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RESEARCH ARTICLE

Effect of feed and holding conditions on survival and growth of juvenile crayfish *Pontastacus leptodactylus* and *Faxonius limosus*

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Abstract. In the European natural environment attempts are undertaken to replace invasive crayfish species with native ones. For this purpose, aquaculture of crayfish is required, but their behaviour and the lack of balanced feeds impede further development. To effectively eliminate invasive crayfish, more knowledge is needed, in context of their similarities and differences from native species. This study investigates two crayfish species, native *Pontastacus leptodactylus* and invasive *Faxonius limosus* in relation to different holding conditions and feeds. Juvenile crayfish were exposed to two types of holding conditions, inside (IN) in tap water and outside (OUT) in water with natural plankton content. In addition, three feeds were tested, sinking chips (F1), flakes (F2) and floating sticks (F3). The feeds composition was similar, but sinking times differed and were quick for F1 and slow for F2 and F3, which provided extended visual and chemical exposure to crayfish. The significant effect of holding conditions on survival was found for both species, with highest results for OUT holding conditions, suggesting important role of natural plankton in survival. Three feeds (F1, F2 and F3), showed no effect for *P. leptodactylus* but F2 and F3 improved growth in *F. limosus*, indicating more efficient utilisation of the detected feed.

Keywords: freshwater crayfish, *P. leptodactylus*, *F. limosus*, feeding, rearing

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Introduction

Freshwater crayfish are important species in ecosystem, specifically in the benthos (Olsson 2005). In Polish inland waters, crayfish species constitute two groups, native and invasive. Native species include noble crayfish, *Astacus astacus*, and narrow-clawed crayfish, *Pontastacus leptodactylus*. The majority of invasive species include spiny-cheek, *Faxonius limosus* and signal crayfish, *Pacifastacus leniusculus*. Sporadically, *Procambarus clarkii* and *Procambarus fallax* f. *virginalis* are found in consequence of releases of aquarium pets into the wild (Solarz et al. 2020). The invasive crayfish (*F. limosus* and *P. leniusculus*) are superseding native ones, due to better resistance to crayfish plague (*Aphanomyces astaci*) and the ability to withstand higher water eutrophication (Abrahamsson and Goldman 1970, Cerenius et al. 2003). Invasive crayfish are more efficient breeders (*F. limosus*) and migrate at a higher intensity (*P. leniusculus*) (Wutz and Geist 2013, Kozák et al. 2015). Native crayfish require human support to maintain and increase their population levels. The common method includes releasing juvenile native crayfish into the environment. Release locations must be free of crayfish plague, and invasive crayfish must either be absent or their populations must be

very low. This procedure requires breeding, hatching and initial rearing of early juveniles, to increase their chances of survival in natural conditions. Crayfish are valued in aquaculture, specifically *A. astacus* and *P. leptodactylus*, due to consumer demand and high market prices of these species (Kozák et al. 2015). A considerable obstacle in crayfish rearing, either for release into the environment or human consumption, is the lack of sufficient and easy to use feeds, for all growth stages.

The feeding preferences of invasive and native crayfish are similar, all species are omnivorous and compete for similar resources, including shelter (Guan and Wiles 1998, Vorburger and Ribi 1999, Ercoli et al. 2021). Their feed preferences change depending on availability, with animal tissue the most favoured and plant when the first is not available (Cronin et al. 2002). Juvenile crayfish are capable of feeding on small organisms, in early life stages. During the larval and early juvenile life stages, crayfish feed on plankton and shift their diet to larger prey and detritus concurrently with growth (Van den Berg et al. 1990, Sierp and Qin 2001, Alcorlo et al. 2004, Meakin et al. 2008). At this stage, the inclusion of animal tissue in the diet is crucial, due to quick growth (Alcorlo et al. 2004).

