

The effect of dietary protein, lipid, and carbohydrate levels on the performance, metabolic rate and nitrogen retention in juvenile European lobster (Homarus gammarus, L.)

Goncalves, Renata; Lund, Ivar; Gesto, Manuel; Skov, Peter Vilhelm

Published in: Aquaculture

Link to article, DOI: 10.1016/j.aquaculture.2020.735334

Publication date: 2020

Document Version Peer reviewed version

Link back to DTU Orbit

Citation (APA):

Goncalves, R., Lund, I., Gesto, M., & Skov, P. V. (2020). The effect of dietary protein, lipid, and carbohydrate levels on the performance, metabolic rate and nitrogen retention in juvenile European lobster (*Homarus gammarus*, L.). *Aquaculture*, *525*, Article 735334. https://doi.org/10.1016/j.aquaculture.2020.735334

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.

- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Manuscript Details

Manuscript number	AQUA_2020_476_R1
Title	The effect of dietary protein, lipid, and carbohydrate levels on the performance, metabolic rate and nitrogen retention in juvenile European lobster (Homarus gammarus, L.)
Article type	Research Paper

Abstract

Releasing hatchery-reared juveniles in the wild can mitigate the general decline in the natural stocks of European lobster, Homarus gammarus, L. However, growth and survival rates in lobster culture are low, presumably due to suboptimal nutrition and feeding. With the aim of determining appropriate nutrient levels, we tested different formulated extruded feeds for the culture of juvenile European lobster. Baseline metabolism (standard metabolic rate, SMR), in combination with the metabolic cost of feeding (specific dynamic action, SDA), and nitrogen retention during digestion and assimilation was investigated for six experimental diets. Diets were formulated to contain two different levels of protein (400 and 500 g kg-1), with three lipid to carbohydrate (L:CHO) ratios (low, medium, and high). These experimental diets were tested over a 32-day period, against a conventional control diet (Antarctic krill, Euphausia superba). During this period, the growth performance of the juveniles was assessed as molting frequency, increments in carapace length and whole body wet weight. At the end of the growth performance trial, oxygen consumption (MO2) and nitrogen excretion rates of individual lobsters were determined prior to and following the ingestion of a single meal. Molting occurred more frequently in juveniles fed with krill and krill resulted in a significantly higher specific growth rate than experimental dry feeds except for the 500-low diet. However, lobsters fed any of the three 500 and the 400-low diets had carapace length increments, SMR, SDA, and nitrogen retention similar to those fed the krill diet. Results suggest that protein is an important macronutrient for juveniles of this species and must be included above 40 %. Also, lobsters have a dietary requirement for carbohydrates ranging from 24% to 35% probably related to the need for glycogen in chitin synthesis. The lower the protein content, the higher the requirement in carbohydrates.

Keywords	Formulated feeds; Antarctic krill; Standard metabolic rate; Specific dynamic action; Growth.
Taxonomy	Marine Biology, Animal Nutrition, Aquatic Species
Manuscript category	Invertebrate Nutrition
Corresponding Author	Renata Goncalves
Corresponding Author's Institution	DTU Aqua
Order of Authors	Renata Goncalves, ivar lund, Manuel Gesto, Peter Skov
Suggested reviewers	Cedric Simon, Adam Powell, Erick Perera, Elena Mente

Highlights:

- Lobster juveniles perform better with 50 than 40% protein formulated extruded feeds.
- Best performance of formulated diets ranging from 24 to 35% in carbohydrate content.
- Dietary carbohydrate has a protein-sparing effect.

1	The effect of dietary protein, lipid, and carbohydrate levels on the
2	performance, metabolic rate and nitrogen retention in juvenile European
3	lobster (Homarus gammarus, L.)
4	
5	Renata Goncalves ^{a,*} , Ivar Lund ^a , Manuel Gesto ^a , Peter Vilhelm Skov ^a
6	^a Tacknight University of Denmark, DTU Agua Saction for Aguaulture. The North See Decearch Centre 0850 Hirtsheld
7	^a Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, 9850 Hirtshars,
/	Denmark
8	
9	* Corresponding author:
10	Email: rego@aqua.dtu.dk;
11	Mobile: +45 52688210
12	DTU Aqua
13	The North Sea Science Park
14	Willemoesvej 2
15	9850 Hirtshals
16	Denmark
17 18	
19	

20 Abstract:

Releasing hatchery-reared juveniles in the wild can mitigate the general decline in the natural stocks of European lobster, Homarus gammarus, L. However, growth and survival rates in lobster culture are low, presumably due to suboptimal nutrition and feeding. With the aim of determining appropriate nutrient levels, we tested different formulated extruded feeds for the culture of juvenile European lobster. Baseline metabolism (standard metabolic rate, SMR), in combination with the metabolic cost of feeding (specific dynamic action, SDA), and nitrogen retention during digestion and assimilation was investigated for six experimental diets. Diets were formulated to contain two different levels of protein (400 and 500 g kg⁻¹), with three lipid to carbohydrate (L:CHO) ratios (low, medium, and high). These experimental diets were tested over a 32-day period, against a conventional control diet (Antarctic krill, *Euphausia superba*). During this period, the growth performance of the juveniles was assessed as molting frequency, increments in carapace length and whole body wet weight. At the end of the growth performance trial, oxygen consumption (MO_2) and nitrogen excretion rates of individual lobsters were determined prior to and following the ingestion of a single meal. Molting occurred more frequently in juveniles fed with krill and krill resulted in a significantly higher specific growth rate than experimental dry feeds except for the 500-low diet. However, lobsters fed any of the three 500 and the 400-low diets had carapace length increments, SMR, SDA, and nitrogen retention similar to those fed the krill diet. Results suggest that protein is an important macronutrient for juveniles of this species and must be included above 40 %. Also, lobsters have a dietary requirement for carbohydrates ranging from 24% to 35% probably related to the need for glycogen in chitin synthesis. The lower the protein content, the higher the requirement in carbohydrates.

Keywords: Formulated feeds; Antarctic krill; Nitrogen retention, Standard metabolic rate; Specific dynamic action;
Growth.

1. Introduction

The European lobster (*Homarus gammarus*) is an economically important decapod crustacean distributed from Northern Norway to Morocco and Eastern Mediterranean (Triantafyllidis et al., 2005). Commercial landings of this species are declining and efforts to enhance natural populations have been made by restocking with hatchery-reared juveniles (Agnalt et al., 2007). Hatchery production of European lobster aims to enhance growth and survival rates by securing optimal water quality, reducing predation, and by improving access to nutritional rich diets (Powell, 2016). Research efforts have been devoted mainly towards improving water quality conditions and the development of novel rearing systems (Daniels et al., 2013, 2015; Drengstig and Bergheim, 2013; Halswell et al., 2016; Middlemiss et al., 2015), but less so towards the development of a species-specific formulated diet. The transition from live or frozen feeds to the use of dry formulated diets may be one way to support a simpler and more sustainable production through the ease of application, reduced cost, and a more consistent nutritional quality (Powell et al., 2017).

The development of a nutritionally balanced formulated feed requires species-specific information on nutritional requirements. A considerable research effort on formulated feed development for spiny lobsters has been made in the last 30 years, the results of which indicate a dietary demand for high protein (>45%), low lipid (<10%), and moderate carbohydrate ($\sim 20\%$) (Williams, 2007). However, the performance of spiny lobsters fed on formulated feeds remains poor, partially as a consequence of a lack of understanding of how they digest and assimilate this type of feeds (Perera and Simon, 2015). There is less literature available for homarid species than for spiny lobsters, and while some nutritional studies have been conducted on American and European lobster, appropriate nutritional levels are still to be defined. Based on the idea that diets should match the dry matter biochemical composition of an organism (Dall et al., 1991), the proximate composition content of *Homarus americanus* post-larvae suggests that an appropriate diet for this species should contain 53% protein, 4% lipid, and 12% carbohydrates (Haché et al., 2015). The observation that European lobster possesses a variety of carbohydrases (Glass and Stark, 1995), is indicative of a digestive capacity of different carbohydrate sources. Furthermore, Powell et al. (2017) identified glycogen deficiencies in the biochemical composition of H. gammarus larvae reared on formulated dry feed. Taken together, these findings suggest that the development of a formulated dry feed for European lobster should consider carbohydrates as a potential non-protein energy source.

