

# Interaction between juvenile narrow-claw crayfish, *Astacus leptodactylus* (Eschscholtz), and common water frog, *Rana esculenta* (L.), tadpoles or common blue damselfly, *Enallagma cyathigerum* (Charpentier), larvae during rearing under controlled conditions

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**Abstract.** Interactions were studied among juvenile narrow-claw crayfish, *Astacus leptodactylus* (Eschscholtz), and common water frog, *Rana esculenta* (L.), tadpoles and common blue damselfly, *Enallagma cyathigerum* (Charpentier), larvae during rearing under controlled conditions. Interactions among the species studied had a positive impact on the survival of the crayfish, but the differences were not statistically significant ( $P \geq 0.5$ ). The juvenile crayfish attacked and consumed the frog tadpoles and damselflies, but the juvenile crayfish very rarely fell prey to them. Only in the initial stage of life and during molting did larval damselflies prey upon juvenile crayfish. After 30 days of the experiment the interaction between crayfish-tadpoles and crayfish-larval damselflies was not noted to have had a statistically significant ( $P \geq 0.05$ ) impact on crayfish growth. Juvenile crayfish aggression toward tadpoles and larval damselflies was often offset by the loss of even both chelipeds. In the crayfish-larval damselfly interaction the loss of both

chelipeds was three-fold more common than it was in the crayfish-tadpole interaction; however, these differences were not statistically significant ( $P \geq 0.5$ ). The effect of intraspecific interaction (crayfish-crayfish) was more a threat in terms of mortality from cannibalism than were interspecific interactions (crayfish-tadpole and crayfish-larval damselfly).

**Keywords:** interaction, *Astacus leptodactylus*, *Rana esculenta*, *Enallagma cyathigerum*, survival, rearing

## Introduction

The narrow-clawed crayfish, *Astacus leptodactylus* (Eschscholtz), is one of the native European crayfish species occurring in Poland (Czerniejewski and Tański 2001). This species originally occurred in the Black and Caspian seas basin, but its area of occurrence has expanded considerably through introductions and migrations (Strużyński 2001, Mastłyński and Andrzejewski 2005). It currently occurs in 27 countries, 14 of which it has been introduced into (Harlioğlu 2004, Souty-Grosset et al. 2006). The species is important economically and ecologically,

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but catches of it have decreased considerably because of overfishing, environmental degradation, disease (crayfish plague), and competition from the alien crayfish species *Orconectes limosus* (Rafinesque) (Białokoz et al. 1996, Strużyński et al. 2001, Śmietana 2001, Śmietana et al. 2004).

The narrow-clawed crayfish primarily inhabits standing waters, but it migrates in streams and canals. It is tolerant of variable environmental conditions, including oxygen deficits and high water temperatures (Ackefors and Lindqvist 1994, Strużyński 2001, Krzywosz et al. 2009). This species can grow to 25 cm in total body length (TL), but the most common individual size encountered measures approximately 15 cm (Kossakowski 1966, Mastyrński and Andrzejewski 2005). Females reach sexual maturity in the third year of life, while males do in the fourth. The breeding season runs from October to November. Females deposit from 60 to 200 eggs during this period and carry them attached to the abdomen for six to eight months until they hatch. The juveniles detach from the female body after the first molting, which occurs on the tenth day post hatch. After a subsequent fifteen or so days, the young leave the mother and begin to live independently (Kossakowski 1966). The first period of crayfish life is marked by high mortality caused by many factors including predation, cannibalism, and environmental conditions (Taugbøl and Skurdal 1992, Ulikowski and Krzywosz 2006). The greatest threats posed to both juvenile and older crayfish are doubtlessly predatory bottom-feeding fish such as perch, *Perca fluviatilis* L., wels catfish, *Silurus glanis* L., burbot, *Lota lota* (L.), and European eel, *Anguilla anguilla* (L.) (Svensson 1993, Czerniejewski and Tański 2001). In the first weeks of life, juvenile crayfish are especially susceptible to attacks from other predatory aquatic organisms such as non-predatory fish, insects and insect larvae, amphibians, and leeches (Blake et al. 1994). Frog tadpoles and larval damselflies occur commonly in most aquatic basins (Juszczyk 1987). In the Polish aquatic environment, the most widespread is the common water frog, *Rana esculenta* (L.). The breeding period of this frog is from April to early June (Juszczyk 1987). The most

