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Growth of juvenile red swamp crayfish (*Procambarus clarkii*) (Decapoda: Cambaridae) reared in groups consisting of either sibling and non sibling individuals

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Abstract. Red swamp crayfish (*Procambarus clarkii* Girard, 1852), which is indigenous to North America, is the most commercially farmed crayfish in the world. Profitability of farming depends mainly on crayfish growth rates. Thus research focused on factors affecting growth is of considerable importance for aquafarming. The objective of the present paper is to determine whether the growth of individual crayfish reared in sibling and non-sibling groups differ. This has not been evaluated previously for this species. The experiment was done in the laboratory in November 2011 and involved the analysis of the growth in terms of length of 96 individuals. Survival of the individuals reared in sibling groups was 98.3% and of those reared in non-sibling groups 96.3%. The differences in growth and number of moults in the two groups were not significant. The observations started two weeks after the juveniles became free-living at which time it is possible that any sibling effect could be indistinct.

Key words. Crustacea, juveniles, astaciculture, growth, *Procambarus clarkii.*

INTRODUCTION

Crayfish grow by periodically moulting their exoskeleton and as in other crustaceans, growth is affected by many factors – e.g. water temperature, light intensity, quantity and quality of nutrients and population density (Aiken 1980, Reynolds 2002). Adult crayfish moult once per year, but juveniles moult more frequently, up to five times per month (Huner & Avault 1976).

Crayfish females in all three families (Astacidae, Cambaridae and Parastacidae) incubate their eggs by carrying them on their swimmerets (pleopods). Unlike most decapod crustacea, crayfish do not have free-living planktonic larvae (Holdich 1992, Gherardi 2002). After hatching from the eggs the larvae remain attached in the 1st and 2nd stages of their development to their mother, siblings or eggs by means of a telson thread and pereopodal hooks. They have strong tendency to aggregate (Holdich 1992, Scholtz & Kawai 2002). The larvae of North American Cambaridae become free-living in 2nd larval stage, but more usually in the 3rd or a later stage (Reynolds 2002, Scholtz & Kawai 2002). Crayfish complete their development without undergoing a metamorphosis before becoming free-living (Gherardi 2002). Initially they forage in the immediate vicinity of their mother and if in danger immediately return to their mother's swimmerets (Aquiloni & Gherardi 2008). Juveniles leave their mother for longer and longer periods and after a few days they stop returning to her (Gherardi 2002, Reynolds 2002). Nevertheless juveniles of some species, including the red swamp crayfish, often remain with their mother for a long time, sometimes up to three months and become free-living in 7th larval stage (Gherardi 2002, Huner 2002).

Crayfish exhibit maternal care (Gherardi 2002) and are classified as subsocial animals (Reznikova 2007). On the other hand, females will kill, eat or abandon her offspring when they reach

the end of the 2nd larval stage if she is stressed or starving (Policar et al. 2011). Although berried crayfish will defend their offspring against attack by other adult conspecifics they are not normally aggressive towards their offspring (Gherardi 2002). Moreover crayfish inhibit the cannibalistic behaviour of their offspring during the whole incubation period by producing a pheromone (Little 1975, 1976). Juvenile crayfish can also locate their mothers by means of this pheromone (Little 1974). Long-term absence of juveniles from the mother's swimmerets induces the termination of the production of the pheromone (Little 1976). If the maternal pheromone becomes imperceptible, both juveniles and their mother become aggressive and cannibalistic (Figler et al. 1997).

In addition to maternal incubation, the eggs can be removed from several crayfish in berry and artificially incubated together (Jones 1995, Perez et al. 1999, Kouba et al. 2010). Karplus et al. (1995) have published the only study that has evaluated the relationship between siblings and non-sibling juvenile crayfish. They studied the red claw crayfish (*Cherax quadricarinatus* von Martens, 1868), which belongs to the family Parastacidae, and report no significant difference. We hypothesize, that the situation in species of other families could be different because they differ in a number of biological features (Scholtz et al. 2003, Buřič et al. 2011). Therefore, we determined the effect on growth in terms of length of rearing juveniles of the red swamp crayfish (*Procambarus clarkii*) in sibling and non-sibling groups.

MATERIAL AND METHODS

All the crayfish used in this study were juvenile red swamp crayfish (*Procambarus clarkii*) collected from 8 females in berry, which were obtained from commercial breeders. Mothers were kept separately in plastic tanks during the period of incubation and 3rd stage juvenile crayfish were replaced with siblings only to grow-out tanks.

Data collection

Total body length (TL) of juveniles was measured from the tip of the rostrum to the posterior end of the telson and it ranged from 11.0 to 19.0 mm with a mean length of 15.3 mm at the beginning of the experiment. Due to the formation of a dominance hierarchy in small groups (Issa et al. 1999), nineteen groups of six individuals were formed (ten groups with siblings and nine with only non-siblings) and released in observation tanks (Faunarium PT-2255, 230×155×170 mm). The tanks were filled with fresh water to a depth of 50 mm and the bottoms covered with 1 cm of pale silica sand (grain size 1.0 mm) as recommended by Issa et al. (1999). Each individual was marked with a unique colour tag (one or two blue, red and yellow points) of nail-polish applied to the dorsal side of the cephalothorax (Mazlum 2007). The presence of the tag was checked every day and any crayfish that moulted was retagged. Number of moults for each individual was recorded. The tanks were then placed under a camera mounted on a tripod (Toshiba Cameleo S20, distance from the tank was 0.5 m) and a digital photograph taken and the total body length (TL) of each individual was measured (Ulikowski & Krzywosh 2009). Crayfish were not weighed. Groups were kept under the same conditions (temperature ~24 °C, 12 hour light-dark cycle, dissolved oxygen 5.0–7.1 $mg \times l^{-1}$, pH 6.7–7.5) and fed *ad libitum* once every 24 hours with TetraMin Crisps (Tetra GmbH, Melle, Germany). This feed contains: protein (min.) 46.0%, fat (min.) 12.0%, fiber (max.) 9.0%, moisture (max.) 9.0%, phosphorus (min.) 1.2% and ascorbic acid (min.) 425 mg/kg. The percentage content of protein in the diet was more than 30% as recommended by Huner & Meyers (1979). The feed was spread over the bottom of each tank to reduce competition for food (Barki et al. 1997). Competition for shelter (Figler et al. 1999) did not occur because none was provided. Stock density corresponded to 170 individuals per m^2, which is that commonly used in astaciculture (Verhoef & Austin 1999). The experiment continued for three weeks at the end of which the length (TL) of each of the crayfish was measured again.

