Comparative survival and growth performance of European lobster *Homarus gammarus* postlarva reared on novel feeds

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Abstract

One approach to ongrow juvenile European lobster, *Homarus gammarus* is to utilize land based rearing systems, incorporating automated feeding, individual culture and provision of stable pelleted feeds, preferably using sustainable ingredients. We initiated three feeding experiments to investigate the general suitability of ingredients produced from seafood byproducts as novel feeds for *H. gammarus*, in terms of promoting survival, development and growth of post-larval lobsters from postlarvae (PL) stage IV to the first juvenile stage (stage V). The first experiment was designed to screen an array of candidate, locally produced, novel protein sources on growth performance parameters. This initial experiment revealed that PL reared on a raw (i.e. wet, unprocessed shrimp) feed used as a reference, showed superior performance to those reared on experimental feeds containing fishmeal, herring protein isolate or mussel meal; however, a novel type of shrimp meal, produced by flocculation from waste water, promoted the best PL performance of any experimental feed. A second experiment was designed to test the effect of drying method and to optimise the form of a wet shrimp reference feed used by lobster hatcheries. This showed that the performance of PL reared on experimental freeze-dried shrimp feed was not significantly different to those reared on the wet, unprocessed shrimp used as a reference feed. However, lobsters offered experimental oven-dried shrimp feed (with or without an immune supplement) resulted in significantly lower survival or growth performance. A third and final experiment was designed in an attempt to improve a candidate Herring-based protein source, by supplementing with nutrients found in shrimp. However, the results showed that PL reared on the wet reference shrimp feed still showed superior growth and survival than those reared on a herring feed alone, or supplemented with additives found in shrimp meal (either glucosamine, astaxanthin or both supplements combined). The high survival and growth, low incidence of moulting problems and high availability of waste shrimp material, suggest that non-heat-treated shrimp products are a promising feed ingredient for post-larval European lobsters.

Introduction

Cultivation of the European lobster (*Homarus gammarus*) currently operates at modest scales. Following larval metamorphosis through 3 pelagic Zoeal stages in upwelling tanks, post-larval lobsters may be ongrown in communal or separate benthic rearing systems (reviewed by Nicosia & Lavalli, 1995). The aim of farming this species could be divided into two complimentary routes: The improvement or remediation (restocking and stock enhancement) of the lobster capture fishery by releasing juvenile lobsters into the wild (Ellis et al., 2015), or the emerging sub-sector of commercial lobster farming (e.g. Drengstig & Bergheim, 2013). Long-term ongrowing of cannibalistic *Hommarus* spp. juveniles has proved challenging to realize and operate at the technical levels and scales necessary for individual rearing, threatening economic viability (Aiken & Waddy 1995). One approach may follow extensive sea-based culture, in which juvenile lobsters obtain nutrition from natural food such as plankton and fouling organisms (e.g. Daniels et al., 2015, Powell & ELCE, 2016). An alternative approach may be to improve the design of land based rearing systems by reducing costs and benefitting from economies of scale (Drengstig & Bergheim, 2013, Powell & ELCE, 2016). Alongside consistent and optimal composition and price, a physically stable dry feed (suitable for automated feeding) would also permit cheaper storage and labour costs (Cho, 1990; Fiore & Tlusty, 2005). With recent interest in expanding *H. gammarus* hatcheries (Drengstig & Bergheim, 2013), future lobster feeds could include a wide range of alternative ingredients to fishmeal (Glencross, Booth and Allan 2007), whilst the use of local raw materials (e.g. seafood industry byproducts) would also improve sustainability (Arnason et al., 2015).

Formulated feeds for juvenile *H. gammarus* are proprietary, confidential within hatcheries and have yet to enter commercial production (European Lobster Centre of Excellence, ELCE, *pers. comm*). Indeed, most contemporary juvenile lobsters, destined for release into the sea, are generally reared for several weeks in Aquahive systems, using live or sterilized copepods (e.g. Daniels et al., 2015; Shellfish Hatchery Systems Ltd, 2017). Prior to this, juvenile lobsters were ongrown in small compartments (e.g. “Orkney Cells”), and were variously fed sterilized mysids, euphausids or *Artemia*

54 *salina*, wet feed such as mussels, squid or periwinkles, and commercially available aquaculture feeds
55 (e.g. Burton, 2003; Schmalenbach, Buchholz, Franke & Saborowski, 2009). Formulated, dry pelleted
56 feeds are widely used in established crustacean sectors, such as Penaeid shrimp hatcheries and
57 ongrowing facilities (Wickins & Lee, 2002). However, these feeds are species-specific, produced with
58 a wide knowledge of nutritional requirements. Total or partial replacement of live or wet feed has been
59 proven for American lobster, *Homarus americanus* larvae and post-larval stages (Fiore & Tlusty,
60 2005; Tlusty, Fiore & Goldstein, 2005), using alternative protein sources (Floreto, Bayer & Brown,
61 2000). More recently, dry pelleted feeds have been used to successfully rear *H. gammarus* larvae
62 (Powell et al., 2017). However, it is challenging to understand the nutritional requirements via
63 observations and changes in biochemical composition, occuring during periods of nutritional and
64 environmental stress, which can change nutrient demand (Anger 1998, Torres et al., 2002).
65 Suboptimal feed can cause a variety of challenges when rearing lobsters, for example "Moult death
66 syndrome" (MDS) which causes mortality by entrapment in the exuviae. Prior studies with *Homarus*
67 spp. juveniles have shown that the incidence of MDS could be reduced by including a source of
68 phosphatidylcholine in the diet, such as lecithin (e.g. Kean et al., 1985). Supplementation of a simple
69 fish based feed with powdered crustacean exoskeleton or a chitin source has also reduced gut bacterial
70 load and increased survival in crabs and shrimp (Powell & Rowley, 2007, Niu et al., 2013). The
71 addition of astaxanthin into formulated feed has also increased growth and survival in crustaceans,
72 including lobsters (Lim, Yusoff, Shariff and Kamarudin 2017).
73
74 In the present study, we aimed to examine the growth, moulting and survival success of recently
75 metamorphosed lobster larvae, reared through postlarval (PL) stage IV to first juvenile stage V, and
76 fed novel feeds through a series of objectives which were formulated as experiments. Objective one
77 compared feeds which incorporated novel types of feed ingredients produced from commercial
78 seafood byproducts, sourced from local industry and processed by predefined methods (mussel,
79 shrimp and herring processors). Objective two was designed to optimize the form of raw shrimp,
80 which was the best performing feed from objective one. Finally, objective three was designed to
81 increase the suitability of other feed protein sources (namely, herring byproducts) by supplementing

with nutrients abundant in crustacean (shrimp) exoskeleton, such as the chitin monomer glucosamine, and the carotenoid astaxanthin.

