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Shrimp waste meal (*Pandalus borealis*) as an alternative ingredient in diets for juvenile European lobster (*Homarus gammarus*, L.)

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ABSTRACT

The use of sustainable ingredients in the formulation of aquafeeds remains ever important. The European lobster (*Homarus gammarus*) is an emerging species for aquaculture, the success of which relies on knowledge of its nutritional requirements and the selection of dietary ingredients that facilitate viable production and future commercialization. In this study, we investigated the feasibility of using shrimp waste meal (SWM) as an ingredient in formulated diets for juvenile lobsters (stages VII – VIII, ~ 164 mg) during an 8-week feeding experiment. The experimental diets were isoproteic (~ 54 % crude protein) and isolipidic (~ 11 % crude fat), in which either 7 %, 14 %, 21 %, or 28 % of the dietary protein was supplied by SWM. Experimental diets were benchmarked against a control diet without SWM (SWM 0 %). Diet performance was evaluated based on survival, body mass gain, carapace length increment, and moulting cycle duration. Nitrogen metabolism was evaluated from the nitrogen excretion rates of individual lobsters, determined before and following the ingestion of a single meal. The exoskeleton colouration of individual lobsters was assessed using digital colour analysis at the end of the growth trial. The results indicate that the inclusion of SWM at 28 % protein significantly enhanced survival and had no effect on growth performance, postprandial nitrogen metabolism, and exoskeleton colouration. Therefore, we recommend the use of SWM, at least up to 28 % of dietary protein, as a potential alternative ingredient in the formulation of future commercial feeds for juvenile *H. gammarus*.

1. Introduction

A global demand exceeding supply and high market prices for European lobster *Homarus gammarus*, L. makes this species an excellent candidate for aquaculture. At present, the farming of *H. gammarus* operates at a modest scale within two complementary segments: as a fishery remediation measure to enhance wild stocks by releasing hatchery-reared juveniles into natural habitats, and as an emerging sector of commercial on-growing for consumption (Hinchcliffe et al., 2021). Developments have been made to improve land-based farming by using advanced robotic systems in RAS (Drengstig and Bergheim, 2013) and at sea by employing sea-based container culture systems (Daniels et al., 2015; Halswell et al., 2018). However, the farming of *H. gammarus* has yet to overcome several major challenges. In particular, slow growth rates and high mortality associated with cannibalistic behaviour (Powell et al., 2017; Scolding et al., 2012) lead to high production costs (Drengstig and Bergheim, 2013). The replacement of fresh or frozen diets, currently being used in hatcheries, with formulated feeds specifically designed for *H. gammarus* would improve the economic viability

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of land-based lobster farming by reducing the feeding costs (Powell et al., 2017). Further economic benefits could be gained from the use of sustainable ingredients (Cottrell et al., 2020; Naylor et al., 2021), such as industrial by-products or waste streams, in the formulated feeds (Hinchcliffe et al., 2020).

Although the inclusion of fishmeal in aquafeeds has been decreasing over the last two decades, it remains the main source of protein in formulated feeds for marine crustacean species (Turchini et al., 2019). Fisheries and aquaculture by-products are increasingly being considered a suitable option to reduce the use of fishmeal in aquafeeds (Stevens et al., 2018). This would provide additional economic advantages through the valorisation of waste products and contribute to the sustainable production of aquatic species (Hua et al., 2019).

The Northern shrimp, *Pandalus borealis*, is the most commercially important Caridea species in the North Atlantic, and accounts for 10 % of the global shrimp production (Paterson, 2003). Global landings of *P. borealis* amounted to ~295.000 tonnes annually between 2010 and 2016 (FAO, 2021). The processing of *P. borealis* leads to large amounts of waste in the form of heads, appendages, and exoskeletons, which accounts for at least 50 % of total production (Dave et al., 2020). Researchers have been focused on the development of value-added products based on bioactive components (e.g. protein, chitin, pigments, lipids, vitamins) from shrimp waste and their applications in pharmaceutical, environmental, food, and animal feed industries (Nirmal et al., 2020). Nevertheless, large quantities of this by-product continue to be disposed of as biological waste or discarded at the sea (Dave et al., 2020) causing environmental concern since the rate of biodegradation of the discarded waste is low (Shahidi and Synowiecki, 1991).

The idea that shrimp waste meal (SWM) represents a potential protein source in aquafeeds is based on its relativey high protein content and a well-balanced amino acid profile (Cruz-Suárez et al., 1993). A protein content of 44.5 % (dry weight basis) and high levels of arginine and lysine have previously been reported for *P. borealis* processing waste (Heu et al., 2003). Arginine and lysine, together with leucine, have been identified as the main amino acids in whole-body tissue of *H. gammarus* juveniles (Mente et al., 2001), indicating a high requirement of these three amino acids for the species. Additionally, SWM is a natural source of astaxanthin, which is frequently used as a feed additive in the formulation of aquafeeds to improve the colouration of many aquatic species (Lim et al., 2018), including lobsters (Barclay et al., 2006). Previous studies on homarid lobsters have shown that individuals reared on experimental formulated diets frequently display different and deviating exoskeleton colouration compared to those fed natural diets (Floreto et al., 2001), which may interfere with survival following restocking or decrease the product value of commercial production. The colouration of homarid lobsters is a function of the carotenoid content in the epidermis, in particular astaxanthin, which at appropriate level confers a natural exoskeleton colour (Tlusty, 2005). Therefore, the inclusion of astaxanthin in formulated diets can successfully revert exoskeleton colour alterations (Crear et al., 2002). Astaxanthin also presents antioxidant characteristics and is considered beneficial to the health of aquatic species (Lim et al., 2018).

In contrast to the numerous good qualities of the SWM, it also contains high levels of chitin. While chitin is known to inhibit nutrient digestibility in fishes (Karlsen et al., 2017), the effect of chitin on the digestive performance of crustaceans is more controversial. Chitinase, a digestive enzyme required to break down chitin, has been documented in most, if not all crustacean species examined, and facilitates the digestion of prey exoskeletons, as well as the own exoskeleton following ecdysis (Ceccaldi, 1989). Although the metabolic cost of moulting cannot be precisely quantified, ecdysis is associated with large losses of nitrogen and carbon (Zoutendyk, 1988), which must be recovered from the diet. Conceivably, it may be advantageous to use chitin rich diets for crustaceans, if intermediate products in the chitin degradation pathway can be used in exoskeleton formation, rather than being synthesized

Table 1
Proximate and amino acid composition of the shrimp waste meal (SWM).