commonly occurring damselfly species in the vicinity of Polish waters is the common blue damselfly, *Enallagma cyathigerum* (Charpentier), which is classified to the damselfly with similarly shaped wings (Odonata, Zygoptera) (Stańczykowska 1986). Adult specimens are observed from May to September, and they breed during this period (Wendzonka 2005). Damselflies spend the larval period in the aquatic environment, which, in the temperate climate, can last from one month to as long as three years (Stańczykowska 1986). Most larval damselflies are predatory (Jura 2004), and they feed almost exclusively on animal food including small crustaceans, frog tadpoles, and even small fish (Johansson and Johansson 1992, Barnett and Richardson 2002, Crumrine and Crowley 2003). Since frog tadpoles, larval damselflies, and juvenile crayfish inhabit similar ecological niches, a variety of interactions may occur among them.

The aim of the current experiment was to verify the hypothesis we propose that the presence of *R. esculenta* tadpoles or *E. cyathigerum* larvae affects the survival, growth, and number of *A. leptodactylus* individuals with missing chelipeds during rearing under controlled conditions.

## Material and methods

The experiment was performed at the Department of Sturgeon Fish Breeding in Pieczarki, which is part of the Inland Fisheries Institute in Olsztyn. The material used in the study comprised juvenile *A. leptodactylus* specimens that had undergone the first molting (juvenile stage 2), *R. esculenta* tadpoles, and larval *E. cyathigerum* with mean total body lengths of  $10.6 \pm 1.0$ ,  $30.3 \pm 4.4$ , and  $14.5 \pm 1.9$  mm, respectively (TL  $\pm$  standard deviation). The tadpoles and damselfly larvae were caught in a pond immediately before the beginning of the experiment, while the juvenile crayfish were obtained from females in breeding ponds that had undergone maternal incubation in a recirculating system under controlled conditions (Ulikowski and Krzywosz 2005). The organisms

collected for the study were placed in 12 tanks with a volume of 10 dm<sup>3</sup> each. A layer of mineral substrate about 10 cm thick and comprised of gravel with a diameter of 5-15 cm was laid on the bottom of the tanks to provide the crayfish with hiding places. In addition to the tanks, the crayfish were also stocked into troughs with 50 partitions (each measuring 12x12x12 cm). Water flow through the tanks and trough partitions was accomplished with a recirculating system fitted with a water purification system (biofilter, settling tank, UV lamp) and thermoregulation.

Two experimental and two control groups were formed:

- Experimental group R+K – comprising 50 juvenile crayfish specimens and 5 tadpoles in each basin (four replicates of this stocking variant);
- Experimental group R+LW – comprising 50 juvenile crayfish specimens and 5 larval damselflies in each tank (four replicates of this stocking variant);
- Control group Rb – comprising 50 juvenile crayfish specimens in each tank (four replicates of this stocking variant);
- Control group Rp – comprising juvenile crayfish specimens stocked individually in each of the 50 trough partitions.

With 50 crayfish specimens stocked into each tank the density was 1000 specimens m<sup>-2</sup>, which is recommended when rearing juvenile crayfish under similar conditions (Ulikowski and Krzywosz 2004). The proportion of the crayfish stock to tadpoles or larval damselflies was determined hypothetically at 10:1. During the experiment, larval damselfly losses were replenished three times in group R+LW (12 specimens in total). The losses were attributed to the metamorphosis of the larvae into adult forms and then the adults flying away, which occurred during the experiment (Stańczykowska 1986).

