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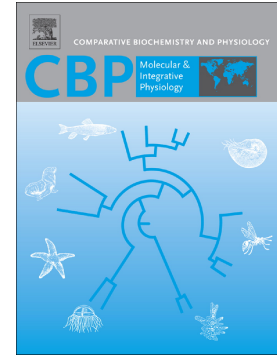
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Interactions of temperature and dietary composition on juvenile European lobster  
(*Homarus gammarus*, L.) energy metabolism and performance

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**Abstract**

Optimal rearing temperatures for European lobster *Homarus gammarus* in aquaculture differ from those prevalent in their aquatic ecosystems and acclimating juveniles to the prevailing temperatures before release may aid in the success of re-stocking programs. As the dietary nutritional composition is important for optimal performance of *H. gammarus*, in this study we aimed to investigate whether juvenile growth and energy metabolism responses to temperature variation could be modulated by the diet. Prior to the trial start, the juveniles were divided into two groups. One was maintained at 19°C and the other gradually adapted to 13°C. From this point and for a 24-day period, juveniles (~ 100 mg) within each temperature group were assigned one of two experimental diets: a carbohydrate-rich (HC) or a protein-rich (HP) extruded feed. Antarctic krill (AK) was used as a control diet within each temperature group. Feed intake, growth, glycogen, glucose, lactate, and protein concentrations of *H. gammarus* in each group were evaluated. Regardless the dietary treatment, feed intake, cephalothorax protein and glucose, and abdominal glycogen and glucose levels decreased at colder temperature. The effect of lower temperature on growth (SGR and molting rate declines) and energy metabolism (reduction on cephalothorax glycogen and protein) was more severe in HC-fed lobsters. Results showed that the impact of lower temperature on juvenile *H. gammarus* can be modulated by diet highlighting the importance of designing optimized diets not only for growth and feed efficiency but also for resilience to environmental variation.

Keywords: Extruded feeds; Carbohydrate; Protein; Glycogen; Glucose.

## 1. Introduction

The European lobster *Homarus gammarus* is a commercially important crustacean widely distributed from northern Norway to Azores and Morocco (Triantafyllidis et al., 2005). Because of its high market price, the species has been subjected to high fishing pressure causing decreases in annual landings particularly during the 1960's and 1970's (Ellis et al., 2015). As a mitigation measure, several experimental stock enhancement projects have been launched across Europe (Agnalt, 2004; Browne and Mercer, 1998; Cook, 1995; Latrouite and Lorec, 1991; Schmalenbach et al., 2011). Despite the relative success of some programs in recovering European lobster natural stocks (Agnalt, 2004), the hatchery-rearing of larvae and juvenile lobsters is not yet a sustainable practice (Beal et al., 2002). The lack of suitable artificial diets capable of reducing feeding costs and simplifying production practices remains as a major issue, together with high mortalities associated to cannibalistic behavior (Hinchcliffe et al., 2020; Powell, 2016; Powell et al., 2017).

The substitution of natural diets for artificial feeds has been investigated in both the American lobster, *Homarus americanus* and the *H. gammarus* (Floreto et al., 2000; Goncalves et al., 2021, 2020; Hinchcliffe et al., 2020; Tlusty et al., 2005). Together, the results establish that juveniles of both species have a high requirement for protein, a moderate requisite for carbohydrates, and a poorer utilization of lipids. The high requirement for protein is also reflected in the fact that, in their natural habitats, homarid lobsters mainly feed on mollusks, crustaceans and polychaetas (Ali and Wickins, 1994). Protein is the main building block for tissues in crustaceans, and therefore, fundamental for somatic growth (Castell and Budson, 1974). In instances of insufficient non-protein energy in the diet, crustaceans will use protein for energy instead of growth (Ward et al., 2003). Hence, an efficient diet must provide adequate non-protein energy to allow the more costly protein sources to be spared for growth (Nelson et al., 2006).

Unlike fishes, crustaceans can make use of high carbohydrate in their diet to meet energy requirements as it is a readily available source of energy for most species (Wang et al., 2017). An efficient digestibility and subsequent use for energy of polysaccharides (starch and dextrin) have been demonstrated for several

crustaceans including the spiny lobsters *Panulirus argus* and *Jasus edwardii* (Rodríguez-Viera et al., 2017; Simon, 2009). (Goncalves et al., 2021, 2020) observed that the increase of carbohydrate content in a formulated extruded feed of 40% protein (as fed) improved *H. gammarus* growth performance. It was also observed for the prawn *Penaeus monodon* that the dietary protein content could be lowered from 50% to 40% without significant effect on growth, if the energy level of the diet was kept constant (Bautista, 1986). Since carbohydrates are in general less expensive than animal protein ingredients (Wang et al., 2016) it is economically attractive to increase the proportion of carbohydrates in formulated feeds for lobsters.

