ELSEVIER

Aquaculture 166 (1998) 259–267

Aquaculture

Culture of the Australian red-claw crayfish (*Cherax quadricarinatus*) in Israel
III. Survival in earthen ponds under ambient winter temperatures

Ilan Karplus [a,*], Myron Zoran [a], Ana Milstein [a], Sheenan Harpaz [a], Yoav Eran [b], Dan Joseph [b], Amir Sagi [c]

[a] *Department of Aquaculture, Agricultural Research Organization, P.O. Box 6, Bet-Dagan 50250, Israel*
[b] *Department of Fisheries, Ministry of Agriculture and Rural Development, Fish and Aquaculture Research Station, Dor, D.N. Hof Hacarmel 30820, Israel*
[c] *Department of Life Sciences, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel*

Accepted 18 May 1998

Abstract

The survival of the tropical crayfish *Cherax quadricarinatus* in earthen ponds under ambient winter temperatures was studied in the temperate zone in the central coastal plain of Israel. Four treatment groups in which sex and size were each tested at two levels were simultaneously run in four replicates over a period of 118 days (28 November 1996–26 March 1997). Minimum and maximum water temperatures were monitored every day, with minimum daily temperatures of under 10°C being recorded on six days. Overall survival was 60%; neither independent nor combined effects of sex and size on survival were found to be significant. Change in weight was minimal and few crayfishes entered baited traps, probably reflecting the reduced motor activity and feeding at low water temperatures. The economic implications of crayfish survival in earthen ponds under ambient winter temperatures, as well as the potential establishment of wild populations are discussed. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Cherax quadricarinatus; Cold tolerance; Red-claw; Crayfish

* Corresponding author. Tel.: +972-3-9683388; Fax: +972-3-9605667; E-mail: vlaqua@volcani.agri.gov.il

PII S0044-8486(98)00290-7

1. Introduction

One of the major constraints for profitable outdoor culture of tropical crustaceans in the temperate zone is growth arrest and mortality at low temperatures. Despite these constraints for aquaculture, tropical species are superior for culture compared to temperate climate species due to their higher growth rates. However, during the cold months in the temperate zones, most tropical species, if not marketed, have to be removed from the grow-out ponds and stored under warmer conditions. Storage in greenhouses is expensive, space limited and problematic due to heavy cannibalism found among many species of crustaceans when maintained at high densities. As a consequence, mainly brood stocks are stored over winter, while the bulk of the population is disadvantageously cultured for only short periods—stocked into grow-out ponds in spring and harvested during autumn (Sandifer and Smith, 1975; Lee and Wickins, 1992).

The tropical Australian red-claw crayfish *Cherax quadricarinatus* is presently cultured both in the tropic and temperate zone regions (Ackefors, 1994; Medley et al., 1994; Karplus et al., 1995). Knowledge of the lower ranges of temperatures that can be tolerated are of particular importance to growers in the temperate zones. Reports on cold tolerance of red-claw are based mainly on personal communications lacking details of the experimental procedures. Different lowest tolerated temperatures have been reported: 14°C (Morrisy et al., 1990), 12°C (Rouse, 1995), 5°C (Merrick and Lambert, 1991) and even 4°C (Semple et al., 1995). Some authors have also elaborated on the importance of the duration of time spent at specific temperatures. Merrick and Lambert (1991) stated that this crayfish survived 5°C for three weeks, but suffered from high mortality when exposed to 9°C and less for a period of two to three months. Similarly, Semple et al. (1995) reported that red-claw could tolerate temperatures as low as 3°C for short periods of time, but would die following prolonged exposure to temperatures below 10°C. The effect of size on cold tolerance in *C. quadricarinatus* was addressed in a short preliminary study using six large ($\bar{x} = 52$ g) and six small ($\bar{x} = 13$ g) individuals. Temperature was slowly reduced from 25°C to 6–7°C over a period of 16 days. Crayfish were exposed to temperatures below 10°C for seven days and to temperatures of around 7°C for two days. At temperatures of 6–7°C, crayfish became immobile and only slowly resumed normal position after being placed on their back. Water warming, in order to return to the original temperature, was relatively rapid and carried out over a period of two days. Both small and large crayfishes survived this exposure to cold temperatures without any mortality (Zoran et al., 1996).

