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Intensification of redclaw crayfish *Cherax quadricarinatus* culture I. Hatchery and nursery system

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Abstract

Intensification of an indoor hatchery and nursery system for the Australian redclaw crayfish *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae) was obtained by increasing the surface area available for the crayfish juveniles and by synchronizing the age of the hatchlings held in each tank. The former improvement was facilitated by distributing an artificial seaweed-like material throughout almost the entire volume of small (275 l) hatching tanks. As the number of egg-bearing females was increased from 3 to 8 per hatching tank, the number of juveniles per liter also increased to as many as 6.5 juveniles/l, without reaching an apparent upper limit. The hatchlings were kept in the tanks for 75 days from the day females were found to be gravid and then harvested and graded according to size. The average juvenile weight at harvest was 0.34 ± 0.04 g. The weight distribution of the juvenile males was not significantly different from that of the juvenile females on the day of harvest, and in both the distribution was positively skewed.

Keywords: Crayfish; Red claw; Crustacea; Hatching system; Nursery system; Artificial substrate; Juveniles; Weight distribution; Intensive culture; Sex ratio; *Cherax quadricarinatus*

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1. Introduction

A steady supply of good quality fry is a basic requirement for an intensive crustacean culture system, allowing it to operate more effectively, at higher capacity and with greater profitability. The development of a closed recirculating water hatchery and nursery system is thus a prerequisite for the controlled production of young disease-free crustaceans. Different hatchery techniques, with varying degrees of intensification, have already been developed for a variety of crustaceans, including shrimps (*Penaeus* species) (Aquacop, 1983b; Jackson et al., 1992), lobsters (*Homarus* species) (Chang and Conklin, 1983), spiny lobsters (*Palinurus* species) (Kittaka, 1997), a freshwater prawn (*Macrobrachium rosenbergii*) (Aquacop, 1983a; Malecha, 1983), a crab (*Cancer irroratus*) (Charmantier-Daures and Charmantier, 1991), and some species of freshwater crayfish (the Australian redclaw crayfish *Cherax quadricarinatus* and *Procambarus clarkii*) (Jones, 1995; Nelson and Dendy, 1979).

At present, commercial production of freshwater crayfish, including *C. quadricarinatus* is largely performed extensively (in earthen ponds) or semi-intensively (in large tanks) (Lawrence and Morrisy, 2000). However, growing interest in the farming of large, high-value freshwater crayfish of the genus *Cherax* in temperate climates has been accompanied by the need to develop semi-intensive and intensive methods (Manor et al., 2002) based on smaller systems and to develop both intensive hatchery production of juveniles and nursery culture of advanced juveniles for pond stocking (Verhoef and Austin, 1999).

Two major factors complicate the mass production of *C. quadricarinatus* juveniles. The first is the fact that *C. quadricarinatus* is a benthic animal, normally living on the bottom and leaving the water column virtually empty of animals, even though the behavior of juvenile crayfish suggests that they may be less benthic than adults (Cukerzis, 1986; Jones, 1995). The second factor is the inability of growers to control and monitor the age of juvenile crayfish in earthen ponds. This situation results in major loses due to predation by the larger animals both on the smaller crayfish and on the animals going through the vulnerable molt stage. As a result, hatching ponds must be continuously harvested.

The first step towards the intensification of a hatchery system for *C. quadricarinatus* was taken by Jones (1995). This author summarized some of the basic requirements and parameters concerning the production of juvenile redclaw crayfish such as environmental conditions, ovary and egg development staging and broodstock male:female ratio. The work presented here addresses some of the problems raised by Jones, i.e. the optimal stocking density and juvenile habitat requirements. The distribution of juvenile weights and sizes on the day of harvesting is also presented.

2. Materials and methods

2.1. Timing of the experiments

The experiments were conducted over four consecutive seasons from the winter of 1997 until the summer of 2000.