While the mentioned studies provide relevant information for the development and optimization of formulated feed for *H. gammarus*, little information is available on the dietary effects on animal resilience to environmental change. This is particularly relevant when developing feeds for hatcheries targeting the production of juveniles for re-stocking purposes. Even if the optimal rearing conditions for dissolved oxygen, pH, and salinity lie within the prevalent conditions at sea, that is not the case for temperature (Kristiansen et al., 2004). Recommended aquaculture rearing temperatures between 18°C and 22°C (Wickins and Lee, 2002) are justified by maximum growth rates within this thermal window (Thomas et al., 2000). However, and at least for releases in the North Atlantic and North Sea region where temperatures oscillate between 11°C to 17°C from May to August (van der Meeren et al., 2000), temporarily rearing juvenile *H. gammarus* at colder temperatures before release would allow a more precise evaluation of their ability to survive in the sea, find out whether improvements are needed, and eventually increase the likelihood for successful restoration.

Beyond growth performance, changes in temperature can also alter the energy utilization in crustaceans causing changes in the metabolite levels of their most important depots – the hepatopancreas and muscle (Jimenez and Kinsey, 2015). These shifts in energy storage may reflect changes not only due to a direct effect of temperature, but also to indirect adjustments, for example, in activity level and feed intake (Jimenez and Kinsey, 2015). In fact, coupled with metabolic rate depression, the decrease in feed intake is

a common response to decreased temperature in crustaceans and, depending on its magnitude, the energy metabolism might be affected (Sacristán et al., 2017). Further, it has also been demonstrated that, in instances of feed intake restriction, the energy metabolism can be modulated by dietary composition (Vinagre and Silva, 1992).

In this context, we aimed to evaluate the ability of juvenile *H. gammarus* to cope with the effects of temperature variation while fed different formulated experimental diets. Therefore, the impact of a high protein feed or a carbohydrate-rich extruded feed were evaluated in relation to a control diet of Antarctic krill (*Euphausia superba*) on the performance and energy metabolism of juvenile *H. gammarus* held at different temperatures. To this end, we monitored growth (moultin rates, specific growth rates, and carapace length increments) and measured glycogen, glucose, lactate, and protein concentrations in juvenile *H. gammarus* held at 13°C and 19°C and fed the different diets.

## 2. Materials and Methods

### 2.1. Experimental animals

Juvenile lobsters were obtained from wild females caught in the Limfjord (North Jutland, Denmark). After hatching, pelagic larvae were communally reared in 500 mL squared tanks, supplied with fresh seawater in a flow through system (17°C temperature, 33-35 PSU salinity, > 90% dissolved oxygen, < 0.1 mg.L<sup>-1</sup> ammonia-N). From hatching, larvae were daily fed with thawed Antarctic krill, *Euphausia superba*, Akudim A/S, Denmark, a common diet used in lobster hatchery farming units (Burton, 2003). When the animals reached the postlarval stage IV, they were transferred to individual compartments in a raceway system. The system consisted of 3D printed PolyLactic Acid (PLA) bioplastic cassette systems (Prusa i3 MK2, Czech Republic) with individual compartments of 200 mL. The cassettes were placed in the raceway that was supplied by a semi-closed recirculation seawater system at a constant flow rate of 330 L h<sup>-1</sup> (19 ± 1°C temperature, 34 ± 1 PSU salinity, > 90% dissolved oxygen, < 0.1 mg.L<sup>-1</sup> ammonia-N). The photoperiod was set at 8h light: 16h dark. The early juveniles were fed daily with thawed Antarctic krill and kept under these conditions for approximately six weeks during which individuals developed into stage > V. Prior to the commencement of the experiment, the juveniles were divided in two groups of 27 individuals each. One group was maintained in the same raceway at 19 ± 1°C temperature. The other was moved to an identical raceway system and adapted to a lower temperature (13 ± 1°C) by decreasing 1°C per day during six days. The chosen acclimation period was within the thermal acclimation interval (3-14 days) suggested by (Camacho et al., 2006) for adult *H. americanus*.

### 2.2. Experimental procedure

At the beginning of the experiment, all lobsters were individually weighted and measured for carapace length. Three homogeneous groups of nine individuals per temperature (initial weight of 101 ± 37 mg per lobster; carapace length of 7 ± 1 mm, mean ± SD) were randomly allocated to the dietary treatments. The same 3D printed cassette system described above was used for the feeding trial. Hence, as each lobster



was held separately, the experimental unit in the present study was the individual lobster. Juvenile lobsters were fed Antarctic krill - AK, a carbohydrate-rich - HC (40% protein and 35% carbohydrate) extruded feed or a protein-rich - HP (50% protein and 26% carbohydrate) extruded feed. The AK was used as a control group. The extruded feeds (Sparos Lda., Portugal) were formulated to be isoenergetic and were extruded as 4 mm pellets (Goncalves et al., 2020). Details on the experimental feed ingredients and proximate composition are provided in Table 1. Each individual lobster was daily fed a pre-weighted pellet (~ 45 mg) or krill piece (~ 40 mg). Juveniles were allowed to eat for 4h, from 9:00 to 13:00. At the end of each meal, uneaten feed was removed and stored at -20°C for feed intake estimation. The daily uneaten feed fraction from groups of three lobsters (minus equal dead) was stored in the same vial until the end of the trial, allowing triplicates per dietary × temperature treatment. Each vial content was then filtered, dried and weighed. The feed intake was estimated applying the formula:

$$FI (\% BW_i^{-1} day^{-1}) = (dF - uF - L) \times BW_i^{-1} \times \Delta t^{-1} \times 100\%$$