Materials and Methods

Broodstock and larval rearing. Adult gravid *H. gammarus* broodstock were sourced and maintained as described in Powell et al., (2017). Larvae were collected and reared to postlarval (PL) stage IV as described in Powell et al., (2017) with modifications. Larvae were procured from one female per experiment to reduce variation, and were stocked sequentially into 4 cylindro-conical hoppers (70L) over 2-3 days at an initial density of 1000-5000 larvae per hopper. Larvae were fed with 1g of "B1" Otohime feed (Marubeni Nisshin Feed Company Ltd, Tokyo, Japan) every 3h (8g/day) and supplemented with *ca.* 2g wet weight, Planktonic AS feed (700-1000µm grade) three times per day. After 14 days, late stage Z3 larvae were placed into floating Aquahive trays (Shellfish Hatchery Systems Ltd, Orkney, UK), with PL (and any remaining moult) randomly but equally recruited (i.e. according to age and specific hopper origin) across 4-5 discrete Orkney Cell matrices (Shellfish Hatchery Systems Ltd, Orkney, UK) on the day of metamorphosis (n=50). Recruited PL were limited to those that possessed both chelae and exhibited no obvious deformities, and which also metamorphosed within 6 days of being moved to Aquahive trays. PL that died within 24h of recruitment, or 24h of subsequent T_0 measuring, were replaced with PL from the same brood. Recruitment across an experiment was completed within 8-9 days.

Ethics statement. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

PL experimental system and experimental design. The same flow through system was used to provide water quality and lighting as described in Powell et al. (2017). For each feed treatment, Orkney Cell matrices (5 x 10 blocks) were labelled alphanumerically and placed inside circular tanks (*ca.* 100 L volume) with an external standpipe of sufficient height to permit *ca.* 100 ml volume of water in each Orkney cell, and *ca.* 30 cm depth of water underneath the matrix. Inflowing water (19°C, 2 L/min across two inflows per tank) from a single header tank was provided equally to all tanks and

monitored every 15 min using a Sensdesk sensor and online recording system (HW group s.r.o., Czech Republic). Each circular tank was also aerated gently from the base (*ca.*1 L/min). The insides of Orkney cells were individually cleaned daily with a large pipette to remove uneaten feed, exoskeletons and dead PL, and were additionally gently flushed from above with excess water, twice per day (09:00 and 17:00). Concentrations of nitrite and ammonium were maintained below 6 and 2.5 µmol/L respectively. Every week, the undersides of the matrix were cleaned using a scrubbing brush, and tank bottoms syphoned to remove debris. PL were fed to apparent excess (up to 2 x 2mm experimental pellets per day, or *ca.* 2 x 3mm cube of defrosted shrimp *Pandalus borealis* abdomen) so that feed particles were always available, and a quantity remained uneaten upon cleaning. The duration of all experiments was designed to rear stage IV PL to juvenile stage V within a 30 day test period. After moulting to stage V, exoskeletal material was retained for 24h, to allow sufficient time for the lobster to ingest the moult. The following three experiments and associated test feeds were conducted:

Experiment one - Screening of byproduct-derived ingredients. PL were offered excess wet shrimp abdomen (R, wet shrimp reference feed), and four additional treatments: isocalorific and isonitrogenous commercial fishmeal (F), or experimental shrimp meal (S; spray-dried), herring meal (H; freeze-dried) or mussel meal (M; oven-dried) based feeds (Table 1b).

Experiment two – Effect of drying method. PL were offered shrimp abdomen (R, wet shrimp reference feed), fed ad libitum, and three additional experimental shrimp based treatments: freeze-dried (FD), oven-dried (OD) and oven-dried with a Bio-Mos® (Mannan Oligosaccharide), a prebiotic with immunostimulant properties (ODS). The latter two feeds were included to ascertain any benefits from a prebiotic, by comparing performance strictly between OD and ODS.

Experiment three – Supplement assessment. PL were offered shrimp abdomen (R, wet shrimp reference feed), and four additional experimental treatments: isocalorific and isonitrogenous freeze-dried herring meal (H), herring meal with Astaxanthin additive (HA), herring meal with Glucosamine additive (HG), and herring meal with both additives (HAG; Table 1c).

Feed production. For experimental feed treatments used in Experiments 1 and 3, isocalorific and isonitrogenous pellets were formulated and produced (Table 1). Three novel protein sources (shrimp by-product meal, herring by-product meal and mussel meal) were used as a replacement for fishmeal and added at an inclusion rate that contributed towards 70% of the total crude protein of the formulated feeds. Shrimp meal was produced on site at a shrimp boiling and peeling company by flocculation of shrimp boiling water with carrageenan according to the principle of Forghani, Bordes, Ström and Undeland (2020). Flocs were separated by flotation and subsequently spray dried (Anhydro Lab S3 spray dryer, Forghani et al. in manuscript). Herring by-product meal was produced by the pH-shift process (see Undeland, Kelleher and Hultin et al., 2002; Hinchcliffe et al., 2019) followed by freeze-drying. Mussel meal was produced from a confidential method by Musselfeed AB (Sweden), comprising an oven-drying process. For control (reference) feeds and material for experiment 2, prior observations showed that juvenile and adult lobsters survived well on an *ad lib* diet of shrimp, *Pandalus borealis,* for *ca.* 1 year. A single batch of freshly caught local shrimp (Gullmarsfjorden, Sweden) were frozen at -20°C and individual shrimp were defrosted daily prior to feeding. The cephalothorax, telson and any eggs were discarded, and small cross sections of abdomen, including both muscle and carapace, were removed. These were offered as wet (reference feed), freeze-dried or oven-dried (100°C for 24h) material, fed directly to PL for experiment 2. For experiment 1 and 3, defrosted shrimp was fed as a wet reference diet only, to allow comparison with growth data across the three experiments. For experiment 3, feeds were formulated using the herring meal, additional ingredients (Table 1) and experimental additions of supplements were then added (astaxanthin, glucosamine, both supplements and neither supplement). Levels of added astaxanthin were based on an extensive review by Lim *et al.,* (2017), in this case a high dose (350 mg/kg^{-1}) was chosen in order to observe a maximum effect since there has been no previous study on astaxanthin in a formulated diet of *H. gammarus.* Similar doses have been used to obtain significantly higher survival in the diets of crustaceans (Yamada *et al.,* 1990). Glucosamine addition was based on the previous study of Nui e*t al.,* (2013). For experiment 1 and 3, each diet was made in a single batch using standard feed ingredients (see table 1) and mixed using a kitchen mixer (Hugin Titanium, Kenwood, London, UK.), with water added dropwise to reach the desired consistency. The resulting paste was processed through a meat grinder (Nima Maskinteknik AB,