In the stage two juvenile *P. leniusculus*, three formulated feeds including commercial trout feed, raw trout feed and *Daphnia pulex* feed showed highest growth and survival for the first one (Celada et al. 1993). All tested feeds were in form of pellets (commercial feed) or pieces bound by agar (raw trout feed and *D. pulex* feed; Celada et al. 1993). Authors conclude that poor survival in the *D. pulex* group, may be the result of low dry matter content. Juvenile *P. leniusculus* fed with formulated feed showed significantly increased survival and growth, when additionally supplied with live *Artemia salina* nauplii and *D. pulex*. The results improved concurrently with dosage increase (Sáez-Royuela et al. 2007).

Laboratory observations of juvenile *Cherax cainii* showed highest preference to consume frozen copepods in the smallest animals. The preference decreased gradually with size increase and concurrent shift to pelleted feed offered together with frozen copepods (Tulsankar et al. 2021a). Inclusion of live plankton in juvenile *C. cainii* diets resulted in improved growth, survival and health. Formulated feed was most favoured, when live plankton was not available, but growth and survival were reduced (Tulsankar et al. 2022). Similarly, juvenile *Procambarus clarkii* growth was significantly faster when feed rations included live zooplankton (Brown et al. 1992). Live plankton provided at optimal, constant density may be a major source of nutrients for crayfish (Tulsankar et al. 2021b).

Environmental factors affecting growth and feeding behaviour require more studies, despite extensive research. Here we analyse the effect of holding conditions in combination with three feeds on the survival and growth of juvenile native (*P. leptodactylus*) and invasive (*F. limosus*) crayfish species. Our study included feeds of different physical properties (sinking chips, flakes and floating sticks) and two types of water (tap water and water similar to the natural, supplied from Łuczański Canal, Giżycko, 54°02'22.3" N; 21°45'26.6" E) used in the experimental conditions. The feeds were tested in an attempt to determine which feed properties are beneficial for juvenile crayfish. The comparison of tap water and water supplied from Łuczański Canal provided insight into juvenile crayfish requirements in context of addition of live plankton to their diet.

Materials and Methods

Experimental animals

First instar juveniles *P. leptodactylus* and *F. limosus* were hatched at the National Inland Fisheries Research Institute, using methods described by Ulikowski and Krzywosz (2004). Twenty specimens in the second juvenile stage (after the first moult) of *P. leptodactylus* and *F. limosus* were allocated to each experimental vessel three to five days after the first moult, prior to the start of the experiment. The initial total length (TL) was 0.95 cm ± 0.01 S.E. and 0.71

Table 1
Experimental feeds composition

Feed	F1 (sinking chips)	F2 (flakes)	F3 (floating sticks)
Analysis			
Protein (%)	41	49	41
Fat (%)	9	10.5	9.2
Fibre (%)	3	3	5.5
Moisture (%)	10.5	6	10.5
Ingredients			
Fish & fish derivatives	+	+	+
Moluscs & crustaceans	+	+	+
Insect larvae meal	+	-	+
Vegetable protein extracts	+	+	+
Algae	+	+	+
Derivatives of vegetable origin	+	+	+
Fruit	+	-	+
Cereals	+	+	+
Yeasts	+	+	+
Oils and fats	+	+	+
Additives			
Vit. A	+	+	+
Vit. D_3	+	+	+
Vit. E	+	+	+
Vit. C	+	+	+
Beta-carotene	-	+	+
L-carnitine	-	+	-
Fe	+	+	+
Zn	+	+	+
Mn	+	+	+
Cu	+	+	+
I	+	+	+
Se	+	-	+
Mo	+	-	+
Astaxanthin	+	+	+
Lecithin	-	+	-
Antioxidants	+	+	+

cm ± 0.01 S.E. for *P. leptodactylus* and *F. limosus*, respectively.

Experimental feeds

The feeds tested in the experiment included three fish feeds: sinking chips (F1), flakes (F2) and floating sticks (F3), as shown in Table 1 (Tropical®, Poland). Rations per replicate were in abundance, 1 g of feed was supplied daily in the afternoon before start of dark phase. The approximate sinking times of feeds were 2.61 s ± 0.37 S.E. for F1, 5.21 s ± 0.48 S.E. for F2, and 12.26 s ± 0.52 S.E. for F3, irrespective of holding conditions.