Where: FI = feed intake, dF = distributed feed, uF = unconsumed feed, L = leaching after 4h, BW<sub>i</sub> = initial body weight, Δt = number of days during which uneaten food was collected. Details on the procedure are described in (Goncalves et al., 2021). The occurrence of new moults and deaths was daily inspected. Dead individuals were daily counted and removed. Moulded exoskeletons were left in the compartments, so the juveniles were allowed to eat them upon moulting. After 24 days kept under the above mentioned condition, each lobster was lethally cold anesthetized, measured and weighted. Individual juveniles were rinsed with Milli-Q water and stored at -80°C until further analysis. The following formulas were used to determine growth performance:

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

Where: SGR = Specific growth rate, BW<sub>f</sub> = final wet body weight, BW<sub>i</sub> = initial wet body weight, Δt = duration of the trial (24 days).

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

Where,  $iCL$  = increment in carapace length,  $CL_f$  = final carapace length,  $CL_i$  = initial carapace length.

### 2.3. Biochemical analyses

After removal of the pleopods, legs, chelipeds, antennae and antennules, each individual juvenile was divided in two different sections – cephalothorax and abdomen. The separation was performed to distinguish different target tissues, since the small size of the animals did not allow for the dissection of specific organs (i.e. hepatopancreas) nor the collection of hemolymph. Thus, it was assumed that the cephalothorax would better represent the metabolite levels in the hepatopancreas and hemolymph and the abdomen would represent the metabolites in the muscle. The frozen cephalothorax and abdomen of each lobster were minced on an ice-cold Petri dish, homogenized by ultrasonic disruption with 10 and 20 volumes of ice-cold Milli-Q water, respectively, centrifuged (10 min at 13000 g) and the supernatant used to assay tissue metabolites. Lactate levels were determined with a colorimetric kit (K-Late 06/18, Megazyme, Ireland). Tissue homogenate glucose was analyzed with colorimetric kit from Merck Millipore (CBA086, Germany). Glycogen levels were assessed by measuring glucose before and after glycogen breakdown by  $\alpha$ -amylglucosidase (Keppler and Decker, 1974). Soluble protein was determined spectrophotometrically at 595 nm using a commercial Bradford-based reagent from Sigma (B6916, St. Louis, USA).

### 2.4. Statistical analysis

Data are expressed as means  $\pm$  SEM unless otherwise specified. Before analysis, parametric assumptions of normality of residuals and homogeneity of variances were tested using the Shapiro-Wilk and Levene's test, respectively. In instances where assumptions were not met, data were square root transformed. Metabolite levels, protein concentration, SGR,  $iCL$ , and FI were subjected to a two-way ANOVA, considering temperature and diet as explanatory variables. Whenever significant differences were identified, means were compared by the Holm-Sidak *post hoc* test. Principal component analysis – PCA – was performed using the metabolite levels. Moulting occurrence was analyzed by using a Kaplan-Meier

procedure. Significance was tested using the Log-rank (Mantel-Cox) test. Whenever significance was detected, a Chi-square table with multiple comparisons was generated to identify differences among treatments. Differences were considered significant when  $p < 0.05$ . The PCA analysis was performed using R version 3.5.1 software and the factoextra version 1.07 package. All other statistical analysis were performed using the IBM SPSS Statistics 25.0 and graphics were generated by GraphPad Prism version 5.0 software package.

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### 3. Results

#### 3.1. Growth performance

At the end of the 24-day experimental period, the cumulative moulting for the HC13 (11%) group was significantly lower compared to all the other treatments ( $\chi^2 = 6.16$ ,  $p = 0.01$ ), except for the HC19 (Fig. 1). Table 2 summarizes the effect of temperature, diet, and their interaction on growth, feed intake, and survival of early juvenile *H. gammarus*. During the experimental period, juveniles grew from an initial mean weight of 101 mg (7.0 mm carapace length) to mean weights ranging from 100 mg to 138 mg (7.6 mm to 8.5 mm carapace length) among treatments. The SGR was significantly affected by the main factor diet – the SGR in lobsters fed the HC feed ( $0.1 \pm 0.2 \% d^{-1}$ ) was 10-fold lower compared with the AK-fed lobsters ( $1.0 \pm 0.2 \% d^{-1}$ ). The negative SGR ( $-0.2 \% d^{-1}$ ) observed for the HC13 group had a major contribution for the overall lower SGR in the HC-fed juveniles. No significant effects were detected for the carapace length increment. The feed intake varied between  $2 \% BW_i d^{-1}$  and  $12 \% BW_i d^{-1}$  and was significantly affected by both main factors - temperature and diet. The dry mass feed intake was higher for both extruded feeds (HP and HC) when compared to the AK diet but the reverse trend was observed when intake was estimated from wet mass (data not shown). Low temperature caused a significant decrease in feed intake in all dietary treatments. The survival of juvenile lobsters fluctuated between 56% (HC13) and 100% (HP13), with most deaths being observed during the moulting process.