The crayfish were fed once daily *ad libitum* with Perla Larva Proactive 4.0 feed (Skretting, France; the proximate composition was protein 62.0%, lipid 11.0%, fiber 0.8%, ash 10.0%), which Ulikowski et al. (2008) determined to be suitable for rearing

juvenile crayfish. The tanks were cleaned of unconsumed feed once weekly.

During the 30 days of the experiment, the water flow through the tanks was, on average,  $0.49 \pm 0.06$  dm<sup>3</sup> min<sup>-1</sup> and the mean water temperature was  $19.2 \pm 0.9^\circ\text{C}$ . The oxygen content in the water at the tank outflows did not fall beneath 6.0 mg dm<sup>-3</sup>. The concentration of total ammonia nitrogen (TAN) at the tank outflows did not exceed 0.02 mg dm<sup>-3</sup>, and that of nitrite (NO<sub>2</sub>) did not exceed 0.002 mg dm<sup>-3</sup>. The water pH ranged from 7.9-8.2. During the experiment the light intensity was measured at the tank and trough water surfaces throughout the natural light cycle. It ranged from 0.004 lx at night to 336.6 lx during the day, while that in the troughs was from 0.005 to 320.5 lx, respectively. The measurements were taken with a L-100 luxometer by Sonopan. In addition to these measurements, the organisms were observed so any differences in behavior among the stocking variants could be recorded.

At both the beginning and end of the experiment, all of the organisms were counted and photographs of them were taken for measurements of total body length (TL). The method developed by Ulikowski and Krzywosz (2009) was used for the measurements aided by the MultiScan 8.0 computer program. The body measurement results served to calculate crayfish body weight with the dependence formula of length-mass ( $W = 0.1306 \text{ TL}^{2.533}$ ) that was developed by the authors mentioned above. Additionally, survival was calculated and the final percentage share of specimens with pairs of chelipeds or the loss of either one or both of them was calculated. The specific growth rate of the crayfish was calculated with the following formula:  $\text{SGR} = 100 \times \ln(w_f) - \ln(w_i) \times t^{-1}$  where: SGR – specific growth rate,  $w_i$  – initial body weight (mg),  $w_f$  – final body weight (mg),  $t$  – experiment duration (days).

The data obtained was analyzed statistically with Statistica 7.1 (Statsoft Polska). Data concerning survival and the percentage share of specimens missing chelipeds was subjected to arcsin transformation prior to analysis (Zar 1984). These values and the hypotheses tested in the study were verified with single

**Table 1**

Impact of interactions between the juvenile crayfish *Astacus leptodactylus* and tadpoles of the common water frog, *Rana esculenta*, or the larval common blue damselfly, *Enallagma cyathigerum*, on the mean values of total body length (TL), body weight (W), and specific growth rate (SGR) of crayfish. N – sample number, SD – standard deviation, Max – maximum value, Min – minimum value, R+K = crayfish-tadpole interaction; R+LW = crayfish-larval damselfly interaction; Rb = crayfish-crayfish interaction; Rp = no interaction

Variants	R+K	R+LW	Rb (control)	Rp (control)
interaction	crayfish-tadpole	crayfish-larval damselfly	crayfish-crayfish	no interaction
N	101	89	101	33
TL (mm)	14.1 <sup>ab</sup>	14.1 <sup>ab</sup>	13.7 <sup>b</sup>	14.7 <sup>a</sup>
SD	1.3	1.2	1.1	1.8
Max	17.8	17.7	17.9	18.6
Min	11.3	11.1	11.5	11.9
W (mg)	107.6 <sup>ab</sup>	107.3 <sup>ab</sup>	100.7 <sup>b</sup>	121.3 <sup>a</sup>
SD	26.0	23.0	22.1	38.7
Max	191.8	190.0	195.4	214.5
Min	60.4	58.3	63.8	69.1
SGR (% day <sup>-1</sup> )	2.4 <sup>ab</sup>	2.4 <sup>ab</sup>	2.2 <sup>b</sup>	2.7 <sup>a</sup>
SD	0.8	0.7	0.7	1.0
Max	4.4	4.4	4.5	4.8
Min	0.6	0.4	0.7	1.0

Mean values with different letter indexes in rows are statistically significantly different (ANOVA, Tukey's test,  $P < 0.05$ )

factor analysis of variance (ANOVA,  $P \leq 0.05$ ). Tukey's post-hoc test was applied to statistically significant differences (LSD,  $P \leq 0.05$ ).