Holding conditions

The experiment lasted for thirty-nine days during the last five days of spring and in summer. The crayfish were cultured in two types of holding conditions: inside (IN) and outside (OUT) (Table 2).

In the type IN, the crayfish were cultured inside laboratory containers (Szkudlarek et al. 2021), in circulating tap water (closed circulation), filtered with a 300 micron gravity-fed sieve filter, at a water exchange rate of six exchanges h^{-1}. The water quality parameters were: temperature – 19.11 °C ± 0.42 S.E.; dissolved oxygen – 100.83% ± 0.18 S.E.; specific conductivity (SPC) 552.87 µS/cm ± 2.58 S.E.

Table 2
Design of the experiment, showing feeds tested (F1 – sinking chips, F2 – flakes, F3 – floating sticks), holding conditions (IN – inside, OUT – outside), crayfish species (*Pontastacus leptodactylus* and *Faxonius limosus*) and the number of replicates × crayfish per replicate.

Feed	Holding system	Species	Number of replicates and number of animals per replicate
F1	IN	*P. leptodactylus*	6 × 20
		F. limosus	6 × 20
	OUT	*P. leptodactylus*	6 × 20
		F. limosus	6 × 20
F2	IN	*P. leptodactylus*	6 × 20
		F. limosus	6 × 20
	OUT	*P. leptodactylus*	6 × 20
		F. limosus	6 × 20
F3	IN	*P. leptodactylus*	6 × 20
		F. limosus	6 × 20
	OUT	*P. leptodactylus*	6 × 20
		F. limosus	6 × 20

The photoperiod was 12:12 L:D, and the light was artificial.

The type OUT was located outside in a shaded area, and the crayfish were cultured in open flow water from Łuczański Canal. The water inlet was located in a small port connected to the canal. The water was not filtered at the inlet and outlet before returning into the canal. Water exchange rate was six exchanges h^{-1}, water quality was ambient and maintained levels: temperature – 24.13 °C ± 3.87 S.E.; dissolved oxygen – 85.66 % ± 0.97 S.E.; SPC – 406.28 µS/cm ± 5.74 S.E. The photoperiod and light were natural, with 17 h 4 min and 16 h 0 min of daylight at the start and at the end of the experiment, respectively.

Water quality was measured daily in the morning with an YSI multiparameter probe (Yellow Spring Instruments, USA). Water supplied from Łuczański Canal to the OUT holding conditions was analysed for plankton content and structure. The analysis was performed ten days after the start of the experiment. Samples for analysis were collected at the water surface near the water inlet and from surrounding area in the port. Sampling was done for day and night plankton composition. Samples were fixed with Lugol's solution, settled for 48 h at 4°C in the dark, decanted and analysed with a microscope (Nikon Eclipse E200). Ciliates were counted on microscopic slides, the rotifers and crustaceans were counted in a Sedgewick-Rafter counting chamber (Kalinowska and Karpowicz 2020). Chlorophyll levels were determined using spectrophotometry (Golterman 1969; Kalinowska and Karpowicz 2020). Water supplied to the OUT holding conditions contained large amounts of plankton, including cladocerans in a size range of 230–700 µm (*Daphnia* sp., *Bosmina* sp., *Chydorus* sp.), copepods in 800–2 000 µm size classes (*Cyclops* spp., *Eudiaptomus* sp.), ciliates in a size range of 10–450 µm (*Paradileptus elephantinus*, *Urotricha* spp., *Rimostrombidium* spp.), rotifers in a size range of 80–150 µm (*Synchaeta* sp., *Keratella cochlearis*, *Polyarthra* spp.), and medium-sized (30–100 µm) algae (*Spirogyra* sp., *Asterionella formosa.*, *Ceratium hirundinella*, *Dinobryon* sp., *Peridinium*/*Gymnodinium* spp.). Crustacean numbers including nauplii (120–150 µm) were 137.5 and 84.5 ind. × L^{-1} and chlorophyll levels were 12.78 and 14.09 µg × L^{-1}, for day and night, respectively.