#### 3.2. Metabolites

The effect of temperature, diet, and the interaction of both factors on the content of metabolites is summarized in Table 3. In general, glycogen and lactate levels were higher in the abdomen than the cephalothorax while glucose and protein contents varied within a similar range in both body sections. There was a significant main effect of the diet on the glycogen content in the cephalothorax, which was lower in the HC-fed lobsters ( $8 \pm 2 \mu\text{mol g}^{-1}$ ) than in the AK-fed group ( $40 \pm 9 \mu\text{mol g}^{-1}$ ). In the abdomen, the level of glycogen was significantly affected by temperature being higher at 19°C ( $183 \pm 44 \mu\text{mol g}^{-1}$ )

than at 13°C ( $77 \pm 23 \mu\text{mol g}^{-1}$ ). There was a main effect of temperature on glucose content in both cephalothorax and abdomen, which was higher at 19°C than at 13°C. No significant differences among treatments were observed for the lactate levels in neither the cephalothorax nor the abdomen. Both the temperature and the interaction temperature  $\times$  diet affected the protein content in the cephalothorax of early juveniles. Thus, within the HC dietary treatment, a significant reduction of 51% in protein content was detected in the cephalothorax of juveniles held at 13°C compared to those maintained at 19°C. Within the 13°C temperature group, the cephalothorax protein content was significantly lower ( $10 \pm 1 \text{ mg g}^{-1}$ ) for the HC-fed juveniles compared to those fed on the AK diet ( $20 \pm 1 \text{ mg g}^{-1}$ ).

To obtain an overall picture of the nutrient partitioning of the lobsters at the end of the trial, metabolite levels measured in the cephalothorax were subject to principal component analysis – PCA (Fig. 2). Two principal components accounted for 83.4% of the variability (PC1 60.9% and PC2 22.5%). From all parameters, glucose (0.58) showed the highest loading in the PC1 while the protein (-0.84) showed the highest loading in the PC2. The clearest separation was observed between the HC fed group at 13°C and all the other groups. While all other groups were evenly distributed in the plot, the HC13 formed a distinct cluster to the upper left area of the plot indicative of a negative correlation mainly with glucose and protein levels.

#### 4. Discussion

This study demonstrated that both temperature and diet type had a significant impact on *H. gammarus* juvenile growth performance and energy metabolism. Lobsters reared on krill (AK) performed generally better compared to those fed on the HC extruded feed while no significant differences were observed between the HP and the AK diets. Results presented here compare well with a previous study performed in juvenile *H. gammarus* reared under similar conditions as our temperature control (19°C) and using the same diets (Goncalves et al., 2020). In the cited work, the authors also observed a significant reduction on the SGR of juveniles fed the HC feed compared to those fed the AK diet, whereas the SGR of HP-fed lobsters was not different from either AK- or HC-fed juveniles. Moreover, the assessed SGR by Goncalves et al. (2020) – 0.5 % day<sup>-1</sup> for the HC feed, 0.6 % day<sup>-1</sup> for the HP feed, and 1.2 % day<sup>-1</sup> for the AK diet – agrees well with the estimated SGR in this study: 0.4 % day<sup>-1</sup>, 0.6 % day<sup>-1</sup>, and 1.2 % day<sup>-1</sup> for the HC, HP, and AK diet reared at 19°C, respectively. The lack of significant differences in cumulative moultings within dietary treatments in lobsters reared at 19°C also corroborates the results reported in Goncalves et al. (2020). However, a significant lower moulting rate (complete moults), caused by the high incidence of deaths during the moulting process, was observed for the HC13 group. The surprisingly negative SGR (-0.22 % d<sup>-1</sup>) recorded for the HC13 group is probably a consequence of this. While the observed negative SGR goes against the well-accepted hypothesis that crustaceans body weight is modulated by water content (Nguyen et al., 2014), it was previously observed that juvenile *H. americanus* lost some weight prior to moulting (Floreto et al., 2000). Our results suggest that juveniles fed the HC feed while held at 13°C could have been progressing until pre-moult stage but most of them did not succeed further in the moulting process. Also, it seems that the higher incidence of “moult death syndrome” – MDS – in the HC13 group might have been caused by suboptimal feeding. High mortalities by entrapment in the exuviae – MDS – previously reported for *H. gammarus* juveniles reared on experimental feeds have been associated to potential nutritional imbalances in those feeds (Hinchcliffe et

al., 2020). So, taken together, results suggest an adaptive response to the lower protein content in the HC extruded feed, in particularly when reared at lower temperatures.

It has been previously established that some protein-sparing effect of carbohydrates exists in crustaceans. For example, (Conklin, 1995) suggested that the protein requirement for *H. americanus* could be as low as 30%, given an appropriate protein source and sufficient non-protein energy in the diet. More specifically, Bautista (1986) demonstrated that the protein content in a diet used for *P. monodon* could be reduced from 50% to 40% without compromising growth by increasing carbohydrate, as long as the energy level was maintained. For the spiny lobster *Panulirus argus*, dietary protein could also be replaced by carbohydrates but was limited by their metabolic capacity to use up to 20% of carbohydrates in their diet (Rodríguez-Viera et al., 2017). If a similar limit applies to juvenile *H. gammarus*, than the HP diet (26% carbohydrate content) was already above their metabolic capacity. Hence, increasing carbohydrates to 35% in the HC feed at the expense of protein would not provide any advantage in terms of protein-sparing. In this study, the protein-sparing effect potential of the HC feed remains unclear. The lack of a dietary effect in the cephalothorax and abdomen protein content in lobsters held at 19°C points to a protein-sparing effect of the HC feed at this temperature. However, the more severe impact of low temperature on the growth and cephalothorax protein level in lobsters fed the HC feed suggests no protein-sparing potential at lower rearing temperatures.