Proximate composition (g kg^{-1})		Amino acid composition (g kg^{-1} SWM)	
Moisture	28	*Essential*	
Crude protein	478	Arginine	27
Crude fat	61	Histidine	10
Ash	335	Isoleucine	18
Chitin	160	Leucine	27
		Lysine	26
		Methionine	10
		Phenylalanine	19
		Threonine	18
		Tryptophan	5
		Valine	21
		ΣEAA	181
Astaxanthin contents (mg kg^{-1})		*Nonessential*	
Esterified astaxanthin	69.0	Alanine	23
Free astaxanthin	3.6	Aspartic acid	40
		Cysteine + Cystine	5
		Glutamic acid	54
		Glycine	21
		Proline	36
		Serine	20
		Tyrosine	16
		ΣNEAA	215
		EAA/NEAA	0.84
		ΣAA	396

de novo from monosaccharides and amino acid precursors. This is supported by previous research showing a beneficial effect of dietary chitin or its derivatives on the growth performance or survival of the white leg shrimp, *Litopenaeus vannamei*; the black tiger shrimp, *Penaeus monodon*; and the shore crab, *Carcinus maenas* (Niu et al., 2013, 2011; Powell and Rowley, 2007). However, increasing levels of dietary chitin has also been reported to have no effect on growth or survival of the black tiger shrimp (Fox, 1993).

Collectively, the reported findings suggest that *P. borealis* processing waste can be used as an alternative protein source in the formulation of diets for *H. gammarus*. In this study, an experiment was conducted to determine whether shrimp meal produced from *P. borealis* processing waste (heads, appendages, and exoskeletons) can be used as an ingredient in formulated feeds to produce *H. gammarus* juveniles. The experiment examined growth performance, survival, feed efficiency, proximate composition, nitrogen metabolism, and exoskeleton colouration of early juvenile lobsters beginning at stages VII to VIII fed one of the five experimental feeds formulated to include different levels of SWM (0–28 % of total dietary protein).

2. Material and methods

2.1. Experimental diets

Five diets were formulated to include different levels of SWM (0 %, 7 %, 14 %, 21 %, and 28 % of total dietary protein) while maintaining equal lipid and protein content. The 28 % SWM inclusion level was the maximum possible to sustain a total protein content of 54 % (dry weight basis) previously reported as adequate for juvenile *H. gammarus* (Goncalves et al., 2021a). The diets were fabricated as soft semimoist diets, to explore the effect on feed intake, as extruded pellets may be too hard (personal observation, Goncalves et al., 2021a). The experimental diets (~ 40 % DM) were formulated to contain 22 % total crude protein – CP (54 % of DM) and 4.3 % total crude fat – CF (11 % of DM). Diets were formulated by combining different proportions of fishmeal (South America, CP 68.1 %, CF 8.9 %, Ash 15 %) and shrimp waste meal (SWM - heads, appendages, and exoskeletons from Northern shrimp *Pandalus borealis,* CP 47.2 %; CF 6.1 %, Ash 33.5 %, Table 1). A fixed amount of squid meal (CP 77.2 %, CF 4.4 %, Ash 8 %) was kept in all experimental diets. The other ingredients used in the formulation of the diets are presented in Table 2. Shrimp waste meal (SWM) and experimental diets were prepared at DTU Aqua facilities (Hirtshals, Denmark). A mixture of heads, appendages, and exoskeletons from the commercially caught Northern shrimp *Pandalus borealis* (Launis A/S, Skagen, Denmark) were oven-dried at 60 °C until constant weight. Dried SWM was pre-grounded to a particle size < 5 mm (Krups Speedy Pro homogenizer) and then milled to a final particle size of 120 μm using an ultra-centrifugal mill (ZM 200, Retsch GmbH, Dusseldorf, Germany). The obtained SWM was stored at − 20 °C until further usage. The dry ingredients of each diet (except carrageenan) were thoroughly mixed and heated to 85 °C. Carrageenan, krill oil, and water were mixed and heated to 85 °C to create a carrageenan emulsion. The dry ingredients were stirred with the carrageenan emulsion until a homogeneous paste was obtained. Water loss by evaporation (estimated by weight difference) was compensated by

Table 2
Ingredients used (g kg^{-1} diet) and proximate composition of the experimental diets.

	Level of SWM as % of dietary protein				
	0	7	14	21	28
Ingredients (as used/as DM, g kg^{-1})					
Fish meal	191/494	172/434	155/386	136/331	118/281
Shrimp waste meal	0/0	35/89	64/159	98/239	132/316
Squid meal	71/185	73/184	73/181	74/180	74/177
Krill oil	26/68	26/65	26/64	26/63	25/60
Maize starch	17/45	16/39	14/35	12/29	10/25
Potato starch	17/45	16/39	14/35	12/29	10/25
Wheat starch	35/91	31/79	28/70	24/59	21/49
Vitamin premix	12/31	12/30	12/30	12/29	12/29
Carrageenan	16/41	16/40	16/40	16/39	16/39
Copper supplement	0.05/0.13	0.05/0.13	0.05	0.05/0.13	0.05/0.13
Proximate composition (as fed/as DM, g kg^{-1})					
Crude protein	235/580	222/551	214/521	207/525	225/517
Corrected crude protein[a]	235/580	220/545	210/510	200/508	216/496
Crude fat	46/114	44/109	42/102	40/102	44/101
Moisture	595	597	589	606	565
Ash	54/133	60/149	67/163	72/183	88/202
NFE[b]	70/171	77/191	88/214	75/190	78/179
Gross energy[c] (KJ g^{-a})	8.1	7.8	7.8	7.3	7.9
Chitin[d]	0/0	6/14	10/25	16/40	21/49

[a] Crude protein corrected for 6.9 % nitrogen in pure chitin (Liu et al., 2012).
[b] Nitrogen-free extract (NFE) calculated as: 100 % - crude protein % - crude fat % - ash % - moisture %.
[c] Coefficients for energy concentration: 21.3 kJ, 39.5 kJ, and 17.6 kJ for protein, lipids, and carbohydrates (NFE), respectively (Cuzon and Guillaume, 1997).
[d] Estimated from chitin content in SWM (16 %) and assuming SWM is the only ingredient containing chitin.

adding additional heated water (85 °C). The fluid-viscous mixture was poured into a steel tray, evenly distributed in a thin layer, and cooled down until the mixture solidified. Finally, moist pellets with a size of 4 × 4 × 4 mm were obtained by pushing a 3D printed plastic grid (i3 MK2, Prusa, Prague, Czech Republic) into the solidified mixture. The proximate composition, carotenoid and chitin content as well as the amino acid composition of the SWM are shown in Table 1. Ingredient inclusion and proximate composition of the experimental diets are presented in Table 2. Amino acid profiles of the experimental diets are presented in Table 3.