The crayfish in both holding conditions were located in metal cages with perforated walls to allow for water circulation (Fig. 1). The cages were in the sets of 9 (3 rows × 3 cages), the dimensions of sets were 48 cm length × 48 cm width × 15 cm depth.

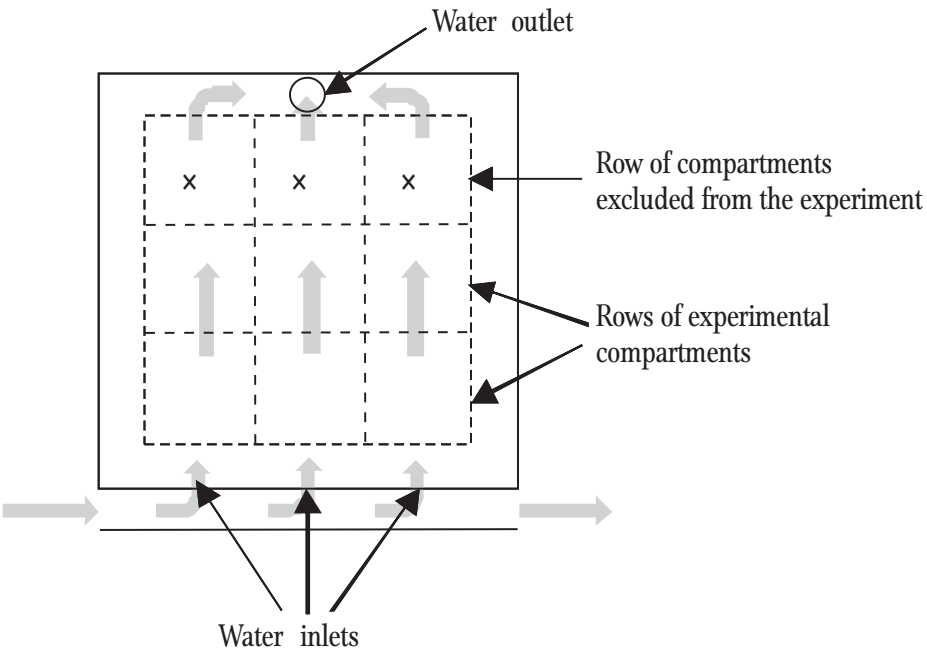

Figure 1. Plan of the experimental system showing a set of nine compartments made of perforated metal. The first and second row of compartments were used in the experiment, the third was excluded. Grey arrows show the direction of water circulation.

The dimensions of individual cage were 16 cm long × 16 cm wide × 15 cm deep. Each cage set was located in a separate open tank, with water flowing from one side. Two rows of cages (the first and second from the water inlets) were used in the experiment, the third row was excluded from the experiment due to technical reasons (difficult access). Each cage set was covered with metal sheets, to prevent the crayfish from escaping.

Growth and survival

At the termination of the experiment, survival (S, %) data was collected, and the crayfish were photographed alongside a scale. Next, Final TL (cm) measurements were taken from photographs with CoolView software. The Final TL (cm) and Initial carapace length (CL, cm) data were used to calculate TL Gain (%).

Statistical analysis

Prior to the analysis, the data homogeneity of variance, were tested with the Shapiro-Wilk W test. Square root transformation was used when normality was not met. The data transformations for the spiny-cheek Final TL (cm) and TL Gain (%) were ineffective, and the analysis was performed without meeting Shapiro-Wilk W test conditions. Levene's test was used for homoscedasticity testing, the data was natural logarithm transformed when variances were not homogeneous. Data was tested with two-way ANOVA, Tukey's HSD test was applied when significant difference was found. Pairwise multiple comparison was used for analysis of significant interactions. The data was analysed using SPSS software v. 29.

