A COMPARISON OF SURVIVAL AND GROWTH IN JUVENILE *ASTACUS LEPTODACTYLUS* (ESCH.) AND *PACIFASTACUS LENIUSCULUS* (DANA) UNDER CONTROLLED CONDITIONS

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ABSTRACT

The aim of this study was to compare survival and growth of juvenile narrow-clawed crayfish (*Astacus leptodactylus*) and signal crayfish (*Pacifastacus leniusculus*) fed only with experimental pellets (45% of proteins, 6% of fat, 20% crude fibre) under controlled conditions. The animals were reared in 12 (50 l) circular plastic tanks, the narrow-clawed crayfish set-ups being designated A1 and A2, the signal crayfish set-ups P1 and P2. The initial stocking densities for each species were 600 (A1, P1) and 1,200 (A2, P2) juveniles stage 2 per square metre for each set-up in three replicates. The experiment lasted 92 days under continuous photoperiod (L:D 24:0; 100 LUX) conditions at a temperature of 22.0 ± 0.1°C and an oxygen saturation > 90%. One shelter (plastic tube) was provided for 2 crayfish in each set-up.

The highest survival rate was obtained for signal crayfish from set-up P1 (47.5%), the lowest for narrow-clawed crayfish from set-up A2 (22.8%). Crayfish survival evidently depends on the stocking density: in set-ups A1 and P1 it was about 16% higher than in set-ups A2 and P2 ($P < 0.05$). Mortality was significantly higher ($P < 0.05$) in both species during the first 30 days of the experiment (48-77% of the total mortality) than during the subsequent two months of the experiment (< 20% of the total mortality).

The final body size was the largest in narrow-clawed crayfish from set-up A1 (799 mg, 29.2 mm) and the smallest in signal crayfish from set-up P2 (534 mg, 26.5 mm). Specimens of narrow-clawed crayfish were larger than signal crayfish, and the specimens of both species in set-ups A1 and P1 were larger than those in set-ups A2 and P2. During the first 30 days of the experiment the specific growth rate (SGR) of both species in all set-ups was twice the value reported during the subsequent two months ($P < 0.05$).

Key-words: *Astacus leptodactylus*, *Pacifastacus leniusculus*, juveniles, survival, growth.

COMPARAISON DE LA SURVIE ET DE LA CROISSANCE DE JUVÉNILES CHEZ *ASTACUS LEPTODACTYLUS* (ESCH.) ET *PACIFASTACUS LENIUSCULUS* (DANA) DANS UN ENVIRONNEMENT CONTROLÉ

RÉSUMÉ

Le but de cette étude était de comparer la survie et la croissance de juvéniles chez l'écrevisse à pattes grêles (*Asatcus leptodactylus*) et chez l'écrevisse signal (*Pacifastacus leniusculus*) maintenues dans un environnement contrôlé et nourris uniquement à l'aide de granulats expérimentaux (45 % de protéines, 6 % de graisses, 20 % de fibres). Les animaux ont été élevés dans 12 bacs circulaires de 50 l. Les bacs contenant les écrevisses à pattes blanches ont été notés de A1 et A2 et ceux contenant les écrevisses signal P1 et P2. Chaque bac a été testé en triplicat. La densité initiale en juvéniles pour les deux espèces était de 600 individus au stade 2 pour A1 et P1 et de 1 200 individus pour A2 et P2 par mètre carré. L'expérience a été conduite pendant 92 jours sous une photopériode continue (jour: nuit 24 : 0; 100 LUX), avec une température de 22,0 ± 0,1 °C et une saturation en oxygène supérieure à 90 %. Dans chaque bac, un abri (tube en plastique) a été fourni pour deux écrevisses.

