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The spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817) as an inhabitant of the Baltic Sea - experimental evidences for its invasion of brackish waters

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Key words: *Orconectes limosus,* invasive species, hatching success, body increments, condition, survival, brackish water colonization

Abstract

The main aim of the present study was to determine the effect of low salinity conditions (3 PSU and 7 PSU) on the hatching success, growth and mortality of *Orconectes limosus*. The results revealed that berried females survived exposure to salinities of 3 PSU and 7 PSU whilst incubating their eggs. The reproductive success reached 100% at both salinities. The length increment of young crayfish per molt was larger at 7 PSU than at 3 PSU – but their condition was affected by higher salinity. The mortality among juveniles reached approximately 50% within 5 weeks of hatching at both salinities.

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INTRODUCTION

The spiny-cheek crayfish *Orconectes limosus* Rafinesque, 1817 is a freshwater crayfish, native to the eastern USA (Pavlović et al. 2006). It appeared in European inland waters following its introduction in 1890 (Leńkowa 1962, Holdich et al. 2009). At that time, *O. limosus* was intended to replace the native European noble crayfish *Astacus astacus* stocks, which had been devastated by the crayfish plague *Aphanomyces astaci*. It has since spread very rapidly and is now recorded in more than 20 European countries (Lodge et al. 2000), where it plays a significant role (Vogt 1999, Holdich 1999, Lozán 2000, Holdich 2002, Holdich et al. 2009). Features, such as rapid maturation, short life-span, high fecundity, two mating periods and resistance to water pollution (Troschel and Dehus 1993, Schulz and Śmietana 2001, Kozak et al. 2006), have enabled the spiny-cheek crayfish to adapt to a wide variety of environmental conditions, and together with its migratory ability, to easily invade new, suitable localities (Ďuriš et al. 2006, Holdich and Black 2007). *O. limosus* has been recorded not only in inland waters but also in Baltic brackish waters with salinities up to 10 PSU, where it had established self-reproducing populations (Leppäkoski and Olenin 2000). The occurrence of the spiny-cheek crayfish in Polish coastal waters (Szczecin Lagoon, Pomeranian Bay, the Gulf of Gdansk, Vistula Lagoon, waters adjacent to Władysławowo, Jastrzębia Góra and Ostrowo) has been recorded since the 1930s (Pieplow 1938, Gajewski and Terlecki 1956, Żmudziński 1961, Leńkowa 1962, Leppäkoski 1984, Gruszka 1999, Szaniawska et al. 2005, Skóra 2007, Zięcik and Zawiliński 2010 – personal communications). Burba (2008) recorded a single individual from the Baltic coast of Lithuania, a country where this species is spreading very fast (Holdich et al. 2009). The fact

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that the spiny-cheek crayfish is capable of living in saline waters for quite a long time is confirmed by barnacles carried by some specimens on their carapaces. Its role in the brackish waters of the Baltic Sea has been defined as a weak impact on communities and ecosystem functioning (Zaiko et al. 2010). So far, numerous studies on the biology and ecology of spiny-cheek crayfish have been undertaken (Lehmann and Quiel 1927; Kulmatycki 1935, 1936; Lieder 1959; Pieplow 1938; Kossakowski 1962; Leńkowa 1962). In addition, fecundity and juvenile production were examined by Stypińska (1972, 1973, 1978), Stucki (2002), Hamr (2002) and Kozak et al. (2006). Geelen (1975, 1978) investigated the expansion of *O. limosus* and Kossakowski (1961) attempted to estimate its economic significance. The growth and size issues have been taken up quite frequently (Pieplow 1938, Orzechowski 1984, Krzywosz et al. 1995, Chybowski 2000).

Nevertheless, all these investigations applied to fresh waters only. There are few elaborations known from being related to the influence of brackish water on the spiny-cheek crayfish. Szaniawska et al. (2006) carried out investigations in saline waters, namely on *O. limosus* morphometry in the Vistula Lagoon, drawing a comparison between fresh and saline waters. Michałowska (2001) investigated changes in osmotic concentration of the crayfish under different salinity conditions. No data on the reproduction and growth of *O. limosus* in brackish waters are available.

