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Intensification of redclaw crayfish *Cherax quadricarinatus* culture II. Growout in a separate cell system

Rivka Manor [a], Ran Segev [b], Marcia Pimenta Leibovitz [b], Eliahu D. Aflalo [a], Amir Sagi [a,*]

[a] *Department of Life Sciences and the Institute for Applied Biosciences, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel*

[b] *Aquaculture Unit Negev-Arava Research and Development, Sapir, D.N. Arava 86825, Israel*

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Abstract

In the process of exploring ways to intensify crayfish culture, a growout system of individual cages (cells) was designed to determine the effects of gender and cell size on the growth of the red claw crayfish *Cherax quadricarinatus.* Cells of three different diameters—large (25 cm), medium (20 cm) and small (16 cm)—were used. When crayfish were stocked at a mean weight of approximately 10 g, growth rate of males was significantly higher than that of females. The growth rate of the males in the large cells was 0.31 ± 0.14 g/day, while that of the females was 0.18 ± 0.09 g/day. The size of the cell had significant influence on the weight of males. Male crayfish in the large and medium cells grew better than those in the small cells. When males were stocked at a higher mean weight (about 23 g), their mean weight after 206 days was higher in the large cells (69.28 ± 15.72 g) than in the small cells (58.11 ± 12.66 g), suggesting that the growth of large males was also affected by cell size. Regardless of cell size, male animals of this species grew faster than females under conditions of individual cells. This intensive culture method appears to present a powerful improvement in yields, by as much as two orders of magnitude, in comparison with communal cultures.
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Keywords: Crayfish; Red claw; *Cherax quadricarinatus*; Individual cell; Intensive culture; Growth

* Corresponding author. Tel.: +972-8-646-1364; fax: +972-8-647-2890
E-mail address: sagia@bgumail.bgu.ac.il (A. Sagi).

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1. Introduction

Freshwater crayfish may be cultured in extensive or intensive systems (Lee and Wickins, 1992; Ackefors and Lindqvist, 1994; Mills et al., 1994), but the current trend favors intensive technologies because they facilitate more accurate control over culture conditions (Lee and Wickins, 1992) and increased yields per volume of water. It is believed that these increased yields are related to the benthic character of some freshwater crayfish and that the total volume of water available is better utilized when it is divided into separate cells.

In crayfish, growth is a complex process that includes protein synthesis and cell proliferation during intermolt periods and a rapid increase in length and weight at the time of molting (Aiken and Waddy, 1992). In culture systems, the process is affected by a wide variety of factors, including stocking density and compartment size (Aiken, 1980; Morrissy, 1992; Morrissy et al., 1995) as well as gender, acclimation, dissolved oxygen concentration, food supply, temperature, photoperiod, sexual maturity and age (Chittleborough, 1975; Aiken, 1980; Hartnoll, 1983; Botsford, 1985).

In compartmentalized rearing systems for freshwater crayfish, space limitation may cause inhibition of growth (Jussila, 1997). The need has thus arisen to define a parameter that would provide a measure of the optimal compartment size for which growth would not be inhibited. The density factor k, which is expressed as the ratio of the size compartment in individual rearing systems to the size of the crayfish, has been tested as a tool to evaluate rearing systems. In previous studies on crayfish, Goyert and Avault (1978), Du Boulay et al. (1995) demonstrated growth was inhibited at $k \leq 50$, and Jussila (1997) found growth inhibition at $k \leq 45$, while in lobsters van Olst and Carlberg (1978) found growth inhibition at $k \leq 33$.

Several crustacean species exhibit bimodal growth patterns in which males grow better than females or vice versa. The red claw crayfish *Cherax quadricarinatus* is one such species: in earthen pond cultures, males weighed more than females at the end of the experimental period (Curtis and Jones, 1995). This difference was clearly manifested in a second growout season of over-wintered populations (Sagi et al., 1997a). The above may be exploited to develop an intensive culture strategy for all-male monosex populations (Curtis and Jones, 1995; Sagi et al., 1997a).

Recently, an attempt was made to culture the marron *C. tenuimanus* in an intensive system comprising individual compartments designed to minimize social contacts and cannibalism (Jussila, 1997). In this study, we set out to test, for the first time, a culture system comprising individual cells for *C. quadricarinatus.* We investigated the differences in growth between male and female animals in an intensive growout system and attempted to determine the optimal individual cell size.