Le taux de survie le plus important a été obtenu pour le lot P1 (47,5 %) d'écrevisses signal et le moins important pour le lot A2 (22,8 %) d'écrevisses à pattes grêles. La survie des écrevisses dépend évidemment de la densité de départ: dans les lots A1 et P1, la survie est supérieure de 16 % par rapport à celle des lots A2 et P2 (P < 0,05). La mortalité a été significativement plus importante (P < 0,05) pendant les 30 premiers jours de l'expérience (48-77 % de la mortalité totale) que pendant les deux derniers mois (< 20 % de la mortalité totale).

Les écrevisses à pattes grêles du lot A1 ont présenté les tailles corporelles les plus grandes (799 mg ; 29,2 mm) alors que les plus petites ont été enregistrées dans le lot P2 (534 mg ; 26,5 mm). D'une façon générale, les écrevisses à pattes grêles étaient plus grandes que les écrevisses signal et les individus des lots A1 et P1 étaient aussi plus grands que ceux des lots A2 et P2. Pendant les 30 premiers jours de l'expérience, le taux de croissance spécifique (SGR) de chaque espèce (A1, A2, P1, P2) était plus important que celui mesuré dans les deux mois suivants (P < 0,05).

Mots-clés : *Astacus leptodactylus*, *Pacifastacus leniusculus*, juvéniles, survie, croissance.

INTRODUCTION

The range of distribution and the number of localities of native noble crayfish (*Astacus astacus* (L.)) and narrow-clawed crayfish (*Astacus leptodactylus* (Esch.)) in Poland have declined drastically in recent decades (KRZYWOSZ, 1994; BIAŁOKOZ *et al.*, 1996). At present, the yields from natural waters are no greater than 2 tonnes per year (MASTYŃSKI and ANDRZEJEWSKI, 2001). This situation is due principally to the degradation and pollution of the natural environment, but several cases of crayfish plague (*Aphanomyces astaci*) and the expansion of spiny-cheek crayfish (*Orconectes limosus* (Raf.)) have also contributed. As a result, populations of noble crayfish and narrow-clawed crayfish are numerous only in SE Poland (KRZYWOSZ, 1994). Since the market value of native crayfish in Europe is higher than the one of imported species, it is more profitable to develop the farming of the former (HOLDICH, 1993), and many crayfish farmers in Europe have done precisely that (ACKEFORS, 1998). Crayfish farming also plays a significant role in the active protection of native species for restocking programmes (ACKEFORS and LINDQVIST, 1994; ACKEFORS, 1998; MASTYŃSKI and ANDRZEJEWSKI, 2001; SKURDAL and TAUGBØL, 2002; CARRAL *et al.*, 2003; ŚMIETANA *et. al.,* 2004).

In Poland signal crayfish (*Pacifastacus leniusculus*) have the fastest rate of growth, reaching marketable sizes (10 cm TL) after 3 years of breeding, which is about one year earlier than our native species (KRZYWOSZ, 1994). The faster growth of the former species has also been reported from other European countries (ABRAHAMSSON, 1971; WESTMAN, 1973; CUKERZIS, 1989; ACKEFORS and LINDQVIST, 1994). But these reports are based on a comparison with crayfish growth under natural conditions, which is susceptible to a variety of environmental and climatic factors. Nevertheless, under controlled conditions it is possible to compare different crayfish species, which may enable these claims to be verified under the same breeding conditions. The results obtained by different authors (TCHERKASHINA, 1977; ULIKOWSKI and KRZYWOSZ, 2004a) show that the growth rate of narrow-clawed crayfish under optimal conditions is comparable to that of the signal crayfish.

The aim of this study was to compare, in a controlled experiment, survival and growth in juvenile narrow-clawed crayfish and signal crayfish fed with pellets *ad libitum*.