Örebro, Sweden) to produce 1.5 mm pellets which were dried (forced air oven; 40 °C, 24 h until no further change in mass) in a drying cupboard. All dry feed used in the three trials were stored in air tight containers at 4°C and used within 7 days.

PL measurements. PL were observed at least twice daily (09:00 and 17:00) to record mortalities and moulting to stage V with relation to stocking day, i.e. age in days since metamorphosis and immediate recruitment. For each treatment, this enabled calculation of survival to stage V, and the time taken to moult (intermoult duration). Alternatively, mortality was recorded. Any moulting complications were also noted upon moulting to stage V (defined as stage IV PL surviving the moulting process, but moulting was incomplete, chelae were lost or other minor deformities were observed). Carapace length (CL) was measured 24h after recruitment, and again within 48h following moult to stage V, in order to calculate moult increment (percentage increase in CL from stage IV to stage V). For CL measurement, lobsters were imaged at *ca.* x 20 magnification using a stereomicroscope (Leica Wild M8, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). Measurements of carapace length (CL) were taken along the midline from the back of the eyesocket to the posterior margin of the carapace, using Dino-Lite software (AnMo electronics Corporation, Taiwan). Lobster wet weight (WW) was also recorded 24 h after recruitment, and again at a known age toward the end of an experiment (30±5 days after metamorphosis). WW recording was advanced or delayed if individuals had moulted and were not fully calcified. Lobsters were blotted dry and weighed on a balance (Mettler Toledo XP205, Barcelona, Spain) to calculate WW increase, and thus growth rate (percentage increase in wet weight per day) and specific growth rate (see below).

Feed composition. All test ingredients were analysed to determine their nutritional profiles before incorporation into experimental diets. Biochemical analysis was conducted as described in Powell et al., (2017), with the exception of energy content which was determined through bomb calorimetry (Parr 6300; Parr Instrument Company, Moline, IL,USA) according to AOAC 1995, with values expressed as MJ kg^{-1}.

189 *Statistical analysis and parameter definitions.* Lobster performance data generated from different feed
190 treatments were analysed and compared, within discrete experiments, using GraphPad-Prism
191 (GraphPad Software Inc San Diego, USA). Lobster survival is displayed as percentage alive
192 following moult from stage IV to stage V. Similarly, moulting complications are shown as a
193 percentage of affected as a proportion of surviving stage IV lobsters. These data were analysed using
194 raw data between feed treatments using Fisher's Exact (i.e. Stage IV moulted *vs* those that did not; or
195 stage V expressing complications *vs* those unaffected). All other parameters shown are mean ±1 SEM
196 and were tested for normality and homogeneity of variances (Kolmogorov-Smirnov test; Bartlett's test
197 respectively) prior to analysis. All percentage data values were arcsine-square-root transformed prior
198 to analysis (i.e. moult increment, the percentage CL increase between stage IV and stage V; and
199 growth rate, the percentage increase in wet weight per day, between start (WT_0) and end (WT_1). In
200 addition to moult increment and growth rate, intermoult duration (number of days required to moult
201 from stage IV to stage V), longevity (number of days required to die prior to successfully moulting to
202 stage V) and SGR ((ln WT_1 – ln WT_0) / production period * 100) were compared between feed
203 treatments, using ANOVA and Tukey post-hoc test if data was parametric, or alternatively Kruskal
204 Wallace and Dunns post-hoc test if data resisted transformation and did not meet parametric
205 assumptions. Individual PL were occasionally checked to ascertain CL increase, and were removed
206 from calculations for average intermoult duration if a moult to stage V had been missed and not been
207 recorded. The incidence of this was n=0-3 per treatment. Feed analytical data is shown for reference
208 only and is not qualitatively compared.

209

210 **Results**

211 *Observations.* All feed pellets and raw shrimp feed were negatively buoyant, and sank provided water
212 surface tension was broken, and subsequently appeared physically stable following 24h immersion.
213 Stage IV PL inspected and manipulated all feeds soon after introduction of feed items to individual
214 cells, although recently metamorphosed individuals occasionally required *ca.* 48h for apparent

weaning to occur. Although manipulation was initially only a few minutes duration, individuals were often seen returning to pellets throughout the day. PL also partially ate moulted exoskeletons in all feed treatments. Feed ingestion was confirmed by the appearance of a dark spot situated in the cephalothorax, posterior to the eyes, approximating to the location of the stomach and hepatopancreas. Toward the end of an experiment and during weighing, lobsters offered feeds containing shrimp meals (S, FD, OD, ODS), and wet shrimp reference feed (R), generally appeared to have a more robust and colourful (green-blue) carapace, and also appeared more aggressive during handling (weighing) compared to most lobsters in the other feed treatments. However, HA and HAG feeds with added astaxanthin (experiment 3) also influenced lobster colour (orange–red colour).