2. Materials and methods

Our custom-designed crayfish growout system of 126 individual cages comprised a 3-m^2 (bottom area) tank filled to a depth of 1 m containing 18 units, each made up of

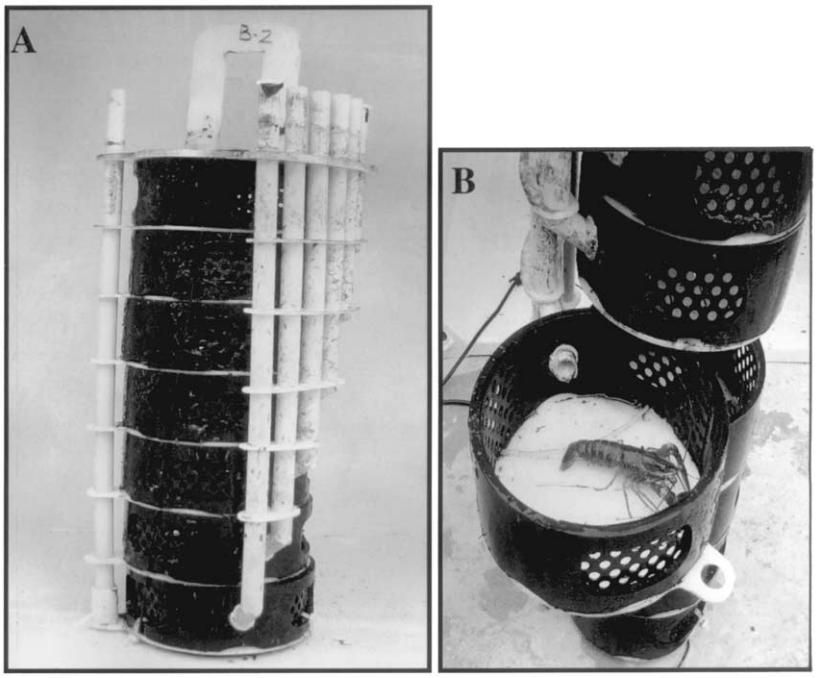

Fig. 1. Seven-story separate cell structure for the intensive growout of *C. quadricarinatus* (A). Close up of an individual cell containing a single crayfish (B).

seven separate circular cells of the same size placed one on top of the other (Fig. 1A). Each unit was elevated 20 cm from the bottom of the tank. The walls and floor of each cell were made of fenestrated polypropylene to facilitate free flow of water (Fig. 1B). Each cell, 11 cm in height, was supplied with its own PVC feeding pipe 2.1 cm in diameter. Units of three different diameters were placed randomly in the tank, the diameters being large, 25 cm; medium, 20 cm; and small, 16 cm, giving bottom areas of 490.9, 314.2 and 201.1 cm^2, respectively. Water temperature was maintained at 27 ± 3 °C. Water quality was assured by circulating the whole volume of water through a 0.3 m^3 biofilter every hour. The biofilter comprised of polypropylene 3 mm diameter bead grains. Fresh water was added to replace 10% of the water per day. The cells were cleaned by running water through the feeding pipes and siphoning off the debris from the bottom of the tank twice a week. The following parameters were monitored twice a week: ammonium (less than 1 ppm), nitrite (less than 1 ppm), nitrate (less than 50 ppm), pH (8.0 ± 0.5) and oxygen (6.5 ± 1 mg/l). Food comprising fish pellets (every day) and wheat grains (twice a week) was supplied through the individual pipes to each cell twice a day. The fish pellets (Zemach Corporation-4714) were 4 mm diameter, 45% protein and 12% fat. The amount of food per animal per day was calculated as 4% of the body weight of the largest animal in the experiment. The animals were weighed (± 0.01 g) approximately once a month and the results per treatment are presented as average $\pm$ SD.

The density factor k was calculated from the equation $k = A/C^2$, where C, carapace length (cm) and A, bottom compartment area (cm^2). Specific growth rate (SGR) was calculated from the equation $SGR = \{[\ln(Wt) - \ln(Wi)] \times 100\}/t$ where Wt, weight at time t (g), Wi, initial weight (g), and t time (days). Growth rate was calculated from the equation, growth rate $= (Wt - Wi)/t$.