Reproduction and growth are two important characteristics expressing the fitness and adaptation of a species to its habitat (Guan and Wiles 1999). To find out whether *O. limosus* can populate Polish coastal waters with salinity up to 7 PSU, aspects of its reproduction and growth in slightly saline waters (3 PSU and 7 PSU) were investigated. The salinity tolerance of developing embryos, hatching success, body length increments, crayfish condition and mortality at water salinities of 3 PSU and 7 PSU were taken into the consideration.

MATERIALS AND METHODS

Experimental site

Females with pleopodal eggs were collected in northeast Poland from the south-western Vistula Lagoon, near the mouth of the rivers Nogat and Szkarpawa. The specimens were caught randomly with fyke nets (circumference 100-140 cm, mesh size 16-20 mm) in May. The water salinity at the collection site was 2 PSU.

Experimental conditions and procedure

In the laboratory, the crayfish were placed in aquaria (0.34 m^2, V=117 dm^3) with 10 cm long PCV tubes for shelter. The salinities used were 3 PSU and 7 PSU. Natural water of 7 PSU was pumped directly from Puck Bay; the water salinity of 3 PSU was prepared by diluting water from the bay with tap water. Ten animals were transferred to each of the two salinity treatments (3 PSU and 7 PSU) with acclimation steps of 1 PSU and 1.5 PSU every other day, starting from the initial salinity of 2 PSU. The water in the aquaria was aerated and filtered mechanically and biologically. A flow-through system enabled the complete renewal of the water every 9 days. Physicochemical parameters – salinity (PSU), temperature (°C), dissolved oxygen (mg dm^{-3} O_2) and pH level – were measured (Table 1).

Table 1

Physicochemical water parameters and the measurement procedure during experiments.

parameter	value	measuring tools	measurement frequency
salinity	3 ±0.5 7 ±0.5	WTW Ecoline LF 170 TetraCon 700 probe	once a day
temperature (°C)	16.5 – 24.5	WTW Ecoline LF 170 TetraCon 700 probe	once a day
dissolved oxygen (mg dm^{-3} O_2)	>7.0	WTW Oxi 197 Ecoline CellOx probe	once a week
pH	6.7 – 8.2	WTW pH 197 SenTix 97 T probe	once a week

The experiment was run under darkness (low light) until the juveniles hatched; after that, 12/12h light/dark regime was applied. When the offspring appeared, every female and her juveniles were located in a separate aquarium. The young crayfish were separated from their mothers after the second molt. One month after hatching, 50 juvenile crayfish (groups of 10 specimens each, obtained from 5 females) from both salinities were weighed individually to the nearest 1 mg, then placed in a Petri dish filled with water (3 PSU or 7 PSU) to be photographed next to a ruler. Every crayfish was measured (total length TL – from the anterior edge of the rostrum to the end of the telson; carapace length CL – from the anterior edge of the rostrum to the posterior edge of the carapace) accurate to 0.5 mm on the basis of the photographs, using the Corel

Draw 11 software. After measurement, each crayfish was put into a plastic, perforated cylinder (5.5 cm long, 4.4 cm wide) submerged in aquaria. The date of every molt was recorded, and three days after the molt, each young crayfish was removed from the cylinder and the measurement procedure repeated. Carapace measurements were divided into two length classes with the following carapace dimensions: 6.0 – 10.0 mm and 11.0 - 15.0 mm – for comparison with data obtained by Orzechowski (1984) in fresh waters. The mean wet weight in mg was established in every class. The remaining crayfish were kept in aquaria for mass culture. The growth investigation lasted 3 months.

The mature females were fed every other day with *Enteromorpha* algae and with walleye pollock fillets twice a week. Young crayfish were fed twice a day with artificial fodder Ovo-vit. Every other day, *Enteromorpha* algae and walleye pollock were served interchangeably.