The standard metabolic rate (SMR) represents the minimum energy expenditure of an ectotherm animal (Rosewarne et al., 2016). Although individuals with higher SMR have higher maintenance metabolism, previous studies also suggest this is indicative of an increased growth potential (Álvarez and Nicieza, 2005; Auer et al., 2015; Reid et al., 2012; Van Leeuwen et al., 2012). The magnitude of the postprandial metabolism, commonly referred to as the specific dynamic action (SDA), depends largely on the size and nutritional composition of a meal. It provides information on the cost and duration of the nutritional processes, including the energy expended towards food handling, absorption and storage of nutrients, deamination of amino acids, protein and lipids synthesis for growth, and synthesis of excretory products (Jobling, 1993). In crustaceans, the mechanical costs of digestion are calculated to be 5% to 8% while protein synthesis accounts for 20% to 37% of SDA (Whiteley et al., 2002). Therefore, SDA determination is a useful performance parameter in nutritional studies. In principle, the higher the fraction of the meal energy allocated to SDA, the less energy will be retained to fuel locomotion, growth, and reproduction (Stieglitz et al., 2018). The present information on the metabolism in European lobster is sparse. Whiteley et al., (1990) compared aquatic and aerial rates of MO₂ (mass-specific oxygen consumption) of this species at a temperature range 10-20°C. Later, Drengstig (2017) reported data of standard metabolism in *H. gammarus* at 20°C. However, to our knowledge, no studies on the effects of dietary composition on SMR or the SDA of European lobster have been performed.

Lobsters excrete the majority of the end product of protein metabolism across the gill epithelium in the form of ammonia (Burger, 1957), while a smaller part of nitrogenous waste is converted into urea in the antennal and maxillary glands (Binns and Peterson, 1969). Wickins (1985) investigated the effect of feeding on ammonia excretion by Homarus gammarus and observed that lobsters exhibited a significant increase in ammonia excretion after a meal. The efficiency with which dietary amino acids are deposited as new tissue can be estimated by the quantification of nitrogen excretion during digestion (Ming, 1985). Amino acids that are deaminated for de novo lipogenesis and glycogenesis, or oxidized for fuel, turn into nitrogenous waste (Skov et al., 2017). Therefore, high nitrogen excretion rates are indicative of reduced protein retention.

This study aimed to investigate the role of protein inclusion levels combined with different non-protein energy sources (lipids and carbohydrates) in the metabolism and growth of European lobster. For that purpose, the present work compared the respiration and nitrogen excretion rates of European lobster juveniles (< 1 g) reared on Antarctic

005
225
226
227
221
228
229
220
230
231
232
202
233
234
225
235
236
237
238
239
240
270
241
242
2/2
243
244
245
240
246
247
2/18
240
249
250
251
201
252
253
200
254
255
256
200
257
258
200
259
260
261
201
262
263
200
204
265
266
200
267
268
260
209
270
271
070
272
273
27/
214
275
276
077
211
278
270
219

2 - Materials and methods

96 2.1. Experimental animals

97 Experiments were conducted at the aquaculture facilities at the Technical University of Denmark, Section
98 for Aquaculture, Hirtshals. All experimental animals were hatched from eggs obtained from wild European lobster
99 females caught along the Skagerrak coast of North Jutland, Denmark. Experimental lobster juveniles were reared
100 individually in cassette systems consisting of 200 mL compartments. Cassettes were placed in raceways supplied by
101 a flow-through semi-closed seawater system at a constant flow rate of 330 L h⁻¹ (18±0.5°C temperature, 34±1 PSU
102 salinity, > 90% dissolved oxygen, <0.1mg L⁻¹ ammonia-N), subjected to a photoperiod cycle of 8h light: 16h dark.
103 Lobsters were fed once daily with thawed Antarctic krill, *Euphausia superba* (Akudim A/S, Denmark).

104 2.2. Growth trial

Animals were held under the above-described conditions for four months from settling, after which they were randomly divided into seven treatment groups (N=10, per diet) while ensuring animals were of similar size across all treatments $(0.86 \pm 0.06 \text{ g})$ wet weight, mean \pm SEM). An experimental period of 32 days before respirometry and nitrogen excretion trials was used for evaluation of growth performance. During this period, each juvenile was individually fed its respective diet in excess each morning, and allowed to feed for 4h before uneaten food was removed. Additionally, juveniles were allowed to feed on their molted exoskeletons. Molt occurrences were recorded daily. At the beginning and end of the experimental period, lobsters were gently blotted dry with a paper towel and body wet weight was recorded to the nearest 0.01g. Carapace length was recorded with a vernier caliper from the base of the eve socket to the posterior edge of the cephalothorax. The following formulas were used:

19
20
114 Cumulative molting (CM, %) =
$$\sum_{i=0}^{n} (Mi \times Lob^{-1}) \times 100$$

322 115 where: i = the day; Mi = number of molts on the day i; Lob = number of lobsters in each treatment.

116 Specific growth rate
$$(SGR, \% day^{-1}) = [ln (BW_f) - ln (BW_i)] \times days^{-1} \times 100$$

326 117 where, $BW_f = final$ wet body weight, $BW_i = initial$ wet body weight.

329 **118** Carapace length increment (iCL, %) = $(CL_f - CL_i) \times CL_i^{-1} \times 100$

- 331 **119** where: $CL_f = final carapace lentgh; CL_i = initial carapace length.$

2.3. Experimental diets

Six formulated dry diets were evaluated using Antarctic krill as a reference diet. The six experimental dry diets were formulated to have two fixed protein levels (400 or 500 g kg⁻¹), and for each protein level, three L:CHO ratio levels (low: 0.3, medium: 0.5, and high: 0.8-1.0). Different protein, lipid, and carbohydrate contents were achieved by altering squid meal, wheat gluten, wheat starch, and fish oil inclusion levels. Experimental diets were extruded as 4 mm pellets and were manufactured by SPAROS Lda (Olhão, Portugal). Proximal analysis of krill and experimental diets were performed in duplicate. Briefly, the diets were finely ground using a Krups Speedy Pro homogenizer and analyzed for crude protein, (i.e. Kjeldahl N × 6.25, ISO 5983-2 (2005), crude fat (Bligh and Dyer, 1959), dry matter and ash (NMKL 23, 1991). Formulation and proximate composition are presented in Table 1.

2.4. Respirometry trial

Measurements were performed on 10 intermolt lobsters per dietary treatment. All animals used were fasted for 48h prior to respirometry measurements to ensure a post-absorptive state. Experiments were performed in 75 mL respirometers supplied with temperature-controlled aerated seawater, using 8 chambers at a time. The bottom of each chamber was equipped with a perforated base plate, under which a magnetic stirrer ensured water mixing in the chamber. Oxygen content was registered every 15 sec using a sensor connected to an optical oxygen meter (FireSting O2, Pyro Science GmbH, Aachen, Germany) installed in each chamber. MO₂ measurements were performed by computerized intermittent flow, in loops of 60 min (consisting of a 5 min flushing period, followed by a 55 min closed period). The oxygen consumption rate was determined by linear regression of the decline in oxygen content during the closed period. The mass-specific oxygen consumption (MO_2) was calculated based on the slope of the regression according to Steffensen (1989) as:

 $MO_2 = \alpha \times V_{resp} \times \beta \times BW^{-1}$

where: $\alpha = \text{slope} (\Delta pO2 \times \Delta t^{-1})$, $V_{\text{resp}} = \text{volume of the chamber minus the volume of the lobster (using a lobster density)}$ of 1), β = oxygen solubility at the experimental temperature, and BW = wet body weight of the lobster.

Standard metabolic rate (SMR) was estimated from the first 48h MO₂ measurements, which was calculated as described by Skov et al. (2011). Briefly, MO_2 measurements were grouped in frequency classes, and SMR was calculated from the most frequently occurring bins and their relative contribution. Following SMR measurements, chambers were opened and a pre-weighed piece of thawed krill or a feed pellet was offered to each lobster. During

feeding, chambers were kept unsealed, the flushing pump was stopped, and external aeration was provided. After a
2h feeding period, the remaining krill or pellet was carefully removed and MO₂ postprandial measurements resumed
for 48h for estimation of SDA response. The uneaten feed fraction was collected, filtered, and dried for voluntary feed
intake (VFI) estimation employing the following formula (Nguyen et al., 2014):

L

where: dF = distributed feed, uF = unconsumed feed, L = leaching after 2h. Leaching was estimated by placing a
pre-weighed quantity of each diet in the chambers under the same conditions as in the feeding period but in this
case, without animals.

For calculation of SDA variables digestion was determined to be completed when MO₂ postprandial measurements plotted over time fell within 15% of the SMR previously recorded for that chamber (Jordan and Steffensen, 2007). According to Secor (2009), the following SDA variables were calculated to describe the postprandial MO₂: SDA_{dur} (h) is the time from feeding until MO₂ converged with the SMR + 15%. The SDA_{cost} (µg O2 g⁻¹) is the post-feeding integrated excess MO2 above SMR. The SDApeak (µg O2 g⁻¹ h⁻¹) is the maximum value of MO₂ above SMR during the SDA course and SDA_{ttp} (h) is the time from feeding to SDA_{peak}. SDA_{coef} (%) is the SDA_{cost} converted to energy using an oxycalorific coefficient of 14.06 J mg⁻¹ O₂ (Dejours, 1981) and divided by the energy content of the meal which was calculated from the estimated feed intake. The scope is the SDA_{peak} divided by the SMR.