2.1. Experiment 1—Growth of males and females in separate cells

Sixty-three female and 63 male crayfish with a mean body weight of 10.7 ± 1.1 g were collected from a nursery system at Tzofar, Israel (latitude 32 °N) (Parnes and Sagi, 2002). Each animal was then held in a separate cell of our growout system for 98 days, from March to July. Males and females were alternated vertically in each of the 18 units, but horizontal and vertical distributions of the animals were random.

2.2. Experiment 2—Effects of cell size and location in the growout system on the growth of males held in separate cells

Since the first experiment showed significantly higher growth rates of male animals, a second experiment, starting with larger males, was designed to test growth performance and the effects of cell size on males grown in the same system. One hundred and twenty-six *C. quadricarinatus* males with a mean body weight of 23.2 ± 3.4 g were collected from an artificial pond at Ben-Gurion University of the Negev, Beer-Sheva, Israel. Each animal was held in a separate cell for 206 days, from August to March. The males were dispersed randomly among the cells. Weights and growth rates were compared between different cell sizes and different vertical locations.

2.3. Statistical analysis

Data is expressed as mean ± SD. Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by the Scheffe test for multiple comparisons. Weight and growth rate values were transformed to natural log (ln) or to square root (when needed) prior to statistical analysis. P values < 0.05 were considered statistically significant.

3. Results

3.1. Experiment 1—Growth of males and females in separate cells

The average weight of the male animals in the experiment became significantly higher than that of the female crayfish from the 36th day of the experiment and remained significantly higher until the end of the experiment ($P < 0.001$, Fig. 2). On the 36th day, the mean weight of the males was 20.5 ± 5.0 g, while that of females was 17.4 ± 3.5 g. At the end of the experiment, the mean weights were 36.6 ± 11.6 g for the males and 26.4 ± 6.9 g for the females. At that time, the weight distribution of the

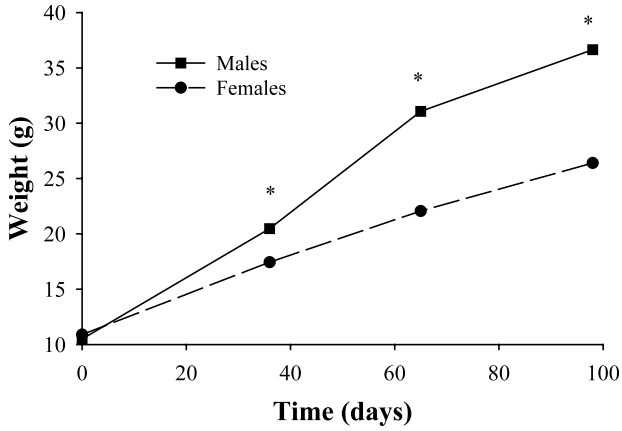

Fig. 2. Growth of *C. quadricarinatus* males and females in separate cells over a period of 98 days (March–July). Asterisk represents significant difference ($P < 0.001$, two-way ANOVA followed by Scheffe's Multiple Comparisons test).

males was wider, ranging from 16.2 to 73.1 g, while female weights ranged from 13.7 to 48.6 g.

The size of the cell had a statistically significant influence on the weight of both male and female animals (Fig. 3A and B). However, from agricultural point of view the differences in females are insignificant. The analysis showed a significant effect of cell size already after 36 days, mean weight being larger in the large and medium cells in comparison to the small cells ($P < 0.01$). Growth inhibition in the small cells was reflected in k value < 22.6. Throughout the experiment, there was no significant difference between the weights of the males in the large and medium cells. At the end of the experiment, the mean weight of the males in the large cells was significantly higher than that of the males in the small cells (40.6 ± 12.7 and 29.7 ± 7.1 g, respectively).

Growth rate of the male crayfish was significantly higher ($P < 0.05$) than that of female animals, regardless of cell size (Fig. 4). The minimum growth rate was 0.06 g/day for males and 0.04 g/day for females and the maximum growth rate was 0.64 and 0.39 g/day for males and females, respectively. The SGR for the males (1.20 ± 0.33) was significantly higher than that for the females (0.87 ± 0.31). Growth rate was significantly affected by cell size (Fig. 4). The growth rate of the males in the large and medium cells was significantly higher ($P < 0.05$) than that of the males in the small cells (0.31 ± 0.14, 0.29 ± 0.11 and 0.19 ± 0.07 g/day, respectively). Female growth rate was 0.18 ± 0.09, 0.15 ± 0.06 and 0.14 ± 0.06 g/day for large, medium and small cells, respectively.