The mortality of crayfish during the whole experimental period was established.

Statistics

The obtained values were expressed as means with standard deviation (±SD). Linear regression (y = ax + b) with the correlation coefficient (r) was used to determine the relationship between the total length (TL) and carapace length (CL), and the exponential function:

$$WW = a \times TL^b$$

with the correlation coefficient (R) to determine crayfish condition and the relationship between the wet weight (WW) and the total length. The significance criterion was set up at $p<0.05$. After data standardization, the Shapiro-Wilk test was employed to verify the normal distribution of the experimental data, after which the significance of the obtained differences was tested with the Mann-Whitney *U*-test at the significance level of 5%. All statistical analyses were performed using *STATISTICA 8.0* (StatSoft, Tulsa).

RESULTS

Reproductive success

Embryos developed normally during acclimation and the entire incubation period at both applied salinities (3 PSU and 7 PSU). No loss or discoloration of eggs was observed. Reproductive success was 100% at both salinities. The total number of freshly hatched crayfish was 1100 at 3 PSU and 827 at 7 PSU. The maximum number of juveniles obtained from one female was 220 at 3 PSU and 183 at 7 PSU. The minimum number of juveniles was 45 at 3 PSU and 16 at 7 PSU. The first juvenile stage molted into the second one with the complete success. The second juvenile stage molted into the third juvenile stage with 98.4% success at 3 PSU and 97.5% success at 7 PSU. The next juvenile stage was achieved with 94.7% success at both salinities.

Growth

Table 2 shows the carapace length, body length and wet weight ranges with means for every 10-specimen group of 1-month old crayfish from the salinity of 3 PSU and 7 PSU.

The mean (±SD) carapace length, total length and wet weight established for 50 crayfish from the salinity of 3 PSU and 7 PSU are presented in Figure 1.

Table 2

The range and mean (±SD) of carapace length, body length and wet weight for the groups of one month old crayfish from the salinity of 3 PSU and 7 PSU.

Salinity (PSU)	Group of juveniles	Carapace length (mm)			Total length (mm)			Wet weight (mg)		
		Min	Mean ±SD	Max	Min	Mean ±SD	Max	Min	Mean ±SD	Max
3	1-10	5.0	5.5 ±0.6	7.0	9.5	10.7 ±1.2	13.5	19	28.6 ±12.3	59
	11-20	5.0	5.5 ±0.4	6.0	9.5	10.7 ±0.9	12.0	21	29.7 ±5.6	38
	21-30	4.5	5.1 ±0.2	5.5	9.0	9.9 ±0.6	11.0	18	22.3 ±2.6	26
	31-40	5.0	5.4 ±0.2	5.5	9.5	10.4 ±0.5	11.0	19	24.6 ±2.6	29
	41-50	5.0	5.2 ±0.2	5.5	9.5	10.1 ±0.4	11.0	19	22.7 ±4.1	32
7	1-10	5.0	5.9 ±0.7	7.0	10.0	11.7 ±1.0	12.5	20	37.1 ±11.0	54
	11-20	5.5	6.1 ±0.6	7.0	10.5	12.0 ±1.1	14.0	26	34.3 ±7.9	53
	21-30	5.0	5.9 ±0.7	7.0	10.0	11.6 ±1.4	14.0	26	34.3 ±7.9	53
	31-40	5.0	6.5 ±1.0	7.5	10.0	12.6 ±2.0	15.0	22	44.3 ±16.3	67
	41-50	5.5	6.0 ±0.6	7.0	10.5	11.7 ±1.2	14.0	25	30.3 ±5.0	40

During the three-month growth experiment, the maximum number of molts per individual was 7 at 3 PSU and 3 at 7 PSU. The maximum increases were 3.5 mm (3 PSU) and 4.0 mm (7 PSU) in carapace length and 6.5 mm (3 PSU) and 7.0 mm (7 PSU) in body length. The intermolt period was similar at both salinities, varying from 6 to 31 days at 3 PSU and from 6 to 34 days at 7 PSU. The mean increase in carapace length was 1.3 ±0.7 mm at 3 PSU and 1.7 ±0.9 mm at 7 PSU, whereas the mean increase in total length was 2.4 ±1.4 mm and 3.4 ±1.6 mm respectively. Differences in total length and carapace