2.2. Broodstock

Male and female *C. quadricarinatus* crayfish were grown in our facility at Ben-Gurion University of the Negev. Broodstock, one male and five females per tank, were held all year round in beige plastic tanks ($70 \times 50 \times 45$ cm) each filled with 100 l of fresh water. The tanks were kept indoors at 27 ± 2 °C and a photoperiod of 14L:10D. The tanks were supplied with pieces of PVC piping (16 cm long, 5 cm diameter) as shelters for the animals. Good water quality was maintained by circulating the water through an immersed gravel biofilter via an airlift. The animals were fed three times a week, twice with a mixture of wheat grains and grated potatoes and carrots, and once with pieces of frozen fish fillets. Food was supplied ad libitum. Broodstock tanks were cleaned by siphoning once a week. Water was added to compensate for water lost during the siphoning process and from evaporation. Ammonia and nitrite levels were monitored on a weekly basis. A stock of males and females were held separately in temperature-controlled large plastic tanks (5 m^3) located in a greenhouse. Dead brooders were replaced. The average weight of females used in the experiments was $51.4 + 18.9$ g (range 21.4–99.5 g). The males were matched to females according to their size and were at least as large as the females.

2.3. Hatchery and nursery system

The hatchery and nursery system and the time schedule of the experiment are presented diagrammatically in Fig. 1. The system consisted of five 275 l fiberglass tanks ($100 \times 60 \times 50$ cm; bottom surface area of about 0.6 m^2), all connected to a single external gravel biofilter, through which tank water was circulated at a rate of 0.6 tank volume per hour. The tanks were painted dark green. A mesh cover over the water outlet (hole size 1.5 mm) of each tank prevented the juveniles from moving from one tank to another. The mesh covers were cleaned 3–4 times a week, but the hatching/nursing tanks themselves were not cleaned until harvest day (75 days from the date females were found to be gravid).

Six plastic elements designed to have a seaweed-like form (Fig. 2) were placed in each tank. Each element was composed of a mass of black high-density polyethylene filaments (a by-product of the plastics industry) of width 3–7 mm and approximate thickness 0.15 mm attached to a plastic base. Each element, which was encased in a piece of plastic fencing to facilitate handling, occupied an approximate volume of 30–40 l and had a surface area of about 8–9 m^2. Since the specific gravity of the plastic material (0.945 g/cm^3) is close to that of water, the black filaments floated