2.2. Experimental animals

All experimental animals were hatched from eggs obtained from wild ovigerous females (body weight 500–700 g) caught in the Limfjord (North Jutland, Denmark). After hatching, lobster larvae were communally reared following the same procedure described in Goncalves et al. (2022). Upon metamorphosis to post-larvae (Rötzer and Haug, 2015), stage IV lobsters were transferred to individual compartments in a raceway system. The system consisted of 3D printed plastic cassette systems with 200 mL perforated compartments to allow water flow. The cassettes were distributed into a raceway (250 × 35 × 6.5 cm, 57 L), supplied by a seawater recirculation system. Water flow rate was kept constant at 330 L h^{-1} (19 °C ± 1 °C temperature, 34 ± 1 g L^{-1} salinity, > 90 % dissolved oxygen, < 0.1 mg L^{-1} ammonia-N). The photoperiod cycle was fixed at 8 h light: 16 h dark by fluorescent lamp. Light intensity at the water surface was 7–8 lux. Post-larvae were fed daily on thawed Antarctic krill (*Euphausia superba*, Akudim A/S, Esbjerg, Denmark) and kept under these conditions for approximately 8 weeks during which individuals developed into stage > VI.

2.3. Growth trial

At the beginning of the experiment, lobsters were individually weighed and carapace length measured. Five groups of 15 individuals (stage VII-VIII, initial weight of 164 ± 3 mg; carapace length 8.2 ± 0.1 mm, mean ± SD) were randomly allocated to the dietary treatments. Individual lobsters in each group were hand-fed one pellet (55 mg average weight) of the assigned diet each morning and allowed to feed for 4 h. Shed exoskeletons during moulting were left in the compartments for the newly moulted juveniles to consume. The presence of shed exoskeletons and mortality were recorded daily. Lobsters were individually weighed and measured every second week. Body weight (BW) was determined to the nearest 0.001 g using a PG503 Delta Range balance (Mettler Toledo, Ohio, USA). Carapace length (CL) was measured to the nearest 0.1 mm with a vernier caliper from the base of the eye socket to the posterior edge of the cephalothorax. The following formulas were used to determine the growth performance:

$$SGR \quad (\% \quad day^{-1}) = \left[ln(BW_f) - ln(BW_i)\right] \times \Delta t^{-1} \times 100\%$$

Where: SGR = specific growth rate; BW_f = final body weight; BW_i = initial body weight; Δt = duration of the growth trial (56 days).

$$iCL \quad (\%) = \left(CL_f - CL_i\right) \times CL_i^{-1} \times 100\%$$

Table 3
Amino acid profile of the experimental diets (g kg^{-1} diet).

	Level of SWM as % of dietary protein				
	0	7	14	21	28
Essential					
Arginine	12	11	11	11	12
Histidine	7	5	5	5	6
Isoleucine	9	8	8	8	9
Leucine	16	14	14	14	15
Lysine	15	14	14	14	14
Methionine	6	5	5	5	5
Phenylalanine	8	7	8	8	8
Threonine	9	8	8	9	9
Valine	10	9	9	9	10
ΣEAA	91	80	84	84	89
Nonessential					
Alanine	13	11	12	12	13
Aspartic acid	18	17	18	19	20
Cysteine + Cystine	2	2	2	2	2
Glutamic acid	27	25	26	26	28
Glycine	14	12	12	12	13
Proline	10	9	10	9	11
Serine	8	7	8	8	9
Tyrosine	8	6	7	7	7
Tryptophan	2	2	2	2	3
Hydroxyproline	3	3	0	0	0
ΣNEAA	105	94	97	97	106
EAA/NEAA	0.86	0.85	0.87	0.86	0.84
ΣAA	196	174	181	181	195

Where: iCL = increment in carapace length; CL_f = final carapace length; CL_i = initial carapace length.

At the end of the trial, juveniles were lethally anesthetized in ice-cold seawater, weighed, measured, individually photographed, rinsed in distilled water, and stored at − 80 °C until further analysis.

2.4. Analytical methods

Dry matter, ash, and crude fat contents were determined gravimetrically, crude protein was estimated using the Kjeldahl N × 6.25 method by Eurofins Steins Laboratory (Vejen, Denmark). Since shrimp waste meal contains chitin, which is partially composed of non-protein nitrogen, corrected crude protein values were calculated (Table 2). The amino acid profile of the diets and SWM and the carotenoid content of the SWM, were also analysed by Eurofins Steins Laboratory (Vejen, Denmark). The SWM chitin content was determined spectrophotometrically following the method described in Tsuji et al. (1969). Proximate composition analysis of lobster juveniles was performed for three individuals per dietary treatment. Whole body juvenile lobsters were freeze-dried and subsequently combusted for 16 h at 500 °C (NMKL 23, 1991) for dry matter and ash content determination, respectively. Protein was determined spectrophotometrically at 750 nm using a commercial Lowry-based microprotein determination kit (BIO-RAD 500–0112, Bio-Rad Laboratories, California, USA). Lipids were extracted with chloroform – methanol (2:1 by volume) according to the Folch method (Christie and Han, 2010) and the lipid content was determined gravimetrically.

