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Original Research Article

Fatty acid profile of cooked leg meat and raw hepatopancreas of red king crab (*Paralithodes camtschaticus*) during three-month live holding without feeding at 5 and 10 °C

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ABSTRACT

The red king crab (RKC, *Paralithodes camtschaticus*) is a highly-valued decapod species. Typically, RKCs undergo a period of live holding (LH), often without feeding, in onshore facilities, allowing for flexible management before export to destination markets. This study aimed to (*i*) gain information on the fatty acid (FA) profile of the cooked leg meat and raw hepatopancreas obtained from RKC harvested in Norwegian waters of the Barents Sea and (*ii*) investigate how these FA profiles are affected by LH without feeding for up to 92 days at 5 or 10 °C. Minor changes were observed in the FA profile of cooked leg meat, which retained its nutritional value in omega−3 FA content. In contrast, the FA composition of raw hepatopancreas was severely affected by the LH time, with substantial changes occurring especially between 41 and 62 days at 10 °C and between 62 and 92 days at 5 °C. Saturated and specific monounsaturated FAs (16:1*n*−7*c* and 18:1*n*−9*t*), as well as 22:5*n*−3*c*, were preferentially utilized at the beginning of the starvation period, followed by the mobilization of C_{18-22} unsaturated FAs. Long-chain highly-unsaturated FAs were preferentially retained during LH, especially 20:4*n*−6*c* and 20:5*n*−3*c*. The information emerging from the present study may be practically exploited for selecting or designing suitable feed for RKC during LH at different temperatures.

1. Introduction

The red king crab (RKC, *Paralithodes camtschaticus*) is a decapod species of paramount commercial importance, caught in Arctic waters but known and appreciated worldwide for its sensory and nutritional properties (Voldnes et al., 2020). Nowadays, most of the RKC harvested along the Norwegian coastal area of the Barents Sea is exported to North American and Asian markets as live crabs (Lian et al., 2021b). Before export, it is common practice for the Norwegian crab industry to stock RKCs in onshore facilities for extended periods (Lorentzen et al., 2018). This enables flexible management and maximizes profitability as it ensures a reliable and consistent supply when the demand and the market prices are high (Lorentzen et al., 2019). Furthermore, operative costs can be reduced when live holding (LH) is carried out without feeding (Lorentzen et al., 2020; Woll & Berge, 2007).

The RKC is a cold-water-adapted species typically found within a temperature span of 2–7 °C (Loher & Armstrong, 2005) but tolerant to a wider temperature range during seasonal migrations (Pinchukov & Sundet, 2011). Water temperature is considered the main factor determining the energetic balance of RKC (Nilssen & Sundet, 2006) as higher temperatures are associated with lower dissolved oxygen levels (Coates & Söderhäll, 2020) and elevated metabolic rates, leading to higher oxygen consumption (Siikavuopio & James, 2015). It has been reported that adult RKC displays a temperature *preferendum* in the coldest end of the gradient (2.5–3.5 °C) (Christiansen et al., 2015). During LH, it is desirable to maintain the metabolic rate of RKC as low as possible; however, temperature-controlled conditions would incur additional costs related to chilling of seawater, which normally ranges 4–10 °C (March–October) along the Norwegian coast of the Barents Sea (Ozhigin et al., 2011).

Typically, the edible part of RKC mainly consists of the meat found in the appendages (i.e., the muscle of the chelipeds and walking legs). In

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addition, in some Asian countries, the hepatopancreas and reproductive organs located in the carapace are consumed as a special delicacy (Voldnes et al., 2016).

The nutritional content of the meat from crab appendages is characterized by high-quality protein, low fat content, and a high level of polyunsaturated fatty acids (PUFAs) (Barrento et al., 2009). PUFAs and especially omega-3 fatty acids are recommended as part of a healthy diet as they carry health-promoting and disease-preventative properties (Larsen et al., 2011).

The hepatopancreas, also referred to as the perigastric organ or digestive gland, is a crucial multifunctional organ (Ponomareva et al., 2021), with primary roles in nutrient uptake and metabolism of intermediary carbon and nitrogen compounds (Rőszer, 2014). Moreover, the hepatopancreas functions as a depot of lipid reserves (Cervellione et al., 2017) which can be utilized and mobilized to target tissues during starvation periods (Sánchez-Paz et al., 2006).

Among lipids, fatty acids serve as both a high-density energy source and essential components of cell membranes as well as precursors of eicosanoids and other biologically active substances (Tocher, 2010). In this way, they play a fundamental role in the growth and survival of crustaceans, especially considering that several decapod species have limited ability to synthesize *de novo* the omega−6 and omega−3 fatty acid families (D'Abramo, 1997). Experimentally induced starvation can reveal the pattern of fatty acid retention and depletion, which may vary considerably among crustaceans (Sacristán et al., 2017). Nonetheless, to the best of our knowledge, studies on the alteration in the fatty acid composition of crab tissues during extended starvation periods are limited to species such as female swimming crab (*Portunus trituberculatus*) (Ding et al., 2017) and juvenile Chinese mitten-handed crab (*Eriocheir sinensis*) (Wen et al., 2006).

The present study was undertaken to (*i*) gain information on the fatty acid profile of the cooked leg meat and raw hepatopancreas obtained from RKC harvested in a Norwegian coastal area of the Barents Sea and (*ii*) investigate how these fatty acid profiles are affected by time and temperature of LH without feeding, with focus on their nutritional value and catabolic pattern, respectively, in the cooked leg meat and raw hepatopancreas. Intermolt RKCs with high meat content were kept without feeding in containers with seawater at two temperatures (5 and 10 °C) for up to 92 days (Lorentzen et al., 2019). Proximate and fatty acid composition were determined on the cooked leg meat and in the raw hepatopancreas obtained from RKCs sampled at the start (i.e., day 0, also referrable to as wild RKCs) and after 41, 62, and 92 days of LH without feeding in both temperature groups.

2. Material and methods

2.1. Harvest and live holding

The present study was carried out on samples obtained from the RKCs in the LH experiment reported by Lorentzen et al. (2019).

Briefly, in December 2016, adult male RKCs were harvested along the northern Norwegian coastal area of the Barents Sea. The RKCs (N = 78, average weight 2.4 kg (± 0.3), range 2.2–2.8 kg) were in the intermolt stage and with high leg meat content (i.e., the cooked meat occupied 87 % of the cross-sectional area of the middle of the *merus,* the most proximal article of the walking legs) at the time of harvest. After an acclimatization period of seven days in tanks with running seawater at 4 °C, a LH study at two temperatures was performed by equally distributing and holding the crabs (n = 60) in six circular tanks (volume 0.7 m^3) supplied with circulating seawater (34 ‰ salinity) at 5 °C (± 0.2, three tanks, n = 10 in each tank) and 10 °C (± 0.2, three tanks, n = 10 in each tank). The seawater was continuously UV treated, filtered through a 150 μm sand filter, and circulated at a flowing rate of 4 $dm^3\ min^{-1}$ (kg crab)$^{-1}$. A temperature of 5 °C represents a near-optimal temperature for the RKC, while 10 °C is close to the highest temperature to which the RKC can be exposed during LH. The crabs were not fed during the LH period.

The LH experiment was performed at the Aquaculture Research Station in Kårvika (Tromsø), where the RKCs were exposed to the natural light cycle and intensity for the time of the year. No cannibalism was observed during the experiment.

The processing and sampling of the RKCs was performed at the start (day 0, n = 18) and at day 41 (n = 9 from the group at 5 °C; n = 9 from the group at 10 °C), 62 (n = 9, 5 °C; n = 9, 10 °C) and 92 (n = 11, 5 °C; n = 7, 10 °C) of the LH study. In total, one and five RKCs perished, respectively, in the 5 °C and 10 °C group, between day 62 and 92 of LH. The molting process was completed by six RKCs held at 10 °C for 62 days, three RKCs held at 5 °C for 92 days, and four RKCs held at 10 °C for 92 days. The samples obtained from molted RKCs were not included in the present study.

The described experiment had been approved by the local responsible laboratory animal science specialist under the surveillance of the Norwegian Animal Research Authority and registered by the Authority, thereby conforming to Directive 2010/63/EU.

2.2. Processing and sample preparation

The RKCs were processed following the procedure that reflects industrial protocols described by Lorentzen et al. (2019). Briefly, the RKCs were weighed and split into two sections (i.e., clusters) from which the chelipeds (i.e., the limbs bearing the claws) were removed and weighed. After splitting, the hepatopancreas was immediately collected from the carapace, weighed, and packed into polypropylene test tubes for storage at −80 °C. The headspace of the tubes was flushed with nitrogen gas before freezing.