During two consecutive years (1994 and 1995), large red-claw specimens were found at the Dor Fish and Aquaculture Research Station (32.7°N, 35.0°E) in open earthen ponds, when drained at springtime, after the cold winter months. These crayfish survived ambient winter temperatures; however, percent survival was not known (Joseph, pers. comm.). These field observations, coupled with the information on cold tolerance of red-claw crayfish and the fact that the water temperature in fish ponds drops below 10°C for only limited periods of time in the central coastal plain of Israel (Gat et al., 1992), suggest that *C. quadricarinatus* may be able to survive ambient winter temperatures in open earthen ponds. This information presents favorable economic potential, but also an environmental hazard due to the risk of these crayfish establishing viable populations in nature.

The ability to culture the Australian red-claw crayfish outdoors throughout the year in the temperate climate zones without the need for overwintering in greenhouses during the colder months would substantially contribute to culture profitability. The aim of the present study was to test the effects of sex and size at stocking on the survival of this species in earthen ponds under ambient winter temperatures.

2. Materials and methods

The effects of sex and size at stocking on the survival and growth of crayfish (*C. quadricarinatus*) in earthen ponds under ambient winter temperatures were studied using a factorial experimental design. Sex and size were each tested at two levels (i.e., male vs. female and large vs. small). The experiment, thus, consisted of four treatment groups (Fig. 1), simultaneously run in four replicates over a period of 118 days (28 November 1996–26 March 1997). In order to control environmental effects related to the spatial location of the 16 experimental ponds, treatments were assigned to ponds following a randomized block design, with all treatments represented in each of the four blocks.

From a pond raised population held over winter within plastic-covered earthen ponds, 1600 crayfish were selected for this study. Only hard-shelled, intact animals were stocked. The large males and females included crayfish that were not smaller than 20 g or larger than 75 g. The small males and females were not smaller than 5 g or larger

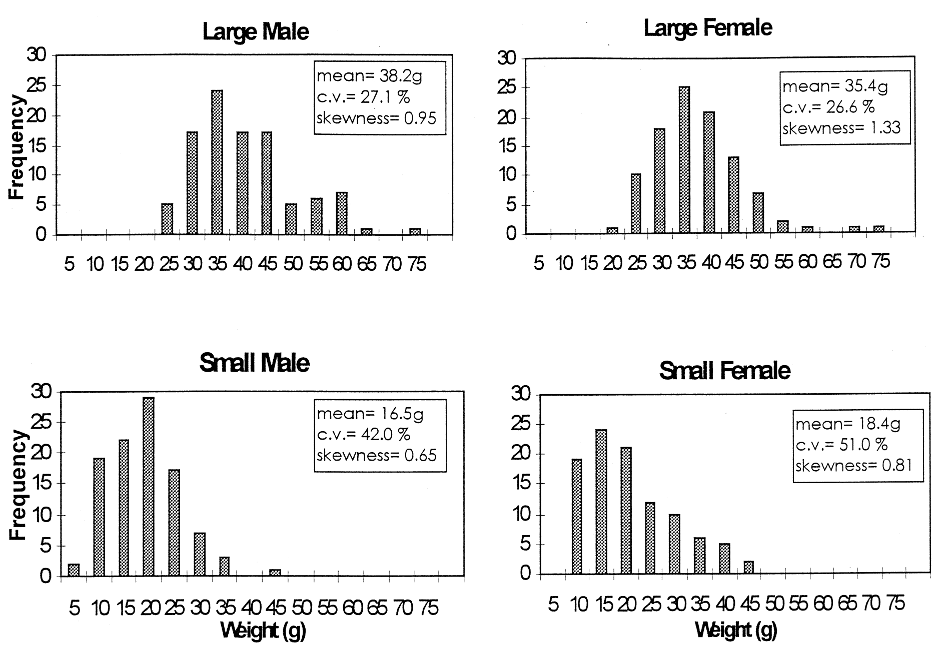

Fig. 1. Size distribution of *C. quadricarinatus* at stocking, each size and sex combination represents a different treatment.