Experiment one – Screening of byproduct-derived ingredients. H. gammarus showed significant differences in survival and moulting success between the five different feeds treatments in experiment 2 (Table 3, Figure 1A). Survival was significantly lower for lobsters offered mussel feed (M), compared to all other treatments other than fishmeal feed (F; Fisher's Exact, $P<0.001$). Mean intermoult duration was significantly shorter for lobsters offered wet shrimp reference feed (R) compared to all other treatments, other than the shrimp feed (S; Kruskal-Wallace, $P<0.001$). Stage IV PL offered wet shrimp reference feed (R) moulted to juvenile stage V more quickly than those offered any other feed (i.e., first moult occurred on day 11), and all survivors had completed moult to stage V earlier than other treatments, (i.e., all moulted by day 20; Figure 1A).

Lobster growth and development was also significantly different between feeds (Table 3). Moult increment was significantly higher for lobsters offered wet shrimp reference feed (R), compared to those offered mussel (M) or fishmeal (F) feeds (ANOVA, $P<0.05$-0.01). Growth rate and SGR was also significantly higher for lobsters offered wet shrimp reference feed than in all other treatments, other than those offered shrimp meal feed (S; Table 3, Kruskal-Wallace, $P<0.05$-0.001). Lobsters offered mussel (M) and herring (H) meal feed showed the lowest growth rate and SGR. Whilst the prevalence of moulting problems across treatments was not significantly different, they occurred in over 5% of lobsters offered herring and fish meal feed and two of mortalities in the herring feed treatment were due to MDS. In contrast, lobsters offered mussel (M), wet shrimp reference (R) and

shrimp meal (S) feed showed zero, or very few moulting problems. Results of proximate composition of all experimental feeds are shown in table 2 for comparison purposes. The reference wet shrimp feed (R) used in the present study had a moisture content of 78% compared to all dry experimental diets (*ca.* 7%) Analysis of dry matter showed that all dry experimental feeds were isonitrogenous and isocalorific, however the reference shrimp diet contained higher protein (*ca.* 68% on a dry weight basis). The total ash content of the herring meal experimental diet was lower, *ca.* 4%, compared to the other experimental dry diets (*ca.* 11%).

Experiment two - Dehydration method. Lobster survival was very high in experiment 2. The majority (over 90%) of individuals successfully moulted to stage V in both reference and all experimental feed treatments, with no apparent mortality due to MDS. There were almost zero moulting complications seen across the experiment (Table 4, Figure 1B). However, lobsters fed both the wet shrimp reference (R) and experimental feeds containing freeze-dried shrimp (FD) showed improved performance, in terms of growth and development parameters, when compared to either of the oven-dried shrimp treatments (OD, ODS), as intermoult duration was significantly shorter, whilst moult increment, growth rate and SGR were significantly higher (Table 4, Kruskal-Wallace, $P>0.001$). Lobsters offered wet shrimp reference feed (R) were not significantly different to those offered freeze-dried shrimp feed (FD) in any performance parameter. Similarly, lobsters offered either oven-dried shrimp feeds (OD, ODS) were not significantly different from each other in any parameter. Analysis of proximate composition in the diets utilized in the present experiment showed that nutritional characteristics presented little variation amongst the three experimental dried shrimp feeds,

Experiment three – Supplement assessment. Lobsters offered wet shrimp reference feed (R), and herring (H) meal feeds containing one additive only (Astaxanthin, HA, or Glucosamine, HG), showed high survival to stage V and were significantly higher than the Herring (H) only diet and Herring containing both supplements (Astaxanthin and Glucosamine combined, HAG). Apparent MDS caused mortality in 4 and 7 individuals in the H and HAG treatments, respectively. Other moulting complications were significantly higher in lobsters offered the three experimental feeds (H, HA and HAG; Fisher's Exact, $P<0.05$) compared to the shrimp reference (R). Compared to the shrimp

reference, moulting duration was significantly prolonged for lobsters offered all experimental feeds other than HA (Kruskal-Wallace, P<0.01-0.001). Lobsters reared on herring (H) meal feed showed the longest intermoult duration compared to the other treatments (Table 5, Kruskal-Wallace, P<0.001). Lobster moult increment was significantly greater for those offered raw shrimp reference feed (R) than for any of the experimental feeds containing herring meal (Table 5, ANOVA, P<0.001), however there was no difference amongst lobsters offered any experimental herring feed. Similarly, growth rate and SGR for lobsters offered wet shrimp reference diet (R) was significantly greater than experimental feeds (Kruskal Wallace, P<0.001). Slowest growth rate and SGR were observed in lobsters reared on herring meal feed (H) without any supplements. Growth rate of lobsters reared on astaxanthin supplemented feed (HA) was significantly higher than Herring feed alone (H; Kruskal Wallace, P<0.01) and the SGR of lobsters reared on supplemented feed (HA and HAG) were also significantly higher than Herring feed alone (H; Kruskal Wallace, P<0.01). Analysis of composition between the dry experimental feeds-in experiment 3 showed that lipid levels in all herring based diets containing the astaxanthin supplement (HA, HAG) were elevated to *ca.*14% compared to herring based feeds which did not contain astaxanthin which had a lipid profile of *ca.* 11%.

Discussion

The present study details satisfactory performance of stage IV PL reared on shrimp feeds, using the described experimental set-up. Despite differences in species, temperature, feed and ration, similar survival and growth parameters were achieved in comparison with related studies rearing juvenile *H. americanus* (e.g. Fiore & Tlusty, 2005).

Experiment one

The results of experiment one suggest that a shrimp meal-based feed promoted an improved growth rate compared to feeds containing mussel meal, herring meal and standard fishmeal, and improved survival compared to fishmeal and mussel meal based feeds. Experimental feeds that included a source of crustaceans or crustacean meal have also tended to improve performance in juvenile *H. americanus* reared on increasing proportions of *Artemia* (Tlusty, Fiore & Goldstein, 2005) krill meal (Floreto,

295 Brown & Bayer, 2001) and for adult animals, crab waste (Skonberg et al., 2001). Tlusty, Fiore &
296 Goldstein (2005) suggested that poorer performing lobster feeds may be lacking in essential nutrients,
297 compared to *Artemia* controls. Indeed, Floreto, Brown & Bayer (2001) correlated better performing
298 feeds containing krill with higher proportions of carotenoids, n-3 PUFA fatty acids and arginine
299 following carcass analysis. Nevertheless, Floreto et al., (2000) successfully reared *H. americanus* on
300 50% soybean meal dry diets without crustacean raw ingredient inclusion; however, no crustacean
301 based diet was used as a reference. In the present study all experimental feeds contained satisfactory
302 arginine levels, but were lower compared to the reference shrimp diets (Table 2). Barrento et al.
303 (2009), investigated the tissue of wild European lobster and found that arginine composition was 0.5-
304 2%, wet weight. For fatty acids, a significant PUFA source was provided by assuring similar levels of
305 fish oil inclusion in all diets to avoid potential deficiency.