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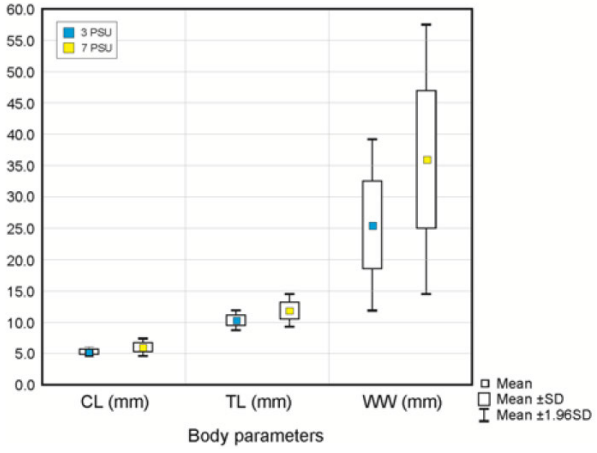

Fig. 1. Mean carapace length - CL (±SD), mean total length – TL (±SD) and mean wet weight – WW (±SD) established for 50 one month old crayfish from the salinity of 3 PSU and 7 PSU.

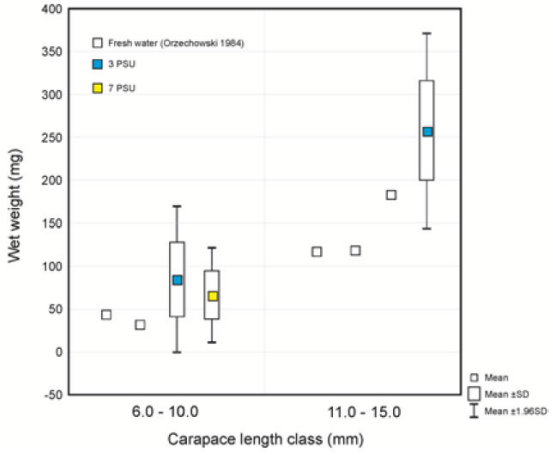

Fig. 2. Growth of crayfish under experimental salinity conditions (3 PSU and 7 PSU) and in fresh water. The mean wet weight and carapace length calculated for two carapace length classes (6.0 – 10.0 mm and 11.0 – 15.0 mm).

length increments between salinities 3 PSU and 7 PSU were statistically significant ($p<0.05$).

The mean carapace length estimated in carapace length class 6 – 10 mm was 7.9 ±1.3 mm at 3 PSU and 7.6 ±1.1 mm at 7 PSU. The mean carapace length in class 11 – 15 mm was 11.6 ±1.0 mm at 3 PSU, no data were available at 7 PSU. Greater mean carapace lengths were recorded in fresh waters: in class 6 – 10 mm, it was 9.4 and 8.4 mm and in class 11 – 15 mm, it was 12.7, 12.8 and 14.5 mm (Orzechowski 1984). Nevertheless, the mean wet weight in carapace length classes of *O. limosus* was the highest at 3 PSU and the lowest in fresh waters (Fig. 2).

The biggest weight gain in mg per molt amounted to 176 mg (46.4% of the premolt weight) at 3 PSU and 105 mg (166.7% of the premolt weight) at 7 PSU, whereas the lowest weight gain per molt came to 2 mg at 3 PSU and 14 mg at 7 PSU, i.e. 5.3 – 10.0% and 25.9% of the premolt weight respectively (Fig. 3). The mean gain on the body weight was 42.1 ±35.7 at 3 PSU and 40.3 ±22.3 at 7 PSU, and it did not differ significantly between salinities ($p<0.05$).

The weight gain per molt was highly variable for the same total length increments, and increased with the increasing total length (Fig. 4).