The clusters were cooked in boiling water for 16 min reaching a core temperature of 92 °C in the *merus* of the largest walking leg of the cluster. The temperature was monitored using K-type thermocouples connected to data loggers (model 175H1, Testo Ltd., Hampshire, UK) placed in the geometric center of the *merus*.

Afterward, the clusters were cooled in ice water with 3.5 % NaCl (w/v) for 21 min until the core temperature was below 4 °C. Samples of cooked leg meat were extracted from the middle part of the *merus* of the second walking leg and packed in polypropylene test tubes, the headspace of which was flushed with nitrogen as described above.

For the sake of simplicity, hereinafter, the samples "raw hepatopancreas" and "cooked leg meat" are referred to as "hepatopancreas" and "leg meat", respectively.

2.3. Hepatosomatic index and cheliped index

The hepatosomatic index (HSI) and the cheliped index (CI) were calculated as:

$$HSI = (W_{Hepatopancreas} / W_{Crab}) \times 100 \quad (1)$$

$$CI = (W_{Chelipeds} / W_{Crab}) \times 100 \quad (2)$$

where $W_{Hepatopancreas}$ is the weight of the raw hepatopancreas, $W_{Chelipeds}$ is the weight of the two raw chelipeds, and W_{Crab} is the live RKC weight.

2.4. Analytical determinations

2.4.1. Proximate analysis

The moisture and the ash content were determined according to standard AOAC (2000) and ISO (1998) methods, respectively. The nitrogen content was quantified using a LECO TruMac analyzer (LECO Corp., St. Joseph, MI, USA), and the protein content was calculated applying a conversion factor of 6.25. The fat content was determined by pulse nuclear magnetic resonance on dried samples equilibrated at 50 °C in a heating block. The proximate analysis was performed on the leg meat and hepatopancreas extracted from four individuals in the wild and day 92/5 °C groups and three individuals in the day 92/10 °C group.

The results were expressed as percentages of wet sample weight.

2.4.2. Fatty acid profile

Fatty acid profile analysis was conducted at the Institute of Food & Health of the University College Dublin (UCD, Belfield, Dublin, Ireland). The leg meat and hepatopancreas samples were shipped in polypropylene test tubes placed in boxes with dry ice, which reached UCD within 16 h. Upon arrival, the samples were immediately stored at −80 °C. Before analysis, the samples were partially thawed by placing the tubes in a refrigerator for 12 h at 4 °C and mashed using an Ultra-Turrax® homogenizer (IKA-Werke GmbH, Staufen, Germany) at 7500 rpm for 15 s.

The fatty acid profile was determined by gas chromatographic analysis of fatty acid methyl esters (FAMEs) prepared by microwave-assisted derivatization in a microwave reaction system (MRS, model MARS 6™, CEM Corp., Matthews, NC, USA) according to the method described by Brunton et al. (2015). Each sample, corresponding to biological material obtained from a single crab, was analyzed in triplicate, and a sample aliquot of approximately 3 g was used for each analytical replicate. Reagents of analytical grade (Sigma-Aldrich) were used for the preparation of FAMEs, which started with a saponification step carried out by heating in the MRS. In detail, samples (3 g) were placed in perfluoroalkoxy reaction vessels (capacity 55 mL) to which 10 mL of 2.5 % (w/v) potassium hydroxide in methanol and 0.1 mL of internal standard (IS) solution (10 mg/mL tricosanoic acid in chloroform) were added. Subsequently, the reaction vessels were heated to 130 °C over a 4 min period and held at this temperature for 4 min. After cooling in ice for 5 min, methyl esterification was carried out by adding into the reaction vessels 15 mL of a solution 5 % (v/v) acetyl chloride in methanol and by subsequently heating them in the MRS to 120 °C over a 4 min period and holding them at this temperature for 2 min. After cooling in ice for 5 min, FAMEs were extracted by adding 10 mL of pentane and about 20 mL of a saturated aqueous sodium chloride solution (NaCl) to the reaction vessels. To facilitate FAMEs extraction, the reaction vessels were upended both after the addition of pentane and the saturated NaCl solution. After phase separation, the top layer (i.e., pentane) was aliquoted into 1.5 mL vials containing 0.2 g anhydrous sodium sulfate for analysis using a gas chromatograph (GC) (model Clarus 580, PerkinElmer Inc., Waltham, MA, USA) fitted with a CP-Sil 88 capillary column (100 m × 0.25 mm, 0.2 μm film thickness) (Agilent Technologies Inc., Santa Clara, CA, USA) and a flame ionization detector (FID). The operating conditions of the GC-FID for separation and quantification of FAMEs were set as reported by Gangopadhyay et al. (2017). Briefly, 0.5 μL of the sample was injected at a split ratio of 1:10 (inlet temperature of 250 °C, carrier gas was hydrogen under a constant flow of 1.25 mL min^{-1}) and separated using a CP-Sil 88 (100 m × 0.25 mm internal diameter, 0.2 μm film; Agilent Technologies). The oven temperature was 80 °C with an initial ramp of 6.2 °C min^{-1} to 220 °C which was held for 3.2 min before a final ramp of 6.3 °C min^{-1} to 240 °C, which was held for 6.5 min. The FID temperature was set to 270 °C.

The FAMEs were identified by comparing their retention times with an authentic analytical standard (Supelco® 37 Component FAME Mix, Sigma-Aldrich). The quantification of each fatty acid was based on an internal standard method, using the software TotalChrom (version 6.3.2, PerkinElmer) for peak area integration. The fatty acid content was calculated as:

$$\text{Fatty acid (mg/g sample)} = A_{FAME} / A_{IS} \times W_{IS} / W_{sample} \times 10 \times \text{purity}_{IS} \quad (3)$$

where A_{FAME} and A_{IS} are the peak areas of each FAME and of the IS, W_{IS} and W_{sample} are the weight of the IS (g) and of the sample (g), and purity_{IS} is the purity of the IS.

The fatty acid analysis was conducted on the samples of leg meat and hepatopancreas obtained from five crabs for each combination of LH time and temperature, except for the crabs held at 10 °C for 62 and 92 days in which only three individuals could be sampled due to molting of the other exemplars in the time/temperature group. Unless otherwise specified, the results were expressed as percentage of total detected fatty acids.

2.5. Statistical analysis

The results were reported as mean values (± standard deviation), and the statistical analyses were performed considering each crab as a biological replicate. Significant differences in the HSI, CI, and proximate composition between time/temperature groups were analyzed by one-way analysis of variance (ANOVA) followed by *post-hoc* pairwise multiple comparisons (Scheffé's test) using the software Statistica™ (vers. 13.5, TIBCO Software Inc., Palo Alto, CA, USA).

The general effects of LH time and temperature on the fatty acid profile were investigated by full factorial ANOVA using the general linear model (GLM) procedure in Statistica™. The explanatory variables LH time and temperature were handled as categorical factors. The data obtained from the RKCs sampled at the start of the LH study (i.e., day 0) were not considered for the full factorial ANOVA. Significant differences between groups were assessed by *post-hoc* pairwise multiple comparisons (Scheffé's test).

Besides, a one-way ANOVA followed by *post-hoc* comparisons (Dunnett's test) was carried out on data grouped by the combination of LH time and temperature to highlight significant differences between the results for day 0 and each of the other time/temperature groups.

To amplify data visualization and interpretation, principal component analysis (PCA) was carried out on fatty acid composition data using the package *FactoMineR* in the software R (vers. 4.0.3) (R Core Team, 2020). The PCA was performed considering only the fatty acids that accounted for at least 0.2 % of the total detected fatty acids in each tissue.

All statistical analyses were carried out at a 95 % confidence level ($\alpha = 0.95$).

3. Results

3.1. Hepatosomatic index, cheliped index, and proximate composition

As shown in Table 1, after the 92-day LH without feeding, the hepatopancreas index (HSI) and the cheliped index (CI) decreased significantly ($p \leq 0.05$) in both temperature groups. The proximate composition of the leg meat showed a decrease in protein which was statistically significant only for the RKCs held at 10 °C, accompanied by a significant reciprocal increase in moisture. At the same time, during

Table 1
Hepatosomatic index (HSI) and cheliped index (CI) of red king crabs sampled at the start and end of the live holding period, reported together with the proximate composition (%) of their cooked leg meat and raw hepatopancreas.