306 Phospholipids, such as phosphatidylcholines, with feed incorporating crab extract have been observed
307 to improve survival and growth in *H. americanus* (Kean et al., 1985). An increased phospholipid
308 content in the shrimp diets, compared to the other experimental sources, may be a reason that MDS
309 was rarely observed (Coutteau et al., 1997). Overall, the shrimp meal-based feed promoted an
310 improved growth rate compared to other protein sources; however, we believe care must be taken
311 when utilizing a raw crustacean diet. It is possible that storage and transport conditions can degrade
312 essential phospholipids (Sasaki and Capuzzo 1984; Fiore and Tlusty 2005). For example, Wickens et
313 al., (1995) observed that *H. gammarus* larvae offered frozen mysids had a higher rate of moulting
314 problems compared to those offered a similar diet supplemented with live *Artemia*. The low ash
315 content displayed by the experimental diet based on herring meal provided an interesting insight into a
316 parameter that is often neglected and originates from a production step utilized in the pH-shift process
317 (Hinchcliffe et al., 2019). The pH-shift process used to produce the protein was identified as a
318 promising technique to produce high quality fishmeal from bone rich by-products by the removal of
319 ash during a separation step (Hinchcliffe et al., 2019).

320

Experiment two

Experiment two, in which all feeds contained raw or processed dried shrimp, resulted in high survival, growth, no MDS and a low incidence of moulting complications. However, intermoult duration was much shorter for lobsters offered wet reference shrimp and freeze-dried shrimp feed only, whilst growth rate and SGR were also significantly higher. The nature of processing an ingredient prior to formulation and subsequent incorporation into a commercial feed often has important consequences (Glencross et al., 2007). For instance, differences in the digestibility of nutrients were observed with increasing heat exposure in canola meal, which caused lower digestibility (Glencross, Hawkins and Curnow 2004). It is well known that protein damage can be sustained during ingredient processing when an intensive heat treatment is applied, e.g. via Maillard reactions, cross linking and polymerization. This in turn can lower digestibility and affect feed pellet palatability (Moskness, Rosenlund and Lie 1995). Previous research has also demonstrated that cuttlefish *Sepia officinalis* offered frozen or freeze-dried grass shrimp (*Palaemonetes varians*) grew faster than those fed oven-dried or boiled shrimp (Domingues, Marquez, Lopez & Rosas, 2009). The authors suggested that the latter preparation techniques likely impacted upon heat labile components, and denatured protein and oxidised fatty acids. Similarly, Gabaudan, Pigott & Halver (1980) found that protein digestibility and metabolizable energy of krill and brine shrimp was reduced in oven-dried, but not freeze-dried samples. In our study, compared to freeze-dried or raw shrimp controls, lobsters offered oven-dried shrimp feeds required a longer duration to moult to stage V, and did not grow so quickly, suggesting suboptimal digestion and presumably reduced nutrient assimilation. Digestibility or feed intake studies with small crustaceans which eat tiny feed particles intermittently are technically challenging, and potentially studies with adult lobsters could be performed to determine feed digestibility and palatability. These results may also suggest that other feeds tested in our study (i.e. oven-dried mussel meal supplied as an industrial byproduct used in experiment one) could be improved if an alternative drying technique was used.

Finally, the comparison of lobsters offered oven-dried feed with or without Bio-Mos ® suggests that an immune supplement conferred no direct advantage to *H. gammarus* PL in terms of survival or

348 growth in this experiment. Since no immune parameters were measured, it is not possible to state how
349 the immune status, and hence any related lobster performance, may have changed. However recent
350 studies (Daniels et al., 2013; Middlemiss et al., 2015) have incorporated probiotics (*Bacillus* spp.) and
351 prebiotics (mannan oligosaccharides) into larval feeds (*Artemia salina*) and culture water of *H.*
352 *gammarus.* Daniels et al., (2013) found improvement in survival, growth and stress tolerance of
353 communally reared larvae in experimental treatments, which used pro- and prebiotics (including Bio-
354 Mos ®) in a green water system (mesocosm). Our study does differ, as we not only used a different
355 life stage, but also reared individually in a "clear water" system without live feeds. Hence, the
356 development of the immune system between larval and postlarval lobsters, and bacterial loading
357 between experimental systems, is likely to have differed. Thus, further studies investigating immune
358 competence or bacterial loading in PL lobsters should be performed, to investigate its potential impact
359 for long term ongrowing operations.

360

361 *Experiment three*

362 Experiment 3 was designed to investigate if a herring meal-based feed could be improved by
363 supplementing with glucosamine (chitin monomer) and/or astaxanthin at high doses, based on the
364 results of experiment 1 which showed that shrimp-based feeds promoted better lobster performance
365 compared to a basic herring meal. Crustacean diets are a source of astaxanthin (Lim et al., 2017), and
366 chitin (Niu et al., 2013) which have both been shown to enhance growth, survival and stress tolerance
367 in crustacean diets (Niu et al., 2013; Lim et al., 2017). Whilst survival of lobsters offered HA and HG
368 feeds were significantly increased compared to those fed herring alone (H), in general survival in all
369 four herring-based feeds were inferior to the wet shrimp reference diet regardless of supplementation.
370 In particular, the incidence of MDS or moulting complications at stage V were not eliminated by any
371 of the supplements. Furthermore, PL fed HAG feed, which contained both supplements, was one of
372 the poorest performing diets in terms of survival and development, indicating that a combination of
373 both supplements at the high doses may have created an antagonistic effect on lobster performance.

374 The observation that most lobsters ate their moult within 24h (and indeed assumed a different colour
375 in HA and HAG treatments) suggest that the glucosamine and astaxanthin supplements are capable of
376 being digested and metabolized. Surprisingly, the results in the current study do not support the
377 hypothesis that supplementation with glucosamine and astaxanthin improve lobster performance.