2.5. Nitrogen excretion

Pre- and postprandial total ammonia nitrogen (TAN) excretion were determined for each individual lobster between the second and fourth week of the growth trial in 130 mL chambers, using 15 chambers at a time. After a 24 h fasting period, each lobster was transferred to a chamber supplied with aeration. Water conditions were maintained within the same limits as in the growth trial (19 °C ± 1 °C temperature, 34 ± 1 g L^{-1} salinity, > 90 % dissolved oxygen). Water samples of 15 mL were collected manually from individual chambers at times 0 h, 2 h, 6 h, 12 h, and 24 h for baseline screening of TAN excretion rates. After this period, lobsters were offered a pre-weighed pellet for 4 h. At the end of the meal, juveniles were transferred into chambers with clean seawater. Water samples were manually collected at times 0 h, 2 h, 6 h, 12 h, and 24 h for the determination of postprandial TAN excretion rates. Collected water samples were filtered (0.2 μm, Filtropur, Sarstedt, Numbrecht, Germany) and stored at 4 °C until analysis. TAN of collected water samples was determined in duplicate according to DS (1975). TAN excretion rate was determined by adapting the formula often used to calculate the oxygen consumption rate (Frisk et al., 2013):

$$TAN \quad (\mu g \quad h^{-1} g \quad BW^{-1}) = V_{ch} \times \Delta[TAN] \times \Delta t^{-1} \times BW^{-1}$$

Where: V_{ch} = volume of the chamber, Δ[TAN] = the change in TAN-concentration over time, Δt = time period between sample collections, BW = body weight.

Feed intake was estimated from the uneaten feed fraction after collection, filtration, and drying, employing the formula:

$$FI \quad (\% \quad BW^{-1} day^{-1}) = (dF - uF - L) \times BW^{-1} \times 100\%$$

Where: FI = feed intake, dF = distributed feed, uF = unconsumed feed, L = leaching after 4 h, BW = body weight. Leaching was estimated by placing a pre-weighed pellet in the chamber under the same conditions as in the feeding period but without animals. Leaching was estimated for 6 pellets of each diet. Nitrogen intake was calculated as 16 % of protein intake estimated from the total amino acid content in each diet (Table 3, Chibnall et al., 1943). The TAN excretion profile was analyzed following a similar approach as in Frisk et al. (2013) using curve fitting and integrals of the area under the curve (AUC) produced with GraphPad Prism version 5.0 software package. From the TAN profile, we quantified the standard TAN excretion rate (STR, μg N h^{-1} g^{-1} BW) estimated by averaging the pre-feeding TAN excretion rates; postprandial peak (μg N h^{-1} g^{-1} BW) - maximum value of TAN excretion rate curve during the postprandial course; time to peak (TTP, h) - the time from feeding to the postprandial peak; factorial postprandial scope - the peak divided by the STR; postprandial duration (h) - the time from feeding until TAN excretion rate converged with the STR; TAN excretion - the post-feeding integrated excess TAN excretion rate above STR. A graphical representation of the above-mentioned variables is provided in Fig. 2.

2.6. Exoskeleton colouration

Exoskeleton colouration was evaluated following a similar procedure described in Tlusty (2005). Briefly, each lobster was photographed with a digital camera (PowerShot G15, Canon, Tokyo, Japan). The camera was set with a focal length of 6.1 mm and pictures were taken at an exposure time of 1/30 s with an aperture of F2.8. Lobsters were prepared for the photograph by wiping excess water off the exoskeleton and placed on the same white background. Pictures were stored in a 4000 × 2664 pixel format. Each image was analysed for RGB scores using ImageJ 1.52 n software (Abramoff, 2004). The RGB scores were measured for each individual at 3 different body locations, including the telson (TEL), the first abdominal segment (FAS), and the dorsal carapace (DC) (Fig. 3). The white background was used to correct for deviation from pure white (100, 100, 100) by standardizing the exoskeleton RGB measurements by the values obtained for the white surface.

2.7. Statistical analysis

Survival data was analysed by a Kaplan-Meier procedure. Log-Rank (Mantel-Cox) test was used to determine significance ($p < 0.05$). A Chi-square table with multiple comparisons was generated to identify differences among dietary treatments. All other analysed parameters are expressed as mean ± SEM, unless otherwise specified. Before analysis, parametric assumptions of normality of residuals and homogeneity of variance were tested using the Shapiro-Wilk and Levene's test, respectively. In instances where assumptions were not met, data were square root or log transformed. Comparisons between dietary treatments were performed using one-way ANOVA followed by the Tukey post hoc test, whenever significance differences were identified. A post hoc power analysis was conducted using the G*Power 3.1 software (Erdfelder et al., 1996). The sample size of 75 and the alpha level of $p < 0.05$ were used for the statistical power analyses. The recommended effect sizes used for the assessment were as follows: small ($\rho = 0.10$), medium ($\rho = 0.30$), and large ($\rho = 0.50$). The estimated statistical power was 0.14, 0.77, and > 0.99 for the detection of small, medium, and large effect size, respectively. All statistical tests, apart from the power analysis, were performed using the IBM SPSS Statistics 25.0, and graphics were generated by GraphPad Prism version 5.0 software package.

3. Results

Results for specific growth rate (SGR), carapace length increment (iCL), cumulative weight gain, feed intake, duration of the moult cycle, and proximate composition of the juveniles are presented in Table 4.

3.1. Survival, growth, and feed utilization

The survival of juvenile lobsters varied from 47 % to 87 % and was significantly affected by dietary treatment (Fig. 1). Feeding lobsters with the SWM 28 % diet resulted in a significantly better survival than that of the SWM 7 % diet ($\chi^2 = 5.44$, $p = 0.02$). During the experimental period, lobsters grew from an initial mean weight of 164 mg (8.2 mm carapace length) to mean weights ranging from 308 to 362 mg (10.2–11.2 mm carapace length). Despite the trend for faster growth in juveniles fed the SWM 28 % compared to those fed SWM 0 % revealed by the cumulative weight gain results, no significant differences were detected among the dietary treatments. The SGR and iCL were not significantly affected by the dietary treatment: SGR varied from 1.05 to 1.25 and the iCL between 24 % and 35 %. The dietary inclusion level of SWM had a significant effect on the feed intake, which varied between 1.61 % BW^{-1} day^{-1} (SWM 14 %) and 2.86 % BW^{-1} day^{-1} (SWM 28 %). However, a post hoc Tukey test failed in identifying differences among dietary treatments. Juveniles completed on average 1.7 moulting cycles for the duration of the experiment. The intermoult period was not affected by the dietary treatment and varied between 26 and 31 days (Table 4).

Table 4
Growth performance and proximate composition of juvenile *Homarus gammarus* fed the experimental diets over an eight-week period.