		Live holding time and temperature		
		Day 0	Day 92	
			5 °C	10 °C
HSI		4.2 ± 1.0^{a}	2.0 ± 0.4^{b}	1.4 ± 0.1^{b}
CI		13.1 ± 0.6^{a}	10.4 ± 1.3^{b}	11.5 ± 1.1^{b}
Proximate composition (%)				
Cooked leg meat	Moisture	78.5 ± 0.7^{c}	80.8 ± 1.2^{b}	82.9 ± 0.6^{a}
	Protein	18.0 ± 1.4^{a}	17.2 ± 0.9^{a}	14.8 ± 0.7^{b}
	Fat	0.79 ± 0.07	0.67 ± 0.02	0.69 ± 0.08
	Ash	1.7 ± 0.2	1.7 ± 0.3	1.4 ± 0.2
Raw hepatopancreas	Moisture	61.1 ± 3.6^{b}	79.1 ± 0.3^{a}	80.2 ± 0.8^{a}
	Protein	13.1 ± 0.6	14.6 ± 0.1	13.9 ± 0.1
	Fat	21.6 ± 3.8^{a}	0.9 ± 0.1^{b}	1.2 ± 0.0^{b}
	Ash	1.9 ± 0.3	2.5 ± 0.2	2.0 ± 0.1

Note. Results are expressed as mean values (± standard deviation). Different superscript letters within the same row indicate significantly different mean values ($p \leq 0.05$, one-way ANOVA followed by Scheffé's test).

LH, the fat content of the hepatopancreas decreased dramatically (by 95 %) in the RKCs held at both temperatures, also in this case compensated by an increase in the moisture content, while the protein levels remained unchanged.

3.2. Fatty acid composition

Among the fatty acids assayed in the leg meat of wild RKCs (i.e., LH day 0), polyunsaturated fatty acids (PUFAs) were present in the highest proportion (> 50 %), followed by monounsaturated (MUFAs) and saturated fatty acids (SFAs) (Table 2). The most abundant fatty acids (i.e., ≥ 2 % of the total detected fatty acids) were palmitic (16:0), stearic (18:0), palmitoleic (16:1*n*−7*c*), oleic (OLA, 18:1*n*−9*c*), *cis*-vaccenic (18:1*n*−7*c*), arachidonic (ARA, 20:4*n*−6*c*), eicosapentaenoic (EPA, 20:5*n*−3*c*), and docosahexaenoic (DHA, 22:6*n*−3*c*) acid.

The fatty acid profile of the hepatopancreas of wild RKCs was characterized by a proportion of SFAs similar to that found in the leg meat but with higher MUFA and lower PUFA levels (Table 3). In addition to the ones listed for the leg meat, the most abundant fatty acids in the hepatopancreas included gondoic (20:1*n*−9*c*), linoleic (LA, 18:2*n*−6*c*), eicosadienoic (20:2*n*−6*c*), and docosapentaenoic (DPA, 22:5*n*−3*c*) acid.

With respect to the most abundant fatty acids, in wild RKCs, the leg meat and hepatopancreas differed mainly in the relative proportions of EPA and PUFAs, which resulted higher in the leg meat, and that of 16:1*n*−7*c*, 18:1*n*−7*c*, and total MUFAs, which were higher in the hepatopancreas.

Among the less abundant fatty acids (i.e., < 2% of total detected fatty acids), the leg meat contained higher relative levels (0.97 % ± 0.11) of heptadecenoic acid (17:1*n*−7*c*) than the hepatopancreas (0.19 % ± 0.07), whereas the opposite was found for myristic (14:0; leg meat 0.87 % ± 0.07, hepatopancreas 1.96 % ± 0.29), elaidic (18:1*n*−9*t*; leg meat 0.36 % ± 0.03, hepatopancreas 0.88 % ± 0.08), dihomo-γ-linolenic (20:3*n*−6*c*; leg meat 0.09 % ± 0.02, hepatopancreas 0.30 % ± 0.03) and docosadienoic (22:2*n*−6*c*; leg meat 0.24 % ± 0.01, hepatopancreas 0.79 % ± 0.06) acid. Notably, γ-linolenic acid (18:3*n*−6*c*) was found only in leg meat (0.15 % ± 0.04).

Considering the most abundant fatty acids detected in the leg meat, the LH without feeding affected only EPA and DHA, which varied significantly as an effect of time. The total amount of fatty acids was significantly affected by the interaction LH time × temperature. Compared to the value observed for wild RKCs, the total fatty acids were significantly lower in the RKCs sampled at 62 days of LH at 10 °C and 92 days of LH at 5 °C.

Compared to the leg meat, the fatty acid composition of the hepatopancreas was more substantially affected by the LH without feeding. In detail, considering the most abundant fatty acids, the relative amount of 18:0, ARA, and total omega−6 fatty acids increased with LH time and temperature under the significant combined effect of these two factors. Furthermore, the interaction LH time × temperature was also significant for 20:1*n*−9*c*, total MUFAs, and DHA, which all showed elevated values at day 42 (both temperatures) and 62 (5 °C) before a sharp decrease observed at day 62 (10 °C) and 92 (both temperatures). The opposite pattern was observed for EPA and total PUFAs. The LH temperature significantly affected the relative proportion of 16:0 and total SFAs, which were significantly lower in the RKCs held at 5 °C for 62 and 92 days compared to their wild counterparts. Besides, the LH time led to a significant increase in the PUFA/SFA ratio, along with a significant decrease in the omega−3/omega−6 ratio and in the relative proportion of 16:1*n*−7*c* and 20:2*n*−6*c*. The total amount of fatty acids detected in the hepatopancreas also varied significantly with the LH time, with the values observed at day 62 (10 °C) and 92 (both temperatures) significantly lower than those found for the other time/temperature groups.

Principal component analysis (PCA) was performed to provide a visual overview of the changes in the fatty acid composition of the leg meat (Fig. 1) and hepatopancreas (Fig. 2) concerning each RKC. For leg

Table 2
Fatty acid composition (% of the total detected fatty acids) of cooked leg meat of the red king crabs sampled during live holding without feeding.

Fatty acid	Live holding time (t) and temperature (T)							Significance (*p*-value)		
	Day 0	Day 41		Day 62		Day 92				
		5 °C	10 °C	5 °C	10 °C	5 °C	10 °C	t	T	t × T
16:0	15.4 ± 3.1	16.0 ± 1.2	16.3 ± 0.4	16.0 ± 1.8	15.9 ± 1.1	15.4 ± 0.3	16.2 ± 0.7	0.799	0.405	0.751
18:0	4.1 ± 0.4	3.8 ± 0.3	4.1 ± 0.1	4.0 ± 0.5	4.3 ± 0.2	3.9 ± 0.3	3.7 ± 0.3	0.241	0.368	0.244
∑SFA[1]	22.1 ± 0.6	22.3 ± 1.3	23.1 ± 0.5	22.3 ± 1.3	22.6 ± 0.7	21.6 ± 0.4	22.3 ± 0.8	0.233	0.134	0.820
16:1*n*−7*c*	3.1 ± 0.6	4.0 ± 1.5	3.8 ± 1.0	2.6 ± 0.4	2.9 ± 1.4	3.5 ± 0.8	4.1 ± 0.7	0.072	0.602	0.738
18:1*n*−9*c* (OLA)	14.5 ± 1.2	13.3 ± 1.0	14.3 ± 0.8	14.7 ± 2.1	15.5 ± 1.1	13.6 ± 0.6	14.9 ± 0.7	0.101	0.041	0.909
18:1*n*−7*c*	6.5 ± 1.2	7.5 ± 1.2	6.6 ± 0.7	6.2 ± 0.4	6.5 ± 1.3	7.9 ± 0.4	6.9 ± 0.2	0.051	0.080	0.197
∑MUFA[2]	27.0 ± 1.4	27.1 ± 1.4	26.8 ± 0.9	26.1 ± 2.9	26.9 ± 2.1	27.0 ± 0.9	27.5 ± 0.5	0.676	0.663	0.783
20:4*n*−6*c* (ARA)	7.6 ± 2.3	7.3 ± 2.8	7.4 ± 2.1	7.6 ± 2.7	10.2 ± 5.4	9.0 ± 1.7	6.9 ± 1.1	0.499	0.879	0.275
∑(*n*−6)	10.7 ± 2.4	10.5 ± 3.0	10.8 ± 2.0	11.0 ± 3.3	13.5 ± 5.6	11.7 ± 2.0	10.2 ± 1.3	0.533	0.727	0.421
∑(*n*−6) (mg/100 g)	50 ± 6	48 ± 14	41 ± 7	55 ± 15	41 ± 15	43 ± 9	39 ± 9	0.506	0.089	0.666
20:5*n*−3*c* (EPA)	26.0 ± 1.4	27.9 ± 2.0	26.6 ± 0.9	27.0 ± 2.0	27.0 ± 1.1	29.7 ± 1.8*	30.7 ± 1.5*	**0.002**	0.900	0.355
22:6*n*−3*c* (DHA)	12.8 ± 2.1	10.9 ± 2.1	11.5 ± 1.4	12.2 ± 2.3	9.0 ± 2.6*	9.1 ± 1.0*	8.8 ± 0.1*	**0.050**	0.197	0.110
∑(*n*−3)	40.3 ± 1.0	40.0 ± 2.2	39.2 ± 1.8	40.6 ± 2.2	37.0 ± 3.0	39.7 ± 1.6	40.1 ± 1.6	0.603	0.134	0.180
∑(*n*−3) (mg/100 g)	193 ± 34	183 ± 24^{ab}	150 ± 17^{ab}	209 ± 37^{a}	116 ± 32^{b}*	144 ± 5^{b}	155 ± 46^{ab}	0.422	**0.003**	**0.006**
∑PUFA[3]	50.9 ± 1.5	50.6 ± 2.4	50.1 ± 0.6	51.6 ± 3.6	50.6 ± 2.6	51.4 ± 0.5	50.2 ± 0.9	0.743	0.321	0.942
∑PUFA[3] (mg/100 g)	243 ± 33	231 ± 25^{ab}	192 ± 16^{b}	265 ± 41^{a}	157 ± 28^{b}*	187 ± 11^{b}*	194 ± 54^{ab}	0.304	**0.001**	**0.005**
∑(*n*−3) / ∑(*n*−6)	3.9 ± 0.9	4.1 ± 1.5	3.7 ± 0.8	4.0 ± 1.2	3.2 ± 1.6	3.5 ± 0.7	4.0 ± 0.6	0.798	0.603	0.506
∑PUFA[3] / ∑SFA[1]	2.3 ± 0.1	2.3 ± 0.2	2.2 ± 0.0	2.3 ± 0.3	2.2 ± 0.2	2.4 ± 0.0	2.3 ± 0.1	0.509	0.162	0.972
EPA + DHA (mg/100 g)	187 ± 33	178 ± 23^{ab}	146 ± 17^{bc}	202 ± 36^{a}	113 ± 32^{c}	141 ± 5^{bc}	153 ± 45^{abc}	0.514	**0.003**	**0.006**
∑FAMEs[4] (mg/100 g)	480 ± 80	460 ± 60^{ab}	380 ± 30^{bc}	510 ± 70^{a}	310 ± 70^{c}*	360 ± 20^{bc}*	380 ± 100^{abc}	0.262	**0.002**	**0.006**