Table 1. Relative increase in length (%) and basic statistics for both sibling and non-sibling groups of juvenile *Procambarus clarkii*

group type	n	mean	median	min.	max.	S.D.
siblings	54	17.51	15.38	0	53.85	13.20
non-siblings	42	18.58	14.84	0	58.33	14.98

Table 2. Frequency table of the number of moults recorded for individuals in non-sib (non-sibling) and sib (sibling) groups of juvenile *Procambarus clarkii*

number of moults	frequency non-sib	sib
0	1	3
1	22	30
2	18	20
3	1	1

Statistical analysis

All the statistical computations and evaluations were made using program Statistica ver. 10 (StatSoft, Inc., 2011). Differences between the initial and final TLs of individuals were expressed as a % of the initial TL.

In addition to basic descriptive statistics (mean, median, minimum, maximum and standard deviation), non-parametric Mann-Whitney U tests for independent groups was used to test for possible differences in the growth of the individuals in the sibling and non-sibling groups.

RESULTS

At the end of three weeks there were altogether sixteen groups of juvenile crayfish, nine sibling and seven non-sibling groups, which provided data on the growth of 96 individuals (54 siblings and 42 non-siblings). Mortality was very low as only three individuals died, one in each of two groups of non-siblings and one in a sibling group. The results for these groups were not included in the analysis. Survival rate of individuals was 98.3% for siblings and 96.3% for non-siblings. Maximum relative increase in length of juvenile crayfish was 53.9% with a mean of 17.5% for siblings and 58.3% with a mean of 18.6% for non-siblings. Minimum relative increase in length of individuals in both types of group was 0%. The basic statistics are summarized in the Table 1. Differences in relative increase in length of sibling and non-sibling juvenile crayfish were not significant (Mann-Whitney U test, p=0.658). Moulting frequency of juveniles in both groups varied from 0 to 3 over the period of the experiment and was not significantly different between sibling and non-sibling groups (Table 2).

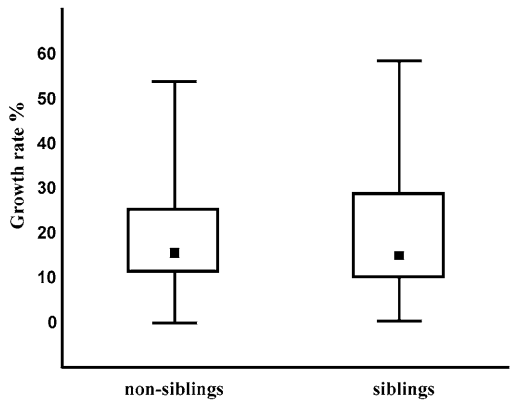

Fig. 1. Box-plot graph of the relative increase in length (%) recorded for individuals in sibling and non-sibling groups of juvenile *Procambarus clarkii.* Median (■), 25–75% (□), minimum and maximum (—).

DISCUSSION

Juvenile red swamp crayfish (*Procambarus clarkii*) reared in sibling and non-sibling groups did not differ significantly in their growth and moulting frequencies. These findings are similar to those of Karplus et al. (1995), who studied a different species of crayfish, namely the red claw crayfish (*Cherax quadricarinatus*). In contrast to that reported in recent studies (e.g. Issa et al. 1999), the mortality recorded in our study was very low. Hence we suggest that juvenile red swamp crayfish are surprisingly not very aggressive either towards siblings or non-siblings. This may be associated with the long period of time juvenile red swamp crayfish spend with their mother in her burrow in the wild (Huner 2002) and as a consequence they tend to be less cannibalistic than most other species of crayfish (Gherardi 2002). In addition, in our experiment they were fed *ad libitum*, which would have reduced the competition for food, and the fact that the food was distributed evenly over the bottom of the tanks meant that juveniles did not have to compete directly for resources as is documented by Barki et al. (1997). Moreover crayfish mothers may adopt juveniles other than their own (Aquiloni & Gherardi 2008). As mothers are tolerant of these juveniles, non-siblings behave in the same way and thus do not inhibit the early growth of other juvenile conspecifics. This may have modified the differences in the effect siblings and non-siblings on the growth of the juvenile red swamp crayfish in our experiment.

Despite the negative result, we cannot completely reject the notion that presence of siblings as opposed to non-siblings has a positive effect of on juvenile crayfish, because it may only occur during the first few days after they become free-living, as in the giant water bug (*Lethocerus deyrollei* Vuillefroy, 1864) (Hemiptera: Belostomatidae) (Ohba et al. 2006). Our observations started two weeks after the juveniles became free-living and therefore they may then no longer respond to siblings and non-sibling juveniles differently.

In order to confirm or reject these assumptions future detailed studies need to focus on the early development and social behaviour of juvenile crayfish.

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