## Results

The highest stock survival was in control group Rp (72.0%), while the lowest was in control group Rb (52.5%). Survival in groups R+K and R+LW was higher by 10.5 and 4.5%, respectively, in comparison to control group Rb (Fig. 1), but these were not statistically significant ( $P \geq 0.5$ ). Different juvenile crayfish behavior was observed in experimental groups R+K and R+LW than in control group Rb. The presence of tadpoles or larval damselflies caused the juvenile crayfish to be less active and to spend more time in hiding places, which meant there was less contact among the species. The juvenile crayfish attacked and ate the tadpoles and larval damselflies, but they fell prey to these organisms less frequently. Only in the first week of the experiment and during molting did larval damselflies eat the juvenile crayfish. Tadpoles were not observed to attack or eat juvenile crayfish even during molting.

The fastest juvenile crayfish growth was noted in control group Rp and the slowest in control group Rb; the differences between the means were statistically significant ( $P < 0.05$ ; Table 1). No statistically significant differences ( $P \geq 0.05$ ) were noted in crayfish growth between the experimental (R+K and R+LW) and control groups (Rp and Rb).

The highest percentage of specimens missing both chelipeds was confirmed in group R+LW, in which they were three-fold more common than in group R+K (Fig. 2), but the differences were not statistically significant ( $P \geq 0.5$ ). The loss of both chelipeds was not noted in either of the control groups (Rb and Rp). Specimens with one missing cheliped in experimental groups R+K and R+LW were noted more than 10 and 15% more frequently than in control groups Rb and Rp, respectively, but this was not statistically significant ( $P \geq 0.5$ ).

## Discussion

Our results confirm the well-known fact that the survival of crayfish reared in individual compartments without contact with other specimens is higher than

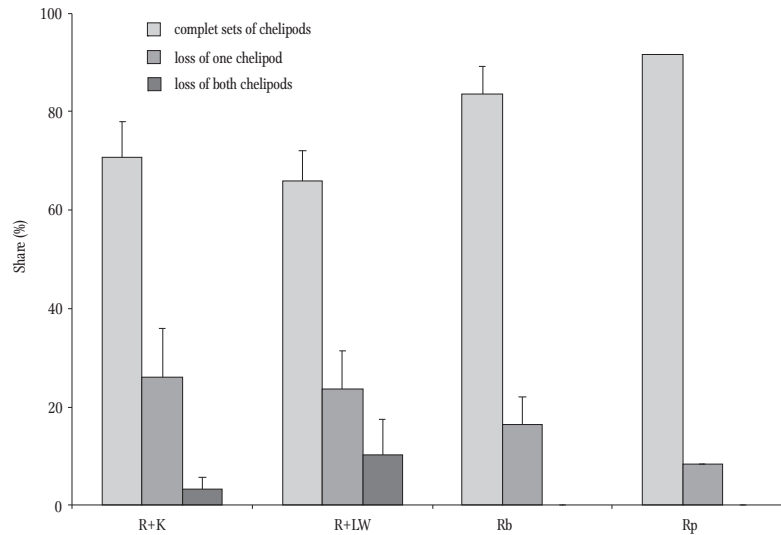


Figure 1. Impact of interactions between the juvenile, crayfish *Astacus leptodactylus*, and common water frog, *Rana esculenta*, tadpoles or larval common blue damselfly, *Enallagma cyathigerum*, on crayfish cheliped loss. Data are mean  $\pm$  SD. R+K = crayfish-tadpole interaction; R+LW = crayfish-larval damselfly interaction; Rb = crayfish-crayfish interaction; Rp = no interaction.