Note. Results are expressed as mean values (± standard deviation). Different superscript letters within the same row indicate significantly different mean values ($p \leq 0.05$, full factorial ANOVA followed by Scheffé's test). Significant factor effects are highlighted in bold ($p \leq 0.05$, full factorial ANOVA). The symbol (*) indicates the mean values which are significantly different ($p \leq 0.05$, one-way ANOVA followed by Dunnett's test) from the mean value observed at day 0.

Abbreviations: OLA, oleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; FAME, fatty acid methyl ester.

[1] Includes also SFAs between 0.2 and 2 % (i.e., 14:0, 15:0, and 17:0) and SFAs < 0.2 % (i.e., 12:0, 20:0, and 21:0).

[2] Includes also MUFAs between 0.2 and 2 % (i.e., 17:1*n*−7*c*, 18:1*n*−9*t*, 20:1*n*−9*c*, and 22:1*n*−9*c*) and MUFAs < 0.2 % (i.e., 15:1*n*−5*c*).

[3] Includes also PUFAs between 0.2 and 2 % (i.e., 18:3*n*−3*c* and 22:5*n*−3*c*) and PUFAs < 0.2 % (i.e., 18:3*n*−6*c*, 20:3*n*−6*c*, 20:3*n*−3*c*, and 22:2*n*−6*c*).

[4] Includes also 12:0, 14:0, 15:0, 17:0, 20:0, 21:0, 15:1*n*−5*c*, 17:1*n*−7*c*, 18:1*n*−9*t*, 22:1*n*−9*c*, 18:3*n*−6*c*, 18:3*n*−3*c*, 20:3*n*−6*c*, 20:3*n*−3*c*, 22:2*n*−6*c*, and 22:5*n*−3*c*.

Table 3
Fatty acid composition (% of the total detected fatty acids) of raw hepatopancreas of the red king crabs sampled during live holding without feeding.

Fatty acid	Live holding time (t) and temperature (T)							Significance (*p*-value)		
	Day 0	Day 41		Day 62		Day 92				
		5 °C	10 °C	5 °C	10 °C	5 °C	10 °C	t	T	t × T
16:0	12.9 ± 1.0	11.5 ± 1.3	12.4 ± 1.9	10.8 ± 0.6*	12.3 ± 0.5	10.8 ± 0.7*	11.5 ± 0.3	0.309	**0.039**	0.788
18:0	4.9 ± 0.6	4.2 ± 0.3^{b}	4.0 ± 0.5^{b}*	4.2 ± 0.5^{b}	6.2 ± 0.1^{a}*	5.7 ± 0.3^{a}*	6.1 ± 0.3^{a}*	**< 0.001**	**< 0.001**	**< 0.001**
∑SFA[1]	21.5 ± 1.9	19.5 ± 1.4	20.5 ± 2.2	18.7 ± 1.2*	21.6 ± 0.8	18.5 ± 0.8*	19.7 ± 0.2	0.253	**0.006**	0.327
16:1*n*−7*c*	8.1 ± 0.5	7.1 ± 2.8	6.0 ± 2.5	4.1 ± 0.5*	3.6 ± 0.3*	2.5 ± 0.2*	4.3 ± 0.4*	**0.002**	0.985	0.219
18:1*n*−9*c* (OLA)	11.6 ± 1.9	14.7 ± 1.6	16.0 ± 1.4*	17.2 ± 3.1*	15.0 ± 1.1	14.1 ± 0.9	14.4 ± 2.3	0.199	0.774	0.172
18:1*n*−7*c*	11.2 ± 0.5	10.2 ± 3.0	7.3 ± 1.8*	7.1 ± 0.4*	7.3 ± 0.2*	8.9 ± 1.4	10.2 ± 1.3	0.054	0.535	0.054
20:1*n*−9*c*	3.0 ± 0.7	6.1 ± 4.0^{abc}	7.7 ± 3.6^{ab}*	9.0 ± 2.8^{a}*	2.4 ± 1.6^{bc}	1.2 ± 0.3^{c}	1.1 ± 0.1^{c}	**0.001**	0.141	**0.018**
∑MUFA[2]	35.4 ± 2.4	39.8 ± 3.5^{a}	38.6 ± 2.7^{ab}	39.7 ± 5.5^{a}	30.3 ± 0.6^{c}	29.1 ± 1.0^{c}*	32.2 ± 2.0^{bc}	**< 0.001**	0.070	**0.005**
18:2*n*−6*c* (LA)	1.4 ± 0.5	1.7 ± 0.6	2.0 ± 0.5	1.7 ± 0.2	2.0 ± 0.1	1.4 ± 0.4	1.6 ± 0.4	0.212	0.135	0.929
20:2*n*−6*c*	2.5 ± 0.9	3.1 ± 0.9	2.9 ± 0.6	3.3 ± 0.8	2.5 ± 1.4	1.4 ± 0.1	1.5 ± 0.3	**0.001**	0.324	0.434
20:4*n*−6*c* (ARA)	4.5 ± 0.7	5.1 ± 2.5^{b}	4.3 ± 2.2^{b}	5.5 ± 1.7^{b}	15.2 ± 1.9^{a}*	15.8 ± 2.2^{a}*	13.0 ± 1.2^{a}*	**< 0.001**	**0.024**	**< 0.001**
∑(*n*−6)	9.5 ± 1.6	11.1 ± 4.0^{b}	10.5 ± 3.2^{b}	12.2 ± 2.6^{b}	20.4 ± 0.4^{a}*	19.0 ± 2.4^{a}*	16.7 ± 0.4^{ab}*	**< 0.001**	0.134	**0.003**
20:5*n*−3*c* (EPA)	16.9 ± 2.5	13.3 ± 3.5^{bc}	10.2 ± 1.1^{c}*	10.0 ± 2.3^{c}*	15.0 ± 3.0^{abc}	19.7 ± 1.6^{a}	18.2 ± 0.6^{ab}	**< 0.001**	0.903	**0.005**
22:5*n*−3*c* (DPA)	2.5 ± 0.7	1.9 ± 0.4	1.9 ± 0.3	1.6 ± 0.2*	1.1 ± 0.3*	0.9 ± 0.2*	1.0 ± 0.2*	**< 0.001**	0.378	0.110
22:6*n*−3*c* (DHA)	13.7 ± 2.3	13.8 ± 3.9^{ab}	17.3 ± 2.1^{a}	16.6 ± 2.9^{ab}	10.7 ± 0.7^{b}	12.5 ± 1.7^{ab}	12.0 ± 2.2^{ab}	**0.047**	0.353	**0.005**
∑(*n*−3)	33.6 ± 3.8	29.6 ± 4.2	30.4 ± 2.3	29.3 ± 4.7	27.7 ± 1.7	33.5 ± 1.7	31.5 ± 2.4	0.075	0.486	0.604
∑PUFA[3]	43.1 ± 2.6	40.7 ± 2.5^{c}	41.0 ± 4.1^{bc}	41.5 ± 4.8^{bc}	48.1 ± 1.5^{ab}	52.4 ± 1.0^{a}*	48.2 ± 2.0^{ab}	**< 0.001**	0.516	**0.012**
∑(*n*−3) / ∑(*n*−6)	3.6 ± 0.9	3.0 ± 1.3	3.1 ± 0.8	2.5 ± 0.6	1.4 ± 0.1*	1.8 ± 0.3*	1.9 ± 0.2*	**0.005**	0.292	0.204
∑PUFA[3] / ∑SFA[1]	2.0 ± 0.3	2.1 ± 0.1	2.0 ± 0.4	2.2 ± 0.2	2.2 ± 0.2	2.8 ± 0.2*	2.5 ± 0.1	**< 0.001**	0.141	0.218
∑FAMEs[4] (g/100 g)	8.9 ± 2.2	8.9 ± 2.3	9.5 ± 3.9	6.3 ± 1.4	1.7 ± 1.2*	0.9 ± 0.1*	0.8 ± 0.2*	**< 0.001**	0.140	0.051