	Level of SWM as % of dietary protein					
	0	7	14	21	28	One-Way ANOVA
Bioassay						
BW_i (mg)	168 ± 18 (15)	162 ± 12 (15)	164 ± 16 (15)	160 ± 12 (15)	167 ± 16 (15)	$F_{4,74} = 0.05$
CL_i (mm)	8.2 ± 0.3 (15)	8.1 ± 1.1 (15)	8.2 ± 0.2 (15)	8.2 ± 0.3 (15)	8.4 ± 0.3 (15)	$F_{4,74} = 0.24$
BW_f (mg)	342 ± 40 (9)	315 ± 26 (7)	309 ± 54 (9)	308 ± 44 (11)	362 ± 47 (13)	$F_{4,48} = 0.31$
CL_f (mm)	11.2 ± 0.5 (9)	10.7 ± 0.3 (7)	10.2 ± 0. 6 (9)	10.3 ± 0.5 (11)	10.8 ± 0.4 (13)	$F_{4,48} = 0.68$
SGR (% d^{-1})	1.14 ± 0.19 (9)	1.24 ± 0.19 (7)	1.25 ± 0.21 (9)	1.05 ± 0.14 (11)	1.25 ± 0.13 (13)	$F_{4,48} = 0.31$
iCL (% CL_i^{-1})	34.87 ± 4.74 (9)	33.64 ± 5.75 (7)	26.91 ± 3.72 (9)	24.08 ± 4.29 (11)	26.02 ± 2.86 (13)	$F_{4,48} = 1.31$
FI (% BW^{-1} day^{-1})	1.75 ± 0.30 (13)	1.91 ± 0.26 (12)	1.61 ± 0.47 (11)	2.56 ± 0.27 (13)	2.86 ± 0.37 (14)	**$F_{4,62} = 2.70$***
Moult cycle (days)	29.3 ± 1.6 (7)	26.0 ± 2.4 (8)	28.3 ± 2.0 (7)	30.7 ± 2.1 (7)	30.9 ± 1.8 (9)	$F_{4,37} = 1.03$
Cumulative weight gain (mg)						
Week 2	17.0 ± 9.3 (15)	26.4 ± 9.7 (14)	19.9 ± 5.9 (13)	25.0 ± 10.0 (14)	41.1 ± 16.1 (15)	$F_{4,70} = 0.71$
Week 4	52.2 ± 10.8 (12)	71.5 ± 8.5 (11)	74.4 ± 16.0 (10)	66.0 ± 10.2 (13)	80.6 ± 13.1 (14)	$F_{4,59} = 1.20$
Week 6	76.8 ± 20.3 (10)	106.0 ± 21.3 (7)	113.0 ± 21.7 (9)	104.2 ± 26.1 (12)	143.5 ± 37.1 (13)	$F_{4,50} = 1.00$
Week 8	158.4 ± 31.2 (9)	156.1 ± 24.7 (7)	152.0 ± 37.4 (9)	146.5 ± 32.7 (11)	186.8 ± 32.7 (13)	$F_{4,48} = 0.31$
Proximate composition (% of DM)						
Dry matter	23.9 ± 1.2 (3)	26.4 ± 2.5 (3)	28.8 ± 1.4 (3)	26.2 ± 1.3 (3)	24.4 ± 2.7 (3)	$F_{4,14} = 1.00$
Protein	29.1 ± 2.2 (3)	24.0 ± 4.1 (3)	30.6 ± 2.2 (3)	32.2 ± 1.1 (3)	31.5 ± 1.7 (3)	$F_{4,14} = 1.74$
Lipid	5.2 ± 1.0 (3)	6.2 ± 0.8 (3)	6.7 ± 1.0 (3)	7.8 ± 0.7 (3)	6.3 ± 0.2 (3)	$F_{4,14} = 1.41$
Ash	34.4 ± 0.4 (3)	31.6 ± 2.3 (3)	35.4 ± 4.9 (3)	32.9 ± 2.9 (3)	31.2 ± 1.7 (3)	$F_{4,14} = 0.40$

BWi = initial wet body weight; CLi = initial carapace length; BWf = final wet body weight; CLf = final carapace length; SGR = specific growth rate; iCL = carapace length increment; FI = dry feed intake. Values are means ± SEM. The values in parenthesis - () - represent the number of replicates of the corresponding calculated parameter. The bold font followed by symbol (*) indicates statistically significant effect of dietary treatment at $p < 0.05$ measured by one-way ANOVA. The absence of superscript letters indicates that no significance differences were detected by the Tukey post hoc test followed by one-way ANOVA.

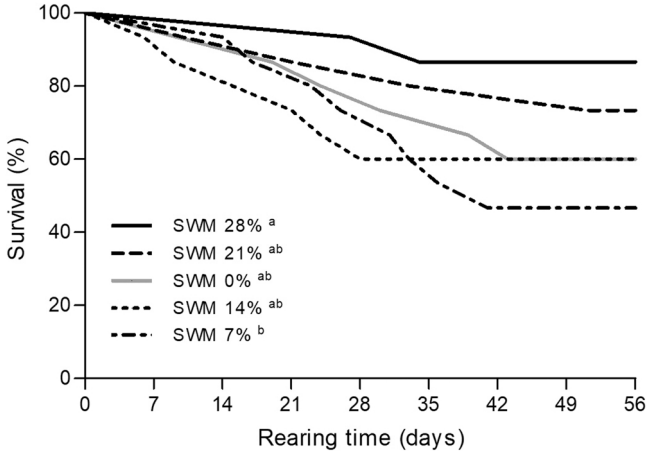

Fig. 1. Survival of *Homarus gammarus* juveniles (% of initial number, N = 15 per group) fed experimental diets containing different levels of shrimp waste meal over an eight-week period. Different letters denote statistically significant differences ($p < 0.05$) determined by Log-rank test.

3.2. Proximate composition

There was no effect of the diet on the proximate composition of the juvenile lobsters (Table 4). The protein content ranged between 24 % and 32 % and lipid content between 5 % and 8 %. The dry matter content varied from 24 % to 29 % and the ash content between 31 % and 35 %.

3.3. Nitrogen excretion

Pre- and postprandial TAN excretion values are summarized in Table 5. No statistical difference was observed in the STR between treatments. The postprandial course of TAN excretion rate was identical among the dietary groups with an increase during the first 5–6 h followed by a decline during the following 9–11 h (Fig. 2). The postprandial peak, scope, time to peak, and duration of TAN excretion rate was similar between diets. Diet significantly affected the N-intake, however, a post hoc Tukey test failed in identifying differences among dietary treatments. The N-excretion/N-intake ratio was between 12 % and 19 % and no differences were observed between diets.