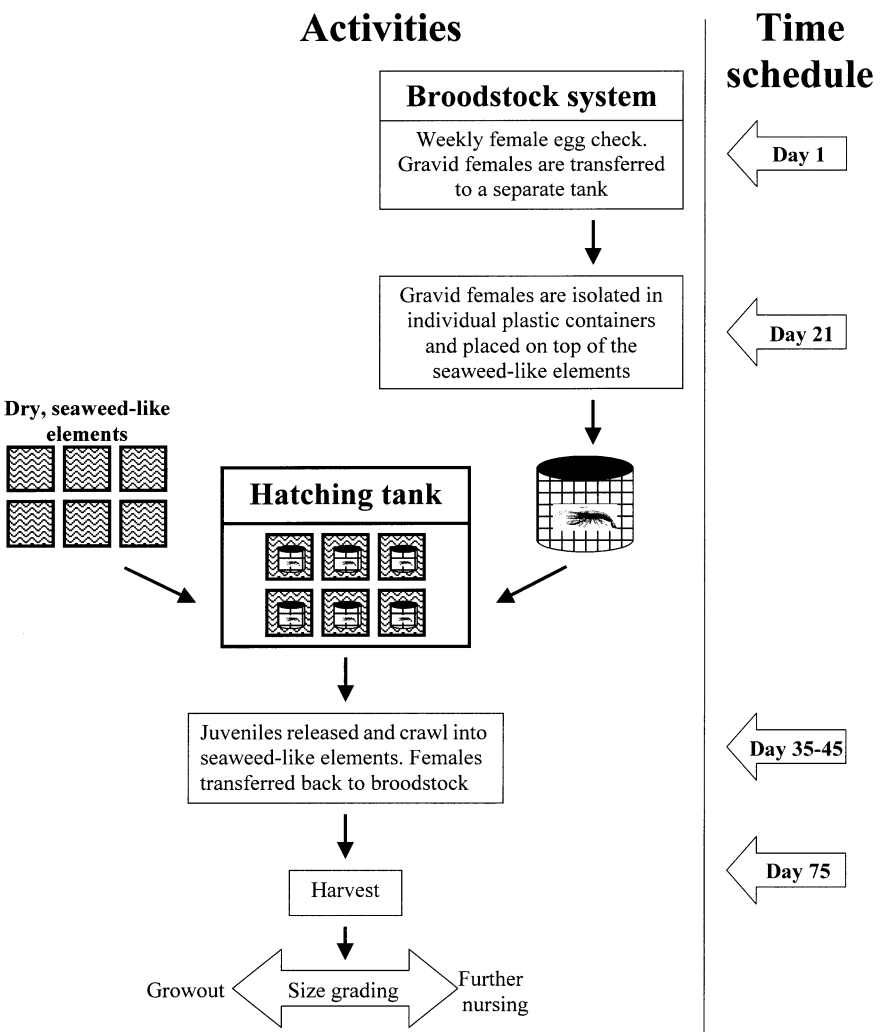

Fig. 1. Flow chart of the sequence of events in the intensive hatchery system. Days are counted from the day the females were found to be gravid. The number of the females represented in the chart was chosen arbitrarily.

such that they were distributed throughout about 80% of the tank volume. The six elements occupying each hatching tank gave the tank an approximate surface area of 50 m^2. Each tank was supplied with three air stones for continuous aeration, one stone between each pair of elements.

After each harvest, the elements were removed from the tanks and left to dry for at least 24 h before being used for the next batch, thus ensuring that no live juveniles remained inside the elements. Similarly, before the tanks were stocked with gravid females, they were thoroughly scrutinized for the presence of juveniles from the previous batch. Failure to follow these procedures resulted in cannibalism of newly

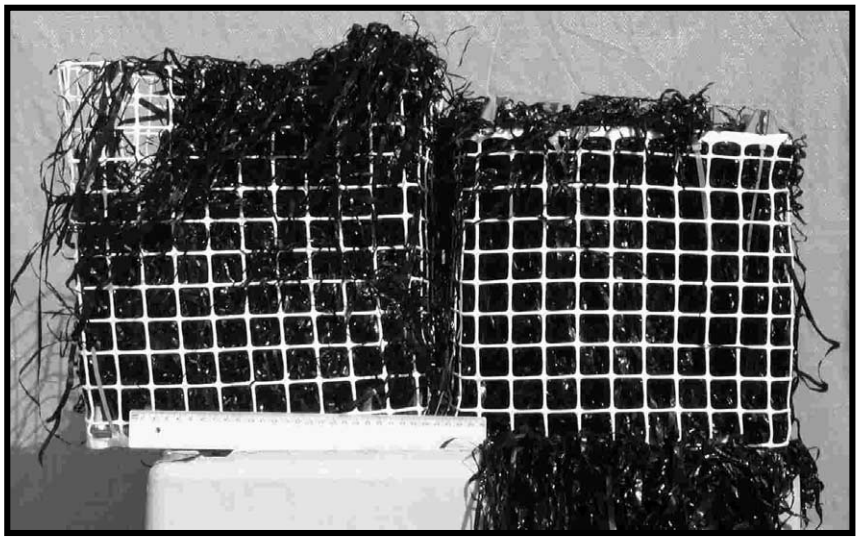

Fig. 2. Two seaweed-like plastic elements designed for intensive *C. quadricarinatus* hatching and primary nursing. The one on the left is oriented in the same way it would be in a hatching tank and the one on the right is turned upside down showing the harvest position.

hatched juveniles by older animals from the previous batch. In general, nursing juvenile crayfish without supplying any shelter results in a rapid disappearance of large numbers of animals due to cannibalism (based on our previous experience, common knowledge and Jones, 1995). Thus, in the present study we did not find it necessary to employ a treatment with no substrate as control.

Water quality and temperature were the same as those for the broodstock system, as was the photoperiod.