During the course of the experiment, one male and two females died, a finding that represents a similar high survival of 98.4 and 96.8% for males and females, respectively.

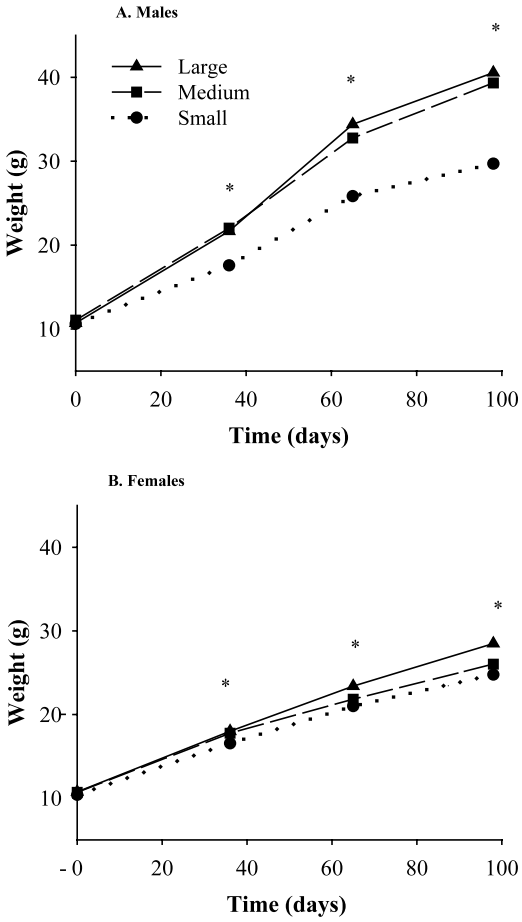

Fig. 3. Growth of *C. quadricarinatus* males (A) and females (B) in separate cells of different sizes over a period of 98 days. Asterisk represents significant difference between the small cell versus the large and medium cells ($P < 0.01$, two-way ANOVA followed by Scheffe's Multiple Comparisons test).

3.2. Experiment 2—Effects of cell size and vertical location on the growth of males in separate cells

The growth rate of males in the large cells in experiment 2 (0.22 ± 0.08 g/day) was significantly lower ($P < 0.05$) than that in the first experiment (0.31 ± 0.14 g/day), while the growth rate in the small cells was similar in the two experiments. Similarly to experiment 1, the average growth rates of males in the large and medium cells (0.22 ± 0.08 g/day) were significantly higher ($P < 0.05$) than that in the small cells (0.17 ± 0.06 g/day) ranging between 0.07 and 0.38 g/day (Fig. 5).

In keeping with the findings of experiment 1, the size of the cell had a marked influence on the weight of the male animals (Fig. 6). The average weight of the males in the large cells became significantly higher than that in the small cells from the 91st

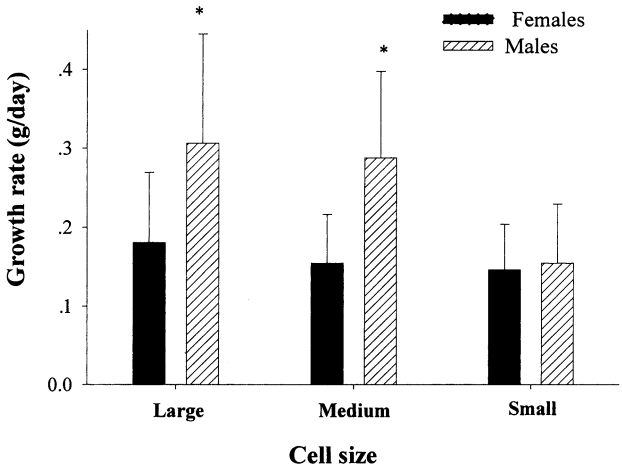

Fig. 4. Growth rate of *C. quadricarinatus* males and females in separate cells of different sizes over a period of 98 days (March–July). Initial mean weight was 10.7 ± 1.05 g. Asterisk represents significant difference between the male animals versus the female animals ($P < 0.01$, two-way ANOVA followed by Scheffe's Multiple Comparisons test).