than 45 g. Groups of 100 crayfish were bulk-weighed before stocking into ponds. Average weight of large male and female crayfish was 40.3 ± 2.6 g and 36.3 ± 1.0 g, respectively. The small male and female crayfish averaged 17.3 ± 1.0 g and 18.6 ± 0.8 g, respectively. One replicate from each treatment was also weighed individually to the nearest 0.1 g prior to stocking (Fig. 1).

The study was carried out at the Dor Fish and Aquaculture Research Station in 16 rectangular 300 m^2 earthen ponds, with a mean water depth of about 1 m. Water was added to the ponds only to make-up for losses due to evaporation and seepage. The pond slopes were lined with stones, which provided ample shelters for the crayfish. The ponds were not aerated throughout the study. Feed consisted of commercial crayfish pellets, provided at 4% of crayfish biomass at stocking and kept at the same level throughout the study. Proximate analysis of diet was: protein 29.3%, fat 5.6%, calcium 4.7%, phosphorus 1.0%, ash content 16.6% and crude fiber 3.3%. Pellets contained vitamin premix 0.5% and 2% guara gum which gave the pellets water stability for at least 3 h. Food was provided manually in the mornings. Minimum and maximum water temperatures were recorded every morning in four ponds, each located within a different block. Water quality (ammonium (NH_4^+), nitrite (NO_2^-), pH and dissolved oxygen) was monitored in the mornings, once every 14 days in all ponds. Oxygen and pH were measured in the field with portable meters. Ammonium and nitrite were determined using colorimetric Hach kits (respective serial numbers 21194/49 and 14078/49). An attempt to sample the crayfish was carried out in the following manner: crayfish were sampled once every 10 days with the aid of wire traps baited with fish, in four ponds, each representing a different treatment group.

Six silver carps (*Hypophthalmichthys molitrix*), each weighing about 1700 g, were stocked on 17 December into each pond to prevent the development of algal blooms. Ten common carps, each weighing about 120 g, were stocked on 29 December into each of the ponds to prevent the development of algal mats by stirring up the sediment, thus, reducing pond transparency (Milstein, 1992).

At the end of the experiment, all ponds were drained and the crayfish were collected manually. Each pond was systematically searched for crayfish, including the turning over of stones by a group of several individuals over a period of three consecutive days. All crayfish were sexed and weighed to the nearest 0.1 g. Crayfish of a different sex from that stocked into a particular pond or smaller than the crayfish stocked into that pond were excluded from the computation of survival and growth of the pond. Crayfish larger than stocked were not excluded, since one cannot rule out growth leading to size differential.

The data were analyzed using SAS (1989) statistical software package. Descriptive statistics (i.e., means, s.d., C.V. and skewness) were computed from raw data. Statistical analyses were based on parametric tests (i.e., two-way ANOVA). Arcsin transformations were applied to percentages and log transformations to daily weight gain.

3. Results

Throughout the entire study, levels of dissolved oxygen were high and close to saturation (80–100%). These results were obtained despite the lack of aeration, probably due to the low water temperature and the very low biological loading of the ponds. Total ammonium (NH_4^+) and nitrite (NO_2^-) levels were low: 0.3–0.6 mg/l and 0.05–0.15 mg/l, respectively. Water pH was stable and slightly alkaline (7.7–8.2). Values of unionized ammonium under our pH values were low (< 0.04 mg/l) and nontoxic.