Statistically significant relationships were found between the total length (TL) and the carapace length (CL), and also between the total length (TL) and the wet weight (WW) for all the taken measurements of crayfish from salinity of 3 PSU and 7 PSU (Fig. 5 a, b). Individual weights increased more quickly in crayfish from 3 PSU than the ones from 7 PSU. The physiological condition of the crayfish was adversely affected, more by the salinity of 7 PSU than of 3 PSU.

Mortality

There was no mortality among stage 1 of juveniles. The total mortality in stage 2 of juveniles was 1.6% at 3 PSU and 2.5% at 7 PSU. Losses among stage 3 of juveniles reached 3.7 and 2.8% at 3 PSU and 7 PSU respectively. The number of juvenile specimens was reduced by approximately 50% within 5 weeks of hatching at both salinities (Fig. 6). The first two weeks of life of the young crayfish was the time of the lowest mortality, but during the next four weeks crayfish mortality increased from nearly 10% to 73% at 7 PSU and almost 80% at 3 PSU.

All females survived the exposure to salinities of 3 PSU and 7 PSU whilst incubating their eggs; their mortality was high mainly after molting. At the salinity of 3 PSU all ten females molted and nine females after molting died while at the salinity of 7 PSU nine females molted and all nine died after. The final mortality at this salinity was 100%.

DISCUSSION

Females with pleopodal eggs were collected from the Vistula Lagoon by the mouth of the rivers Nogat and Szkarpawa, where the salinity at the time of

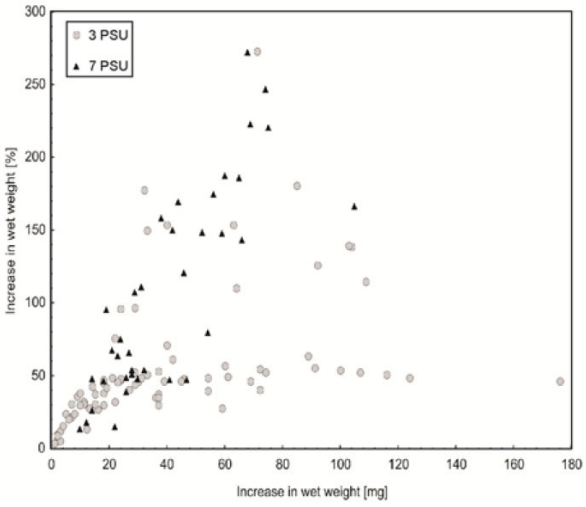

Fig. 3. Weight gain per molt of crayfish at the salinity of 3 PSU (n = 79) and 7 PSU (n = 37), expressed in mg and as % of the premolt weight.

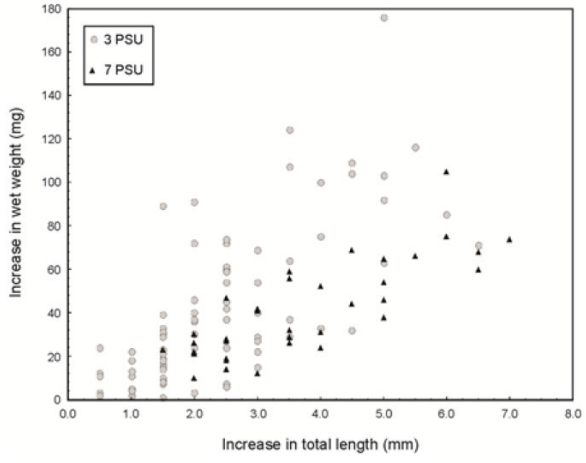

Fig. 4. Weight gain per molt of crayfish at the salinity of 3 PSU (n = 79) and 7 PSU (n = 37) with reference to total length increments.

sampling was 2 PSU. Two salinities were applied in the experiment: 3 PSU (the mean salinity of the Vistula Lagoon) and 7 PSU (the salinity of the Gulf of Gdansk). During acclimation to the experimental salinities, no eggs were observed to drop off the females, nor did any mortality occur. According to Kinne (1971), eggs, embryos and reproducing females are particularly sensitive to salinities in the 5-8 PSU range. However, the lack of egg deformation and the 100% survival rate of females in the reproductive period at both experimental salinities (3 PSU and 7 PSU) show that the spiny-cheek crayfish