2.4. Hatching procedures

The broodstock females were checked once a week for presence of eggs. Each week gravid females were transferred to a separate holding tank, where they remained undisturbed for 3 weeks from the time of egg laying. Thereafter, females having intact, healthy embryos were transferred to the hatching tanks, in which each animal was held individually in a perforated cylindrical plastic basket (18 cm diameter, 23 cm height, 5 mm hole size diameter). This procedure prevented cannibalism by the female on craylings released from other mothers (Levi et al., 1999). Each basket was supplied with a piece of a PVC pipe (7.5 cm diameter, 13 cm length), as a shelter for the brooding female. The basket bottom surface area (approximately 250 cm^2) was less than half of that reported by Levi et al. (1999), but did not have any observable effect on the release time of the juveniles. Each basket containing a female was placed in a net bag (5 mm hole size) to prevent the female from drawing the seaweed-like material into the basket and becoming tangled up in it. This arrangement was necessary, since entanglement could prevent movement of the female's abdomen and

hence fanning of the craylings. Each tank was stocked with three to eight females that laid their eggs not more than 1 week apart. The release of juveniles was monitored once a week, and females that had released craylings were returned to the broodstock tank.

2.5. Nursing procedures

Feeding of the juveniles started as soon as the first female of those occupying a tank was seen to release craylings. The juveniles were fed three times a week with a mix of grated potatoes and carrots and commercial fish pellets. Food was supplied ad libitum. At the end of the nursing period, the juveniles were harvested from the tank by taking the seaweed-like elements out of the tank, placing them upside down above a plastic container and flushing them out with water. The juveniles crawled out of the element and fell into the container. The juveniles from each tank were then separated by size in a grader composed of five plastic trays placed one on top of the other, each tray being fenestrated with holes of different sizes (11/9/7/5 or 3 mm diameter). The animals were placed in the upper box and moved down through the holes according to their size. Thereafter, they were counted and weighed. A representative batch was sexed by means of a stereomicroscope, and each animal of the batch was weighed individually.

2.6. Data analysis

Data were analyzed by means of the Basic Statistics and SigmaPlot 5.0. Statistical analysis was based on parametric tests: Lilliefors test for normality, one-way ANOVA followed by LSD test. Where the data had a non-parametric character, a suitable non-parametric test was applied (Mann–Whitney U Test).

3. Results

A quantitative evaluation of the distribution of the animals inside the seaweed-like elements was not performed, but a qualitative visual inspection of the hatching tanks at different times of the day was undertaken. In a young batch, juveniles were not seen on top of the seaweed-like elements, but as time advanced, some larger animals were observed near the top of the tank, crawling on the seaweed-like material. Pushing aside the seaweed-like material revealed juvenile animals all the way down towards the bottom of the tank.

As can be seen from Fig. 3, an increase in the number of females stocked per hatching tank brought about a steady rise in the number of juveniles harvested per tank, with no apparent plateau. There was a significant difference ($***P < 0.001$) between the numbers of juveniles from tanks stocked with three vs. eight females (600 ± 15 vs.1500 ± 181, numbers are Mean $\pm$ S.D.). The numbers of juveniles harvested from tanks stocked with four to seven females were not significantly different from one another. The number of juveniles produced per liter of tank

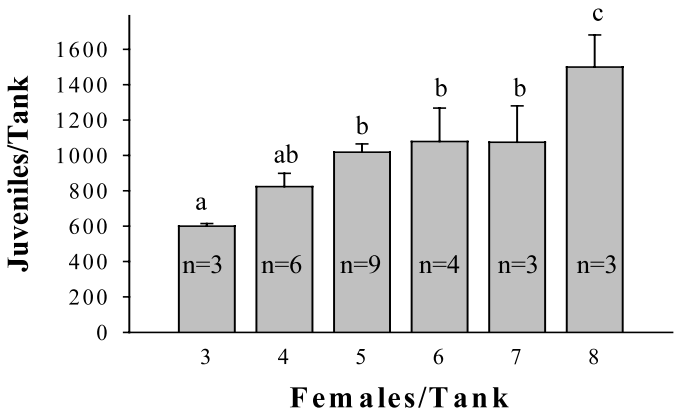

Fig. 3. Number of *C. quadricarinatus* juveniles harvested per intensive hatching tank as a function of the number of gravid females introduced into the tank. Bars represent SE; different letters indicate significant differences between groups ($P < 0.05$, one-way ANOVA followed by LSD test).

volume increased from 2.2 ± 0.1 for three females per hatching tank to 5.5 ± 0.7 juveniles per liter for eight females per tank (***$P < 0.001$). The average number of juveniles produced per female was 193.2 ± 40.8 and did not differ significantly between batches having different numbers of females (Fig. 4).