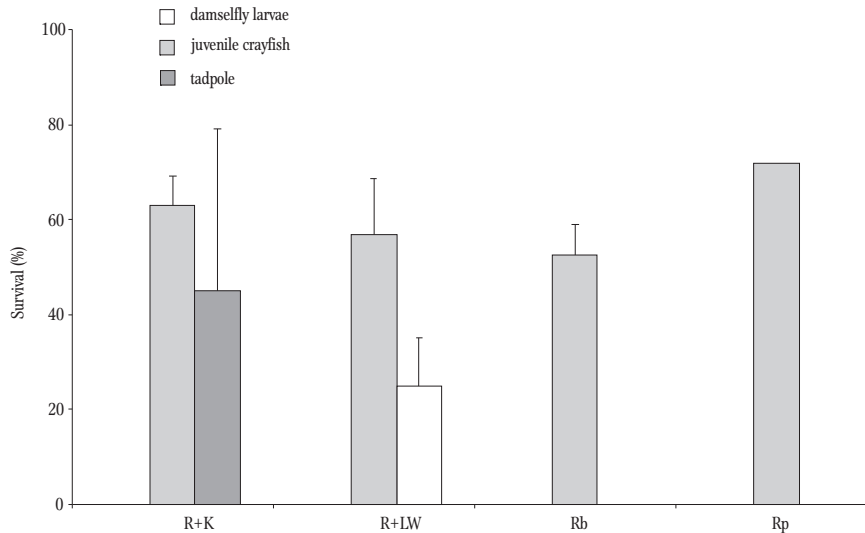


Figure 2. Impact of interactions between the juvenile, crayfish *Astacus leptodactylus*, and tadpoles of the common water frog, *Rana esculenta*, or the larval common blue damselfly, *Enallagma cyathigerum*, on the survival (mean  $\pm$  SD) of these animals. See Fig. 1 for descriptions of variants.

that of those reared in tanks in which there are crayfish-crayfish interactions (Ulikowski and Krzywosz 2004). According to Ulikowski and Krzywosz (2004), the consequence of these interactions is increased cannibalism that intensifies as stocking density increases. In our studies of *Astacus leptodactylus* the effect of intraspecific interaction (crayfish-crayfish) is more dangerous because of cannibalism than is interspecific interaction between crayfish and tadpoles or larval damselflies.

The interaction between crayfish and other organisms has been discussed previously by other authors. Hirvonen (1992) reports that the predatory *Notonecta lutea* Müller can lower the survival of juvenile crayfish *Pacisastacus leniusculus* Dana, and that crayfish caught by these predators often sacrifice a cheliped to escape predation. In our studies a similar increase of sacrificed chelipeds was confirmed in crayfish-larval damselfly interactions, which could indicate the deployment of this defense mechanism by juvenile



crayfish specimens. Another defense mechanism employed by juvenile crayfish against attacking larval damselflies is to use strong abdominal contractions to escape the predators' grasp. This behavior was described by Herberholz et al. (2004), according to whom this behavior is particularly effective when attacks are staged from the front or side. Strong abdominal contractions make escape easier, but sometimes at the cost of losing a limb that is grasped by a predator. According to Gydemo et al. (1990), the presence of hiding places in basins decreases the mortality of juvenile noble crayfish, *Astacus astacus* (L.), caused by the predatory larval damselfly, *Aeschna grandis* (L.). Witzig et al. (1986) observed interactions between juvenile *Procambarus clarkii* (Girard) and the larval damselfly, *Anax junius* (Drury), and confirmed that the frequency of larval attacks depends on water temperature and prey size. As water temperature increased, so did the frequency of attacks, the victims of which were smaller specimens and those that were molting. The observations conducted for the current study were similar; only in the first week of the experiment and during moulting did juvenile crayfish fall prey to larval damselflies.