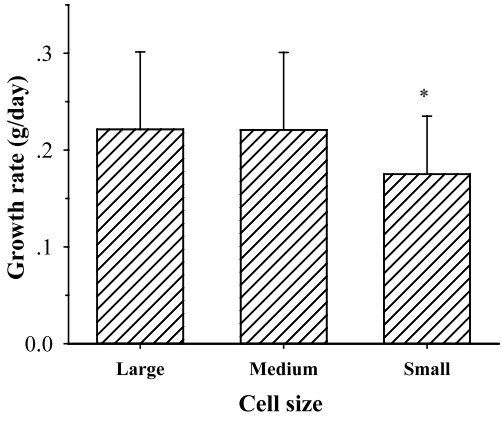

Fig. 5. Growth rate of *C. quadricarinatus* males in separate cells of different sizes over a period of 206 days (August–March). Initial mean weight was 23.2 ± 3.4 g. Asterisk represents significant difference between the small cell versus the large and medium cells ($P < 0.05$, two-way ANOVA followed by Scheffe's Multiple Comparisons test).

day of the experiment and remained significantly higher until the end of the experiment ($P < 0.05$). At the end of the experiment, the mean weight of the males in the large cells was 69.28 ± 15.72 g and that of the males in the small cells was 58.11 ± 12.66 g. On the other hand, the mean weight of the males in the medium cells became significantly higher than that of animals in the small cells only from the 147th day of the experiment ($P < 0.05$) and at that stage was not significantly different from that of the males in the large cells. The SGR of males in the large (0.51 ± 0.13) and

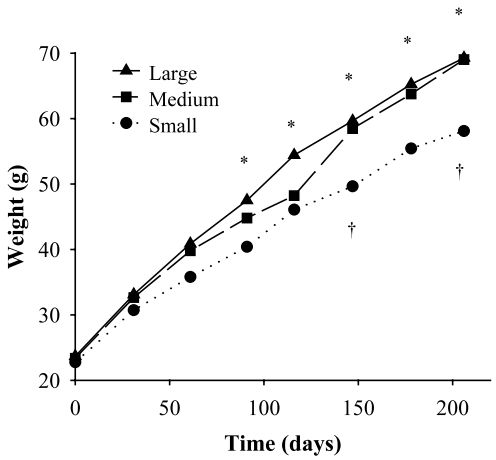

Fig. 6. Growth of *C. quadricarinatus* males in separate cells of different sizes over a period of 206 days. Asterisk represents significant difference between large and small cells; dagger represents significant difference between medium and small cells ($P < 0.05$, two-way ANOVA followed by Scheffe's Multiple Comparisons test).

medium (0.51 ± 0.11) cells was significantly higher ($P < 0.05$) than the SGR of males in the small cells (0.45 ± 0.12). The weights of males on the 206th day of the experiment ranged from 35.8 to 108.4 g.

The vertical location of the cell seemed to affect the growth rate of the male animals (Fig. 7). Growth rate of males in bottom cells was significantly higher ($P < 0.01$) in comparison to males in the upper cells (0.272 ± 0.89 and 0.158 ± 0.57 g/day, respectively).

During the course of experiment 2, 34 males died, which represents 73.0% survival (Fig. 8). It seems that the mortality was random in the different cell sizes, since in the

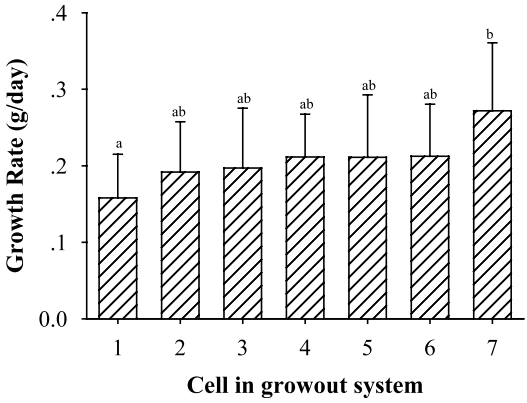

Fig. 7. Growth rates of *C. quadricarinatus* males in cells of different vertical locations. Cell 1 is the upper and cell 7 is the lowest. Different letters represent significant difference between groups ($P < 0.01$, two-way ANOVA followed by Scheffe's Multiple Comparisons test).