During the first three months of the study, maximum and minimum water temperatures in the ponds decreased by about 8°C (Fig. 2). Minimum temperatures of below 10°C were recorded for six days during the end of January and the first half of February. From then on, temperatures began to rise, with the warming-up pace being more rapid than that of the cooling-down. The difference between daily minimum and maximum temperatures was ≤ 5°C. Water temperatures in the four monitored ponds were similar, with differences being not consistent and not exceeding 2°C. Daily minimum water temperatures have been monitored for many years at the Dor Fish and Aquaculture Research Station. The number of days over the last nine years, with minimum water temperatures of ≤ 10°C, during the same period that our overwintering experiment was carried out, is presented in Fig. 3. It can be seen that some winters were relatively milder than the winter of 1996/1997, with no records of 10°C (winter of 1993/1994), while others had many days of temperatures of ≤ 10°C (winter 1991/1992). The winter of 1996/1997, during which the present study was carried out, may, thus, be considered as a representative one for the region with regard to the number of days with minimum water temperatures of ≤ 10°C.

The effects of sex and size on the survival of red-claw crayfish in earthen ponds under ambient winter temperatures are summarized in Tables 1 and 2. Neither independent nor combined effects of sex and size on survival were significant. Similarly, the

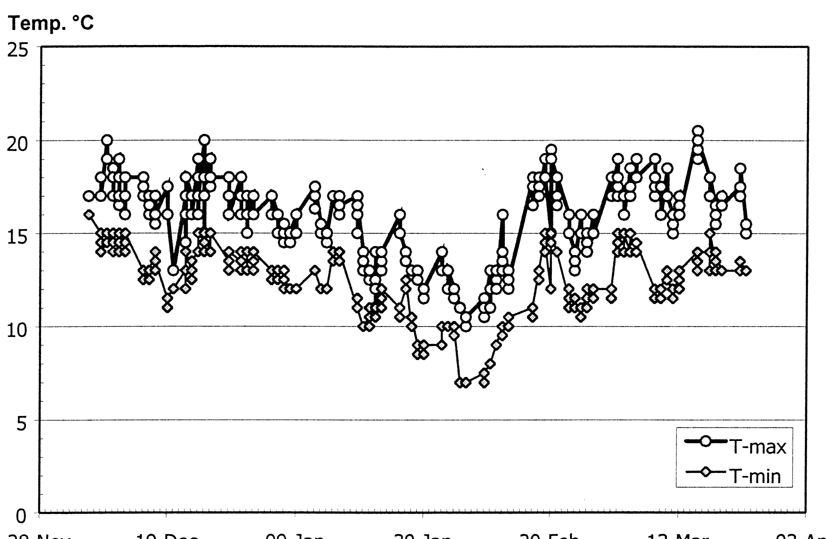

Fig. 2. Minimum and maximum water temperatures recorded at the Fish and Aquaculture Research Station, Dor, in four ponds during the period of overwintering crayfish. (December 1996–March 1997).