In the present study, the feed intake was calculated on a dry weight basis, which explains the general lower intake recorded for the AK diet compared to both – HC and HP – feeds. That is because the AK diet had a much lower dry matter content (~ 11%) than the extruded feeds (~ 90%). Nevertheless, we observed a reduction in feed intake for all dietary groups at 13°C. The decrease in feed intake is a common response of poikilothermic animals to lower temperatures (Thomas et al., 2000; Tully et al., 2000) and it has been previously demonstrated for *H. gammarus* juveniles (Small et al., 2016). Unlike the HC-fed lobsters, juveniles fed the AK diet and the HP feed were able to sustain moulting at the same rate when held at both temperatures even if a higher moulting rate would imply a higher metabolism and,

therefore, greater energy demand to maintain homeostasis (Sacristán et al., 2017). Hence, the similar degree in decreasing intake at 13°C for lobsters fed both extruded feeds coupled with the lower moulting rate observed for lobsters fed the HC feed and held at 13°C corroborates the previously mentioned direct effect of the diet composition.

The lower glycogen level at both temperatures in the cephalothorax tissue homogenates of juveniles fed the HC feed support the hypothesis of a higher energy demand for the HC feed digestion or a limited nutrient assimilation, which may require the mobilization of more glycogen reserves. Hepatopancreatic glycogen is the primary source of energy for crustaceans (Vinagre and Silva, 1992). It can be rapidly converted into glucose to generate energy (Sacristán et al., 2017). Lipids can also be mobilized as an additional source of energy, but more frequently during prolonged periods of food deprivation (Watts et al., 2014). Conversely, the dietary treatment did not affect the glycogen levels in the abdominal tissues. Lower glycogen levels in the muscle of animals in poorer condition, such as the juveniles fed the HC diet, would be expected but that was not the case. It has been previously suggested that decapod crustaceans do not mobilize the tail muscle energetic resources in the same degree as the hepatopancreas (Sacristán et al., 2017). The authors suggested that the observed tail muscle glycogen preservation may reflect its utility as a fuel in searching for food and/or tail flip escape reaction. We observed that glycogen levels were 2 to 3 times and 6 to 11 times higher in the abdomen compared to the cephalothorax, at 13°C and 19°C respectively, supporting the above-mentioned hypothesis. Despite the lack of dietary effect on the abdominal glycogen reserves, there was a temperature effect. Glycogen reserves in the abdomen decreased 96%, 60%, and 32% at the low temperature for lobsters fed the HC feed, the HP feed and the AK diet, respectively. The decrease of glycogen level in the abdomen may be the result of lower feed intake at 13°C. Previous research has shown that a glycogen drop in adult *H. gammarus* (Albalat et al., 2019) and *H. americanus* (Stewart et al., 1972) held at lower temperatures reflected lower feed intake.

Glucose levels were reduced in both – cephalothorax and abdominal – tissues at lower temperature, following a similar trend to the glycogen levels in the abdomen. In the cephalothorax, the glucose content



decreased by 91%, 53%, and 21%, while the abdominal glucose levels were reduced by 85%, 45%, and 12% for the HC, HP, and AK groups, respectively. Results pointed towards a more pronounced glucose reduction in *H. gammarus* fed the HC feed, followed by the HP feed, and ultimately the AK diet, even if this trend was not clearly reflected in statistically significant differences. The reduction in glucose levels at lower temperatures is likely related to the decrease in feed intake observed in animals held at 13°C. Another possibility is the increased mobilization of glucose from glycogen to sustain the higher metabolism at higher temperatures (Thomas et al., 2000). Yet, the higher impact of low temperature in glycogen and glucose reserves of juveniles fed the HC in comparison to HP cannot be explained by feed intake differences since they were fairly similar for both extruded feeds. In crustaceans, the response of carbohydrate metabolism to feed intake restriction can be modulated by the diet composition (Vinagre and Silva, 1992). Glucose and glycogen levels were marginally affected by food deprivation in the crab *Chasmagnathus granulata* previously fed a protein-rich diet, while glycogen was hardly detectable and glucose was reduced 57% in crabs previously fed a high-carbohydrate diet (Oliveira et al., 2004). Similar results were obtained for the crab *Neohelice granulata* (Sarapio et al., 2017). The same hypothesis was also demonstrated for the shrimp *Litopenaeus vannamei*: when fed a low carbohydrate diet it was observed an increase in PEPCK activity, an enzyme that allows synthesis of glucose from pyruvate derived from amino acid metabolism (Rosas et al., 2002). Taken together, results suggest that, during feed restriction, gluconeogenesis and glyceroneogenesis are the main pathways involved in metabolic homeostasis in individuals previously fed a high-protein diet (Sarapio et al., 2017). On the other hand, glycogen mobilization might be more important for crustaceans adapted to carbohydrate-rich diets (Vinagre et al., 2020). Our results suggest that juvenile lobster *H. gammarus* might use a similar strategy to adapt their carbohydrate metabolism in relation to the diet received. Additionally, the lack of dietary effect on the protein levels of lobsters held at 19°C coupled with no alterations in the lactate concentration reinforces this hypothesis – the HC-fed animals were consuming their carbohydrate reserves to sustain aerobic metabolism and preserving their proteins.