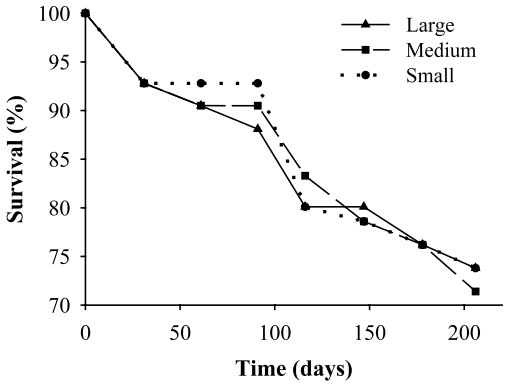

Fig. 8. Survival of male *C. quadricarinatus* throughout the experiment in separate cells of different sizes over a period of 206 days.

medium cells the survival was 71.4% (12 males died) and in the large and small cells the survival was 73.8% (11 males died in each cell size). In the large cells, mortality was higher in the lower cells (nine of the 11 males that died were housed in the two lower stories). In the medium and small cells, the vertical distribution of the mortality seemed random.

4. Discussion

We demonstrated that in our intensive separate-cell system *C. quadricarinatus* males grew faster than females and that growth was affected by cell size. Growth potential of the male animals in the large cells was almost double that of the female crayfish (0.16 vs. 0.31 g/day, Fig. 4). These results suggest that in an all-male monosex culture yields could be as much as 25% higher than those in a mixed population culture. Culture of monosex populations is, indeed, a common procedure in farmed animal husbandry and has been applied successfully in fish aquaculture (Mires, 1977; Tayman and Shelton, 1978). In studies on the cultured crustacean *Macrobrachium rosenbergii*, it was found that marketable yields were increased by culturing all-male populations (Sagi et al., 1986; Cohen et al., 1988; Hulata et al., 1988). In the light of these findings, a number of studies have been conducted on sex heritability in crustaceans with the aim of developing a technology to produce monosex populations as an aquacultural procedure (Sagi and Cohen, 1990; Malecha et al., 1992). The physiological mechanism that confers an advantage in terms of growth is thought to be mediated by the androgenic gland, which is responsible for male sexual differentiation and for the control of sexual plasticity in crustaceans (Charniaux-Cotton and Payen, 1988; Sagi, 1988; Payen, 1990; Sagi et al., 1997b). In *M. rosenbergii*, for example, the androgenic gland has been shown to play a role in growth mediation (Sagi et al., 1997b). In *C. quadricarinatus*, the androgenic gland has been identified (Khalaila et al., 1999), but further study is needed to reveal its

nature and function with the ultimate aim of developing a technology to produce monosex populations and promote growth rates.

In the present study, the density factor k value was used as an indicator of the time at which the cell size became a factor inhibiting growth. In the medium and large cells (314.2 and 490.9 cm^2, respectively), growth was not inhibited within the weight range of crayfish in this study (mean weights of 10–65 g), which suggests that these cell sizes are probably optimal or even too large. In the small cells (201.1 cm^2) growth of *C. quadricarinatus* was inhibited only when k declined below 22.6. In contrast, previous studies have shown that growth was inhibited when k fell below 33 for the lobster *Homarus americanus* (van Olst and Carlberg, 1978), below 50 for the crayfish *Procambarus clarkii* (Goyert and Avault, 1978) and below 45 for *C. tenuimanus* (Jussila, 1997). It thus seems that under the conditions of the present study, *C. quadricarinatus* is more tolerant to higher densities than other species of crustaceans. This is in keeping with the results of Du Boulay et al. (1995) in which growth of *C. quadricarinatus* was not impaired up to 20 g when k values were 39–45. Despite the fact that growth of the males in the small cells was 25% lower than that in the large and medium cells (0.17 and 0.22 g/day, respectively, Fig. 5), yield analysis (Table 1) showed that per given area the yield of the small cells was double (though the crayfish were smaller). There was a tendency for better growth in the lower cells with a significantly higher growth in the lowest compared to the upper cell (Fig. 7), probably due to getting additional feed. Further study on the optimal cell size and shape to be used in a commercial system is needed in light of a cost and market analysis, in addition to evaluation of growth performances. Currently, efforts are being made to design better structures that will solve agro technological problems, such as the need to provide a system for observing the animals throughout the growout period, ways in which to deal with accumulating debris on the bottom of the pond and in the compartment units, and means of feeding each single compartment separately.