MATERIAL AND METHODS

The test animals were juveniles stage 2 (juveniles after the first moult) from both crayfish species. According JONSSON (1990) the signal crayfish juveniles may hatch about 2-4 weeks earlier than the native crayfish juveniles, may have a marked impact on growth during the first year after hatching. Similar observations were made in a comparison of narrow-clawed crayfish and spiny-cheek crayfish (SCHULZ and ŚMIETANA, 2001). Therefore, in order to obtain juveniles narrow-clawed crayfish at the same stage of development of signal crayfish, egg development was accelerated under controlled conditions. Narrow-clawed crayfish females carrying eggs were transferred from the breeding ponds (4th May) to tanks in a re-circulating system. Water was heated from 10° to 20°C over a period of 2 days. The signal crayfish females were taken from the ponds at a later date (20th May). The females of both species were kept in separate parts of the tanks (120 cm^2) at a constant water temperature of 20°C until the 22nd of May. The eggs were removed from the pleopods on the 22nd of May and incubated further, each species separately, in Weiss apparatuses at a water temperature of 21°C. The juveniles of both species hatched between 25th and 27th May, and the first moult took place between 4th and 7th June. The average total body length (TL – from the beginning of the rostrum to the end of the telson) and individual body weight of narrow-clawed crayfish and signal crayfish were similar, the respective measurements being 12.0 ± 0.7 mm and 29 ± 3 mg, and 11.0 ± 0.5 mm and 28 ± 3 mg.

The two species were reared separately in independent, identical water re-circulating systems, each of which consisted of 6 (50 l) circular plastic tanks (0.17 m^2) equipped with a thermoregulator and a biological water filter. Each tank contained shelters for crayfish (two crayfish per shelter) constituted from 18 mm diameter garden hose cut into 2-3 cm segments. There were two initial stocking densities – 600 and 1,200 specimens per square metre for each species in three replicates. The narrow-clawed crayfish setups were designated A1 and A2, the signal crayfish set-ups P1 and P2. The experiment lasted for 92 days under continuous photoperiod (L:D 24:0; 100 LUX) conditions. The crayfish were fed with experimental pellets (45% proteins, 6% fat, 20% crude fibre) provided *ad libitum* every 12 hours.

Water temperature and oxygen content were measured daily with an OxyGuard electronic device. Every two to three days water samples were collected for analysis: pH was determined with a *PM 600* electronic device, ammonia nitrogen $N-NH_4$ (Nessler method) and nitrite nitrogen $N-NO_2$ (sulphanil method) were determined colorimetrically on a *Spekol 11* spectrophotometer. The average water temperature was 22.0 ± 0.1°C, and the oxygen content of the water was > 90%. At pH 7.9-8.3, the ammonia nitrogen content did not exceed 0.1 mg $N-NH_4$/l, and nitrite nitrogen was no greater than 0.04 mg $N-NO_2$/l.

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After 30, 61 and 92 days all the crayfish were caught and counted. The whole stock was weighed to the nearest 0.1 g. The average individual crayfish body weight was calculated by dividing the stock biomass from each tank by the number of specimens it contained. Each of the tank stocks was photographed, and the length of 10-20 specimens from every tank was measured to the nearest 0.1 mm using the *MultiScan* computer program. At the end of the rearing period the same measurements were taken using 30 specimens from each setup. The initial measurements were similar but only 20 specimens were taken from each species. The specific growth rate (SGR) was calculated using the equation.

$$SGR = (lnW_f - lnW_i)\ 100/t$$

Where:

lnW_f is the natural logarithm of the final body weight,

lnW_i is the natural logarithm of the initial body weight, and

t is the time (days) between lnW_f and lnW_i.

The growth results, SGR, and mortality were analysed statistically using the *Statistica 5.1* computer program. One-way analysis of variance (ANOVA) was applied, and the post-hoc averages were compared using Tukey's test at a significance level of $P < 0.05$.

RESULTS

After 92 days of rearing the survival rate was highest in the signal crayfish from setup P1 (47.5%) and lowest in the narrow-clawed crayfish from set-up A2 (22.8%). In all the setups the final survival of the signal crayfish was higher than the one of the narrow-clawed crayfish (Table I), but these differences were not significant ($P > 0.05$). Crayfish survival was clearly dependent on the stocking density: in set-ups A1 and P1 survival was about 16% higher than in set-ups A2 and P2 ($P < 0.05$).