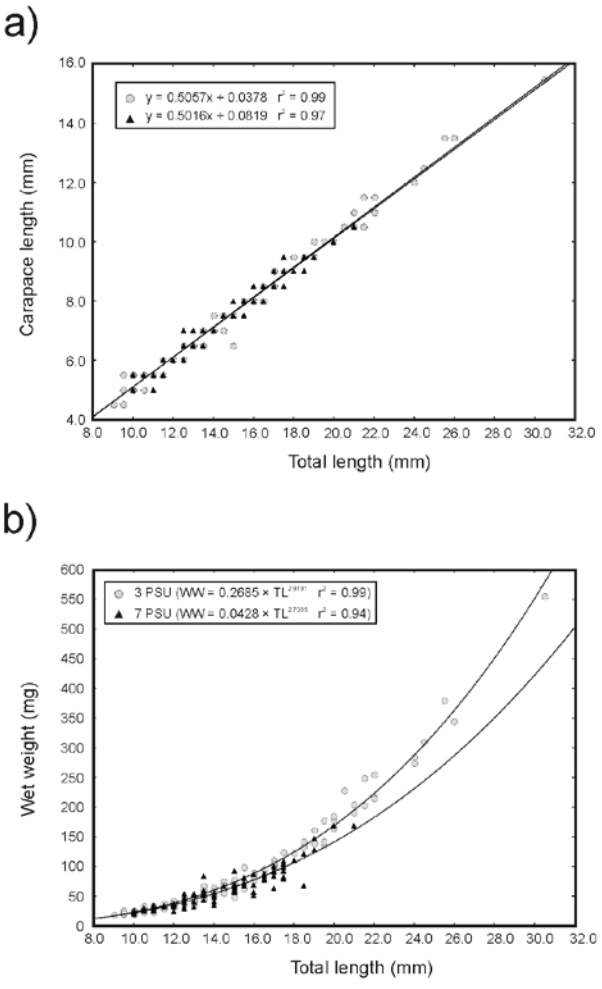

Fig. 5. Relationship between: a) TL - CL and b) TL – WW for all the taken measurements of crayfish at the salinity of 3 PSU (n = 142) and 7 PSU (n = 101).

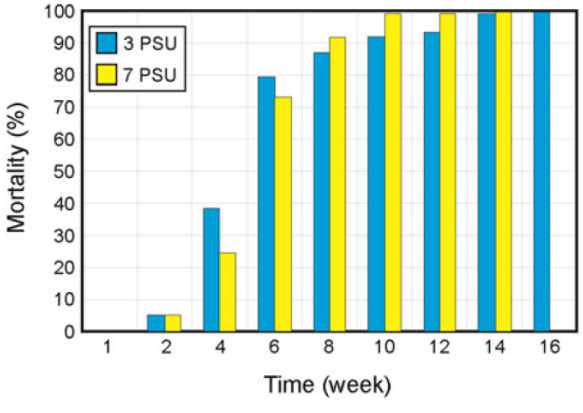

Fig. 6. Mortality in young crayfish at the salinity of 3 PSU (n = 1100) and 7 PSU (n = 827).

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is resistant to such changes in the salinity, and that it displays a certain adaptation to low salinities, even to those regarded as critical. At both salinities, 100% reproductive success was achieved. From 16 to 220 juveniles in the first developmental stage were obtained. A considerable number of crayfish developed through all three first juvenile stages to metamorphose into the next juvenile form. The reproduction of freshwater crayfish in brackish waters was also investigated by Holdich et al. (1997). These authors successfully reproduced narrow-clawed crayfish *Astacus leptodactylus* and signal crayfish *Pacifastacus leniusculus* at the salinity of 7 PSU. The first juvenile stage molted into the second stage, which was able to survive at this salinity for at least two weeks. However, at salinities of 14 PSU and 21 PSU in both species, many eggs became discolored about one week before the expected hatching date and failed to develop further; moreover, juveniles, which did manage to hatch perished within a few hours.