Protein content in the cephalothorax was lower at 13°C suggesting a correlation between protein reserves exhaustion and feed intake. In instances of poor condition, crustaceans can consume the main protein present in the hemolymph – haemocyanin – as energy resource (Watts et al., 2014). The significant interaction effect between the diet and temperature points to a more severe effect of low temperature on the HC group. In fact, results showed a significant decline of 51% in the HC group and a non-significant reduction of 22% in HP fed lobsters held at 13°C compared to 19°C. No reduction was detected in AK fed juveniles. The depletion of protein in the cephalothorax of *H. gammarus* fed the carbohydrate rich feed reflects the lower dietary protein content in the HC feed. It might also be that *H. gammarus* suppress protein synthesis to reduce the costly cellular ATP consumption as previously shown in cichlid fish *Astronotus ocellatus* (Lewis et al., 2007). Neither the diet nor the temperature affected the protein content of the abdominal tissues of the juvenile *H. gammarus*. The abdomen in most lobster species is a muscular structure that supports swimming movement (Duffin, 2007). It has been previously observed that food restriction did not cause extensive degradation of myofibrillar protein (actin and myosin) in the tail of *H. americanus* (D'Agaro et al., 2014). A prudent utilization of muscle protein in less severe nutrient restriction state may be an adaptive strategy to avoid the usage of high costly macromolecules, which could represent an energetic saving in case of prolonged periods without food (Sánchez-Paz et al., 2007).

## 5. Conclusion

This study showed that the resiliency of juvenile *H. gammarus* to the effects of temperature variation on growth and energy metabolism can be modulated by the dietary composition. The more pronounced effect of low temperature on growth and energy metabolism of the HC fed lobsters compared to those fed on krill may be related, at least partially, with the adaptation to a new dietary type. Yet, the more severe impact of low temperature on HC fed individuals compared to HP, suggests that protein-rich feeds may offer some advantage in comparison to high-carbohydrate feeds. Despite the trend for decreased growth and a more pronounced decline in glycogen, glucose, and protein reserves at lower temperatures in lobsters fed the HP feed than those fed the AK diet, we did not find significant differences between both

diets. Further studies considering long adaptation to the extruded feeds before exposure to low temperature are required to broaden this point. There is, however, statistical evidence that animals fed the HP feed were more resilient to low temperature than HC fed animals, as suggested by the difference in moulting rates and the PCA analysis, which identifies a distinct cluster for the HC13 group indicative of a negative correlation with metabolites, in particular, protein and glucose. Although wheat has been identified as one of the best potential carbohydrate sources for the spiny lobster *Jasus edwardsii* (Simon and Jeffs, 2011), future studies should consider other sources (e.g. dextrin, cooked and pregelatinized starches, mussel glycogen) in formulated feeds for *H. gammarus* to further explore better carbohydrate assimilation and hence, improve the potential for protein-sparing. Findings from this study highlight the importance of using well-optimized diets, not only for growth and feed efficiency but also for resilience to environmental change. This is particularly relevant when developing feed products for hatchery units targeting the production of juvenile *H. gammarus* for restocking programs.

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**Table 1.** Formulation and chemical composition of experimental diets (adapted from Goncalves et al., 2020).

	AK	HC	HP
<i>Ingredients (g 100 g<sup>-1</sup> as fed)</i>			
Antarctic krill	100		
Fish meal		15.0	15.0
Squid meal		12.5	25.5
Krill meal		25.0	20.0
Wheat gluten		2.0	5.0
Wheat meal		17.3	17.3
Wheat starch		22.9	14.1
Fish oil		2.2	0.0
Soy lecithin		1.0	1.0
Vitamin & minerals premix		2.0	2.0
Astaxanthin <sup>a</sup>		0.15	0.15
<i>Proximal composition (g 100 g<sup>-1</sup> as fed)</i>			
Moisture	91.6	7.8	8.6
Ash	1.2	6.8	6.9
Crude protein	5.8	40.0	49.7
Crude fat	1.0	10.7	8.6
Carbohydrates <sup>x</sup>	0.5	34.7	26.3
Gross energy (KJ g <sup>-1</sup> ) <sup>y</sup>	1.8	19.0	18.7
Protein/Energy (g MJ <sup>-1</sup> )	32.6	21.0	26.5

<sup>a</sup> Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland.

<sup>x</sup> Estimated by difference: Carbohydrates (%) = 100 – (Crude protein + Crude fat + Ash).

<sup>y</sup> Gross energy (MJ kg<sup>-1</sup>) = (Protein content × 21.3 kJ g<sup>-1</sup> + Lipid content × 39.5 kJ g<sup>-1</sup> + Carbohydrate content × 17.6 kJ g<sup>-1</sup>) / 1000 kJ MJ<sup>-1</sup> (Cuzon and Guillaume, 1997)

**Table 2.** Growth, feeding and survival of *Homarus gammarus* after held for 24 days at two different temperatures and for each temperature fed three different diets.