378 Previous studies have observed that *H. americanus* colour is influenced by the addition of carotenoids
379 in the diet (see review by Lim et al., 2017), although in the spiny lobster *Panulirus ornatus* such
380 supplementation did not markedly improve survival or growth (Barclay, Irvin, Williams & Smith,
381 2006). The addition of crustacean-derived chitin to a basic fish diet improved survival in adult shore
382 crabs *Carcinus maenas* (Powell & Rowley, 2007). Earlier studies demonstrated that the chitin or
383 glucosamine supplements were not as effective as whole shrimp meal (Conklin, Devers & Bordner,
384 1977) suggesting that our Herring feed with added supplements was still deficient, compared to the
385 shrimp reference diet. Niu et al., (2013) tested the addition of chitin, chitosan and glucosamine on the
386 growth and stress performance on the black tiger shrimp, *Penaeus monodon* at inclusion levels of
387 0.4% and concluded that dietary intake of chitin or chitosan could enhance growth performance and
388 resistance to stress in *P. monodon*, but not the inclusion of glucosamine. In contrast to this, the
389 substitution of glucosamine with equal amounts of chitin or chitosan did not produce the same growth
390 promoting response in shrimp (Kanazawa et al., 1970, Kitabayashi et al., 1971; Clark, Lawrence and
391 Swakon, 1993). Clearly therefore, there is further research is needed to understand digestion and
392 assimilation of exoskeletal nutrients in crustaceans.

393 *Future scope*

394 Whilst the nutritional requirements of *H. gammarus* have not yet been established, reported optimum
395 protein levels for *H. americanus* fed artificial formulated feeds have varied widely in the literature.
396 Yet, there still remains a paucity of research testing various protein levels in diets for *H. gammarus*
397 and *H. americanus*. For our study, we designed feeds with a high inclusion level of protein (60%) to
398 compare with raw shrimp reference feed, and the maximum suggested for *H. americanus* (Castell and
399 Budson, 1974) to avoid potential malnutrition in low protein commercial diets (Tlusty and Fiore

2005). Future consideration should also be paid to the interaction between phospholipid requirements and the protein source in aquaculture feeds (See review by Coutteau et al., 1997). In juvenile *H. americanus,* diets based on casein, showed high levels of mortality due to MDS, which were alleviated by supplementation with dietary soybean lecithin (Conklin et al., 1980). However, no phospholipid requirement was found for lobsters when purified crab protein rather than casein, was used as the primary protein source (Kean et al., 1985). Schmalenbach et al., (2009) reared juvenile *H. gammarus* on *Artemia salina,* Brown Crab *Cancer pagurus,* and the isopod *Idotea emarginata,* and achieved a very high survival rate. Brown Crab was considered cost effective for *H. gammarus* and *H. americanus* to utilize due to locally abundant fishery discards (Skonberg et al., 2001; Schmalenbach et al.2009). Therefore, the interaction between protein source and phospholipid levels may have important implications for formulation of practical diets. A comparative study using similar phospholipid sources added to both the commercial fishmeal, experimental diets and crustacean based diets would allow a better interpretation of the results we observed in the present study.

The high protein content of the reference shrimp compared to experimental dry diets also suggests a need for a comparative study investigating differing protein concentrations in diets based on crustaceans and fishmeal. Overall, the results of the present study suggest that the shrimp processing sector represents an undervalued resource that can be upgraded to feed ingredients, which may not require the addition of valuable supplements. Further development could likely investigate the differences between freeze-dried abdomen (i.e. a potential human grade food unsuitable for animal feed), shrimp meal created from steaming water (experiment 2) and other byproducts such as head and carapace waste resulting from a “peeled” product.

In conclusion, our study confirms the usefulness of the method of Tlusty, Fiore & Goldstein (2005) to screen an array of candidate feeds relatively quickly, studying young lobsters. However we would advocate longer term trials, greater than a few months, to proceed using the best performing feeds. This study also provides a breakdown of lobster feed composition, and a method to make satisfactory dry feed (e.g. freeze-dried feed, experiment 2) which gave identical performance to raw shrimp feed, and may assist home aquarists and the restocking subsector. Although it is challenging to understand

the ecological and nutritional needs of juvenile *H. gammarus,* the results of our study show that a diet containing a proportion of shrimp, created from local industry by-products, was the best source of a sustainable lobster feed for the emerging lobster aquaculture sector.

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Conflict of interests

None

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request

Author contributions

JH and AP wrote the manuscript, performed statistical analysis and were responsible for data collection and experimental designs. ML, AV and IU assisted in diet design and feed manufacture. KS, IU, ML and SE assisted with experimental design. All authors have read and approved the final manuscript.

References

Aiken D.E. & Waddy S.L. (1995) Chapter 8. Aquaculture. In: Biology of the lobster *Homarus americanus* (ed. by J.R. Factor, J), pp 153-176. Academic Press, Inc. San Diego, USA.

Arnason J, Larsen B.K., Björnsson B.Th., Sundell K., Hansen A-C., Lindahl O., Kalsdottir S. & Bjornsdottir R. (2015) Local fish feed ingredients for competitive and sustainable production of high-quality aquaculture feed, LIFF. Nordic Innovation Publication 2015:02. http://nordicinnovation.org/Global/_Publications/Reports/2015/NI_LIFF_report_DEF_NOV2015.pdf

AOAC, 1995. Official Methods of Analysis of AOAC International, 16th ed. Association of Official Analytical Chemists, Washington, DC

Barclay MC, Irvin SJ, Williams KC, Smith DM. 2006. Comparison of diets for the tropical spiny lobster *Panulirus ornatus*: astaxanthin-supplemented feeds and mussel flesh. *Aquaculture Nutrition* ***12,*** *117–125*

Barrento, S., Marques, A., Teixeira, B., Vaz-Pires, P., & Nunes, M. L. (2009). Nutritional quality of the edible tissues of European lobster Homarus gammarus and American lobster Homarus americanus. *Journal of agricultural and food chemistry, 57(9), 3645-3652.*

