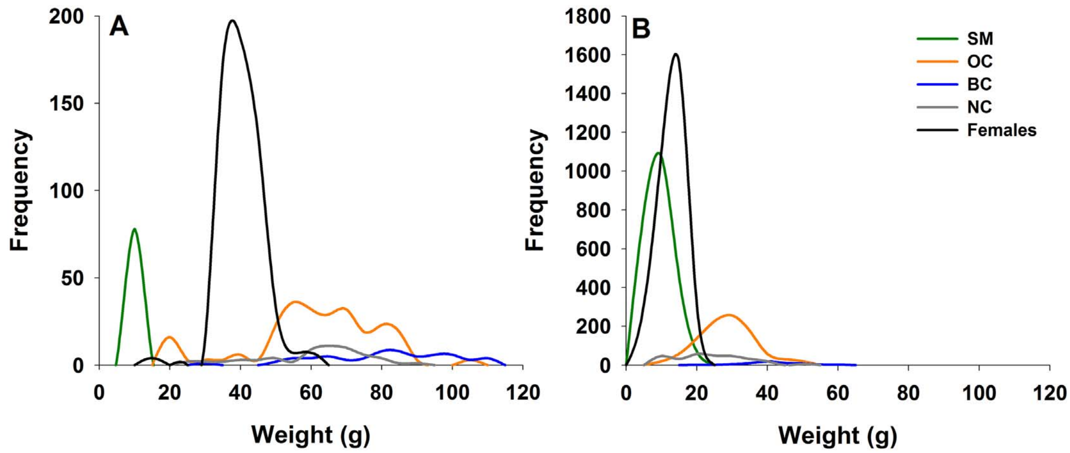

Fig. 3. Weight distribution of male morphotypes and females in a mixed population under extensive and intensive stocking densities, showing small males (SM), orange-claw males (OC), blue-claw males (BC) and no-claw males (NC) stocked at: (A) extensive density (n = 439) and (B) intensive density (n = 560). Frequency was normalized to the total number of animals that were harvested from each pond. Each plot represents one pond out of three.

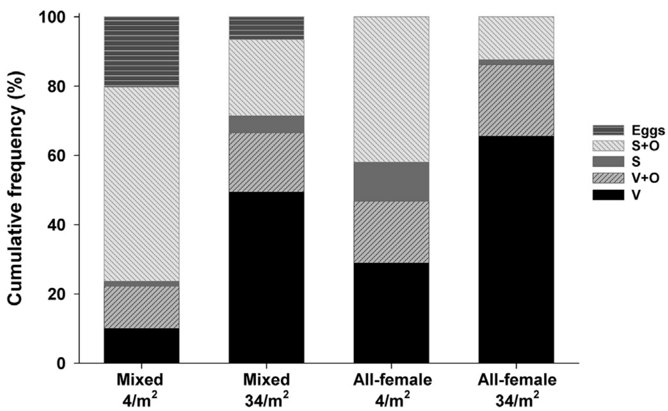

Fig. 4. Cumulative frequency of female reproductive stages in mixed and all-female populations at extensive and intensive stocking densities. The figure shows the distribution of female reproductive stages in samples of: mixed populations at extensive (n = 207) and intensive (n = 200) stocking densities and all-female populations at extensive (n = 310) and intensive (n = 400) stocking densities. Each bar represents one pond out of three. To convert the frequency to percentage, the frequency of each reproductive stage was divided by the total number of females. V - virgin, V + O - virgin with developed ovary, S - spent, S + O - spent with developed ovary, and Eggs - gravid.