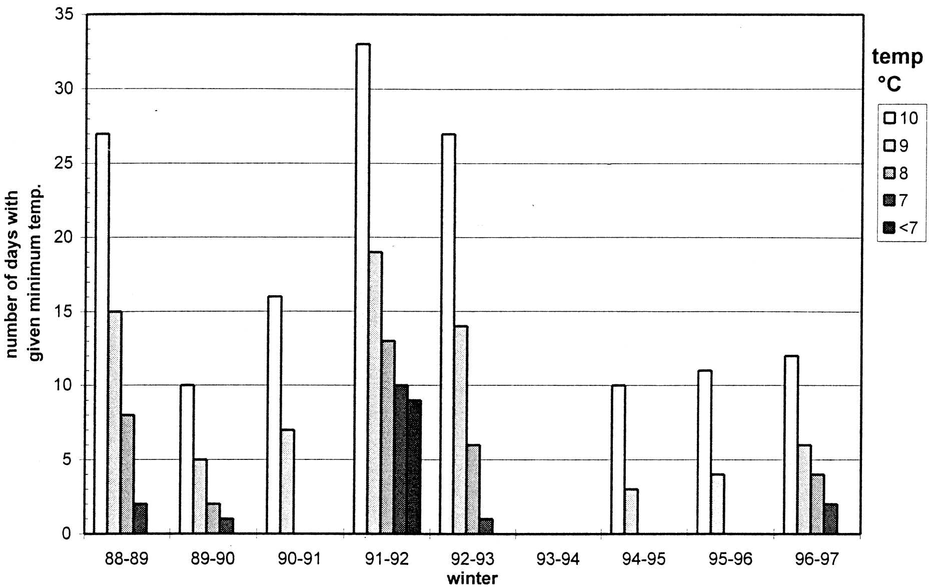

Fig. 3. Number of recorded days with given minimum water temperatures during December–March of nine winters. Data was recorded at the Fish and Aquaculture Research Station, Dor.

spatial distribution of the ponds evaluated by contrasting blocks, had no effect on survival. The mean number of crayfish per pond, recovered on three consecutive days, decreased gradually (33.8 ± 13.9, 14.6 ± 8.7 and 4.3 ± 5.5 for days 1, 2 and 3, respectively). About 8% of all crayfish recovered from a pond were excluded from the computation of survival of treatment groups (Table 2), since they were of a different sex or of a smaller size than those stocked into a specific pond. However, an overall 60% survival was attained with the inclusion of these crayfishes in the computation.

Neither independent nor combined effects of sex and size had a significant effect on the growth of crayfish in open earthen ponds during the winter (Tables 1 and 2). Overall change in weight was minimal. There was a tendency for higher growth rates in small crayfish as compared to large ones ($P = 0.075$), but even the growth rate of the small

Table 1
Mean survival (% ± s.d.) and growth (g day^{-1} ± s.d) according to sex and size at stocking of *C. quadricarinatus* in earthen ponds under ambient winter temperatures

Treatment		Variable	
Sex	Size	Survival	Growth
Male	Small	49 ± 17.4	$+0.074 \pm 0.08$
	Large	58.3 ± 8.5	-0.002 ± 0.02
Female	Small	44.8 ± 6.8	$+0.017 \pm 0.02$
	Large	58.5 ± 11.8	-0.005 ± 0.005

Table 2
Results of two-way ANOVA (F values) of the effect of sex (male vs. female) and size (large vs. small) on survival and growth of *C. quadricarinatus* in earthen ponds under ambient winter temperatures

Effect	*df*	Variable	
		Survival	Growth
Block	3, 9	0.39	1.26
Sex	1, 9	0.10	0.02
Size	1, 9	3.32	4.03
Sex × Size	1, 12	0.46	1.67

All effects were nonsignificant.

crayfish was negligible (Table 1). None of the crayfish collected from the ponds at the end of March had a soft shell, indicating that probably, under ambient winter temperatures, the lack of growth was coupled with an arrest in moulting activity. During the entire study, very few crayfish entered the baited traps (seven crayfish), probably reflecting the reduced motor activity and feeding at low water temperatures.

4. Discussion

The major finding of this study is that the tropical red-claw crayfish can fairly well survive ambient winter temperatures in open earthen ponds in the temperate zones. This finding has substantial implications concerning profitability of culturing this tropical species in Israel and in other countries with similar mild winters. A previous study suggested that growing crayfish for a second grow-out season may increase yields (Sagi et al., 1977). However, overwintering of large numbers of crayfish in greenhouses to be stocked in ponds the following year is expensive and inefficient. The present study suggests that such expensive procedures are, in fact, not necessary. Maintaining crayfish in earthen ponds during the winter under low ambient temperatures results in a complete growth arrest. However, there is also an absence in moulting activity, thus, avoiding many of the mortalities of newly moulted soft-shelled individuals that occur in the heated greenhouses when crayfish are maintained at very high densities.