164 2.5. Nitrogen excretion trial

Following oxygen consumption measurements, each lobster was transferred to a 130 mL seawater container supplied with aeration. Water samples of 15mL were collected manually from individual chambers at time 0h and 48h for baseline screening of total ammonia and nitrogen excretion rates. After this period, lobsters were offered a pre-weighed pellet or krill piece for 2h. After the meal, lobsters were transferred into containers with fresh seawater. Water samples were manually collected at time 0h and 48h for the determination of total postprandial ammonia and nitrogen excretion rates. Collected water samples were filtered (0.2µm, Filtropur Sarstedt, Numbrecht, Germany) and stored at 0°C until analysis. Total nitrogen and ammonia nitrogen of collected water samples were determined in duplicate according to ISO 11905-1 (1997) and DS (1975), respectively. The voluntary feed intake was calculated from the

uneaten fraction that was collected, filtered, and dried. N intake was calculated as 16% of protein intake (Chibnall etal., 1943). Total postprandial nitrogen excretion was calculated using the following formulas:

- 2 175 $N_{pre-feeding} = [(N_{48h} \times V_{48h}) (N_{0h} \times V_{0h})] \times BW^{-1}$
- $N_{post-feeding} = [(N_{48h} \times V_{48h}) (N_{0h} \times V_{0h})] \times BW^{-1}$
- 59 177 $N_{excreted} = N_{post-feeding} N_{pre-feeding}$

 00 178where: $N_{excreted}$ = total postprandial nitrogen excreted (mg total nitrogen per mg wet weight), $N_{pre-feeding}$ = pre-feeding 62 179nitrogen excretion (mg total nitrogen per mg wet weight), $N_{post-feeding}$ = post-feeding nitrogen excretion (mg total 63 180nitrogen per mg wet weight), N_{48h} = total nitrogen concentration at time 48h (mg total nitrogen per mL); N_{0h} = total 65 181nitrogen concentration at time 0h (mg total nitrogen per mL), V_{48h} = volume of the chamber at time 48h (mL), V_{0h} = 67 182volume of the chamber at time 0h (mL), BW = wet body weight of the lobster. Nitrogen retention was expressed as 69 183percentage of total N intake.

184 2.6. Data analysis and statistics

Data are expressed as means \pm SEM unless otherwise specified. All dietary treatments were subjected to a one-way ANOVA to test the experimental formulated dry feeds against the control diet (krill). Whenever significant differences were identified, comparisons against the krill diet were conducted using the Dunnett t-test. Data from experimental formulated dry feed treatments were subsequently subjected to a two-way ANOVA, considering protein level and L:CHO ratio as variables. Following a two-way ANOVA and whenever significant differences were identified, means were compared by the Holm-Sidak post hoc test. Data were checked for normal distribution and homogeneity of variances and, when necessary, log-transformed. Data expressed as a percentage were arcsin transformed. Carapace length increment was log(x+2) transformed due to a high frequency of null observations. Statistical significance was set at $p \le 0.05$. All statistical tests were performed using the IBM SPSS Statistics 25.0 and graphics were generated by GraphPad Prism version 5.0 software package. The linear regression of the decline in oxygen content was computed using R version 3.5.1 software (R Core Team, 2018).

3. Results

3.1. Growth performance

Observation during feeding showed that *H. gammarus* juveniles were attracted to all experimental diets and actively manipulated the offered feed. Minimum cumulative molting during the 32-day growth trial was recorded as 10% for the group of animals fed the 400-high diet. Maximum cumulative molting (90%) was observed for lobsters fed the krill diet (Figure 1). SGR was significantly higher ($F_{6,69} = 4.90$, p < 0.001) in krill-fed lobsters in comparison to lobsters fed experimental dry diets, with the exception of the 500-low diet (Figure 2A). Among experimental diets, SGR was unaffected by protein level ($F_{1,59} = 2.63$, p = 0.11), L:CHO ratio ($F_{2,59} = 2.62$, p = 0.08), or the interaction of the two variables ($F_{2,59} = 1.05$, p = 0.36). Nevertheless, protein content caused a significant positive effect on the carapace length increment (iCL) increment (Figure 2B). The iCL increment in lobsters fed the 500-high diet was significantly higher compared to animals fed the 400-high diet ($F_{1,59} = 6.12$, p = 0.02). The animals fed the 400-medium and 400-high experimental diets presented a significantly lower iCL in opposition to the krill-fed animals $(F_{1,69} = 2.89, p = 0.02)$. Voluntary feed intake expressed as dry weight was higher $(F_{6,71} = 3.19, p = 0.01)$ for the 400-low and 500-medium diets compared to the krill. No significant effect on feed intake for protein or L:CHO ratio was detected on experimental diets. However, the interaction of both was statistically meaningful ($F_{2,62} = 3.33$, p = 0.04). The L:CHO ratio did not affect the 500 group of diets but in the 400 group, the 400-medium diet had a significantly lower VFI compared to the 400-low (Figure 3).

3.2. Metabolic rates

The pre- and postprandial metabolic data for lobsters fed control and experimental diets are presented in Table 2. Individuals that did not ingest sufficient feed to induce a clear postprandial metabolic response were omitted from the analyses. Comparing the results of all treatments, animals fed the 400-medium and 400-high diets had significantly lower SMR compared to the krill-fed animals (Table 2, Figure 5). Within experimental diets, SMR was significantly affected by protein level and L:CHO ratio, but unaffected by the interaction of protein \times L:CHO. With the exception of the 400-low, animals fed diets with 40% protein were observed to have significantly lower SMR values compared to the 50% protein diets (Table 2, Figure 5). The amount of ingested energy was significantly higher for lobsters fed on the 400-low and 500-medium diets compared to the krill-fed group (Table 2). No significant differences in meal energy were observed between experimental diets. SDA_{dur} was significantly longer in lobsters fed 400-low when compared to krill-fed lobsters. Within experimental diets, duration of SDA was affected by protein

level and the interaction of protein × L:CHO, but was unaffected by L:CHO ratio (Table 2, Figure 4). Results showed
lobsters fed the 500-low diet had a shorter SDA response compared to the animals fed on the 400-low diet.

226 <u>3.3. Nitrogen retention</u>

The effects of dietary treatment on nitrogen budgets are shown in Table 3. As for the respirometry trials, animals that did not feed sufficiently were not included in the analysis. Within the experimental dietary groups, protein level, L:CHO ratio, and the interaction of the two, all significantly affected nitrogen intake. N intake was generally higher for the 50% protein diets with no effect of L:CHO. Among the 40% protein diets, N intake was highest for the 400-low diet. Animals fed the 400-medium and 400-high diets presented a lower N intake in comparison to krill. No significant differences were observed for the total nitrogen excreted. Comparing all the dietary treatments, nitrogen retention was significantly lower in animals fed the 400-high diet against lobsters fed krill. Within the experimental diets the % total N retention was significantly affected by protein level, L:CHO ratio, and the interaction of protein × L:CHO ratio. Nitrogen retention was higher for the 50% protein diets with no effect of L:CHO. The lowest N retention was observed in the 40% protein, with a significant decrease with increasing L:CHO ratios (Table 3). The contribution of ammonia to the total nitrogen excreted varied between 64% and 88% among dietary treatments (data not presented) but no statistical differences were found ($F_{6.33} = 1.37$, p = 0.27).

Discussion

The present study demonstrates a limited successful growth of European lobster juveniles fed on formulated dry feeds. Results indicate that SGR of European lobster juveniles fed the 500-low diet (50% protein, 9% lipid, 26% carbohydrate) was not significantly lower than the control group fed on krill. However, the cumulative molting rate was lower in all treatment groups fed formulated experimental diets. Results suggest that the poor growth performance of H. gammarus fed on formulated feeds remains one of the principal obstacles in the development of sustainable aquaculture of this species. Further optimization of formulated diets in terms of mechanical and chemical digestion is imperative. Improving pellet size, format, and texture, and supplementing feeds with additives (digestible binders, pH buffers, and exogenous enzymes) need to be addressed in future research.

Results from this study establish that the dietary regime affects the SMR in European lobster juveniles. Animals fed the 400-medium (40% protein, 15% lipid, and 31% carbohydrate) and 400-high diets (40% protein, 23% lipid, and 23% carbohydrate) showed the lowest SMR. According to Biro and Stamps (2010) a higher SMR is associated with a larger metabolic capacity. In the same study, the authors suggested that individuals with high metabolism were able to process larger meals. Therefore, under this hypothesis, SMR is expected to produce a positive impact on performance (Burton et al., 2011). Our results showed that the juveniles fed the 400-medium and 400-high experimental diets presented the poorest performance in terms of cumulative molting, SGR, and CL increment, confirming Biro and Stamps (2010) hypothesis. The voluntary feed intake for the group of animals fed these two diets was also the lowest, which most likely contributed to the poorest growth performance. Protein synthesis, a crucial process in growth, is strongly affected by the feed intake. Previous studies in several crustacean species have demonstrated that protein synthesis rates generally decrease in starved or less frequently fed animals (Carter and Mente, 2014). The reason why the feed intake was lower for the 400-medium and 400-high diets remains unclear but it might be related to lower palatability of these two diets, or that high lipid levels cause faster satiation. The growth compensation for animals fed the 400-low diet, i.e., low lipid (11%) and high carbohydrate (35%) is in agreement with the effect observed between SMR and L:CHO ratio. This result supports our initial hypothesis that carbohydrate represent an important macronutrient for H. gammarus especially in diets with reduced protein content. In fact, carbohydrates are important for crustacean species as glycogen is an essential precursor of chitin synthesis, serving a critical role during the molt cycle (Wang et al., 2016).