Average juvenile weight on harvest day was 0.34 ± 0.04 g. Fig. 5 presents the size distribution of a representative batch of juveniles sorted in the size grader on harvest day. The majority of the juveniles (68.4%) were small enough to slip through the tray with the smallest holes (3 mm in diameter); 23.0% of the juveniles in the sample were stopped by the 5 mm holes, 7% by the 7 mm holes, and only about 1.6% by the 9 mm holes. A non-normal distribution pattern was found for this batch (Lilliefors **$P <$ 0.01: Skewness = 1.652). All the batches that were sorted by this method showed the same positively skewed pattern.

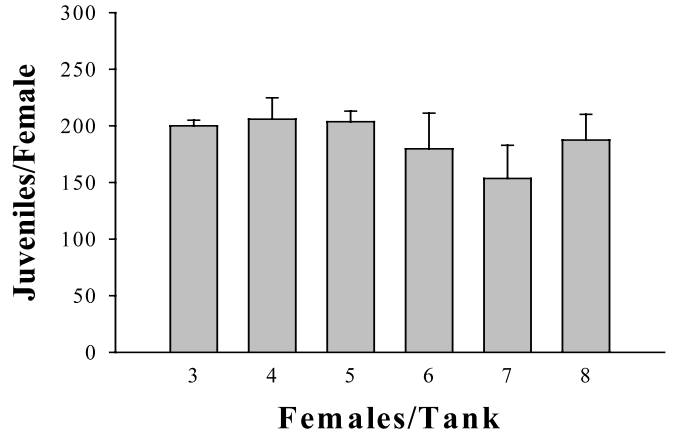

Fig. 4. Number of *C. quadricarinatus* juveniles produced per female as a function of the number of gravid females stocking the hatching tank.

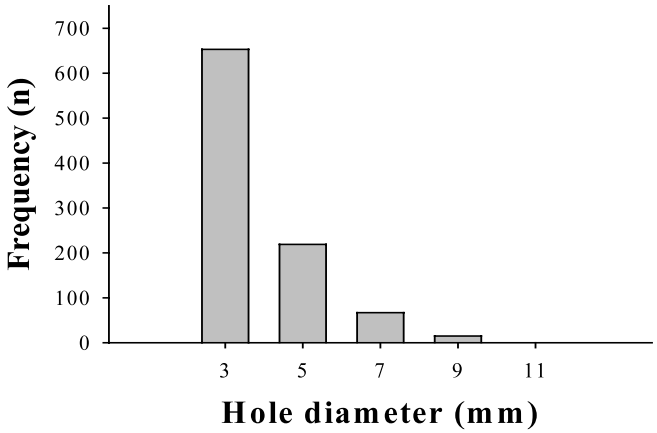

Fig. 5. Size frequency distribution of a representative *C. quadricarinatus* batch at the end of an intensive primary nursing period. The population was graded for size in a five-tray grader with holes ranging from 3 to 11 mm. (The population consists of 954 juveniles, which are the offspring of five females in the hatching tank).

Fig. 6 presents detailed weight frequency distribution diagrams for juvenile males (Fig. 6A) and females (Fig. 6B) from a representative batch in which the animals were weighed individually on harvest day. The weight of the females did not differ significantly from that of the males ($U = 75\,618$, d$f = 1$: $P > 0.05$) and both were found to have a non-normal, positively skewed distribution (Lilliefors $^{**}P < 0.01$: skewness was 1.249 and 1.654 for males and females, respectively). Average male and female weights of 0.54 ± 0.02 g ($n = 432$) and 0.49 ± 0.02 g ($n = 376$), respectively, did not differ significantly from one another. The batch ratio of males to females was 1.149:1, a value that did not differ significantly from the expected ratio of 1:1.

4. Discussion

Intensification and synchronization of a hatchery and nursery system are both prerequisites for the intensive mass culture of all aquatic organisms, including crayfish. The currently used methods for the culture of *C. quadricarinatus* have two major disadvantages: (1) the large amount of water required because the juveniles are benthic, i.e. use only of the bottom surface area; and (2) the difficulty of managing synchronized batches in large ponds. Production rates are thus low.