Note. Results are expressed as mean values (± standard deviation). Different superscript letters within the same row indicate significantly different mean values ($p \leq 0.05$, one-way ANOVA followed by Scheffé's test). Significant factor effects are highlighted in bold ($p \leq 0.05$, full factorial ANOVA). The symbol (*) indicates the mean values which are significantly different ($p \leq 0.05$, one-way ANOVA followed by Dunnett's test) from the mean value observed at day 0.
Abbreviations: OLA, oleic acid; LA, linoleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; FAME, fatty acid methyl ester.
[1] Includes also SFAs between 0.2 and 2 % (i.e., 14:0, 15:0, and 17:0) and SFAs < 0.2 % (i.e., 12:0, 20:0, and 21:0).
[2] Includes also MUFAs between 0.2 and 2 % (i.e., 17:1*n*−7*c*, 18:1*n*−9*t*, and 22:1*n*−9*c*) and MUFAs < 0.2 % (i.e., 15:1*n*−5*c*).
[3] Includes also PUFAs between 0.2 and 2 % (i.e., 18:3*n*−3*c*) and PUFAs < 0.2 % (i.e., 18:3*n*−6*c*, 20:3*n*−6*c*, 20:3*n*−3*c*, and 22:2*n*−6*c*).
[4] Includes also 12:0, 14:0, 15:0, 17:0, 20:0, 21:0, 15:1*n*−5*c*, 17:1*n*−7*c*, 18:1*n*−9*t*, 22:1*n*−9*c*, 18:3*n*−6*c*, 18:3*n*−3*c*, 20:3*n*−6*c*, 20:3*n*−3*c*, and 22:2*n*−6*c*.

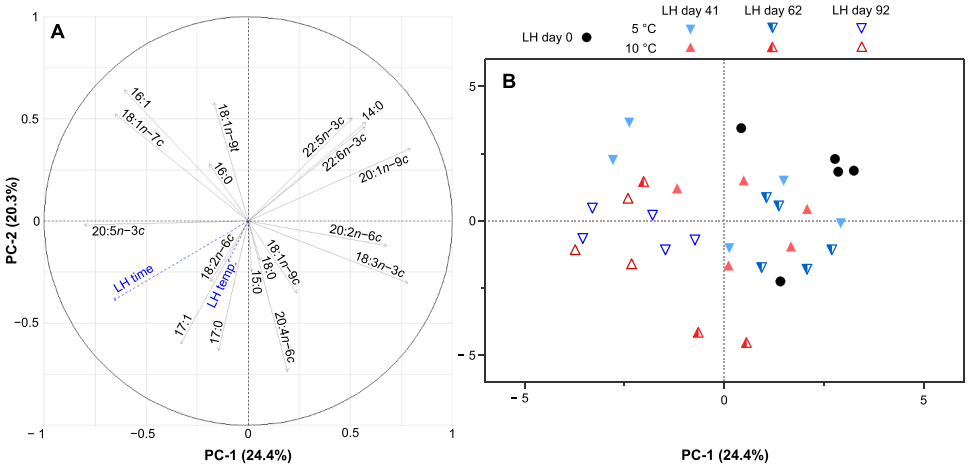

Fig. 1. Loading (A) and score (B) plot obtained by principal component analysis of the fatty acid composition of cooked leg meat. The LH time and temperature are represented as supplementary explanatory variables (i.e., factors).

meat data, the first (PC-1) and the second (PC-2) principal components accounted cumulatively for 44.7 % of the variance, as illustrated by the loading plot in Fig. 1A. The effect of LH time (standardized loadings: PC-1 − 0.66, PC-2 − 0.39) accounted for a larger share of variance compared to the effect of LH temperature (standardized loadings: PC-1 − 0.10, PC-2 − 0.20). The score plot given in Fig. 1B revealed clustering of the observations relevant to RKCs held for 92 days at both temperatures. For hepatopancreas data, 62.0 % of the variance was described by the first two principal components as depicted by the loading plot in Fig. 2A. The total variance could be ascribed mainly to the effect of LH time (standardized loadings: PC-1 − 0.58, PC-2 − 0.61) rather than to that of LH temperature (standardized loadings: PC-1 − 0.04, PC-2 − 0.04). As illustrated by the score plot given in Fig. 2B, the RKCs held for 62 days at 10 °C and for 92 days at both temperatures clustered along PC-1.

4. Discussion

4.1. Wild red king crab

The proximate composition of the leg meat and hepatopancreas of wild RKC (i.e., LH day 0) reflected their biological function as reserves compartments of protein and fat, respectively (Sánchez-Paz et al., 2006). Similar proportions of proximate composition of leg meat have been

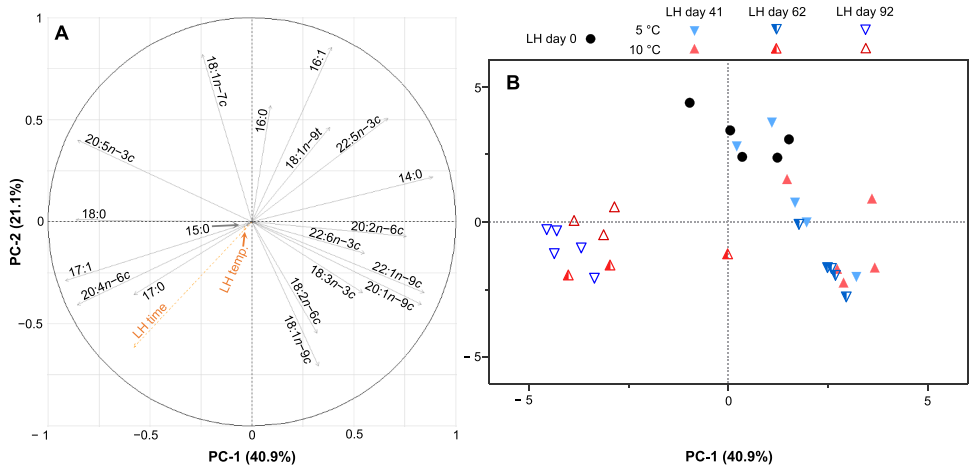

Fig. 2. Loading (A) and score (B) plot obtained by principal component analysis of the fatty acid composition of raw hepatopancreas. The LH time and temperature are represented as supplementary explanatory variables (i.e., factors).

previously reported for cooked intermolt RKC (Krzeczkowski et al., 1971; Lian et al., 2021b) and southern king crab (*Lithodes santolla*) (Lorenzo et al., 2021; Risso & Carelli, 2012). Concerning the hepatopancreas, the lipid content of our samples was, respectively, similar to and lower than that of RKCs harvested in the summer and autumn in different Alaskan locations (Reppond, 2009). The hepatopancreas lipid content and fatty acid profile have been proposed as valuable indicators in assaying the health status of stocks of various Arctic crabs (Reppond, 2009; Solstad et al., 2021).

The fatty acid profile of the leg meat and hepatopancreas differed essentially in the relative level of MUFAs, which was higher in the hepatopancreas, and of PUFAs, which was higher in the leg meat. This is probably due to the fact that MUFAs function as long-term storage lipids, while PUFAs, especially the highly unsaturated and long-chain ones, are mainly regarded as structural lipids (Sacristán et al., 2017). Moreover, the variability in the results between samples of different individuals was lower for leg meat compared to hepatopancreas, which can be explained by the fact that the hepatopancreas is inherently a more heterogeneous tissue matrix and responds more sensitively than the leg muscle to variations experienced by decapod crustaceans in their natural environment (Barrento et al., 2009; Munian et al., 2020).