3.4. Exoskeleton colouration

Two individuals representing the extremes in RGB scores – the lightest and the darkest individuals among all individuals analysed – are presented in Fig. 3 for comparison. High RGB scores translate into lighter colour while low scores translate into a darker colouration of the exoskeleton. Despite tendencies towards lower scores (darker colouration) in all three colours (Red, Green, and Blue) with increasing SWM dietary content, in particular for the FAS and DC body locations, no significant differences were observed between dietary treatments (Table. 6).

Table 5
Overview of pre- and postprandial total ammonia nitrogen excretion (TAN) of juvenile *Homarus gammarus* fed the experimental diets.

	Level of SWM as % of dietary protein					
	0	7	14	21	28	One-Way ANOVA
STR (μg h^{-1} g^{-1}BW)	11.6 ± 1.3	9.6 ± 1.4	8.4 ± 0.7	8.7 ± 0.9	8.2 ± 0.7	$F_{4,62} = 1.66$
Postprandial peak (μg h^{-1} g^{-1} BW)	44.5 ± 5.4	36.8 ± 5.0	42.5 ± 7.2	43.0 ± 4.2	43.1 ± 3.4	$F_{4,62} = 0.34$
Factorial postprandial scope	4.2 ± 0.5	4.4 ± 0.7	5.3 ± 1.0	5.3 ± 0.5	5.8 ± 0.7	$F_{4,62} = 0.99$
Time to peak (h)	4.8 ± 0.5	5.2 ± 0.5	5.7 ± 0.6	4.8 ± 0.5	4.9 ± 0.5	$F_{4,62} = 0.53$
Duration (h)	13.9 ± 1.6	13.5 ± 1.8	12.8 ± 1.3	16.2 ± 1.6	14.5 ± 1.7	$F_{4,62} = 0.64$
TAN excretion (μg g^{-1} BW)	195.3 ± 37.7	164.2 ± 29.1	191.3 ± 40.1	247.3 ± 33.8	244.9 ± 37.4	$F_{4,62} = 1.03$
N-intake (μg g^{-1} BW)	1310 ± 255	1323 ± 177	1116 ± 335	1880 ± 197	2046 ± 264	$\mathbf{F_{4,62} = 2.65}$*
N_{exc}/N_{int} (%)	12.3 ± 3.8	12.2 ± 2.0	18.5 ± 4.3	13.5 ± 1.8	11.8 ± 1.6	$F_{4,62} = 1.45$
N	13	12	11	13	14	

Visual explanation of the different variables is showed in Fig. 2. STR = Standard ammonium nitrogen excretion rate; Nint = nitrogen intake. Values are means ± SEM. N represents the number of replicates per treatment. The symbol (*) indicates statistically significant effect of dietary treatment at $p < 0.05$ measured by one-way ANOVA. The absence of superscript letters indicates that no significance differences were detected by the Tukey post hoc test followed by one-way ANOVA.

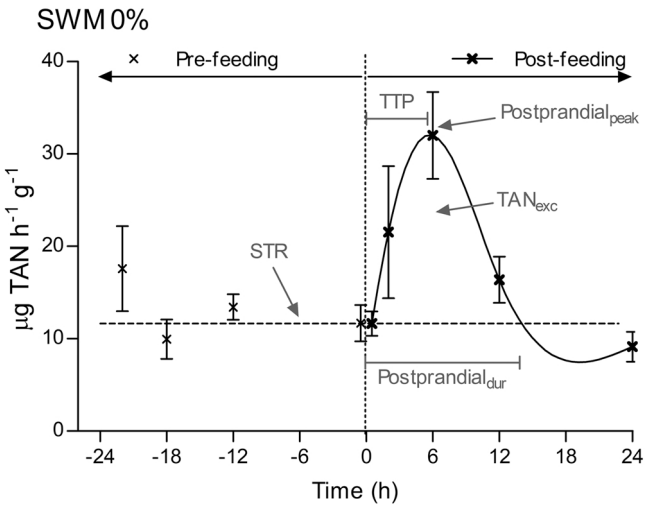

Fig. 2. Representative plot of pre- and postfeeding total ammonia nitrogen excretion rates (ug TAN h^{-1} g^{-1} BW) over time in *Homarus gammarus* juveniles fed one of the experimental diets (SWM 0 %). The juveniles were fed at 0 h (vertical dashed line). The dashed horizontal line represents the standard TAN excretion rate (STR). The postprandial TAN excretion variables mentioned in Table 5 are visually explained. Data points represented as mean ± standard error.

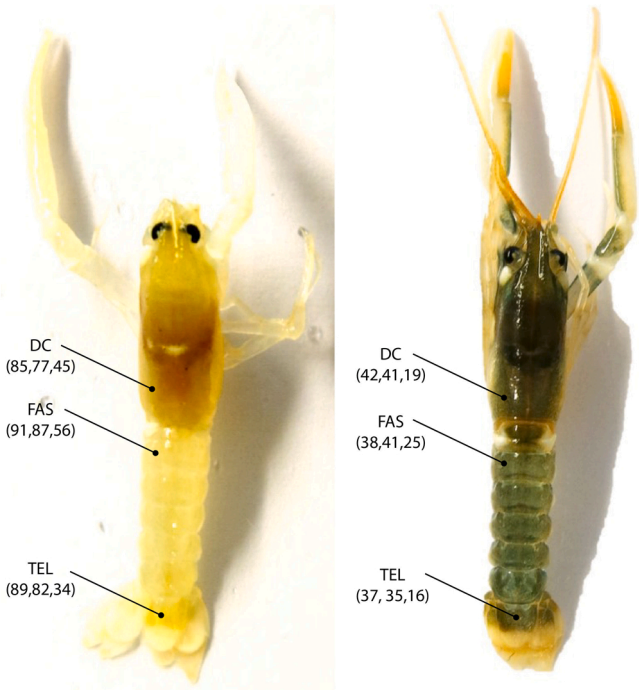

Fig. 3. Body locations of *Homarus gammarus* juveniles examined by digital colour analysis. TEL = telson; FAS = first abdominal segment; DC = dorsal carapace. Example of two specimens; light colour and dark colour juveniles and respective (R,G,B) scores.