Some years ago, it was reported that intensive culture of crayfish juveniles for stocking into extensive systems is done, particularly in Europe (Du Boulay et al., 1993). A later study, described the stocking of 10–200 m^2 ponds with juveniles at densities of 50–100 animals per m^2 of pond bottom surface area (Jussila, 1997). One of the first successful trials with artificial volume occupying substrates for high density rearing of crustacean juveniles was performed with the freshwater prawn *M. rosenbergii* (Smith and Sandifer, 1979). However, the prawns used at that study were already after primary nursing and the density reported was lower then 1.1 juvenile

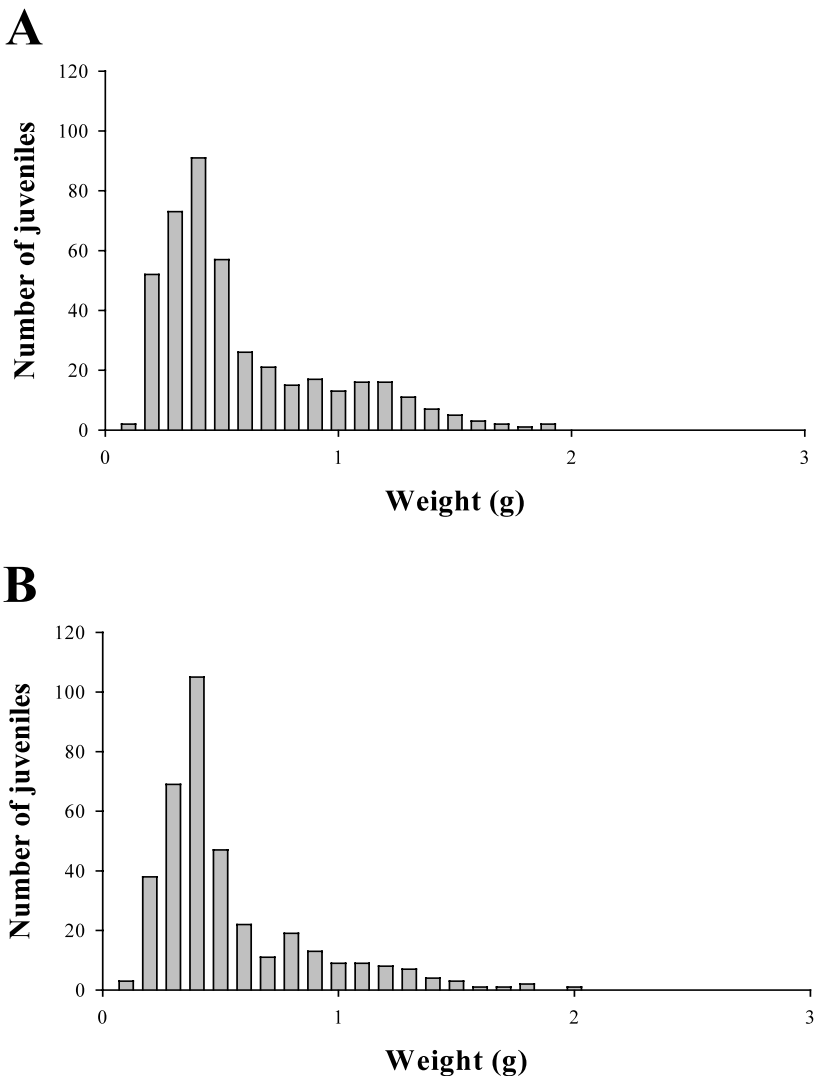

Fig. 6. Representative weight frequency distribution of *C. quadricarinatus* juvenile males (A) and females (B) from an intensive system at the end of the primary nursery period. (The population consists of 808 juveniles, which are the offspring of four females in the hatching tank.)