The examples outlined above of interactions between larval damselflies and juvenile crayfish specimens that have been reported by various authors refer to damselflies of the suborder Anisoptera, which are larger and stronger than larval damselflies from the suborder Zygoptera that were used in the present study. The larval damselfly behavior in the tanks was similar to that in the natural environment as is described by other authors (Stańczykowska 1986, Johansson and Johansson 1992). These larvae, which are usually nearly motionless, settle near the bottom and wait for prey to appear within reach of their mouthparts (Stańczykowska 1986, Johansson and Johansson 1992). Our observations indicated that larval damselflies only attacked smaller or molting crayfish. We confirmed that after the subsequent molt (third juvenile stage) crayfish began to attack larval damselflies. The size correlation of predator-prey between the species shifted as the crayfish grew. Following the second molt (third juvenile stage) they could have been too large to be

potential prey for larval damselflies. The crayfish assumed the role of predator, and the larval damselflies became their prey. Some of the decreasing larval damselfly predation pressure on juvenile crayfish was attributed to the final stage of their larval development (larval skins were found after metamorphosis). According to Stańczykowska (1986), larval damselflies stop feeding a few days prior to metamorphosis.

In the current study no instances were observed of *R. esculenta* tadpoles attacking and eating juvenile *A. laptoctylus* specimens, not even during molting. However, juvenile crayfish did prey on the tadpoles of this frog but not as often as on larval damselflies. The greater juvenile crayfish predation on larval damselflies than on tadpoles could also have stemmed from differences in the sizes of the potential prey (the larval damselflies were smaller than the tadpoles). We also confirmed that the tadpoles avoided the near-bottom areas of the tanks that the juvenile crayfish occupied, which meant they had limited possibilities for obtaining food and feeding. Nyström and Åbjörnsson (2000) also observed that tadpoles limited feeding in the presence of *P. lenisculus* and that their mortality increased. Axelsson et al. (1997) confirm that juvenile *A. astacus* and *P. lenisculus* consumed the eggs and tadpoles of various frog species, and that the quantity of frog eggs consumed by *P. lenisculus* increased as water temperature rose. However, *A. astacus* consumed more tadpoles than did *P. lenisculus*. Both crayfish species sporadically preyed on toad, *Bufo bufo* (L.), tadpoles. Gherardi et al. (2001) confirmed that the adults of the crayfish species *Procambarus clarkii* (Girard) and *Austropotamobius pallipes* (Lereboullet) consumed the tadpoles of the common toad, *Bufo bufo* Laurenti, and two frog species, *R. esculenta* and *Rana italica* Dubois, whether or not they were delivered alive or dead. Starved crayfish were quicker to begin predation and consumption than were satiated crayfish (Gherardi et al. 2001), and the crayfish *P. clarkii* was more active in this respect than was the crayfish *A. pallipes*. Rodriguez-Perez et al. (2014) also confirms that the crayfish *P. clarkii* had a negative impact on the

survival of the Mediterranean tree frog, *Hyla meridionalis* Boettger, in ponds, and that crayfish predation pressure on a population of *H. meridionalis* increased as the crayfish stocking density increased.

In summation, we conclude that in interactions between juvenile crayfish specimens of *A. leptodactylus* and *R. esculenta* tadpoles or larval damselflies, *E. cyathigerum*, it is the crayfish that most frequently plays the role of predator with tadpoles and larval damselflies falling prey to them. Only in the initial stage of life and during molting are juvenile crayfish consumed by larval damselflies. However, no instances were recorded of juvenile crayfish specimens of *A. leptodactylus* being preyed upon by *R. esculenta* tadpoles. Juvenile crayfish lost both chelipeds three times more frequently from crayfish-larval damselfly interactions than as a result of crayfish-tadpole interactions. However, interspecific interaction (crayfish-crayfish) posed a greater threat because of cannibalism than did interactions with either the larval damselfly larvae or tadpoles.

**Author contributions.** D.U. designed the research; D.U. and I.P. performed the research; D.U., I.P., and Ł.Ch. analyzed the data; D.U., I.P., Ł.Ch., T.K. and P.T. wrote the paper.

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