It is well known that the fatty acid composition of decapod crustaceans, including RKC, can be affected directly or indirectly by a series of factors, including the sex, maturity stage, time from ecdysis, physiological state, and the specific availability of food items in their environment (Stevens & Jewett, 2014). Adult RKCs are omnivores, opportunistic, and generalist predators, feeding on the most abundant benthic organisms (Sundet, 2014). Studies on the feeding behavior and predatory effect of RKC in the Barents Sea showed that RKC feeds preferably on mollusks (e.g., bivalves and gastropods) and echinoderms (e.g., sea urchins), but also on worms (e.g., polychaetes and sipunculids), crabs, and fish (Britayev et al., 2010; Jørgensen & Nilssen, 2011). The foraging activity of RKC is linked to the composition of benthic communities in various locations of the Barents Sea (Anisimova & Lubin, 2008; Falk-Petersen et al., 2011). As an example, Dal'nezelenetskaja Bay was found to be substantially more densely populated by sea urchins than other areas of the Kola region (Pavlova, 2009), whereas in the Varangerfjord area (Norwegian coast), the soft-bottom fauna was particularly impoverished of echinoderms, larger mollusks, and polychaetes (Oug et al., 2011).

The fatty acid profile of the leg meat and hepatopancreas of RKC harvested in Norwegian waters of the Barents Sea was similar to that reported for RKC harvested in other areas, although with some differences in the relative SFA, MUFA, and PUFA levels. Specifically, compared to our results, in RKC harvested in the Russian waters of the Barents Sea (Dal'nezelenetskaja Bay), the raw leg muscle of male individuals contained higher proportions of SFAs (increase (↑) by 5.5 percentage points (p.p.)) and PUFAs (↑ 4.3 p.p.) but lower MUFAs (decrease (↓) by 9.8 p.p.) (Dvoretsky et al., 2021). In contrast, the hepatopancreas (female and male individuals considered together) contained higher proportions of SFAs (↑ 7.4 p.p.) but lower content in MUFAs (↓ 7.7 p.p.) and around the same level of PUFAs (Dvoretsky et al., 2020). Latyshev et al. (2009) analyzed male RKCs harvested in the Tartar Strait (Sea of Japan), and compared to our study, they found lower proportional levels of SFAs (↓ 4.0 p.p.) and PUFAs (↓ 2.3 p.p.) in the raw leg muscle and lower relative content in PUFAs (↓ 4.0 p.p.) in the hepatopancreas although with a proportion (6.5–7.0 %) of detected fatty acids not assigned to any of the saturation classes. Notably, compared to Dal'nezelenetskaja Bay RKCs, our leg meat samples were lower in relative content (↓ 2.9 p.p.) in 18:0 but richer (↑ 3.0 p.p.) in OLA (Dvoretsky et al., 2021), whereas our hepatopancreas samples presented a lower content in 18:0 (↓ 3.0 p.p.) (Dvoretsky et al., 2020). Furthermore, compared to Sea of Japan RKCs, higher relative levels of ARA (↑ 3.5 p.p.) and 16:1*n*−7*c* (↑ 3.5 p.p.) were found in our leg meat and hepatopancreas samples, respectively (Latyshev et al., 2009).

Decapod crustaceans have a limited ability to biosynthesize linoleic (omega−6) and linolenic (omega−3) PUFA families (Munian et al., 2020). Dietary essential fatty acids (EFAs) such as DHA, EPA, and ARA are typically synthesized by primary producers such as phytoplankton and microalgae and then bioaccumulated through higher trophic levels of the food web (Parrish, 2009). Among potential RKC preys, it has been reported that polychaetes are deficient in DHA (Thomas et al., 2020), sea urchins are rich in EPA (Rocha et al., 2019), and macroalgae are high in ARA levels (Copeman et al., 2012), whereas echinoderms such as brittle stars (Ophiuroidea) provide high levels of EPA and low levels of DHA and ARA (Graeve et al., 1997). The fatty acid profile of RKC harvested in different geographical areas or at different depths will reflect the composition of the species that constitute the food base of the crab in the specific biocenosis (Dvoretsky et al., 2020). This trophic relationship will result particularly evident for the EFAs.

4.2. *Effect of time and temperature during live holding without feeding*

It is well documented that long-term starvation is tolerated by decapod crustaceans (Albalat et al., 2019; Siikavuopio et al., 2019; Watts et al., 2014), including king crab species (Sacristán et al., 2019; Siikavuopio et al., 2016). In the absence of food resources, crustaceans must rely on their energetic reserves (i.e., glycogen, protein, and fat) to

maintain metabolic functionality and survive (Mcleod et al., 2004). The changes in tissue composition during starvation can indicate both the sequence of utilization and the level of depletion of various body reserves, with a large diversity of responses exhibited among crustaceans (Sacristán et al., 2017).

Examining the HSI and proximate composition results (Table 1), it is clear that RKC resorted massively to utilizing the fat reserves stored in the hepatopancreas during LH without feeding, irrespective of the temperature. This agrees with the recent findings of Sacristán et al. (2020), showing that lipids constitute the most important energetic reserve in adult *L. santolla*. Nonetheless, it should be noted that, at the same time, RKC catabolizes the muscle tissue of the appendages as shown by the decrease in the CI (Table 1) and by the leg meat content data reported by Lorentzen et al. (2019) for the RKCs, object of the same LH experiment. It has been hypothesized that muscle atrophy can be a route for crustacean decapods for mobilizing proteins not only to obtain energy from amino acid catabolism but also to ensure the availability of building blocks for the synthesis of the enzymes required for metabolic pathways (Sacristán et al., 2017). Supporting this hypothesis, in the present study, hepatopancreas protein levels remained unchanged throughout the LH period. Accordingly, no significant changes were reported in the relative protein content of hepatopancreas of false southern king crab (*Paralomis granulosa*) and *L. santolla* after short (12 days) and long (60 days) starvation periods, respectively (Comoglio et al., 2008; Comoglio et al., 2005; Sacristán et al., 2019).

The LH temperature significantly affected the relative protein levels of the leg meat, as significantly lower values were found in the RKCs at 10 °C compared to 5 °C at the end of the LH period (Table 1). Correspondingly, Albalat et al. (2019) reported that high LH temperature (12 °C) exacerbated the changes in the proximate composition of European lobster (*Homarus gammarus*) tissues during starvation, indicating that temperature is a crucial environmental factor eliciting higher energy requirements upon the combination with the absence of feeding. Starved RKCs appear to be susceptible to the influence of temperature with regard to changes in relative proximate composition and meat content of walking legs (Lorentzen et al., 2019), especially if compared to other crab species potentially interacting in the same habitat, such as snow crab (*Chionoecetes opilio*) (Hardy et al., 2000; Lorentzen et al., 2020).

Among lipids, the metabolism during starvation is mainly centered around fatty acids, as they are key constituents of triacylglycerols (TAGs), the major storage lipid class in crustaceans, and phospholipids (PLs), building blocks of cellular membranes. Fatty acids play a prominent role in the provision of energy with interspecific differences in their pattern of utilization or retention (Copeman et al., 2012).

The present study showed that, during starvation, the changes in terms of the absolute content and in the profile of the fatty acids in the muscle tissue were small, especially if compared with the hepatopancreas. This confirms the contention, previously reported also for other crab species, that the hepatopancreas is the central organ for the catabolism of fatty acids during starvation (Ding et al., 2017; Wen et al., 2006). In detail, in the leg muscle, the fatty acids with the highest and fastest degree of depletion were 14:0, 20:1*n*−9*c*, DHA, and DPA, whereas the highest levels of retention over time were observed for 17:0, 17:1*n*−7*c*, LA, and EPA (Fig. 1A). In the hepatopancreas, 17:0, 17:1*n*−7*c*, 18:0, and ARA were spared from the catabolism mainly at the expense of 14:0, 16:0, 16:1*n*−7*c*, 18:1*n*−9*t*, and DPA, which were rapidly utilized during starvation (Fig. 2A). It is interesting to note that 17:0 and 17:1*n*−7*c* were among the retained fatty acids in both tissues during starvation. These fatty acids are typically of bacterial origin (Copeman et al., 2012), and it can be hypothesized that they were incorporated by RKCs after feeding on biofilms that possibly foul the containers used for LH.