prawns per liter of tank water, nevertheless trials which followed the above study showed good growth response of the juvenile prawns to artificial substrate (Cohen et al., 1983; Tidwell et al., 1998, 1999). Trials with artificial substrates for juvenile crustaceans production were performed on commercially important penaeid species showing improved survival but not growth in the marine shrimp *P. vannamei,* and no effect in *P. semisulcatus* and *Metapenaeus monoceros* (Sandifer et al., 1987; Kumlu and Eroldogan, 2000; Kumlu et al., 2001). The current study shows that the

calculated surface area of a typical pond used in the above methods (Jussila, 1997) was applied into a small tank via the use of our material, thus enabling the culture of a very large population of juvenile crayfish. The six artificial seaweed-like elements in the hatching tank provided the crayfish juveniles with a habitat of about 50 m^2, a value >80 times bigger than the area of the tank bottom (i.e. 0.6 m^2). Despite the apparently crowded situation, the stocking densities, even at the highest initial stockings of eight gravid females per tank, remained lower than 40 juveniles/m^2, a value comparable with that reported by Jussila (1997). Since the surface area available to the juveniles in our system was distributed throughout the tank volume, we could calculate juvenile densities per liter of tank water rather than the conventional per square meters. Our calculation showed that the number of juveniles per liter of water was high, being 2.2–5.5, and it is this favorable ratio that gives our system its high production ability. The young crayfish related to the seaweed-like material as a walking surface as they would normally do to the bottom of the tank. In addition, the black seaweed-like material excluded light from the tank, thus providing the animals with the darkness to which they are accustomed. As a result of these two properties of the artificial surface, the juveniles utilized almost the entire volume of the tank.

The second disadvantage of the older methods, i.e. the lack of synchronization of the batches of crayfish juveniles, may lead to the dominance of a few large animals over a large group of small ones and hence to cannibalism by the large animals on the smaller ones or to growth retardation in the latter. Previous experimentation with *C. quadricarinatus* juveniles indicated that cannibalism is often high (Jones, 1995). Since cannibalism may even occur under ideal conditions that allow the juvenile crayfish to molt and grow rapidly, their vulnerability during ecdysis is considerably increased. Synchronization of the age of the juveniles in a batch can partially overcome this problem, which was addressed in our system in two ways: firstly, by thoroughly scrutinizing the hatching tanks for juveniles from a previous batch and secondly by stocking the tanks with gravid females that were, at most, a week apart in their dates of egg laying. In this way, we managed to obtain synchronized batches of juveniles, to minimize loses due to cannibalism, and to harvest more uniform populations.

The phenomenon of a non-normal, positively skewed distribution of juvenile crustaceans upon harvest has been reported for the marine shrimp, *P. vannamei* (Kalagayan et al., 1991), for the freshwater prawn, *M. rosenbergii* (Madhusoodana et al., 1998) and for the crayfish *C. quadricarinatus* (Curtis and Jones, 1995). For *P. vannamei* these findings were attributed to a viral infection, which also slowed the growth of the shrimps, and for *M. rosenbergii* to the complex social organizational hierarchy of the prawns. In the case of the crayfish, positively skewed distributions were found for mixed and monosex, i.e. all-male or all-female populations. The reason for the non-normal distribution found in *C. quadricarinatus* in the present study may lie in the genetic make-up of the animals or in the social structure of the population. This work also shows that, like in other crustaceans, there is no difference in the growth performance of males and females at the early juvenile stage, unlike the case in the adult population (Sagi et al., 1997).

The results reported in the present study clearly show that the numbers of juveniles harvested from our hatching and nursery system may be increased even further, particularly since the efficiency of the system (i.e. the number of juveniles produced per female) did not decline, as could be expected, as the number of females was increased (Fig. 3). However, it should be emphasized that simultaneously placing more than eight females per hatching tank was impossible in our system due to technical reasons. Thus, examination of its biological limit was not applicable. However, a working crayfish farm that adopted and scaled-up the above system is currently operational with high yields (Ben, personal communication). Improving the diet and feeding regime of the juvenile crayfish could probably enhance the productivity of the system. Previous work has indeed shown that under artificial conditions reasonable survival and growth of juvenile crayfish has been achieved with formulated diets (Celada et al., 1989).

In summary, taking advantage of a simple seaweed-like plastic material that provides an enormous surface area in a body of water, we succeeded in producing large quantities of crayfish juveniles in a small volume of water. This space-saving system also enabled us to synchronize the age of the juveniles, thus producing a more uniform population compared to that cultured in a traditional pond.

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