only a minority of the harvested prawns. These large BC males dominate the social hierarchy of the three known male morphotypes (Kuris et al., 1987) and are known to suppress the growth of the majority of the population, both the females and the other males. This inhibition might result from competition for food and territory (Barki et al., 1991; Barki et al., 1992). Another consideration is the fertilization of females in the mixed ponds; the gravid females were relatively smaller than the prawns in the all-female ponds (7% smaller than the females in the all-female ponds under extensive stocking) and hence less marketable (Nair et al., 2006). In contrast, in the absence of males, the energy that would have been invested in reproductive-related processes was directed towards somatic growth in the all-female ponds (Malecha et al., 2010; Sagi and Aflalo, 2005). The final consideration is that the discarding of the unfertilized eggs may have promoted an increase in the frequency of in-tandem ovarian development cycles, which in this species is characterized by a reproductive preparatory molt that could have resulted in an additional size increment (Cavallo et al., 2001). This notion is supported by a previous study showing that removal of eggs resulted in the induction of earlier molting (Scudamore, 1948).

One important finding of this study relates to the amounts of feed required. Previously reported FCR values for *M. rosenbergii* range from 1.8 to 3.1 (Hossain and Islam, 2006; New, 1988). While our results of 2.2 to 3.0 fall within this range, some discussion of this aspect of the study is likely to be useful. As prawns are commonly fed according to total biomass, the quantities required for very young prawns are minute. Since it is not possible to distribute such small amounts of feed evenly over the ponds, particularly at the perimeters, food was supplied in excess to the extensive ponds in the first two months of the experiment. For the intensive ponds, we generally underestimated the survival rates on which feed quantities were calculated. As a result, the food supply to these ponds was not sufficient, which clearly affected the average final body size at harvest. Thus, to summarize, although our calculated FCR values were within range, it seems that there is potential for improvement of the feeding regime and hence the animals' final weight at harvest, thereby reducing the FCR.

Yet another factor dictating the size of the total crop is the stocking density (Cuvin-Aralar et al., 2007; Marques et al., 2000; Ranjeet and Kurup, 2012; Willis and Berrigan, 1977), with the stocking density being negatively correlated with body size. The higher frequency of large animals in general and egg-carrying females in particular in the extensively stocked ponds suggests that a density of $4/m^2$ facilitated improved growth performance and increased the chances of successful mating events. In comparison, for the density of $34/m^2$, the total crop was higher (for both the mixed and all-female cultures), but the mean weight of each animal was approximately 75% lower, which is a valuable consideration for the grower. In addition, the findings that there were many more small virgin females in the intensively stocked ponds, both mixed and all-female, and many more small males in the mixed ponds suggest that at a density of $34/m^2$ both somatic growth and female sexual maturation might be inhibited (Cohen et al., 1981).

To summarize, unlike previous attempts to study all-female populations produced by hand segregation (Malecha, 1986; Sagi et al., 1986), our study constitutes the first large-scale experiment on *M. rosenbergii* all-female aquaculture, in which true all-female populations were generated by our novel biotechnology (Levy et al., 2016). While a previous study argued that all-female aquaculture would be more profitable strictly under intensification (Malecha, 2012), our results showed significant advantages for culturing all-female populations at all stocking densities. While for penaeid shrimps, culture of all-female populations seems to be the biotechnology of choice, as females are significantly larger than males (Gopal et al., 2010; Hansford and Hewitt, 1994; Moss and Moss, 2006), our results show that even in cultured crustaceans, such as *M. rosenbergii*, in which males reach higher – even premium – weights at harvest (Aflalo et al., 2006; Nair et al., 2006), it might be more advantageous to grow all-female cultures due to their narrower size distribution. The dual advantages of a marked improvement in size uniformity, which leads to more marketable sized animals, and the absence of labor-intensive practices, such as selective harvesting, position all-female culture as a promising aquaculture product. In a broader sense and beyond the scope of the prawn industry, all-female monosex cultures can provide the means to protect proprietary elite lines, as in the shrimp industry, which will be further stabilized by eliminating/reducing production of inferior low-quality inbreeds. This will not only incentivize breeders but will also lead to higher quality of seed stock for growers in an industry suffering from inferior seed quality.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.aquaculture.2017.07.039.

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