The cold tolerance of *C. quadricarinatus* in earthen ponds cannot be compared directly to that found in controlled laboratory studies due to the fact that the response of crustaceans to low temperatures is simultaneously influenced by a large number of environmental factors such as light, salinity, pressure and dissolved gases and internal factors such as moult state, nutritional condition, size and sex (Kinne, 1970). Despite our inability to control many of the above described variables, the relative high survival obtained in this field experiment is in accord with the robust cold tolerance of this species as reported in earlier laboratory studies (Merrick and Lambert, 1991; Semple et al., 1995; Zoran et al., 1996).

Several reasons may be put forward to explain why not all crustaceans stocked into ponds were recovered. (1) Death due to low temperatures. (2) Bird predation (over the entire experimental period, several crayfishes in which beak perforations could clearly be seen, were found on the pond banks). Mortality due to cannibalism is rather unlikely due to the low rate of moulting which renders crayfishes susceptible to predation. (3) Migration of individuals, in the absence of fences, away from the monitored ponds into adjacent ones or draining canals. Evidence for such migration in this study is revealed in the presence of small individuals (5.2 ± 6.9 crayfish) in the treatment groups stocked with large-sized animals. (4) Inability to collect live crayfish which remained under large boulders even when the ponds were drained. Yet, due to the extensive efforts of collecting crayfish from ponds, the number of recovered crayfish probably represents fairly accurately those that survived the winter.

It is not possible to estimate the relative contribution of each of the above causes to crayfish survival. Only field experiments on cold tolerance carried out utilizing cages would provide certainty of recovery, while preventing bird predation. However, cages would also prevent the crayfish from burrowing in the sediment under stones, providing them with a possible warmer microhabitat which may contribute to their survival.

The lack of an effect of stocking size on cold tolerance in *C. quadricarinatus*, as revealed in this study, is similar to results of a preliminary laboratory study carried out on this species (Zoran, pers. comm.). Possibly, the lack of moulting activity in both small and large individuals made them more comparable, differing from the usually higher moulting rate of smaller individuals which renders them more susceptible to extreme environmental conditions.

The lack of growth, the low moulting rate and low rate of trapping in baited traps all point towards the obvious reduction in metabolic activity of *C. quadricarinatus* at low temperatures. Crayfish probably hide under rocks, rather immobile with minimal energy expenditure. Under low temperatures, it is not recommended to feed crayfish. However, only laboratory experiments with controlled temperatures and feeding rates will allow us to determine at what temperature feeding rate should be reduced and when feeding should be stopped.

Besides the positive economic impact of alien crayfish (Ackefors, 1997), there is a growing concern regarding the environmental consequences of their introduction (Holdich, 1988, 1997). Israel has no indigenous species of crayfish. Previous introduction for culture of a tropical crustacean—the freshwater prawn *Macrobrachium rosenbergii* to Israel in the early 70s did not establish a natural reproducing population in the environment. A case of establishment of a wild population of this species was recently recorded in the tropical zone (Pereira et al., 1996). Unlike *M. rosenbergii*, *C. quadricarinatus* is not dependent on brackish water for reproduction, and the results of the present study show that it may survive year-round under local climatic conditions. Thus, in the event that *C. quadricarinatus* escapes into the natural habitat, it will probably survive and could disperse and establish itself beyond the limits of the aquafarms. In this respect, introduction of *C. quadricarinatus* into Israel's southern part, in which the introduction sites are isolated from natural water sources by the desert, seems safer. A potential controller of crayfish—the freshwater crab *Potamon potamios* (Karplus et al., 1995), is found in most freshwater bodies of the northern part of the country. Predation of juvenile red-claw by adult *P. potamios* was observed in aquaria (Karplus, pers. obs.). Nevertheless, the results of this study suggest extra caution when introducing *C. quadricarinatus* into a temperate zone.

Acknowledgements

The statistical advice of Mrs. M. Zahrchi is gratefully acknowledged. This study was supported by the Department of Fisheries—Ministry of Agriculture and Rural Development, Israel.

References

Ackefors, H., 1994. Recent progress in Australian crayfish culture. J. World Aquacult. Soc. 25, 14–19.