Mortality in both species was significantly high during the first 30 days of the experiment (48-77% of the total mortality) but significantly lower during the next two months (Figure 1A). The temporal mortality pattern was different in both species, although the final mortality depended only on the initial stocking density ($P < 0.05$) and did not vary between the two species.

The final body size was the largest in the narrow-clawed crayfish from set-up A1 (799 mg, 29.2 mm), and the smallest in the signal crayfish from set-up P2 (534 mg, 26.5 mm). Specimens of narrow-clawed crayfish were larger than signal crayfish, and the specimens of both species in set-ups A1 and P1 were larger than in set-ups A2 and P2. Again, the differences were not significant ($P > 0.05$) owing to the high variation in juvenile size within each set-up (Table I).

In the first 30 days of the experiment the specific growth rate (SGR) in all set-ups was twice as high as during the following two months ($P < 0.05$). During the first month of the experiment narrow-clawed crayfish grew faster ($P < 0.05$) than signal crayfish. During the second, the growth rate was similar, and during the third signal crayfish grew faster ($P < 0.05$) than narrow-clawed crayfish (Figure 1B).

Even though the initial stocking densities in set-ups A1 and P1 were half the size of A2 and P2, the biomasses in the two groups of set-ups gradually converged, so that the final total biomass in all set-ups was similar (Figure 1C). The biomass of narrow-clawed crayfish increased during the third month of rearing only in the set-up with the lower initial stocking density. In both set-ups the final biomasses reached a similar maximum
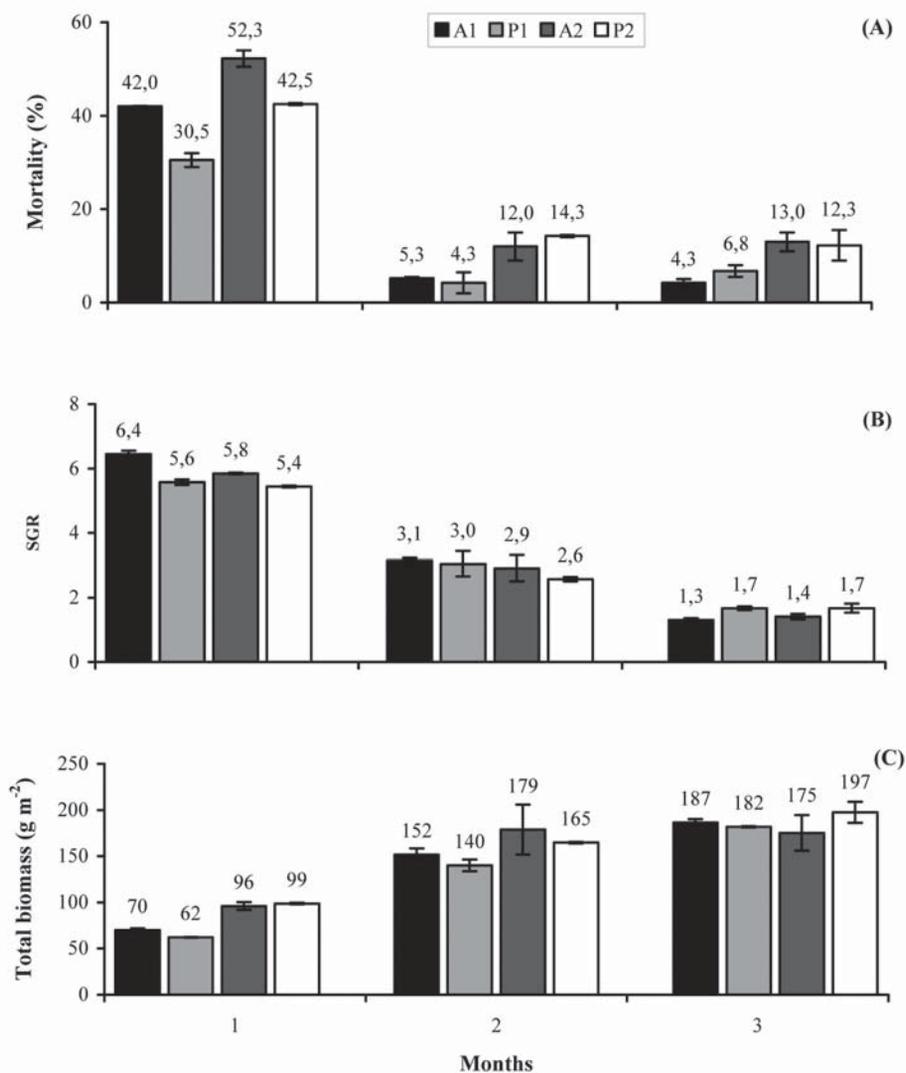
Figure 1