To enable us to compare growth rates obtained in different experiments (i.e. between the two experiments in the present study or growth rates in communal tanks vs. individual cells), we used a growth rate index, the SGR. This is a useful index for comparing results obtained in different studies because of its simplicity of computation and its exponential nature (Evans and Jussila, 1997). The significantly higher SGR of males in the large cells in the first experiment (1.37) compared to that in the second experiment (0.51) (Table 1) might be due to the different seasons in which the two experiments were conducted and/or to the different ages and sizes of the crayfish at the beginning of the two experiments. In some studies photoperiod has been shown to have minimal effect on crayfish growth (Saez-Royuela et al., 1996), while in others both growth and survival were improved when the photoperiod was increased (Westman, 1973; Mason, 1979; Taugbol and Skurdal, 1992). The higher SGR of males in the first experiment is probably a function of the mean body weight at the time of stocking (10.7 g in the first experiment vs. 23.2 g in the second), since growth rate is known to decline with age in freshwater crayfish (Lowery, 1988).

Table 1
Comparison between different *C. quadricarinatus* growout studies in Israel

Culture	Effective density (ind./m^2)	Average initial weight (g)	Survival (%)	Growth rate (SGR)	Yield (kg/m^2)
Polyculture (Karplus et al., 1995)	0.75	2.9	25.1 at 92 days	2.69	0.006
Intensive polyculture (Karplus et al., 2001)	20	7.1	60 at 133 days	1.11	0.214
Monoculture first season (Sagi et al., 1997a)	5.5	1.05	60 at 160 days	2.32	0.142
Monoculture second season (Sagi et al., 1997a)	1	42	78 at 226 days	0.52	0.107
Superintensive culture First experiment (present study)	142.7 large 348.1 small	10.3 large 10.1 small	Large 100 Small 95.2 both at 98 days	1.37 large 1.1 small	4.547 large 8.121 small
Superintensive culture Second experiment (present study)	142.7 large 348.1 small	23.7 large 22.8 small	Large 73.8 Small 73.8 both at 206 days	0.51 large 0.45 small	7.759 large 15.889 small

The comparison was made between earthen pond cultures and superintensive culture in separate cages. The results of the polyculture and monoculture second growout season pertain to males. The results from the superintensive culture pertain to males in large and small cells.

To determine the efficiency of the growout system used in this study vs. that of communal cultures, we compared our system with other systems tried in Israel with the same stock of *C. quadricarinatus*. The factors showing the most marked differences between the communal cultures and the intensive individual cultures were the effective density and the yield (Table 1). The most noteworthy advantage of the intensive culture method seems to be lying in the possibility of increasing the effective density by one (Karplus et al., 2001) or two (Karplus et al., 1995; Sagi et al., 1997a) orders of magnitude. Similarly, there was an enormous improvement in yield compared to conventional practice. Table 1 shows that with the intensive culture method the yields are 55 (in the large cells) or 112 times (in the small cells) larger than that in conventional practice (monoculture first season, Sagi et al., 1997a). Other factors, such as survival and growth rate, did not show marked differences (Table 1). The comparison presented in Table 1 might seem problematic because of the different conditions in the various studies, particularly communal vs. individual cell cultures. To minimize the effects of the differences between the studies and hence to facilitate comparison between them we calculated the yield per unit area for our study conservatively, i.e. by dividing the amount of biomass by the area of the square into which the circular cell would exactly fit. In this way, we take into account the unutilized space between the circular cells. The effective density was similarly reduced by allocating approximately 30% of the area to the filter and other non-productive components. Despite these allowances made for the area in this study, both the yields and the effective density were much higher in our superintensive culture than in the communal cultures.

The advantages of the superintensive system could be further demonstrated by comparing the yields with the experiment done by Curtis and Jones (1995). This comparison shows that the intensive culture has an advantage of several orders of magnitude compared to culture of all males in earthen ponds. Based on the advantages of the intensive culture, which were demonstrated in terms of effective density and yields, we recommend that *C. quadricarinatus* be cultured in all-male populations in individual cells having a size that gives the optimal results. To develop a commercially viable intensive crayfish culture system, it is necessary to develop an optimal superintensive culture system that takes into account the expenditures of such system and the expected income.

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