Burton, C.A. (2003) Lobster hatcheries and stocking programmes: an introductory manual. Sea Fish *Industry Authority Aquaculture Development Service. Seafish Report, SR552*

Castell, J. D., & Budson, S. D. (1974). Lobster nutrition: the effect on Homarus americanus of dietary protein levels. *Journal of the Fisheries Board of Canada, 31(8), 1363-1370.*

Cho, C.Y. (1990). Fish nutrition, feeds and feeding: With special emphasis on salmonid aquaculture. *Food Reviews International,* ***6,*** *333-357.*

Conklin, D.E., Devers K., Bordner C. (1977). Development of artificial diets for the lobster, *Homarus americanus. Journal of the World Aquaculture Society,* ***8,*** *841-852.*

478

479 Conklin, D. E. (1995). Chapter 16. Digestive physiology and nutrition. In J. R. Factor (Eds.) Biology of
480 the lobster *Homarus americanus* (pp. 441–458). San Diego, USA: Academic Press.
481

482 Coutteau, P., Geurden, I., Camara, M. R., Bergot, P., & Sorgeloos, P. (1997). Review on the dietary
483 effects of phospholipids in fish and crustacean larviculture. *Aquaculture, 155(1-4), 149-164.*
484

485 Daniels C.L., Wills B., Ruiz-Perez M., Miles E., Wilson R.W. & Boothroyd D.P. (2015). Development
486 of sea based container culture for rearing European lobster (*Homarus gammarus*) around South West
487 England. *Aquaculture,* ***448,*** *186-195.*
488

489 Domingues P.M., Marquez L., Lopez N, Rosas, C. 2009. Effects of food thermal treatment on growth,
490 absorption, and assimilation efficiency of juvenile cuttlefish, *Sepia officinalis.Aquaculture*
491 *International, 17:283.*

492 Drengstig A. & Bergheim A. (2013) Commercial land-based farming of European lobster (*Homarus*
493 *gammarus* L.) in recirculating aquaculture system (RAS) using a single cage approach. *Aquacultural*
494 *Engineering,* ***53,*** *14–18.*

495 Ellis C.D., Hodgson, D.J., Daniels, C.L., Boothroyd, D.P., Bannister, R.C.A. & Griffiths, A. G.F.
496 (2015) European lobster stocking requires comprehensive impact assessment to determine fishery
497 benefits. *ICES Journal of Marine Science,* ***72****, 35–48.*

498 Fiore, D.R. & Tlusty, M.F. (2005) Use of commercial *Artemia* replacement diets in culturing larval
499 American lobsters (*Homarus americanus*). *Aquaculture,* ***243,*** *291-303.*

500 Floreto, E.A.T., Bayer, R.C., Brown P.B. (2000). The effects of soybean-based diets, with and without
501 amino acid supplementation, on growth and biochemical composition of juvenile American lobster,
502 *Homarus americanus Aquaculture,* ***189,*** *211-235.*

503 Floreto, E.A.T., Brown P.B., Bayer, R.C. (2001). The effects of krill hydrolysate-supplemented soya-
504 bean based diets on the growth, colouration, amino and fatty acid profiles of juvenile American
505 lobster, *Homarus americanus. Aquaculture Nutrition, **7,** 33–43.*

506 Gabaudan J., Pigott JM., Halver JE. (1980). Effect of processing on protein ingredients for larval diets:
507 Biological evaluation. *Journal of the World Aquaculture Society, 11, 424–432*

508 Glencross, B. D., Booth, M., & Allan, G. L. (2007). A feed is only as good as its ingredients–a review
509 of ingredient evaluation strategies for aquaculture feeds. *Aquaculture nutrition*, *13*(1), 17-34.

510 Glencross, B., Hawkins, W., & Curnow, J. (2004). Nutritional assessment of Australian canola meals.
511 I. Evaluation of canola oil extraction method and meal processing conditions on the digestible value of
512 canola meals fed to the red seabream (Pagrus auratus, Paulin). *Aquaculture Research, 35(1), 15-24.*

513 Hinchcliffe, J., Carlsson, N. G., Jönsson, E., Sundell, K., & Undeland, I. (2019). Aquafeed ingredient
514 production from herring (Clupea harengus) by-products using pH-shift processing: Effect from by-
515 product combinations, protein solubilization-pH and centrifugation force. *Animal Feed Science and*
516 *Technology, 247, 273-284.*

517 Kanazawa, A., Shimaya, M,. Kawasaki, M., Kashiwada, K,. (1970). Nutritional Requirements of
518 Prawn-I. Feeding on Artificial Diet. *Bulletin of the Japanese Society of Scientific Fisheries Vol. 36,*
519 *No. 9,949-954.*

520 Kean, J.C., Castell, J.D., Boghen A.G., D'Abramo L.R., Conklin D.E. (1985). A re-evaluation of the
521 lecithin and cholesterol requirements of juvenile lobster (*Homarus americanus*) using crab protein-
522 based diets. *Aquaculture, **47,** 143-149.*

523 Kitabayashi, K., Kurata, H., Shudo, K., Nakamura, K., & Ishikawa, S. (1971). Studies on formula feed
524 for kuruma prawn. I. On the relationship among glucosamine, phosphorus and calcium. Bull. Tokai
525 *Reg. Fish. Res. Lab, 65, 91-107.*

526 Lim, K. C., Yusoff, F. M., Shariff, M., & Kamarudin, M. S. (2017). Astaxanthin as feed supplement in
527 aquatic animals. *Reviews in Aquaculture*.
528

529 Linnane, A., Ball, B., Mercer, J.P., Browne, R., vn der Meeren, G., Ringvold, H., Bannister, C.,
530 Mazzoni, D., Munday, B. (2001). Searching for the early benthic phase (EBP) of the European lobster:
531 a trans-European study of cobble fauna. *Hydrobiologica, **465,** 63-72.*

Middlemiss, K.L., Urbina M.A., Wilson R.W. (2016) Effects of seawater alkalinity on calcium and acid–base regulation in juvenile European lobster (Homarus gammarus) during a moult cycle. *Comparative Biochemistry and Physiology, Part A,* ***193****, 22–28.*