Studies on the SDA in lobster species are scarce, and, to our knowledge, there is no information for European lobsters. In this work, we observed that feeding caused a rise in oxygen consumption 2 to 3 times above SMR levels in European lobster juveniles fed the different diets. In a previous study, in 3.2 g Homarus americanus fed on formulated diets, Koshio et al. (1992) reported an SDA scope of 1.5. The smaller size of the lobsters tested in this study can explain the difference in the SDA scope, as the animal size is known to influence the SDA variables (McCue, 2006). In the present study, the time to achieve the SDA peak ranged between 8h to 11h, with elevated oxygen consumption rates lasting for 17 to 24h. The SDA duration was significantly longer for the 400-low experimental diet compared to krill, which is likely related to the higher meal energy or protein intake (Secor, 2009). SDA duration in southern rock lobster (Jasus edwardsii) fed squid was longer than what we observed in European lobster juveniles. Crear and Forteath (2000) reported that 750 g J. edwardsii took 42h to return to the pre-feeding oxygen consumption level, while Radford et al. (2004) observed that SDA response in 16 g animals of the same species lasted 30h. In this study, SDA coefficient results showed that juveniles fed on the tested diets spend between 3.4% to 7.0% of the meal energy on digestive processes. These results are in agreement with the findings by Crear and Forteath (2000) who reported an SDA coefficient of 6.6% in J. edwardsii. Nevertheless, the amount of meal energy allocated to SDA reported for crustacean species is highly variable even within the same species. For example, Houlihan et al. (1990) observed an SDA_{coef} of 13.3% for 37 g Carcinus maenas while Wallace (1973) reported an SDA_{coef} of 3.4% in 10 g individuals of the same species. Collectively, these findings suggest that the duration of the SDA response and the SDA coefficient increases with increasing body sizes.

In this study, we observed that juveniles excreted the majority of the nitrogenous waste in the form of ammonia (64-88%). Results reported here suggest that the mechanism for nitrogen excretion in H. gammarus is similar to other aquatic crustacean species, namely, the *H. americanus* (Burger, 1957) and *J. edwardsii* (Binns and Peterson, 1969). Total nitrogen budget results showed that the nitrogen retention of juveniles fed the 400-high diet (40% protein, 23% carbohydrate, and 23% lipid content) was significantly lower than in juveniles fed the other experimental or control diets. The severely reduced N intake in this group of animals induced a negative nitrogen balance, i.e., nitrogen excretion exceeded the nitrogen intake. The incapacity of this group of animals for nitrogen retention suggested that, rather than protein deposition, animals were periodically undergoing tissue protein catabolism (Guo et al., 2012).

Results from this study suggest that European lobster juveniles with low SMR and low nitrogen retention have a reduced growth capacity. Nevertheless, the estimated SMR and nitrogen retention were highly affected by the feed intake of the different tested diets. Therefore, the results presented in this study should be interpreted with caution. Moreover, the growth performance indices were calculated over a 32-day period, which could be considered relatively short, particularly in the case of crustacean species. In these animals, wet weight changes follow a typical pattern through the molt cycle. The highest increase occurs in the brief period of rapid water uptake at ecdysis. Further moderate gains are related to carapace mineralization and tissue growth. Finally, during the intermolt period, there is a relative stabilization of fresh weight until the onset of the successive ecdysis (Nguyen et al., 2014). As follows, nutritional studies targeting evaluation of growth performance should allow at least one complete molt cycle per individual.

SMR and nitrogen retention results from this study corroborate the hypothesis that juvenile H. gammarus perform better fed on 500 against 400 g kg⁻¹ protein content in their diet. This level agrees with the protein content (52% DM) in European lobster larvae reported by Powell et al. (2017) supporting the idea that diets should meet the organism's biochemical composition. The results of this study show that protein is a fundamental nutrient in formulated dry feeds. However, its inclusion can potentially be reduced when compensated with appropriate carbohydrate levels. Carbohydrates are the least expensive energy source for aquatic animals (Wang et al., 2016) and therefore, this is an important opportunity for the production of sustainable and economically viable formulated feeds for this species.

310

785		
786 787 788	311	Acknowledgements
789 790	312	This study was partly financed by a Fisheries local action group (FLAG), and the ENV Foundation with support from
791 792	313	the Ph.D. school at DTU Aqua. The authors would like to thank Ole Larsen, Rasmus Jensen, and Jens Nedergaard for
793 794	314	their assistance in taking care of the lobster systems, and to Ulla Sproegel and Brian Moeller for their help with the
795 796	315	laboratory analysis. A special thanks to Tiago Malta for his assistance with raw data transformation. The final
797	316	manuscript also benefitted from the valuable recommendations of anonymous reviewers.
798		
800		
801		
802		
803		
804		
805		
806		
807		
808		
809		
01U 811		
812		
813		
814		
815		
816		
817		
818		
819		
820		
02 I 822		
823		
824		
825		
826		
827		
828		
829		
03U 921		
832		
833		
834		
835		
836		
837		
838		15
839		

841									
842 843									
844	318	References							
845	040								
846 847	319	Agnalt, A.L., Kristiansen, T.S., Jørstad, K.E., 2007. Growth, reproductive cycle, and movement of berried European							
848	320	lobsters (Homarus gammarus) in a local stock off southwestern Norway. ICES J. Mar. Sci. 64, 288–297.							
849	321	https://doi.org/10.1093/icesims/fs1020							
850 951		· · · · · · · · · · · · · · · · · · ·							
852	322	Álvarez, D., Nicieza, A.G., 2005. Is metabolic rate a reliable predictor of growth and survival of brown trout (Salmo							
853	222	trutte) in the wild? Can I Figh A guet Sei (2 (12 (10 https://doi.org/10.1120/604.222							
854	323	trutta) in the wild? Can. J. Fish. Aquat. Sci. 62, 643–649. https://doi.org/10.1139/104-223							
855 856	324	Auer S.K. Salin K. Rudolf A.M. Anderson G.I. Metcalfe N.B. 2015. The optimal combination of standard							
857	524	Auci, S.K., Saini, K., Rudon, A.W., Anderson, G.J., Wettane, W.D., 2015. The optimal combination of standard							
858	325	metabolic rate and aerobic scope for somatic growth depends on food availability. Funct. Ecol. 29, 479-486.							
859 860	326	https://doi.org/10.1111/1365-2435.12396							
861									
862	327	Binns, R., Peterson, A.J., 1969. Nitrogen excretion by the spiny lobster Jasus edwardsi (Hutton): the role of the							
863	220	enternal aland Riel Rull 126 147 152 https://doi.org/10.2207/1520800							
864 865	520	antennai gianu. Bioi. Bun. 150, 147–155. https://doi.org/10.2507/1559809							
866	329	Biro, P.A., Stamps, J.A., 2010, Do consistent individual differences in metabolic rate promote consistent individual							
867									
868	330	differences in behavior? Trends Ecol. Evol. 25, 653–659. https://doi.org/10.1016/j.tree.2010.08.003							
870	221	Dick E.C. Duor W.L. 1050. A ranid method of total linid outraction and murification. Can. I. Diochem. Dhusial							
871	331	Bigi, E.G., Dyer, w.J., 1939. A tapid method of total lipid extraction and purification. Can. J. Biochem. Physiol.							
872	332	37, 911–917.							
873 874									
875	333	Burger, J., 1957. The general form of excretion in the lobster, Homarus. Biol. Bull. 113, 207–223.							
876	224	Destan T. Killer C.C. Americana J.D. Meterle, N.D. 2011 What serves interest if a matrix is mating							
877 878	334	Burton, 1., Killen, S.S., Armstrong, J.D., Metcalfe, N.B., 2011. What causes intraspecific variation in resting							
879	335	metabolic rate and what are its ecological consequences? Proc. R. Soc. B Biol. Sci. 278, 3465-3473.							
880	336	https://doi.org/10.1098/rspb.2011.1778							
881									
883	337	Carter, C.G., Mente, E., 2014. Protein synthesis in crustaceans: A review focused on feeding and nutrition. Cent.							
884	220	Even J. Disl. 0, 1, 10, https://doi.org/10.2479/s11525.012.0124.0							
885	330	Eur. J. Biol. 9, 1–10. https://doi.org/10.2478/811555-015-0154-0							
886	339	Chibnall A C Rees M W Williams E D 1943 The total nitrogen content of egg albumin and other proteins							
888									
889	340	Biochem. J. 37, 354–359.							
890	044	Come D.L. Everyth, C.N.D. 2000. The officiation of interior is forther an everything by the							
892	341	Creat, D.J., Forteath, G.N.K., 2000. The effect of extrinsic and intrinsic factors on oxygen consumption by the							
893									
894		16							
090									