Furthermore, the depletion of specific SFAs and MUFAs, along with DPA in both tissues, is in agreement with the pattern of fatty acid mobilization reported for leg muscle and hepatopancreas of *P. trituberculatus* and juvenile *E. sinensis* during a starvation period of 30 and 70 days, respectively (Ding et al., 2017; Wen et al., 2006). SFAs and MUFAs are associated primarily with storage lipids (i.e., TAGs), which can be catabolized via the β-oxidation pathway, acting as energy reserves (Auerswald et al., 2009). The DPA is an omega−3 EFA; hence its relative decrease with LH time can be considered a direct effect of the absence of feeding. Although not investigated as beyond the scope of the study, it should be highlighted that mitochondrial β-oxidation of fatty acids can lead to the formation of intermediary metabolites that, if accumulated above critical concentration thresholds, may affect the flavor of the meat (Yuan et al., 2021; Zhuang et al., 2016).

Notably, the other major EFAs, namely ARA, EPA, and DHA, classically mainly associated with membrane lipids (i.e., PLs), showed contrasting alteration patterns during LH and between the sampled tissues. In the leg muscle, ARA and EPA were conserved while DHA was mobilized. In the hepatopancreas, ARA was selectively retained; EPA first was preferentially utilized until day 62 at 5 °C and day 41 at 10 °C before being conserved until the end of the LH period, whereas the opposite trend was observed for DHA. This may be linked to overcompensation mechanisms that maximize the sequestering of different EFAs according to their biochemical function during LH without feeding (Thériault & Pernet, 2007). ARA and EPA are precursors to eicosanoids, a class of biologically active hormones that includes prostaglandins, leukotrienes, and thromboxanes, responsible for a series of physiological processes ranging from ionic regulation to stress response (Beder, 2015). Given its structure, DHA ensures the functionality and integrity of biological membranes, facilitating rapid conformational changes in membrane proteins (Thériault & Pernet, 2007). Moreover, DHA constitutes a large part of the lipoprotein complexes that act as carriers in the mobilization of lipids (Glencross, 2009). Wang et al. (2021) have suggested that high hepatopancreas DHA levels are required to maintain essential functions in mud crab (*Scylla paramamosain*) with some capacity for the synthesis of DHA from EPA via the Sprecher pathway (Sprecher, 2000). It can be hypothesized that the proportions of EPA and DHA fluctuate in relation to different levels of physiological stress induced by prolonged LH without feeding, as shown by the capacity of whiteleg shrimp (*Litopenaeus vannamei*) to retain these two EFAs to modulate cellular and biochemical parameters under stressful conditions (Mercier et al., 2009).

In both tissues, the data variation explained by the effect of LH time was, in general, prominent compared to that accounted for by temperature (Fig. 1A and 2A). Nonetheless, in the space delimited by the PC-1 and PC-2 axes, the hepatopancreas samples for the groups day 62/10 °C, day 92/5 °C, and day 92/10 °C clustered together and separately from the observations relevant to the other time/temperature groups (Fig. 2B). This indicates that, in the hepatopancreas, high temperature (10 °C) was an important factor in accelerating changes in the fatty acid profile occurring between day 42 and 62, which at a lower temperature (5 °C) would occur at a later stage of the LH (between day 62 and 92). Specifically, the major changes observed in these time intervals at the two different temperatures were a drastic depletion in the total fatty acid content and a decrease in the omega−3/omega−6 ratio driven mainly by the sharp increase in the relative levels of ARA. It is interesting to note that, by contrast, the omega−3/omega−6 ratio of the leg meat was not affected by LH without feeding. Moreover, in the hepatopancreas, the same time intervals were the turning point for a series of $C_{18–22}$ unsaturated fatty acids (i.e., OLA, LA, 18:3*n*−3*c*, 20:1*n*−9*c*, 20:2*n*−6*c*, and 22:1*n*−9*c*), which first were preferentially retained but then utilized (Fig. 2B).

As reported by Lorentzen et al. (2019), RKCs in the same LH experiment showed a significant decrease in the leg meat content as early as at LH day 42. A marked drop in meat content was observed between 42 and 62 days of LH for the RKCs kept at 10 °C, whereas in the corresponding RKCs kept at 5 °C the leg meat content decreased sharply only between 62 and 92 days of LH. Combining this information with the results of the present study, it can be hypothesized a shift in the catabolic strategies adopted by RKC to cope with starvation in correspondence to a critical

LH time. This specific time window is identifiable between 42 and 62 days at 10 °C and between 62 and 92 days at 5 °C. During LH without feeding, first RKC preferentially mobilizes SFAs and MUFAs, such as 16:1*n*−7*c*. Parallelly, RKC catabolizes the muscle tissue of walking legs and then, as the starvation period continues, a larger share of hepatopancreas fatty acids, including increasing proportions of C_{18} PUFAs and other longer-chain unsaturated fatty acids. In this way, high-energy lipids are oxidized to supply the additional energy demand required by LH without feeding conducted for a prolonged time or for a shorter time but at a high temperature. This catabolic pattern appears to be in line with the findings described initially by Ritar et al. (2003) for spiny lobster (*Jasus edwardsii*) in the larval stage, in which lipid and protein metabolic reserves were mobilized in an orderly sequence leaving the breakdown of highly-unsaturated fatty acids to the last stage prior to death.

4.3. Nutritional value of cooked leg meat

Crab meat is particularly appreciated for its nutritional value, conferred primarily by the high content in PUFAs, especially in the omega−3 fatty acids EPA and DHA (Gökoğlu, 2021). Humans cannot synthesize these fatty acids in quantity sufficient to guarantee optimal physiological performance (Arts et al., 2001). Aquatic food products rich in omega−3 fatty acids are therefore recommended as part of a healthy diet in light of nutritional benefits, including positive effects on the cardiovascular system (WHO/FAO, 2011) and against neurodegenerative diseases such as Alzheimer's disease (Lorente-Cebrián et al., 2015), along with anti-inflammatory and immunomodulatory properties (e.g., among others, therapeutical potential against inflammatory bowel disease and beneficial interplay with gut microbiota) (Parolini, 2019).

In this regard, a daily intake of 250–1000 mg of EPA and DHA together with a general omega−3/omega−6 ratio close to 1 or higher is recommended (Saini & Keum, 2018). Moreover, according to European food legislation, food products in which the sum of EPA and DHA is at least 80 mg per 100 g and per 100 kcal meet the requirement for bearing the label claim *high in omega–3 fatty acids* (Commission Regulation (EU) No. 116/2010), which may be appealing from a marketing standpoint. The leg meat samples of the present study were characterized by good nutritional value and eligible for the above-mentioned label claim (EPA + DHA = 186 ± 33 mg 100 g^{-1}). It is noteworthy that the levels of EPA + DHA remained over the threshold for the omega−3 label claim throughout the LH period, with individual crab values ranging 93–238 mg 100 g^{-1}. At the same time, LH without feeding did not significantly alter the omega−3/omega−6 ratio, with individual crab values varying between 1.7 and 6.4.

As summarized in Table 4, in our samples, the omega−3/omega−6 ratio and relative and absolute EPA and DHA levels of leg meat were in line with those reported in the literature for edible tissue extracted from the appendages of king crabs and other commercially important crab species harvested in Europe.

4.4. Implications for live holding management

The optimization of LH practices is a key element for the success of the Norwegian crab industry operating with live export of adult RKC. In this regard, LH management should be directed to maintaining acceptable animal welfare status while ensuring quality levels closer to those typical of wild RKC (Siikavuopio & James, 2015). An understanding of the mechanisms linked to the resistance of RKC to starvation is a relevant tool not only to investigate the feasibility of conducting LH without feeding but also for the development of tailored feeding protocols (Calvo et al., 2017).

The present study has provided insights into the nutritional requirement linked to fatty acid utilization during LH without feeding. Notably, the cooked leg meat, which constitutes the primary edible tissue in RKC, maintained its nutritional quality features relative to the fatty acid composition throughout the entire three-month starvation period. Parallelly, the monitoring of the changes in the fatty acid composition of the hepatopancreas revealed a specific utilization pattern where SFAs and specific MUFAs, such as 16:1*n*−7c and 18:1*n*−9*t*, as well as DPA, were preferentially catabolized at the beginning of the starvation period, followed by a shift to a higher mobilization of $C_{18–22}$ unsaturated fatty acids at a later stage. This indicated that RKC would require to be supplied with feed containing not only adequate levels of EFAs but also the specific fatty acids matching the utilization sequence elucidated by LH without feeding.

While the information provided by the present study constitutes a first background piece of knowledge for feed development, a better understanding of the biochemical processes involved in the starvation-coping mechanisms is warranted. Besides, an important aspect to consider in crab LH practices is the impact that starvation or feeding may have on the flavor profile of the meat due to the potential presence

Table 4
Overview of literature data on nutritionally important fatty acids in edible tissues extracted from the appendages of king crabs and other commercial crab species harvested in Europe.