4. Discussion

The main findings from this study were that the dietary inclusion of SWM had a significant positive effect on the survival of *H. gammarus* juveniles, and neither negatively nor positively influenced growth performance. The highest survival (87 %) was observed for the group of lobsters fed the SWM 28 %, while the lowest survival (47 %) was recorded for the SWM 7 % dietary group. This finding suggests that the chitin or astaxanthin content in the SWM is potentially nutritionally beneficial for *H. gammarus*, since the SWM was the only source of both chitin and astaxanthin in the diet. Calculating dietary chitin levels (from 0 % to 4.9 %, Table 2), this shows a positive correlation between SWM inclusion and chitin content in the experimental diets.

Table 6
RGB colour scores at three different body locations of *Homarus gammarus* juveniles fed experimental diets containing different levels of shrimp waste meal over an eight-week period.

	Level of SWM as % of dietary protein					
Body location examined	0	7	14	21	28	One-Way ANOVA
TEL						
R	79.3 ± 3.7	76.7 ± 3.5	74.4 ± 3.7	74.3 ± 3.9	75.5 ± 4.6	$F_{4,48} = 0.23$
G	74.1 ± 7.8	72.5 ± 3.3	69.0 ± 4.8	67.9 ± 3.4	71.5 ± 4.2	$F_{4,48} = 0.35$
B	40.6 ± 4.7	41.1 ± 3.3	38.9 ± 4.7	34.6 ± 3.6	40.4 ± 3.5	$F_{4,62} = 0.46$
FAS						
R	79.1 ± 2.9	77.6 ± 2.9	75.2 ± 3.0	74.9 ± 4.0	74.5 ± 4.4	$F_{4,48} = 0.26$
G	77.4 ± 2.7	76.8 ± 2.3	74.2 ± 2.2	72.5 ± 3.2	72.7 ± 3.9	$F_{4,48} = 0.48$
B	53.1 ± 2.4	54.6 ± 2.8	51.1 ± 2.4	47.9 ± 2.7	48.5 ± 2.9	$F_{4,62} = 1.04$
DC						
R	72.2 ± 2.1	71.1 ± 2.6	69.5 ± 2.8	67.9 ± 2.9	66.8 ± 3.3	$F_{4,48} = 0.58$
G	65.0 ± 2.1	63.4 ± 2.5	63.3 ± 2.0	59.3 ± 2.3	60.0 ± 2.9	$F_{4,48} = 0.95$
B	39.0 ± 2.7	36.2 ± 2.8	35.6 ± 2.6	31.1 ± 1.8	33.5 ± 2.4	$F_{4,62} = 1.46$
N	9	7	9	11	13	

Body locations are as identified in Fig. 3 (TEL = telson; FAS = first abdominal segment; DC = dorsal carapace).Values represented as mean ± standard error. N represents the number of replicates per treatment.

Dietary incorporation of chitin and its derivatives has been previously reported to increase survival in crustaceans (Niu et al., 2013; Powell and Rowley, 2007). The addition of chitin derivate – glucosamine – to a feed composed of herring meal improved *H. gammarus* postlarvae survival but not growth (Hinchcliffe et al., 2020), thus corroborating the findings in this study. Chitin is a natural polymer present in the exoskeletons of crustaceans. Chitinase (EC 3.2.1.14), NAGase (EC 3.2.1.30), chitobiase (EC 3.2.1.29), and lysozyme (EC 3.2.1.17) degrade chitin into glucosamine (Stoykov et al., 2015) that can be used for the synthesis of the new exoskeleton during the moulting process (Fox, 1993). The moulting process is a critical period for lobsters where increased mortality is particularly likely (Anger et al., 1985; Goncalves et al., 2021b). Hence, the potentially higher bioavailability of glucosamine in lobsters fed the SWM 28 % could partly explain the lower mortality observed in this dietary group. Additionally, it has been demonstrated that chitin has an immune stimulatory activity (Tsai and Hwang, 2004) and can eliminate potentially pathogenic bacteria from the gut via attachment of these microorganisms to chitin-binding proteins (Holt et al., 2019; Vaaje-Kolstad et al., 2005). It has been observed that crabs (*Carcinus maenas*) fed 5 % chitin supplemented diets had a lower number of bacteria in the hepatopancreas (Powell and Rowley, 2007). The authors concluded that enhanced survival was related to a bacteria purge from the gut caused by dietary chitin.

The increased survival observed in the SWM 28 % group may also be related to a strong antioxidant capacity of astaxanthin. The supplementation of herring meal feed with astaxanthin increased the survival and growth of *H. gammarus* postlarvae (Hinchcliffe et al., 2020). Astaxanthin is present in the exoskeleton of several crustaceans, including the shrimp *P. borealis* (Dave et al., 2020), the species used to manufacture SWM and incorporated in our experimental diets. The antioxidant capacity of astaxanthin is caused by its strong electron-donating capability enabling the protection of the cells from free oxygen radicals (Lim et al., 2018).

Despite the trend for faster growth in lobsters fed the SWM 28 % diet compared to those fed the SWM 0 %, the results showed no significant improvement in growth, neither in terms of SGR nor carapace length increment. In this study, the SGR ranged between 1.05 % day^{-1} and 1.25 % day^{-1} among dietary treatments, which was slightly lower than previously observed for juvenile *H. gammarus* (1.72 % day^{-1}) in a study using an extruded feed of similar macronutrient ratios (Goncalves et al., 2021a). One possible explanation for the difference in growth rates is the use of a younger stage and smaller initial size (stage V to VI, ~ 90 mg wet body weight) animals in the former study compared to individuals in this study (stage VII – VIII, ~ 164 mg wet body weight). The higher SGR observed by Goncalves et al. (2021a) could also be related to a higher dry mass feed intake of the juveniles fed the extruded feed (5.3 % BW^{-1} day^{-1}) compared to the semimoist diets used here (1.6–2.9 % BW^{-1} day^{-1}). The observed differences are likely related to the higher DM content of the former extruded feed (92 %) compared to the semimoist diets used here (~ 40 %). It was initially hypothesized that lobsters may be thrashing and tearing feed more easily when reared on diets with a softer consistency than the extruded feeds previously used, and that this may result in increased feed intake. However, it appears instead that lobster juveniles fed semimoist diets reduced their feed intake (on a DM basis), possibly due to induced satiation from a mechanical expansion of the gut.