southern rock lobster, Jasus edwardsii. J. Exp. Mar. Bio. Ecol. 252, 129-147. https://doi.org/10.1016/S0022-0981(00)00243-4 Cuzon, G., Guillaume, J., 1997. Energy and protein: energy ratio., in: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition. World Aquaculture Society, Baton Rouge, LA, pp. 51–70. Dall, W., Hill, B.J., Rothlisberg, P.C., Sharples, D.J. (Eds.), 1991. Digestion and Assimilation, in: The Biology of the Penaeidae (Advances in Marine Biology, 27). Academic Press. Daniels, C.L., Merrifield, D.L., Ringø, E., Davies, S.J., 2013. Probiotic, prebiotic and synbiotic applications for the improvement of larval European lobster (Homarus gammarus) culture. Aquaculture 416-417, 396-406. https://doi.org/10.1016/j.aquaculture.2013.08.001 Daniels, C.L., Wills, B., Ruiz-Perez, M., Miles, E., Wilson, R.W., Boothroyd, D., 2015. Development of sea based container culture for rearing European lobster (Homarus gammarus) around South West England. Aquaculture 448, 186–195. https://doi.org/10.1016/j.aquaculture.2015.05.026 Dejours, P., 1981. Principles of comparative respiratory physiology. Elsevier, Amsterdam, The Netherlands. Drengstig, A., 2017. Metabolic rates in hatchery-reared European lobster juveniles (Homarus gammarus L.). J. Aquac. Mar. Biol. 5, 3-6. https://doi.org/10.15406/jamb.2017.05.00134 Drengstig, A., Bergheim, A., 2013. Commercial land-based farming of European lobster (Homarus gammarus L.) in recirculating aquaculture system (RAS) using a single cage approach. Aquac. Eng. 53, 14–18. https://doi.org/10.1016/j.aguaeng.2012.11.007 DS, 1975. DS 224 Water Analysis - Determination of Ammonia-Nitrogen. Glass, H.J., Stark, J.R., 1995. Carbohydrate digestion in the European lobster Homarus gammarus (L.). J. Crustac. Biol. 15, 424-433. Guo, Z., Zhu, X., Liu, J., Han, D., Yang, Y., Lan, Z., Xie, S., 2012. Effects of dietary protein level on growth performance, nitrogen and energy budget of juvenile hybrid sturgeon, Acipenser baerii $\bigcirc \times A$. gueldenstaedtii ♂. Aquaculture 338–341, 89–95. https://doi.org/10.1016/j.aquaculture.2012.01.008

954		
955 956	366	Haché, R., Pelletier, C.J., Dumas, A., 2015. Selected nutrient profiles in first larvae and postlarvae of American
957 958	367	lobster (Homarus americanus). Aquac. Int. 23, 929–941. https://doi.org/10.1007/s10499-014-9852-9
959 960	368	Halswell, P., Daniels, C.L., Johanning, L., 2016. Sea based container culture (SBCC) hydrodynamic design
961 962	369	assessment for European lobsters (Homarus gammarus). Aquac. Eng. 74.
963 964	370	https://doi.org/10.1016/j.aquaeng.2016.08.003
965 966	371	Houlihan, D., Waring, C., Mathers, E., Gray, C., 1990. Protein Synthesis and Oxygen Consumption of the Shore
967 968	372	Crab Carcinus maenas after a Meal. Physiol. Biochem., Soc. Integr. Comp. Biol. 63, 735-756.
969 970	373	ISO 11905-1, 1997. Water Quality - Determination of Nitrogen. Part 1: Method using Oxidative Digestion with
971 972 973	374	Peroxodisulfate.
973 974	375	ISO 5983-2, 2005. Animal Feeding Stuffs - Determinatio of Nitrogen Content and Calculation of Crude Protein
975 976 977	376	Content - Part 2: Block Digestion / Steam Distillation Method.
978	377	Jobling, M., 1993. Bioenergetics: feed intake and energy partitioning. Fish Ecophysiol. 1-44.
979 980 981	378	https://doi.org/10.1007/978-94-011-2304-4_1
982 983	379	Jordan, A.D., Steffensen, J.F., 2007. Effects of ration size and hypoxia on specific dynamic action in the cod.
984 985	380	Physiol. Biochem. Zool. 80, 178-185. https://doi.org/10.1086/510565
986 987	381	Koshio, S., Castell, J.D., O'Dor, R.K., 1992. The effect of different dietary energy levels in crab-protein- based diets
988	382	on digestibility, oxygen consumption, and ammonia excretion of bilaterally eyestalk-ablated and intact
989 990 991	383	juvenile lobsters, Homarus americanus Aquaculture. Aquaculture 108, 285–297.
992 993	384	McCue, M.D., 2006. Specific dynamic action: A century of investigation. Comp. Biochem. Physiol A Mol. Integr.
994 995	385	Physiol. 144, 381–394. https://doi.org/10.1016/j.cbpa.2006.03.011
996 997	386	Middlemiss, K.L., Daniels, C.L., Urbina, M.A., Wilson, R.W., 2015. Combined effects of UV irradiation, ozonation,
998 999	387	and the probiotic Bacillus spp. on growth, survival, and general fitness in European lobster (Homarus
1000 1001	388	gammarus). Aquaculture 444, 99–107. https://doi.org/10.1016/j.aquaculture.2015.03.028
1002 1003	389	Ming, F.W., 1985. Ammonia excretion rate as an index for comparing efficiency of dietary protein utilization among
1004 1005	390	rainbow trout (Salmo gairdneri) of different strains. Aquaculture 46, 27-35.
1006 1007 1008		18

1009							
1010							
1011	391	Nguyen NTB Chim L Lemaire P Wantiez L 2014 Feed intake molt frequency tissue growth feed					
1012							
1013	392	efficiency and energy budget during a molt cycle of mud crab juveniles, Scylla serrata (Forskål, 1775), fed on					
1014	202	different practical diets with graded levels of soy protein concentrate as main source of prote. Aquaculture					
1015	575	unierent practical diets with graded levels of soy protein concentrate as main source of prote. Aquaculture					
1017	394	434, 499-509. https://doi.org/10.1016/j.aquaculture.2014.09.014					
1018							
1019	395	NMKL 23, 1991. Gravimetric Determination in Meat and Meat Products.					
1020							
1021	396	Perera, E., Simon, C., 2015, Digestive physiology of spiny lobsters: Implications for formulated diet development.					
1022							
1023	397	Rev. Aquac. 7. https://doi.org/10.1111/raq.12066					
1024							
1026	398	Powell, A., 2016. New developments in European lobster aquaculture. Aquac. Eur. 41, 5-12.					
1027							
1028	399	Powell, A., Hinchcliffe, J., Sundell, K., Carlsson, N.G., Eriksson, S.P., 2017. Comparative survival and growth					
1029	100						
1030	400	performance of European lobster larvae, Homarus gammarus, reared on dry feed and conspecifics. Aquac.					
1031	401	Res. 48, 5300–5310. https://doi.org/10.1111/are.13343					
1032							
1033	402	R Core Team 2018 R: A language and environment for statistical computing R Foundation for Statistical					
1035							
1036	403	Computing.					
1037							
1038	404	Radford, C.A., Marsden, I.D., Davison, W., 2004. Temporal variation in the specific dynamic action of juvenile					
1039	405	New Zeeland reak labeters Jague edwardeii Comp. Dischem Dhusiel - A. Mel Integr. Dhusiel 120, 1, 0					
1040	405	New Zealand lock lobsters, jasus edwardsh. Comp. Biochem. Physiol A Mol. Integr. Physiol. 139, 1–9.					
1041	406	https://doi.org/10.1016/j.cbpb.2004.02.015					
1043							
1044	407	Reid, D., Armstrong, J.D., Metcalfe, N.B., 2012. The performance advantage of a high resting metabolic rate in					
1045	400						
1046	408	juvenile salmon is habitat dependent. J. Anim. Ecol. 81, 868–875. https://doi.org/10.1111/j.1365-					
1047	409	2656.2012.01969.x					
1048							
1043	410	Rosewarne, P.J., Wilson, J.M., Svendsen, J.C., 2016. Measuring maximum and standard metabolic rates using					
1051							
1052	411	intermittent-flow respirometry: A student laboratory investigation of aerobic metabolic scope and					
1053	412	environmental hypoxia in aquatic breathers. J. Fish Biol. 88, 265–283, https://doi.org/10.1111/ifh.12795					
1054	112						
1055	112	Sacar S.M. 2000 Specific dynamic action: A raviau of the nectorendial metabolic response. I Comp. Dhysiol. P.					
1050	415	Secor, S.M., 2009. Specific dynamic action. A feview of the postprandial metabolic response. J. Comp. Physiol. B					
1057	414	Biochem. Syst. Environ. Physiol. 179, 1-56. https://doi.org/10.1007/s00360-008-0283-7					
1059							
1060	415	Skov, P.V., Duodu, C.P., Adjei-Boateng, D., 2017. The influence of ration size on energetics and nitrogen retention					
1061							
1062		19					
1063							
1004							