Results

S results were significantly higher in the OUT holding conditions for both species ($F = 29.216$; d.f. 1,35; $p < 0.001$, and $F = 53.248$; d.f. 1,35; $p < 0.001$ for *P. leptodactylus* and *F. limosus*, respectively) (Fig. 2). Feed and the interaction feed×holding conditions showed no effect on S in both species ($F = 0.965$; d.f. 2,35; $p = 0.392$ and $F = 1.737$; d.f. 2,35; $p = 0.193$ for *P. leptodactylus*, and $F = 2.743$; d.f. 2,35; $p =$

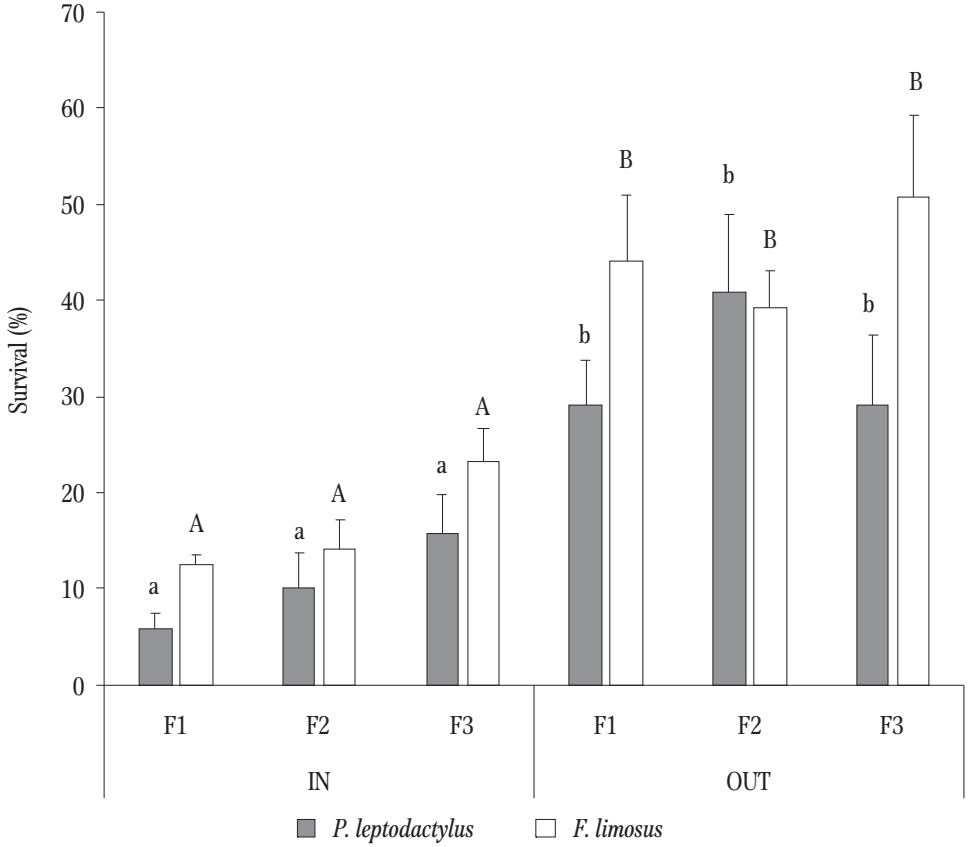

Figure 2. Effect of holding conditions IN (inside) and OUT (outside) and feed type F1 (sinking chips), F2 (flakes), F3 (floating sticks) on Survival in juvenile *Pontastacus leptodactylus* and *Faxonius limosus*. Values are mean ± SE, significant differences in response to the holding conditions are marked with superscripts, lowercase for *P. leptodactylus* and uppercase for *F. limosus*.