Temp	Diet	CL <sub>i</sub> (mm)	BW <sub>i</sub> (mg)	N	CL <sub>f</sub> (mm)	BW <sub>f</sub> (mg)	iCL (% CL <sub>i</sub> )	SGR (% d <sup>-1</sup> )	N	FI (% BW <sub>i</sub> d <sup>-1</sup> )	N	Survival (%)
13°C	HC	6.9 ± 0.3	99.7 ± 11.0	9	7.7 ± 0.3	114.7 ± 3.5	3.6 ± 3.6	-0.22 ± 0.12	5	6.9 ± 1.4	3	55.6
	HP	6.8 ± 0.3	96.7 ± 9.5	9	7.6 ± 0.3	99.5 ± 8.5	12.5 ± 3.8	0.16 ± 0.18	9	7.0 ± 0.9	3	100
	AK	7.3 ± 0.3	104.3 ± 12.1	9	8.5 ± 0.3	138.2 ± 13.9	12.9 ± 3.2	0.78 ± 0.29	8	1.7 ± 0.1	3	88.9
19°C	HC	7.1 ± 0.4	105.4 ± 15.1	9	8.1 ± 0.4	121.9 ± 23.8	11.9 ± 5.4	0.37 ± 0.33	6	10.8 ± 0.7	3	66.7
	HP	6.9 ± 0.3	100.2 ± 15.2	9	8.0 ± 0.7	122.1 ± 26.5	13.1 ± 3.1	0.58 ± 0.33	6	11.9 ± 1.5	3	66.7
	AK	7.1 ± 0.3	99.7 ± 13.6	9	8.2 ± 0.6	131.1 ± 23.8	15.4 ± 4.3	1.18 ± 0.38	7	2.4 ± 0.3	3	77.8
Two-Way ANOVA												
Temp		F <sub>1,53</sub> = 0.00	F <sub>1,53</sub> = 0.02		F <sub>1,40</sub> = 0.22	F <sub>1,40</sub> = 0.24	F <sub>1,40</sub> = 1.32	F <sub>1,40</sub> = 3.74		F <sub>1,17</sub> = 15.57 ** (19°C > 13°C)		
Diet		F <sub>2,53</sub> = 0.69	F <sub>2,53</sub> = 0.06		F <sub>2,40</sub> = 0.85	F <sub>2,40</sub> = 0.91	F <sub>2,40</sub> = 1.27	F <sub>2,40</sub> = 4.91* (AK > 13°C)		F <sub>2,17</sub> = 35.92 *** (HC, HP > AK)		
Temp × Diet		F <sub>2,53</sub> = 0.24	F <sub>2,53</sub> = 0.09		F <sub>2,40</sub> = 0.40	F <sub>2,40</sub> = 0.34	F <sub>2,40</sub> = 0.44	F <sub>2,40</sub> = 0.05		F <sub>2,17</sub> = 2.57		

CL<sub>i</sub> = initial carapace length; CL<sub>f</sub> = final carapace length; iCL = increment in carapace length; BW<sub>i</sub> = initial body weight; BW<sub>f</sub> = final body weight; SGR = specific growth rate; FI = dry mass feed intake.

Values are means ± standard error.

\* p < 0.050; \*\* p < 0.010; \*\*\* p < 0.001

**Table 3.** Concentration of glycogen, glucose, lactate and protein in the cephalothorax and abdominal muscle tissues of *Homarus gammarus* after held for 24 days at two different temperatures and for each temperature fed three different diets.