Mortality (A), SGR (B), and total biomass (C) of juveniles narrow-clawed crayfish and signal crayfish reared during three months after hatching under controlled conditions.

A1 – narrow-clawed crayfish with initial density 600 individuals per square meter.
P1 – signal crayfish with initial density 600 individuals per square meter.
A2 – narrow-clawed crayfish with initial density 1,200 individuals per square meter.
P2 – signal crayfish with initial density 1,200 individuals per square met**er.**

Figure 1

Mortalité (A), taux de croissance spécifique (SGR) (B) et biomasse totale (C) des juvéniles d'écrevisses à pattes grêles et d'écrevisses signal élevées pendant trois mois dans un environnement contrôlé après l'éclosion.

A1 – écrevisse à pattes grêles avec une densité initiale de 600 individus par mètre carré.
P1 – écrevisse signal avec une densité initiale de 600 individus par mètre carré.
A2 – écrevisse à pattes grêles avec une densité initiale de 1 200 individus par mètre carré.
P2 – écrevisse signal avec une densité initiale de 1 200 individus par mètre carré.

level of about 185 g per square metre. Nevertheless, the biomass of the signal crayfish also increased in the third month of the rearing experiment, finally reaching a maximum of 197 g per square metre. This similar final biomass was due to the higher mortality of crayfish in set-ups A2 and P2 than in set-ups A1 and P1, due to the higher initial stocking densities in the former set-ups. At higher stocking densities more individuals lost chelae and walking legs. More signal crayfish than narrow-clawed crayfish sustained this kind of mutilation.

DISCUSSION

The results obtained from our experiment show a similar growth rate in both species during the first three months after hatching. Similar observations were made with older individuals of both species after six months of rearing in our previous investigations (ULIKOWSKI and KRZYWOSZ, 2004a), where the SGR was 0.71 in narrow-clawed crayfish and 0.74 in signal crayfish. The different growth rates of these species in breeding ponds and natural waters may be due to a variety of environmental factors and interspecific differences. According to LOZAN (2000), 33% of the activity of the signal crayfish takes place during daylight, while this figure is only 16% in the narrow-clawed crayfish. In natural environments, therefore, signal crayfish are likely to find food more effectively than native crayfish. Obviously, then, narrow-clawed crayfish are more active at night. They are also much more mobile, capable of covering a distance of 1587 metres in 24 hours, whereas the signal crayfish moves only 190 metres in the same time (LOZAN, 2000).

After 92 days the two experimental species achieved relatively good growth: mean body lengths varied from 27.1 to 29.2 mm TL. These results suggest that the conditions for the crayfish during the whole experiment had been optimal. CELADA *et al.* (1993), for example, reported that the mean carapace length (CL = ~ 50% of TL) varied from 7.2 to 7.5 mm after 110 days of breeding in plastic basins, even though the juvenile signal crayfish these authors examined were kept at much smaller initial densities (50 animals per square metre) and their final sizes were ~ 50% smaller than in our experiment. The same applies to juvenile survival: CELADA *et al.* (1993) reported a diet-dependent survival from 5.3% to 38.7% after 110 days of breeding, while in our experiment the survival of signal crayfish ranged from 31.0% to 47.5%.