1065								
1066								
1067	416	in tilapia (Oreochromis niloticus). Aquaculture 473, 121–127.						
1068								
1069	417	https://doi.org/10.1016/j.aquaculture.2017.02.007						
1070								
1072	418	Skov, P.V., Larsen, B.K., Frisk, M., Jokumsen, A., 2011. Effects of rearing density and water current on the						
1073	410	nominatory, physical acts and have stale as in might be transf. On each making my bigs of high terms another						
1074	417	respiratory physiology and naematology in rambow trout, Oncornynchus mykiss at high temperature.						
1075	420	Aquaculture 319, 446–452. https://doi.org/10.1016/j.aquaculture.2011.07.008						
1076								
1077	421	Steffensen, J.F., 1989. Errors in aquatic respirometry 1989. Fish Physiol. Biochem.						
1078								
1079	422	Stieglitz J.D. Benetti D.D. Grosell M. 2018 Nutritional physiology of mahi-mahi (Coryphaena hippurus):						
1081		2						
1082	423	Postprandial metabolic response to different diets and metabolic impacts on swim performance. Comp.						
1083	474	Biochem Physiol -Part A Mol Integr Physiol 215 28-34 https://doi.org/10.1016/j.chpa.2017.10.016						
1084	121	Dioenem: 1 hysioi. 1 at 17 1461. http:// hysioi. 215, 26 54. https://doi.org/10.1010/j.copu.2017.10.010						
1085	125	Triantafullidis A Apostolidis A D Katsaras V Kally E Marcar I Hughas M Jarstad K E Tsalou A						
1086	423	manalymuis, A., Apostonuis, A.I., Katsates, V., Keny, E., Mercer, J., Hugnes, M., Jørstau, K.E., Tsolou, A.,						
1007	426	Hynes, R., Triantaphyllidis, C., 2005. Mitochondrial DNA variation in the European lobster (Homarus						
1089	107	commence) through out the range Mar Diel 14(202 225 https://doi.org/10.1007/-00227.004.1425.2						
1090	427	gammarus) inroughout the range. Mar. Biol. 146, $223-235$. https://doi.org/10.100//s00227-004-1435-2						
1091	100							
1092	428	Van Leeuwen, T.E., Rosenfeld, J.S., Richards, J.G., 2012. Effects of food ration on SMR: Influence of food						
1093	429	consumption on individual variation in metabolic rate in juvenile coho salmon (Onchorhynchus kisutch). J.						
1094	100							
1096	430	Anim. Ecol. 81, 395–402. https://doi.org/10.1111/j.1365-2656.2011.01924.x						
1097								
1098	431	Wallace, J.C., 1973. Feeding, starvation and metabolic rate in the shore crab Carcinus maenas. Mar. Biol. 20, 277–						
1099	432	281. https://doi.org/10.1007/BF00354271						
1100								
1101	433	Wang, X., Li, E., Chen, L., 2016. A Review of Carbohydrate Nutrition and Metabolism in Crustaceans, N. Am, J.						
1102		······································						
1104	434	Aquac. 78. https://doi.org/10.1080/15222055.2016.1141129						
1105								
1106	435	Whiteley, N.M., AL-Wassia, A.H., Taylor, E.W., 1990. The effect of temperature, aerial exposure and disturbance						
1107	436	on oxygen consumption in the lobster. Homarus gammarus (I) Mar Behav Physiol 17, 213-222						
1108	-00	on oxygen consumption in the looster, <i>nomarus gummarus</i> (1.). Mai: Denay: 1 hysioi: 17, 215–222.						
11109	437	https://doi.org/10.1080/10236249009378772						
1111								
1112	438	Whiteley, N.M., Robertson, R.F., Meagor, J., El Haj, A.J., Taylor, E.W., 2002. Protein synthesis and specific						
1113	400	the sector is sector of the formation of the Distance Dis						
1114	439	dynamic action in crustaceans: effects of temperature. Comp. Blochem. Physiol. Part A Mol. Integr. Physiol.						
1115	440	128, 593-604. https://doi.org/10.1016/s1095-6433(00)00337-8						
1110								
1118		20						
1119		20						
1120								

1121		
1122		
1123	1/1	Wicking J.F. 1085 Ammonia production and ovidation during the culture of marine provens and lobsters in
1124	441	wickins, J.F., 1765. Annionia production and oxidation during the culture of marine prawits and loosters in
1125	442	laboratory recirculation systems. Aquae, Eng. 4, 155–174, https://doi.org/10.1016/0144-8609(85)90011-1
1126	• • • •	
1127		
1128	443	Williams, K.C., 2007. Nutritional requirements and feeds development for post-larval spiny lobster: A review.
1120		
1120	444	Aquaculture 263, 1–14. https://doi.org/10.1016/j.pain.2007.10.001
1404		
1131		
1132		
1133		
1134		
1135		
1136		
1137		
1138		
1139		
1140		
1141		
1142		
1143		
1144		
1145		
1146		
1147		
1148		
1149		
1150		
1151		
1152		
1152		
1155		
1104		
1155		
1150		
1157		
1158		
1159		
1160		
1161		
1162		
1163		
1164		
1165		
1166		
1167		
1168		
1169		
1170		
1171		
1172		
1173		
1174		
1175		
1176		

Table 1. Formulation and chemical composition of experimental diets.

1100									
1181		Protein level	400 g kg	-1		500 g kg	1		
1182		L:CHO ratio	Low	Medium	High	Low	Medium	High	Krill
1183		Ingredients (g kg ⁻¹)							
1184		Antarctic krill							1000.0
1185		Fish meal ^a	150.0	150.0	150.0	150.0	150.0	150.0	
1186		Squid meal ^b	125.0	125.0	125.0	255.0	255.0	255.0	
1187		Krill meal [°]	250.0	250.0	250.0	200.0	200.0	200.0	
1188		Wheat gluten ^d	20.0	20.0	20.0	50.0	50.0	50.0	
1190		Wheat meal ^e	172.5	172.5	172.5	172.5	172.5	171.5	
1191		Wheat starch ^f	229.0	171.0	89.0	141.0	93.0	30.0	
1192		Fish oil ^g	22.0	80.0	160.0	0.0	48.0	112.0	
1193		Soy lecithin ^h	10.0	10.0	10.0	10.0	10.0	10.0	
1194		Vitamin & minerals premix i	20.0	20.0	20.0	20.0	20.0	20.0	
1195		Astaxanthin ^j	1.5	1.5	1.5	1.5	1.5	1.5	
1196		Proximal composition (g kg ⁻¹ as fed,)						
1197		Moisture	78.0	81.0	82.0	86.0	81.0	71.0	916.1
1198		Ash	68.1	68.0	66.2	68.70	68.2	66.3	11.6
1200		Protein	400.0	397.0	385.0	497.0	495.0	481.0	58.2
1200		Lipids	107.0	147.0	233.0	85.8	119.0	172.0	9.6
1202		Carbohydrates x	346.9	307.0	233.8	262.5	236.8	209.7	4.5
1203		L:CHO ratio	0.3	0.5	1.0	0.3	0.5	0.8	2.1
1204		Gross energy (KJ. g ⁻¹) ^y	19.0	19.8	21.6	18.7	19.5	20.8	1.8
1205		Protein/Energy (g MJ-1)	21.0	20.1	17.8	26.5	25.4	23.1	32.6
1206									
1207	117	a Microporco: 70 00/ CD 9 70/	CE Trop	aga Fiskoin	ductri AS	Norwow			

447 ^a Micronorse: 70.9% CP, 8.7% CF, Tromsø Fiskeindustri AS, Norway.
1208

- 1209 448 ^b Squid meal: 83% CP, 4% CF, Sopropêche, France.
- **449** ° Krill meal: 61.1% CP, 17.4% CF, Aker Biomarine, Norway.
- ¹²¹³ 1214 **450** ^d VITAL: 80.4% CP, 5.6% CF, Roquette, France.
- **451** ^e Wheat meal: 11.7% CP, 1.6% CF, Molisur, Spain. 1217
- ¹²¹⁸ 1219 452 ^f Meritena 200: 0.4% CP, 0.1% CF, 90% starch, Tereos, France.
- 1221 **453** ^g Fish oil: 98.1% CF, 16% EPA, 12% DHA, Sopropêche, France.
- **454** ^h P700IPM, Lecico GmbH, Germany.