Millamena, O. M., Bautista-Teruel, M. N., Reyes, O. S., & Kanazawa, A. (1998). Requirements of juvenile marine shrimp, Penaeus monodon (Fabricius) for lysine and arginine. *Aquaculture, 164(1-4), 95-104.*

Mente, E., Houlihan, D.F., Smith, K. (2001). Growth, Feeding Frequency, Protein Turnover, and Amino Acid Metabolism in European Lobster *Homarus gammarus* L. *Journal of Experimental Zoology,* ***289,*** *419–432.*

Moksness, E., Rosenlund, G., & Lie, Ø. (1995). Effect of fish meal quality on growth of juvenile wolffish, Anarhichas lupus L. *Aquaculture Research, 26(2), 109-115.*

Nicosia, F & Lavalli K. (1999) Homarid Lobster Hatcheries: Their History and Role in Research, Management, and Aquaculture. *Marine Fisheries Review,* ***61****, 1-57.*

Niu, J., Lin, H. Z., Jiang, S. G., Chen, X., Wu, K. C., Liu, Y. J., ... & Tian, L. X. (2013). Comparison of effect of chitin, chitosan, chitosan oligosaccharide and N-acetyl-d-glucosamine on growth performance, antioxidant defenses and oxidative stress status of Penaeus monodon. *Aquaculture, 372, 1-8.*

Powell A, ELCE (2016). New developments in European lobster aquaculture. *Aquaculture Europe, 41(2), 5-12.*

Powell A, Hinchcliffe J, Sundell K, Carlsson N-G, Eriksson SP. (2017). Comparative survival and growth performance of European lobster larvae, *Homarus gammarus*, reared on dry feed and conspecifics. *Aquaculture Research, online (doi: 10.1111/are.13343).*

Powell A & Rowley F. 2007. The effect of dietary chitin supplementation on the survival and immune reactivity of the shore crab, *Carcinus maenas.* Comparative Biochemistry and Physiology Part A: *Molecular and Integrative Physiology,* ***147,*** *122-128.*

Schmalenbach I, Buchholz F, Franke H-D, Saborowski R. (2009).Improvement of rearing conditions for juvenile lobsters (*Homarus gammarus*) by co-culturing with juvenile isopods (*Idotea emarginata*) *Aquaculture* ***289****, 297–303.*

Skonberg DI, Donahue DW, Bayer RC, Floreto E Riley JG. (2001). Quality evaluation of American lobsters fed diets containing crab processing waste. *Journal of Aquatic Food Product Technology* ***10,*** *17–29.*

Tlusty MF, Fiore DR, Goldstein JS. (2005). Use of formulated diets as replacements for Artemia in the rearing of juvenile American lobsters (*Homarus americanus*). *Aquaculture* ***250****, 781– 795.*

Tlusty MF & Hyland C. 2005. Astaxanthin deposition in the cuticle of juvenile American lobster (*Homarus americanus*): implications for phenotypic and genotypic coloration. Marine Biology **147,** 113-119.

Wickins JF & Lee D O'C. (2002). Crustacean farming, ranching and culture. *Blackwell Science, Oxford. 434pp.*

Yamada, S., Tanaka, Y., Semeeshima, M. & Ito, Y. (1990) Pigmentation of prawn (Penaeus japonicus) with carotenoids. I. Effect of dietary astaxanthin, b-carotene and cantaxanthin on pigmentation. *Aquaculture, 87, 323–330.*

Tables

Table 1. Composition of feeds in experiments 1, 2 and 3. Figures provided to 2 decimal places. Shading shows ingredient not used in particular experiment.

Table 2. Chemical composition of feeds utilized in experiments 1, 2 and 3. Figures provided to 2 decimal places. Shading shows ingredient not examined in particular experiment. (DM= dry matter, GE= general energy content, CP= crude protein, Ca= calcium, AA= amino acids. Experiment 1, reference= wet shrimp diet, F= fishmeal based, M= mussel meal based, S=Shrimp meal based, H= herring based. Experiment 2, reference= wet shrimp diet, FD= Freeze-dried shrimp, OD= Oven-dried shrimp, ODS=Oven-dried shrimp with supplement. Experiment 3, reference= wet shrimp diet, H= herring based, HA=Herring + Astaxanthin, HG= Herring + Glucosamine, HAG= Herring+ Astaxanthin + Glucosamine).

Table 3. Experiment one. *Screening of byproduct-derived ingredients.* Comparison of survival and growth parameters for *H. gammarus* post-larvae. Data shown as basic survival percentage, or mean average ± 1 SEM. Different superscript letters denote statistically significant difference inside column values at $P<0.05$ or less. Survival measured by Fishers exact. Intermoult duration, SGR and growth rate measured by Kruskal Wallis and moult increment measured by ANOVA.

Table 4. Experiment two*. Effect of drying method.* Comparison of survival and growth parameters for *H. gammarus* post-larvae. Data shown as raw percentage survival , or mean average ± 1 SEM. Different superscript letters denote statistically significant difference inside column values at $P<0.05$ or less. Survival measured by Fishers exact. Intermoult duration, SGR and growth rate measured by Kruskal Wallis and moult increment measured by ANOVA.

Table 5. Experiment three. *Supplement assessment.* Comparison of survival and growth parameters for *H. gammarus* post-larvae. Data shown as basic survival percentage, or mean average ± 1 SEM. Different superscript letters denote statistically significant difference inside column values at $P<0.05$ or less. Numbers in brackets denote number of mortalities caused by MDS. Survival measured by Fishers exact. Intermoult duration, SGR and growth rate measured by Kruskal Wallis and moult increment measured by ANOVA.

Figures

Figure 1. *Homarus gammarus* post-larvae. Cumulative survival and intermoult duration of postlarval stage IV successfully moulting to juvenile stage V, across three feed experiments. **A.** Experiment one, R= Reference shrimp diet, F= fishmeal based, M= mussel meal based, S=Shrimp meal based, H= herring based. **B.** Experiment two, FD=Freeze-dried, Wet= Raw shrimp, OD= Oven-dried, ODS = Oven-dried with immune supplement. **C.** Experiment three, H= Herring without supplement, HA= Herring with astaxanthin, HG= Herring with glucosamine, HAG=Herring with astaxanthin and glucosamine. Graph lines end on the day of the last PL to moult or die, according to specific feed treatment.