Current studies showed that both embryos and early juvenile specimens of *O. limosus* show high tolerance to water salinity of up to 7 PSU, higher than the signal and the narrow-clawed crayfish. Nevertheless, it would be very interesting to investigate aspects of the spiny-cheek crayfish reproduction at higher salinities as it has been done for *A. leptodactylus* and *P. leniusculus*.

Besides the reproduction, growth is the other important characteristic of the species, expressing its condition and adaptation to a given environment (Guan and Wiles 1999). Whereas the salinity did not affect the reproduction of *O. limosus*, it did influence the individual growth of the crayfish in terms of both body length increase and weight increase. The experiment showed that *O. limosus* achieved smaller body dimensions at 3 PSU than at 7 PSU during the same period of time. Nonetheless, the relationship between the wet weight and the total length indicates that for the same body lengths the body weight was lower. Therefore, the salinity of 7 PSU reduced the overall condition of the crayfish. Presumably, while the salinity of 7 PSU stimulates the growth of body length in crayfish, it adversely affects the overall physiology of typical freshwater organisms, in this case, reducing the condition of the crayfish, although to only a slight degree in comparison with 3 PSU. Szaniawska et al. (2005) report the positive effect of salinity on the size of adult spiny-cheek crayfish living at the salinity range of 2-4 PSU. In the present experiment, individual carapace lengths in one-month old crayfish ranged from 4.5 to 7.0 mm at 3 PSU and from 5.0 to 7.5 mm at 7 PSU. At 3 PSU the large majority of animals had carapaces up to 5.5 mm long, whereas at 7 PSU more than half of the crayfish had carapaces longer than 5.5 mm.

Salinity is probably the factor that determines the individual number of molts in crayfish. In the growth experiment at salinity of 3 PSU, one individual molted seven times in three months. One also has to take into account the period of one month prior to placing the crayfish into the tanks, during which the animals molted several times. In its first year of life, the spiny-cheek crayfish molts up to nine times (Krzywosz et al. 2002). The rate of molting recorded in the present work indicates that salinity increases the frequency of molting and probably also the rate of length increase, since after every molt the crayfish were a little longer. The mean increase in the body length in the spiny-cheek crayfish during the first four months of life was 2.4 ±1.4 mm at salinity of 3 PSU and 3.4 ±1.6 mm at 7 PSU. Westman et al. (1993) obtained, in fresh water under laboratory conditions, the mean total length increment of 5.5 and 4.6 mm in the first-year male and female signal crayfish, respectively. In the European crayfish (*A. astacus*), the same authors obtained the mean body length increments of 5.3 mm in males and 4.0 in females. Nevertheless, the shorter time of this growth investigation in comparison to literature data must be taken into account.

At both experimental salinities, the crayfish growth was highly differentiated. According to Świerczyński (2000), such divergence in the growth of individual crayfish may be due to the inhomogeneous growth surface, by which is meant the total effect of environmental conditions. The body weight increment varied a great deal. At the salinity of 3 PSU this increment per molt varied between 2 and 176 mg, and at 7 PSU between 14 and 105 mg. The body weight increment per molt expressed as a percentage of the premolt mass ranged from 5.1 to 273.1% at 3 PSU and from 13.9 to 272.0% at 7 PSU. The growth and weight gain takes place during the molt itself, when the crayfish uptakes water into its tissues, thus increasing in size, weight and volume. The weight gain per molt is normally between 30 and 60% of the premolt weight. During the intermolt, the crayfish replaces its water content with tissue growth, but the weight gain during the long intermolt is normally less than 5% of the immediate postmolt weight (Wolf 2004). Also, the water in the haemolymph decreases during the intermolt (Sardá and Cros 1984). Such an abnormally