1225i Vitamins (IU or mg kg⁻¹ diet): DL-alpha tocopherol acetate, 200 mg; sodium menadione bisulphate, 50 mg; retinyl

456 acetate, 40000 IU; DL-cholecalciferol, 4000 IU; thiamine, 60 mg; riboflavin, 60 mg; pyridoxine, 40 mg;

1234 1235457cyanocobalamin, 0.2 mg; nicotinic acid, 400 mg; folic acid, 30 mg; ascorbic acid, 1000 mg; inositol, 1000 mg; biotin,1237 12384586 mg; calcium pantothenate, 200 mg; choline chloride, 2000 mg, betaine, 1000 mg. Minerals (g or mg kg ⁻¹ diet):1239 1239459copper sulphate, 18 mg; ferric sulphate, 12 mg; potassium iodide, 1 mg; manganese oxide, 20 mg; sodium selenite,1241 12414600.02 mg; zinc sulphate, 15 mg; sodium chloride, 800 mg; excipient wheat gluten, Premix Lda., Portugal.1242 1242461 ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland.1244 1246 1246462 ^x Carbohydrate (%) = 100 - (Crude protein % + crude lipid % + moisture % + ash %)1247 1248 1246463 ^y Gross energy (MJ kg ⁻¹) = Protein content × 21.3 kJ g ⁻¹ + Lipid content × 39.5 kJ g ⁻¹ + Carbohydrate content × 17.61249 1250464kJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997)1251 125212561256 125612671261 126212621262 12631264126412621265 1266126712641262126512661266126712681267
 457 eyanocobalamin, 0.2 mg; nicotinic acid, 400 mg; folic acid, 30 mg; ascorbic acid, 1000 mg; inositol, 1000 mg; botin, 458 6 mg; calcium pantothenate, 200 mg; choline chloride, 2000 mg, betaine, 1000 mg. Minerals (g or mg kg⁻¹ diet): copper sulphate, 18 mg; ferric sulphate, 12 mg; potassium iodide, 1 mg; manganese oxide, 20 mg; sodium selenite, 0.02 mg; zinc sulphate, 15 mg; sodium chloride, 800 mg; excipient wheat gluten, Premix Lda., Portugal. 460 0.02 mg; zinc sulphate, 15 mg; sodium chloride, 800 mg; excipient wheat gluten, Premix Lda., Portugal. 461 ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. 462 * Carbohydrate (%) = 100 - (Crude protein % + crude lipid % + moisture % + ash %) 463 * Gross energy (MJ kg⁻¹) = Protein content × 21.3 kJ g⁻¹ + Lipid content × 39.5 kJ g⁻¹ + Carbohydrate content × 17.6 464 kJ g⁻¹) / 1000 kJ MJ⁻¹ (Cuzon and Guillaume, 1997) 465 465 466 467 468 468 469 469 460 460 460 460 460 461 462 462 463 464 464 465 465 465 465 466 466 467 468 468 469 469 469 469 460 460 460 460 461 462 461 462 462 463 464 465 465 465 466 466 467 468 468 468 469 469 469 469 469 469 469 460 460 461 462 463 464 465 465 465 466 466 467 468 468 468 469 469 469 469 469 469
12374586 mg; calcium pantothenate, 200 mg; choline chloride, 2000 mg, betaine, 1000 mg. Minerals (g or mg kg ⁻¹ diet):1239459copper sulphate, 18 mg; ferric sulphate, 12 mg; potassium iodide, 1 mg; manganese oxide, 20 mg; sodium selenite,12414600.02 mg; zinc sulphate, 15 mg; sodium chloride, 800 mg; excipient wheat gluten, Premix Lda., Portugal.1242461 ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland.1244462* Carbohydrate (%) = 100 – (Crude protein % + crude lipid % + moisture % + ash %)1247463* Gross energy (MJ kg ⁻¹) = Protein content × 21.3 kJ g ⁻¹ + Lipid content × 39.5 kJ g ⁻¹ + Carbohydrate content × 17.61249464kJ g ⁻¹) / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997)12511254125512561256125712581254125912611261126212611262126112611262126112631261126412611265126612661267
 459 copper sulphate, 18 mg; ferric sulphate, 12 mg; potassium iodide, 1 mg; manganese oxide, 20 mg; sodium selenite, 460 0.02 mg; zinc sulphate, 15 mg; sodium chloride, 800 mg; excipient wheat gluten, Premix Lda., Portugal. 461 ⁱ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. 462 ^x Carbohydrate (%) = 100 – (Crude protein % + crude lipid % + moisture % + ash %) 463 ^y Gross energy (MJ kg⁻¹) = Protein content × 21.3 kJ g⁻¹ + Lipid content × 39.5 kJ g⁻¹ + Carbohydrate content × 17.6 464 kJ g⁻¹ / 1000 kJ MJ⁻¹ (Cuzon and Guillaume, 1997) 453 454 455 456 456 457 458 458
1240 460 0.02 mg; zinc sulphate, 15 mg; sodium chloride, 800 mg; excipient wheat gluten, Premix Lda., Portugal. 1242 i ² Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. 1244 i ² Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. 1244 i ³ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. 1244 i ⁴ Carobhydrate (%) = 100 - (Crude protein % + crude lipid % + moisture % + ash %) 1247 i ⁴ Gross energy (MJ kg ⁻¹) = Protein content × 21.3 kJ g ⁻¹ + Lipid content × 39.5 kJ g ⁻¹ + Carbohydrate content × 17.6 1248 464 kJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1250 i ⁴ KJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1251 i ⁵ KJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1256 i ⁵ KJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1257 i ⁵ KJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1258 i ⁵ KJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1260 i ⁵ KJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1261 i ⁵ KJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1262 i ⁵ KJ g ⁻¹ / 1000
 461 ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Garophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Garophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Garophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Garophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Garophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Garophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland. ¹ Garophyll Pink 10% CWS, 10%
 461 - Catophyn Pink 10% CwS, 10% astatannin, DSM Nutritional Products, Switzerland. 462 * Carbohydrate (%) = 100 - (Crude protein % + crude lipid % + moisture % + ash %) 463 * Gross energy (MJ kg⁻¹) = Protein content × 21.3 kJ g⁻¹ + Lipid content × 39.5 kJ g⁻¹ + Carbohydrate content × 17.6 464 kJ g⁻¹) / 1000 kJ MJ⁻¹ (Cuzon and Guillaume, 1997) 1251 1252 1253 1254 1255 1256 1257 1258 1260 1261 1262 1263 1264 1264 1265 1265 1266 1266 1266 1267 1268
1245 462 * Carbohydrate (%) = 100 - (Crude protein % + crude lipid % + moisture % + ash %) 1247 7 1248 463 * Gross energy (MJ kg ⁻¹) = Protein content × 21.3 kJ g ⁻¹ + Lipid content × 39.5 kJ g ⁻¹ + Carbohydrate content × 17.6 1249 464 kJ g ⁻¹) / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1251 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1252 1253 1254 1254 1255 1255 1256 1257 1258 1259 1260 1261 1261 1262 1263 1264 1264 1264 1265 1264 1264 1264 1265 1264 1266 1264 1268 1268
 463 ^v Gross energy (MJ kg⁻¹) = Protein content × 21.3 kJ g⁻¹ + Lipid content × 39.5 kJ g⁻¹ + Carbohydrate content × 17.6 464 kJ g⁻¹ / 1000 kJ MJ⁻¹ (Cuzon and Guillaume, 1997) 454 455 455 456 457 458 459 450 450 451 452 453 454 454 455 454 454 455 454 454 455 454 454 454 454 454 455 454 455 454 454 454 455 454 <li< td=""></li<>
1248 463 > Gross energy (MJ kg ⁻¹) = Protein content × 21.3 kJ g ⁻¹ + Lipid content × 39.5 kJ g ⁻¹ + Carbohydrate content × 17.6 1249 464 kJ g ⁻¹) / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1251 1252 1253 1254 1254 1255 1255 1255 1256 1256 1257 1258 1258 1259 1260 1261 1262 1263 1264 1264 1265 1264 1266 1264 1267 1268
1249 464 kJ g ⁻¹ / 1000 kJ MJ ⁻¹ (Cuzon and Guillaume, 1997) 1251 1 1252 1 1253 1 1254 1 1255 1 1256 1 1257 1 1258 1 1259 1 1260 1 1261 1 1262 1 1263 1 1264 1 1265 1 1266 1 1267 1 1268 1
1251 1252 1253 1254 1255 1256 1257 1258 1259 1260 1261 1262 1263 1264 1265 1266 1267 1268
1252 1253 1254 1255 1256 1257 1258 1259 1260 1261 1262 1263 1264 1265 1266 1267 1268
1253 1254 1255 1256 1257 1258 1259 1260 1261 1262 1263 1264 1265 1266 1267 1268
1254 1255 1256 1257 1258 1259 1260 1261 1262 1263 1264 1265 1266 1267 1268
1250 1257 1258 1259 1260 1261 1262 1263 1264 1265 1266 1267 1268
1257 1258 1259 1260 1261 1262 1263 1264 1265 1266 1267 1268
1258 1259 1260 1261 1262 1263 1264 1265 1266 1267 1268
1259 1260 1261 1262 1263 1264 1265 1266 1267 1268
1260 1261 1262 1263 1264 1265 1266 1267 1268
1261 1262 1263 1264 1265 1266 1267 1268
1263 1264 1265 1266 1267 1268
1264 1265 1266 1267 1268
1265 1266 1267 1268
1266 1267 1268
1267 1268
1200
1269
1270
1271
1272
1273
1274
1275
1277
1278
1279
1280
1281
1283
1284
1285
1286 23
1287

Table 2. Overview of pre and postprandial metabolism responses for European lobsters fed six different experimental and one control diet.