Temp	Diet	Glycogen (glycosil units, $\mu\text{mol.g}^{-1}$ )	Glucose ( $\mu\text{mol.g}^{-1}$ )	Lactate ( $\mu\text{mol.g}^{-1}$ )	Protein ( $\text{mg.g}^{-1}$ )	N
<i>Cephalothorax</i>						
13°C	HC	3.0 ± 0.3	7.9 ± 2.1	36.3 ± 2.6	10.0 ± 1.0 <sup>b,x</sup>	5
	HP	23.8 ± 9.6	49.1 ± 18.5	63.2 ± 15.6	14.5 ± 1.7 <sup>ab</sup>	9
	AK	45.7 ± 12.3	51.8 ± 12.5	46.1 ± 7.4	19.9 ± 2.0 <sup>a</sup>	8
19°C	HC	13.9 ± 1.7	84.8 ± 19.1	52.2 ± 5.0	20.4 ± 2.4 <sup>y</sup>	5
	HP	30.5 ± 12.0	103.5 ± 25.4	81.7 ± 20.2	18.5 ± 1.2	6
	AK	30.5 ± 14.7	65.4 ± 28.1	51.1 ± 13.0	18.1 ± 2.5	5
Two-Way ANOVA						
Temp		$F_{1,37} = 0.32$	$F_{1,37} = 8.79^{**}$ (19°C > 13°C)	$F_{1,37} = 1.49$	$F_{1,37} = 7.05^*$ (19°C > 13°C)	
Diet		$F_{2,37} = 4.21^*$ (AK > HC)	$F_{2,37} = 1.18$	$F_{2,37} = 2.21$	$F_{2,37} = 1.85$	
Temp × Diet		$F_{2,37} = 1.48$	$F_{2,37} = 1.23$	$F_{2,37} = 0.17$	$F_{2,37} = 4.54^*$	
<i>Abdomen</i>						
13°C	HC	6.7 ± 3.2	9.1 ± 2.7	155.5 ± 22.8	14.2 ± 2.2	5
	HP	70.1 ± 37.1	25.9 ± 7.3	146.1 ± 42.6	12.6 ± 2.0	9
	AK	129.4 ± 42.5	31.0 ± 12.2	131.2 ± 23.5	15.7 ± 2.2	8
19°C	HC	151.8 ± 60.1	60.4 ± 18.9	204.6 ± 66.7	16.8 ± 3.5	5
	HP	202.5 ± 62.6	47.0 ± 9.0	217.3 ± 57.8	15.8 ± 1.8	6
	AK	191.0 ± 116.5	35.4 ± 10.9	143.9 ± 51.5	14.4 ± 3.8	5
Two-Way ANOVA						
Temp		$F_{1,37} = 8.23^{**}$ (19°C > 13°C)	$F_{1,37} = 10.89^{**}$ (19°C > 13°C)	$F_{1,37} = 1.90$	$F_{1,37} = 0.52$	
Diet		$F_{2,37} = 1.63$	$F_{2,37} = 0.29$	$F_{2,37} = 0.34$	$F_{2,37} = 0.13$	
Temp × Diet		$F_{2,37} = 1.56$	$F_{2,37} = 2.00$	$F_{2,37} = 0.48$	$F_{2,37} = 0.48$	

Values are means ± standard error.

\*  $p < 0.050$ ; \*\*  $p < 0.010$ ; \*\*\*  $p < 0.001$

Means in the protein column with a different superscript “a” or “b” are significantly different within the 13°C temperature group. A different superscript “x” or “y” indicates significant differences between temperatures within the HC dietary treatment.

### Figure captions:

**Figure 1.** Cumulative moults of *Homarus gammarus* (% initial numbers) fed on different diets and maintained at different temperatures over a 24-day period. Different letters denote statistically significant difference between moult curves at  $p < 0.05$ .

**Figure 2.** Principal component analysis of parameters measured in the cephalothorax of *Homarus gammarus* after held for 24 days under different temperature conditions and fed different diets ( $n = 5$  to 9, see table 2). The PC1 separated the metabolites and protein levels horizontally and explained 60.9% of the variance. The PC2 separated the variables vertically and explained 22.5% of the variance. The contribution of the variables (metabolites and

protein) are represented by the arrows and the stronger the correlation of a variable to PC1 and PC2 the longer the arrow.

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**Highlights:**

The European lobster response to temperature variation can be modulate by diet.

Glycogen and glucose levels decreased for European lobsters held at low temperatures.

Juveniles reared on a high-CHO (36%) extruded feed grew slower.

Glycogen level was lower in juveniles fed a CHO-rich extruded feed.

Protein content decreased with low temperature in lobsters fed a high-CHO feed.

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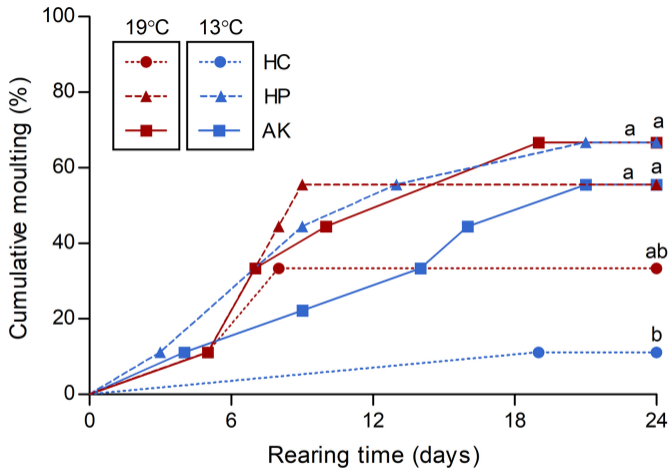


Figure 1

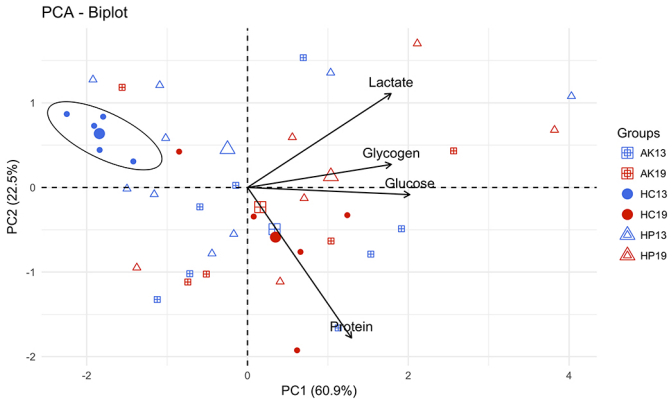


Figure 2