0.081 and F = 0.465; d.f. 2,35; p = 0.633 for *F. limosus*). The holding conditions had a significant effect on *P. leptodactylus* Final TL (F = 25.771; d.f. 1,96; p < 0.001) and TL Gain (F = 25.771; d.f. 1,96; p < 0.001), with improved growth in the IN holding conditions (Fig. 3). There was a significant interaction for feed×holding conditions for *P. leptodactylus* Final TL (F = 4.964; d.f. 2,96; p = 0.009) and TL Gain (F = 4.964; d.f. 2,96; p = 0.009), but means comparison showed not significant results. Feed showed no effect on Final TL (F = 0.532; d.f. 2,96; p = 0.589) and TL Gain (F = 0.532; d.f. 2,96; p = 0.589) in *P. leptodactylus*. Feed had a significant effect on Final TL (F = 10.521; d.f. 2,115; p < 0.001) and TL Gain (F = 10.521; d.f. 2,115; p < 0.001) in *F. limosus*, with the highest results for F3 and F2 (Fig. 3). There was no effect found for the holding conditions and the interaction feed×holding conditions in *F. limosus* Final TL (F = 0.933; d.f. 1,115; p = 0.336 and F = 3.071; d.f. 2,115; p = 0.050) and TL Gain (F = 0.933; d.f. 1,115; p = 0.336 and F = 3.071; d.f. 2,115; p = 0.050).

Discussion

A significant correlation was found between holding conditions and survival for juveniles of two competing crayfish species, *P. leptodactylus* and *F. limosus*. Our study demonstrates that the OUT holding conditions, utilising water with natural plankton was essential in survival of both species. This result corresponds with findings of Sáez-Royuela et al. (2007), that juvenile *P. leniusculus* gained highest survival when supplied with live *Artemia* nauplii. Similarly, in juvenile *C. cainii* and *P. clarkii*, highest survival was obtained in crayfish supplied with live plankton (Brown et al. 1992, Tulsankar et al. 2021b,