Protein (g.kg ⁻¹)	L:CHO	SMR ($\mu g O_2 g^{-1} h^{-1}$)	SDA_{peak} (µg O ₂ g ⁻¹ h ⁻¹)	Scope	$SDA_{up}(h)$	SDA_{cost} (µg O_2 g ⁻¹)	$SDA_{dur}(h)$	SDA_{coef} (%)	Meal energy (J)	~
400	Low	75.6 ± 10.4 ^{ab}	249.4 ± 44.6	3.3 ± 0.4 *,a	9.0 ± 1.5	2079.6 ± 554.4	23.6 ± 0.5 *,a	3.9 ± 1.5	580.0 ± 130.2 *	41
	Medium	51.2 ± 6.1 *,bc	137.6 ± 16.6	$2.7\pm0.2^{\rm ~ab}$	10.3 ± 0.9	704.2 ± 291.3	19.0 ± 1.7 ^{ab}	6.1 ± 2.5	245.1 ± 55.3	-
	High	42.7 ± 5.0 *,c	135.1 ± 27.2	3.2 ± 0.5 *,a	10.8 ± 1.3	581.9 ± 229.6	20.0 ± 2.2 ^{ab}	4.8 ± 3.6	278.9 ± 99.1	
500	Low	97.9 ± 11.9 ª	214.0 ± 33.1	$2.2 \pm 0.2^{\text{b}}$	9.8 ± 0.7	1099.3 ± 247.0	18.0 ± 0.4 b	3.5 ± 0.6	445.1 ± 160.9	
	Medium	80. 7 ± 4.0^{a}	224.2 ± 45.5	2.7 ± 0.5^{b}	11.3 ± 1.2	1683.8 ± 750.5	20.7 ± 0.8 ^{ab}	3.4 ± 1.2	473.0 ± 42.7 *	
	High	73.1 ± 6.5^{a}	149.5 ± 22.5	2.0 ± 0.2^{b}	9.6 ± 0.7	621.0 ± 233.7	17.2 ± 1.3 ^{ab}	2.5 ± 1.0	438.7 ± 110.1	
Control (Krill)	_	107.3 ± 19.3	186.3 ± 17.6	1.9 ± 0.2	7.6 ± 0.2	779.7 ± 229.5	17.2 ± 1.4 ^{ab}	7.0 ± 1.7	103.6 ± 5.6	
¹ One-Way ANC	NA AV	$F_{6,37}=5.27^{**}$	$F_{6,37}=1.99$	$F_{6,37}=2.83^*$	$F_{6,37}=1.50$	$F_{6,37}=1.45$	$F_{6,37}=2.90^{*}$	$F_{6,37}=0.58$	$F_{6,37}=2.76^*$	
² Two-Way ANC	AVC									
Ρ		F _{1,32} =17.70***	$F_{1,32}=0.63$	$F_{1,32}=7.69^{**}$	$F_{1,32}=0.39$	$F_{1,32}=0.39$	$F_{1,32}=4.50^{*}$	$F_{1,32}=0.51$	$F_{1,32}=1.19$	
L:CHO		$F_{2,32}=6.79^{**}$	$F_{2,32}=3.39^*$	$F_{2,32}=0.08$	$F_{2,32}=2.82$	$F_{2,32}=2.82$	$F_{2,32}=1.38$	$F_{2,32}=0.21$	$F_{2,32}=0.50$	
P x L:CHO		$F_{2,32}=0.15$	$F_{2,32}=1.73$	$F_{2,32}=1.95$	$F_{2,32}=1.36$	$F_{2,32}=1.36$	$F_{2,32}=4.21^*$	$F_{2,32}=0.13$	$F_{2,32}=2.57$	

alues are mean \pm standard error.

¹ Superscript * indicate dietary groups significantly different from control (Krill).

² Means in the same column with a different superscript letter are significantly different.

* p < 0.05; **p < 0.01; ***p < 0.01

1333 1334	470	Table 3. Dietary effects on nitrogen budgets in juvenile European lobster.						
1335		Protein (g.kg ⁻¹) L:CHO		N _{intake} (µg.mg WW ⁻¹)	N _{excreted} (µg.mg WW ⁻¹)	N _{retention} (%)		
1336 1337		400	Low	806.44 ± 269.46 ª	65.80 ± 43.32	86.02 ± 9.54 ^{ab}		
			Medium	65.32 ± 23.35 *,b	30.41 ± 9.63	41.58 ± 15.47 ^b		
1338			High	42.73 ± 8.50 *,b	55.17 ± 25.91	-22.08 ± 38.85 *,c		
1339		500	Low	839.33 ± 153.47 ª	71.02 ± 13.42	90.85 ± 1.36 ª		
1340			Medium	1166.83 ± 387.53 a	72.78 ± 11.34	91.98 ± 2.17 ª		
1341			High	873.94 ± 298.23 ª	127.34 ± 71.63	87.12 ± 4.49 ª		
1342		Control (Krill)		657.18 ± 74.53	190.85 ± 87.83	73.30 ± 12.11		
1344 1345		¹ One-Way ANOVA		F _{6,33} =17.14***	F _{6,33} =1.00	F _{6,33} =6.96 ***		
		² Two-Way ANOVA						
1346		P L:CHO P x L:CHO		F _{1,29} =52.21***	F _{1,29} =2.71	F _{1,29} =19.03***		
1347				F _{2,29} =9.49**	F _{2,29} =0.07	F _{2,29} =6.68**		
1348				F _{2,29} =9.64**	F _{2.29} =0.38	F _{2,29} =5.79**		

1349 471 Values are mean ± standard error.

1351 472 ¹ Superscript * indicate dietary groups significantly different from control (krill).

 $\begin{array}{c} 1352 \\ 1353 \end{array} \quad {}^{2} \text{ Means in the same column with different superscript letter are significantly different.} \end{array}$

474 * p < 0.05; **p < 0.01; ***p < 0.001

Ν

Fig. 1. Cumulative molting of European lobster juveniles (% of initial numbers) fed the different diets.

Fig. 2. Specific growth rate (A) and carapace length increment (B) after a 4-week period for European lobster juveniles fed on different diets. Data represents the mean \pm SEM of 10 animals per treatment. Dietary treatments that were significantly different from control (krill) are marked with an asterisk. Different letters indicate significant differences between experimental formulated diets.

Fig. 3. The effect of dietary treatment on voluntary feed intake. Estimated individual feed intake values from both respirometry and nitrogen excretion trials were pooled. The box includes observations from the 25th to the 75th percentile and the whiskers above and below the box indicate the 10th and 90th percentiles. The horizontal line within the box represents the median value and the symbol (+) indicates the mean. Dietary treatments significantly different from control (krill) are marked with an asterisk. Different letters indicate significant differences among experimental formulated diets.

Fig. 4. Representative plots of pre and post-feeding metabolic rates (μ g O₂ g⁻¹ h⁻¹) over time in lobsters fed experimental (A, B, and C) and control (D) diets. The solid line represents SMR and the dashed line represents SMR + 15%. The lobsters were fed at 0h (vertical dashed line). The SDA variables accounting for MO2 postprandial metabolism are visually explained in panel D.

Fig. 5. The effect of dietary treatment on the standard metabolic rate of European lobster juveniles. The box includes observations from the 25th to the 75th percentile and the whiskers above and below the box indicate the 10th and 90th percentiles. The horizontal line within the box represents the median value and the symbol (+) indicates the mean. Dietary treatments significantly different from control (krill) are marked with an asterisk. Different letters indicate significant differences among experimental formulated diets.











Declaration of interests

 \boxtimes The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit / Author	Renata Goncalves	Ivar Lund	Manuel Gesto	Peter Vilhelm Skov
Conceptualization	х	Х	Х	Х
Methodology	х	Х	х	Х
Validation	х			Х
Formal analysis	х			Х
Investigation	х			
Resources		Х	х	Х
Data Curation	Х			
Writing – Original draft	Х			
Writing – Review and Editing		Х	х	Х
Visualization	Х			
Supervision		Х	Х	Х
Project administration		Х	х	
Funding acquisition		х		