The dietary inclusion of shrimp waste meal has been previously determined to improve the growth and feed efficiency of different shrimp species (*P. vannamei* and *P. monodon*) (Cruz-Suárez et al., 1993; Fox et al., 1994). In the present study, the positive effect of SWM inclusion on growth was less evident, although the comparison between the cumulative weight gain per week in the treatment SWM 28 % and SWM 0 % suggests a trend for faster growth at the higher SWM inclusion level. One possibility to explain the dissimilar results to previous studies could be the different species or source (heads, appendages, exoskeletons) and the processing methods used to obtain the shrimp waste meal ingredient. For example, Tacon (1996) reported that shrimp heads contained higher levels of protein and lipids than exoskeletons, whereas exoskeletons had higher levels of ash and crude fibre. Another possibility to explain the lack of significant growth improvement could be the restriction in energy intake caused by the semimoist diets due to the mechanical satiation mentioned in the previous paragraph. Such restrictions may have limited their ability to unfold the growth potential, which could potentially be overcomed by feeding the juveniles multiple times a day. It can not be ruled out that the duration of the study might have limited the detection of significant differences in the growth of the juvenile lobsters among the different dietary treatments.

The dietary inclusion rate of shrimp waste meal did not affect the postprandial total ammonia nitrogen excretion, suggesting a

similar capacity for protein utilization of the different protein sources (Frisk et al., 2013). That is because the amount of postprandial nitrogen excreted as ammonia depends largely on the amount of protein assimilated after feeding (Ponce-Palafox et al., 2017). The estimated ratio N-excreted/N-intake varied between 12 % and 19 % corresponding to 81–88 % N-retention, excluding eventual faecal losses. A slightly higher N-retention (92 %) was estimated for juvenile *H. gammarus* tested under the same conditions and fed an extruded feed of similar protein, lipid, and carbohydrate ratios (Goncalves et al., 2020). However, in the latter, N-retention was estimated from the excretion of total nitrogen instead of TAN, which justifies the slightly higher observed retention. A decreasing trend in the STR (pre-feeding TAN excretion rates) with increasing SWM inclusion suggests a potential for lower amino acid catabolism of metabolic origin and, consequently, a higher availability of proteins that can be used for growth (Rosas et al., 2001). However, despite the trend, no significant differences were detected among dietary treatments, which can also explain the lack of significant effect of SWM inclusion on lobster growth performance.

It was hypothesized that SWM, as a natural source of astaxanthin, would affect the exoskeleton colouration of juvenile lobsters. This has previously been demonstrated in postlarvae *H. gammarus* offered feeds containing shrimp meals or feeds with added astaxanthin, resulting in a more colourful exoskeleton (Hinchcliffe et al., 2020). However, despite the decreasing trend in RGB scores (lower scores indicating darker and more natural colour), improvements in the exoskeleton colouration of juvenile *H. gammarus* in response to SWM inclusion did not occur. It has previously been observed that the use of formulated diets with no astaxanthin supplementation to grow lobsters induces a shift towards a progressively lighter colour (Barclay et al., 2006; Crear et al., 2002; Floreto et al., 2001). Whether in commercial on-growing for consumption or as a fishery remediation measure, the loss of natural colour in hatchery-reared lobsters is undesirable. It decreases the commercial value of the final product and interferes with survival in their natural habitat by decreasing camouflage from predators (Tlusty, 2005). Therefore, it is of great interest if the supplementation of diets with additives can boost body pigmentation, and astaxanthin is the most commonly carotenoid used among several aquatic species (Lim et al., 2018). A carotenoid level of 115 mg kg^{-1} was required in formulated diets to produce juvenile lobster *Jasus edwardsii* of similar colour to wild-caught individuals (Crear et al., 2002) while a specification for juvenile *Panulirus ornatus* of 80 mg kg^{-1} total carotenoid (50 mg kg^{-1} free astaxanthin) was considered appropriate to ensure good colouration (Barclay et al., 2006). If the requirement for juvenile *H. gammarus* is within the same range, then the carotenoid content in the tested diets was clearly insufficient. The estimated total carotenoid content in the processed SWM was 73 mg kg^{-1} (of which only 3.9 mg kg^{-1} was free astaxanthin), which is considerably lower than previously reported values for *P. borealis* by-products (284 mg kg^{-1} in Dave et al., 2020). Astaxanthin has a highly unsaturated molecular structure and hence, a high sensitivity to heat, light, and oxidative conditions (Lim et al., 2018). Consequently, the exposure of the ingredient to different thermal treatments during the boiling of the shrimp or subsequently, during the drying process of the SWM, may have caused damage to, or losses of, astaxanthin.

Currently, the use of SWM in aquafeeds at an industrial scale is challenged by the rapid loss of nutritional quality of the raw material and its transport from landing areas or processing plants usually located in remote regions. This requires the shrimp by-products to be processed at sea,in facilities close to the landing areas or shrimp processing factories, and an efficient transport network. Thereby, additional social benefits could be provided through the valorisation of shrimp waste products by creating new downstream processing job opportunities in rural areas, which would contribute to the sustainability of the aquaculture industry. While the results presented here are encouraging for the future valorisation of shrimp waste as an ingredient for crustacean aquafeeds, there is still room to improve the potential of SWM, especially concerning the levels of astaxanthin, protein, and ash in the processed ingredient. Future studies should consider pre-treatment techniques so that the preservation of carotenoid levels in the SWM and diets can be maximized. The lower protein content and higher ash content in the SWM compared to squid and fishmeal limits its use as 1:1 substitution of high-quality protein sources. The application of techniques during raw material processing that allow the concentration of protein in the SWM would enable its dietary incorporation at higher levels.

5. Conclusion

The inclusion of SWM up to 28 % of dietary protein in formulated feeds for juvenile *H. gammarus* did not affect growth, the postprandial nitrogen metabolism, nor improved the exoskeleton colouration. Rather, the inclusion of SWM at the highest level (28 %) improved survival. The question remains which component in the SWM was responsible for improving survival, but the high chitin level and lower than expected astaxanthin content in the SWM point to a major role of the former, although further studies should explore this hypothesis. The high moisture content (~60 %) in our experimental feeds might have limited the growth potential of the lobster juveniles. Further investigations on the incorporation of SWM in feeds with a higher dry matter content would be relevant, although our results clearly demonstrate the potential of SWM as a protein source for *H. gammarus* juveniles.

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