In our study, mortality was highest during the first month (48-77% of the total mortality) in both species, but fell considerably during the ensuing two months. Similarly, SÁEZ-ROYUELA *et al.* (1995) reported the highest mortality in signal crayfish juveniles during the first 40-60 days following female departure. NYSTRÖM (1994) listed the following main factors affecting the survival of signal crayfish juveniles: stocking density, number of shelters, light intensity, accessibility and quantity of food. The results of our investigations revealed the negative effect of higher stocking densities on growth and survival in both species. Different authors have described similar effects (ACKEFORS *et al.*, 1989; GYDEMO and WESTIN, 1989; ACKEFORS and LINDQVIST, 1994; MACKEVICIENE *et al.*, 1996). High stocking densities intensify mutual aggression and cannibalism, resulting in increased mortality and in a larger number of individuals with lost legs. A similar effect was observed after four weeks of rearing of narrow-clawed crayfish juveniles (ULIKOWSKI and KRZYWOSZ, 2004b), where survival was 70%, 58% and 47.8% from respective initial stocking densities of 300, 600 and 1,200 animals per square metre. The results of our experiment confirmed the impact of initial density on crayfish survival during the first months of breeding. CELADA *et al.* (1993) observed the same effect in signal crayfish. These authors succeeded in achieving a high survival rate of 50-70% in signal crayfish juveniles when they were kept individually in cylindrical compartments for 80 days of the breeding period under laboratory conditions. In breeding ponds, given favourable conditions, the chances of surviving the first year of life are high (45-90%) for both signal crayfish (KRZYWOSZ, 1994; KOZAK *et al.*, 1998) and narrow-clawed crayfish

(TCHERKASHINA, 1977). Most authors therefore recommend rearing crayfish in small ponds (CUKERZIS, 1989; HOLDICH, 1993, 2002; ACKEFORS and LINDQVIST, 1994; ACKEFORS, 1998) with initial stocking densities of juveniles stage 2 of no more than 100 individuals per square metre.

Although the stocking densities in our investigations were higher, the final results of growth and survival in narrow-clawed and signal crayfish were similar to those obtained by other authors (CELADA *et al.*, 1989; ACKEFORS and LINDQVIST, 1994; SÁEZ-ROYUELA *et al.*, 1995). It seems that laboratory conditions (shelter density) reduce the considerable stress experienced by newly moulted crayfish as they constantly try to avoid competition with the other crayfish present in the basins (TAUGBØL 1989). Our results show that it is possible to breed crayfish at high initial densities and to obtain relatively high survival rates, whereas under natural conditions, usually no more than 10% of the same species survive the first year of life (TCHERKASHINA, 1977; ULIKOWSKI, 1996). This is the effect of very much greater number of biotic and abiotic stressors under natural conditions than under breeding conditions (LEWIS, 2002). To summarise our results on survival: both crayfish species can be bred at relatively high initial densities, although an initial density of 600 individuals per square metre appears to promise better results than 1,200 individuals per square metre.

The lack of significant differences in the final biomass in all setups shows that the rate of growth of the signal crayfish, reported as being a fast-growing species, is similar to the one of the native narrow-clawed crayfish under the same laboratory conditions. The same applies to SGR and survival. The only differences, displayed in the mortality pattern, may have been induced by behavioural differences between the species. During the experiment the signal crayfish were more aggressive than the narrow-clawed crayfish, and most individuals of the former species lost chelae or walking legs. Many authors showed that signal crayfish are more aggressive than our native species (SÖDERBÄCK, 1991; LOZAN, 2000; LEWIS, 2002). It has been observed that when signal crayfish are bred together with narrow-clawed crayfish, they exhibit greater aggression towards their conspecifics, while at the same time avoiding clashes with narrow-clawed crayfish (ULIKOWSKI AND KRZYWOSZ, 2004a).

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