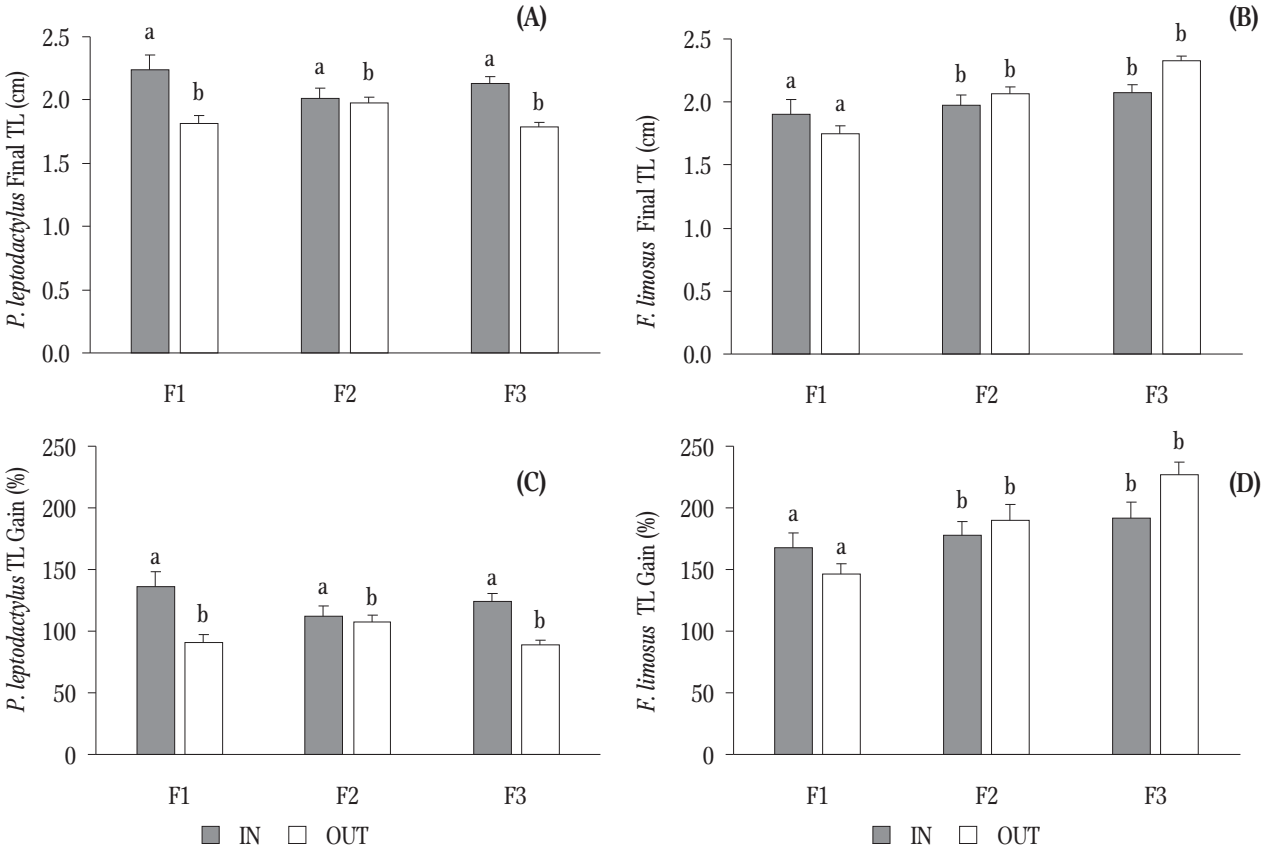

Figure 3. Effect of holding conditions; IN (inside) and OUT (outside) and feed type; F1 (sinking chips), F2 (flakes), F3 (floating sticks) on growth Final total length (Final TL) and total length Gain (TL Gain) in juvenile *Pontastacus leptodactylus* (A, C) and *Faxonius limosus* (B, D). Values are mean ± SE, significant differences are marked with superscripts.

Tulsankar et al., 2022). These results indicate that live plankton is essential in juvenile crayfish survival. Tulsankar et al. (2021b) conclude that it may be the main source of nutrients for juveniles. Live plankton spreads quickly throughout the water in tanks and becomes available to all crayfish. This mitigates seizing the whole feed supply by dominant specimens, when delivered to the small area of the tank floor (Austin et al. 1997). In consequence crayfish survival and growth relies more on feed composition and is less dependent on behaviour and hierarchy. Crayfish feeding behaviour at the juvenile life stage includes active searching and ingesting zooplankton. This behaviour decreases concurrently with growth (Meakin et al., 2008). The increase of crayfish body weight requires increasing the nutrient supply, which leads to shift to larger prey concurrently with growth. This conclusion corresponds with observations of *Cherax tenuimanus* consumption of live plankton. In early juveniles, plankton constitutes a significantly higher percentage of body weight in comparison to larger juveniles. At the same time, capture efficiency of zooplankton for both remains similar (Meakin et al., 2009). In context of growth, our study showed conflicting results for the presence of plankton in OUT holding conditions, in comparison with no plankton in IN holding conditions for both species. *Pontastacus leptodactylus* showed significantly slower growth in the OUT holding conditions and no effect was observed for *F. limosus*. This indicates that content of plankton contributed to survival in both species, which may be the result of more efficient moulting /less failed moults. At the juvenile life stage, crayfish moult frequently, and mortalities

caused by moult failure are common. This observation may indicate the importance of plankton inclusion in successful moulting of juvenile crayfish, but without observable effect on growth at this stage for *F. limosus*. Further research is required to investigate the role of feeding on live plankton in moulting physiology.

For *P. leptodactylus*, slower growth, in terms of TL in the OUT holding conditions may have led to lower cannibalism. In decapods, this behaviour mainly occurs during or shortly after the moult of cannibalised specimen (Buřič et al., 2021; Kropielnicka-Kruk et al., 2022). In decreased growth, moulting is less frequent, leading to less opportunities for cannibalism.

The three feed types tested (F1, F2 and F3), showed low pronounced results, with no effect in *P. leptodactylus* and improved growth in *F. limosus* for F2 and F3. The tested feeds were similar in composition and different in physical characteristics. F1 sank quickly, F2 and F3 floated and sank slowly. This provided extended visual exposure of F2 and F3 and a more uniform distribution of attractants in the water. Attractants leak quickly from feeds and provide short window of attraction to crayfish (Kropielnicka-Kruk et al., 2022). The improved growth of *F. limosus* fed with F2 and F3, may indicate their quick response and capability to utilise available feed. This may be one of aspects, making *F. limosus* an efficient invader that supersedes native species, including *P. leptodactylus* (Dick et al., 2017; Grimm et al., 2020).

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