Ackefors, H., 1997. Alien crayfish: review of their positive impact, The Introduction of Alien Species of Crayfish in Europe, How to Make the Best of a Bad Situation?, An International Workshop. Florence, Italy, 24–27 September 1997, p. 10, Abstract.

Gat, Z., Horowitz, T., Karni, O., 1992. Pond water temperatures and ambient temperatures in the coastal region during the winter of 1991–92, as a background to the understanding of *Tilapia* mortality. Fish. Fishbreed. Isr. 25, 198–207, Hebrew with an English abstract.

Holdich, D.M., 1988. The dangers of introducing alien animals with particular reference to crayfish, Freshwater Crayfish VII, xv–xxx.

Holdich, D.M., 1997. Negative effects of established crayfish introductions, The Introduction of Alien Species of Crayfish in Europe, How to Make the Best of a Bad Situation?, An International Workshop. Florence, Italy, 24–27 September 1997, pp. 18–20, Abstract.

Karplus, I., Barki, A., Cohen, S., Hulata, G., 1995. Culture of the Australian red-claw crayfish (*Cherax quadricarinatus*) in Israel: I. Polyculture with fish in earthen ponds. Isr. J. Aquacult. 47, 6–16.

Kinne, O., 1970. Temperature. Animals: invertebrates. In: Kinne, O. (Ed.), Marine Ecology, Vol. 1, Wiley-Interscience, London, pp. 407–514.

Lee, D.O'C., Wickins, J.F., 1992. Crustacean Farming. Blackwell Scientific Publications, 392 pp.

Medley, B.P., Jones, C.M., Avault, J.W., 1994. A global perspective of the culture of Australian red-claw crayfish, *C. quadricarinatus*: production, economics and marketing. World Aquacult. 25, 6–13.

Merrick, J.R., Lambert, C.N., 1991. The Yabby, Marron and Red-claw: Production and Marketing. J.R. Merrick Publications Artarmon, N.S.W., Australia. 186 pp.

Milstein, A., 1992. Ecological aspects of fish species interactions in polyculture ponds. Hydrobiology 231, 177–186.

Morrisy, N.M., Evans, L.E., Huner, J.V., 1990. Australian freshwater crayfish: aquaculture species. World Aquacult. 21, 113–122.

Pereira, G., Eganez, H., Monente, J., 1996. First record of a reproductive wild population of *Macrobrachium rosenbergii* (De Man) (Crustacea, Decapoda, Palaemonidae) from Venezuela. Acta Biologica Venezuelica 16, 93–95.

Rouse, D.B., 1995. Australian crayfish culture in the Americas. J. Shellfish Res. 14, 569–572.

Sagi, A., Milstein, A., Eran, Y., Joseph, D., Khalia, I., Abdu, U., Harpaz, S., Karplus, I., 1977. Culture of the Australian red-claw crayfish (*C. quadricarinatus*) in Israel: II. Second grow-out season of overwintered populations. Isr. J. Aquacult., Bamidgeh 49, 222–229.

Sandifer, P.A., Smith, T.I.J., 1975. Freshwater prawns. In: Huner, J.V., Brown, E.E. (Eds.), Crustacean and Mollusk Aquaculture in the United States. Avi Publishing, Westport, CT, pp. 63–125.

SAS Institute, 1989. SAS User's Guide: Statistics, 4th edn., Vol. 1. SAS Institute, Cary, NC, 943 pp.

Semple, G.P., Rouse, D.B., McLain, K.R., 1995. *C. destructor*, *C. tenuimanus* and *C. quadricarinatus* (Decapoda: Parastacidae): a comparative review of biological traits relating to aquaculture potential. Freshwater Crayfish VIII, 495–503.

Zoran, M., Milstein, A., Peretz, Y., Zohar, G., 1996. Resistance to low temperature of *C. quadicarinatus*. Fish. Fishbreed. Isr. 29, 10–11, Hebrew with an English abstract.