high body weight increment during the experiment can probably be explained by the greater uptake of water during molting (Chatterji et al. 2004). The effect of salinity on the growth expressed as the body weight increments has also been determined in other crayfish species. Loyacano (1968) states that the growth of the red swamp crayfish *Procambarus clarkii* is reduced at salinities of 10 and 20 PSU, while the study of Sharstein and Chafin (1979) reports that water salinity of up to 12 PSU stimulates this process. In *P. leniusculus* the growth is gradually reduced at salinities of around 5, 9 and 18 PSU in comparison with fresh waters (Rundquist and Goldman 1978). Salinities over 6 PSU affect the growth in the yabby *Cherax destructor*, whereas lower salinities stimulate it (Mills and Geddes 1980). It is likely that the differences in the evolutionary history and osmotic behavior of different freshwater crayfish govern the different growth potentials of these crayfish in brackish waters (Mills and Geddes 1990). It is proved that the crayfish growth may be stimulated in low salinities, possibly because less energy is being used for osmoregulatory purposes (Newsom and Davis 1994).

The mean body weights obtained in the experiment in relation to the length classes 6 – 10 mm and 11 – 15 mm are twice as large as those reported by Orzechowski (1984) for *O. limosus* from the Koronowo Reservoir in 1971-72. The comparison of the growth of the early developmental stages of *O. limosus* in fresh and brackish waters (3 PSU and 7 PSU) indicates that waters with a salinity of 3 PSU offer the best conditions for its growth.

Mortality of the crayfish is linked directly with their growth. The molting season is the time when crayfish are completely defenseless until the new carapace hardens. Moreover, the ionic and osmotic regimes are disturbed at that time (Wheatly and Gannon 1995). At both salinities in the first two weeks of life, crayfish mortality was low. At that time, there was little size differentiation between the crayfish, and cannibalism had not yet come into play. After about five weeks, however, the overall numbers of crayfish in both salinities had fallen by nearly 50%. Orzechowski (1984) reports that crayfish numbers are severely reduced in the first six weeks of life. Our results from brackish water corroborate this statement. The survival of juveniles may be affected by the insufficient number of shelters (Figler et al. 1999), higher molting frequency (Barki et al. 1997) and high density promoting cannibalism (Ulikowski et al. 2006). Young crayfish are very sensitive organisms (Świerczyński 2000). The number of young crayfish might be also dramatically reduced due to measurements taken during the experiment (Ulikowski and Krzywosz 2009). Mortality among adult females was very high directly after molting. This trend was observed in females from both salinities (3 PSU and 7 PSU). Such a high mortality could have been favored by such factors as stress, diet and probably their lower physiological plasticity compared to juveniles. In addition, egg-laying will have weakened the females, especially under the altered salinity conditions, which could have affected their postmolt survival – molting is complex and energy-demanding process. Nevertheless, it should be remembered that the females were perfectly tolerant of the experimental salinities until the inception of molting. The experiment suggests that *O. limosus* may decrease its survival capability to salinity changes with age, while the highest survival was recorded in embryos and the first three juvenile stages. This situation may be related to reduced osmoregulatory abilities in older crayfish. The opposite statement concerning the signal and narrow-clawed crayfish was proposed by Holdich et al. (1997). Rundquist and Goldman (1978) and Anson and Rouse (1994) revealed that early-stage juveniles of *P. leniusculus* and *C. quadricarinatus* respectively, do not tolerate low salinities as older ones do, and thus their colonization in brackish waters is limited.

CONCLUSIONS

To summarize, colonization of brackish habitats with the salinity of up to 7 PSU by *O. limosus* is highly probable. This is indicated by the normal development of embryos, high breeding success, the high survival of early juvenile forms, and also by the tolerance of experimental salinities by older juveniles and adults at least for a few months.

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Program of implementing the elements of modern education forms at the University of Gdansk, the task – Research grants for PhD students – a chance for the economic development conducted in the framework of the Human Capital Operational Programme, action 4.1.1., Improving the quality of educational offer of tertiary education institutions.

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