Species	Harvest area	Sample	EPA (%)	DHA (%)	EPA + DHA (mg/100 g)	∑(*n*−3) / ∑(*n*−6)	References
	North Cape, Barents Sea, Norway	Cooked leg meat	26.0 ± 1.4	12.8 ± 2.1	186.5 ± 33.1	3.9 ± 0.9	Present study
Red king crab (*Paralithodes camtschaticus*)	Dal'nezelenetskaja Bay, Barents Sea, Russia	Raw leg muscle	27.9 ± 0.7	13.2 ± 0.3	117.6	3.7 ± 0.1	Dvoretsky et al. (2021)
	Tartar Strait, Sea of Japan	Raw leg muscle	31.1	9.8	na	7.0	Latyshev et al. (2009)
Southern king crab (*Lithodes santolla*)	San Jorge Gulf, Argentina	Cooked claw, leg, and shoulder meat	21.8 ± 0.5	13.5 ± 0.5	269.0	3.6	Risso & Carelli (2012)
Atlantic spider crab (*Maja brachydactyla*)	English Channel	Raw claw and leg muscle	22.1 ± 1.2	12.5 ± 0.4	69.7 ± 2.1	5.2 ± 0.1	Marques et al. (2010)
Blue crab (*Callinectes sapidus*)	Akyazan Lagoon, Turkey	Cooked claw meat	10.6 ± 0.2	5.9 ± 0.1	na	2.3	Çelik et al. (2004)
Edible crab (*Cancer pagurus*)	Scottish coast and English Channel	Raw muscle	21.4 ± 2.5	11.4 ± 1.2	na	3.8 ± 0.4	Barrento et al. (2010)
	Irish Sea, Ireland	Cooked claw meat	24.3 ± 2.2	11.8 ± 0.6	107.0 ± 10.0	4.4 ± 0.4	Lian et al. (2021a)
Green crab (*Carcinus mediterraneus*)	Tunisian Mediterranean coast	Raw claw muscle	9.1 ± 0.5	10.3 ± 0.5	na	1.5	Cherif et al. (2008)
Vernal crab (*Liocarcinus vernalis*)	Adriatic Sea, Italy	Raw edible tissue	24.2 ± 2.0	16.5 ± 1.9	224.6 ± 61.0	4.8 ± 0.4	Balzano et al. (2017)

Note. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; na = not available.

of different taste- and odor-active compounds forming via different metabolic pathways (Ma et al., 2017; Xu et al., 2021; Yuan et al., 2021; Zhuang et al., 2016). In light of these considerations, future studies should, therefore, not only have a focus on the hepatopancreas histochemistry and lipid class composition but also stretch into the area of metabolomics combined with the study of volatile organic compounds for assessing the impact of LH on the eating quality of the meat.

5. Conclusion

Limited quantitative and qualitative alterations could be observed in the fatty acid profile of cooked leg meat, which retained its nutritional value in terms of omega−3 fatty acid content throughout the live holding (LH) period at both temperatures.

In contrast, the fatty acid composition of raw hepatopancreas was severely affected by the time of LH without feeding, with substantial changes occurring especially between 41 and 62 days of LH at 10 °C and between 62 and 92 days of LH at 5 °C. Saturated fatty acids, docosapentaenoic acid (22:5*n*−3*c*), and specific monounsaturated fatty acids, such as palmitoleic (16:1*n*−7*c*) and elaidic (18:1*n*−9*t*) acid, were preferentially utilized at the beginning of the starvation period, followed by the mobilization of a series of $C_{18–22}$ unsaturated fatty acids. As expected, essential long-chain highly-unsaturated fatty acids were preferentially conserved during LH, especially arachidonic (20:4*n*−6*c*) and eicosapentaenoic acid (20:5*n*−3*c*).

The reported changes in the fatty acid profile may constitute baseline information that can be exploited for selecting or designing suitable feed for red king crab during LH at different temperatures.

CRediT authorship contribution statement

Federico Lian: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Sten I. Siikavuopio:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Sabine M. Harrison:** Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Birthe Vang:** Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Nigel P. Brunton:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Supervision. **Margrethe Esaiassen:** Conceptualization, Writing - original draft, Writing - review & editing, Supervision. **Grete Lorentzen:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

Albalat, A., Johnson, L., Coates, C.J., Dykes, G.C., Hitte, F., Morro, B., Dick, J., Todd, K., Neil, D.M., 2019. The effect of temperature on the physiological condition and immune-capacity of European lobsters (*Homarus gammarus*) during long-term starvation. Front. Mar. Sci. 6, 281 https://doi.org/10.3389/fmars.2019.00281.

Anisimova, N., Lubin, P., 2008. The red king crab and benthos communities. In: Sundet, J.H., Berenboim, B. (Eds.), Research on the Red King Crab (Paralithodes camtschaticus) from the Barents Sea in 2005–2007, pp. 28–32. IMR/PINRO Joint report series 3-2008. Retrieved April 01, 2021 from: https://imr.brage.unit.no/imr-xmlui/bitstream/handle/11250/106651/jrs_2008_3.pdf?sequence=1&isAllowed=y.

AOAC, 2000. Method No. 950.46. Official Methods of Analysis of AOAC International, 17th edition. AOAC International., Gaithersburg, MD.

Arts, M.T., Ackman, R.G., Holub, B.J., 2001. "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. 58 (1), 122–137. https://doi.org/10.1139/f00-224.

Auerswald, L., Pape, C., Stübing, D., Lopata, A., Meyer, B., 2009. Effect of short-term starvation of adult Antarctic krill, *Euphausia superba*, at the onset of summer. J. Exp. Mar. Biol. Ecol. 381 (1), 47–56. https://doi.org/10.1016/j.jembe.2009.09.011.

Balzano, M., Pacetti, D., Lucci, P., Fiorini, D., Frega, N.G., 2017. Bioactive fatty acids in mantis shrimp, crab and caramote prawn: their content and distribution among the main lipid classes. J. Food Compos. Anal. 59, 88–94. https://doi.org/10.1016/j.jfca.2017.01.013.

Barrento, S., Marques, A., Teixeira, B., Anacleto, P., Vaz-Pires, P., Nunes, M.L., 2009. Effect of season on the chemical composition and nutritional quality of the edible crab *Cancer pagurus*. J. Agric. Food Chem. 57 (22), 10814–10824. https://doi.org/10.1021/jf9025597.

Barrento, S., Marques, A., Teixeira, B., Mendes, R., Bandarra, N., Vaz-Pires, P., Nunes, M.L., 2010. Chemical composition, cholesterol, fatty acid and amino acid in two populations of brown crab *Cancer pagurus*: ecological and human health implications. J. Food Compos. Anal. 23 (7), 716–725. https://doi.org/10.1016/j.jfca.2010.03.019.

Beder, A., 2015. The Effects of Dietary Essential Fatty Acid Enrichment on the Nutrition and Condition of Red King Crab (*Paralithodes Camtschaticus*) Larvae. M.Sc. Thesis Retrieved April 01, 2021 from:. University of Alaska Fairbanks, Fairbanks, AK, USA https://scholarworks.alaska.edu/bitstream/handle/11122/6363/Beder_uaf_0006N_10434.pdf?sequence=1&isAllowed=y.

Britayev, T.A., Rzhavsky, A.V., Pavlova, L.V., Dvoretskij, A.G., 2010. Studies on impact of the alien red king crab (*Paralithodes camtschaticus*) on the shallow water benthic communities of the Barents Sea. J. Appl. Ichthyol. 26, 66–73. https://doi.org/10.1111/j.1439-0426.2010.01494.x.

Brunton, N.P., Mason, C., Collins, M.J., 2015. Rapid microwave assisted preparation of fatty acid methyl esters for the analysis of fatty acid profiles in foods. J. Anal. Chem. 70 (10), 1218–1224. https://doi.org/10.7868/s0044450215100047.

Calvo, N.S., Stumpf, L., Cortés-Jacinto, E., Castillo Díaz, F., López Greco, L.S., 2017. Mobilization of energetic reserves during starvation in juveniles of different size of the red claw crayfish *Cherax quadricarinatus*. Aquac. Nutr. 24 (3), 952–960. https://doi.org/10.1111/anu.12631.

Çelik, M., Türeli, C., Çelik, M., Yanar, Y., Erdem, Ü., Küçükgülmez, A., 2004. Fatty acid composition of the blue crab (*Callinectes sapidus* Rathbun, 1896) in the north eastern Mediterranean. Food Chem. 88 (2), 271–273. https://doi.org/10.1016/j.foodchem.2004.01.038.

Cervellione, F., McGurk, C., Berger Eriksen, T., Van den Broeck, W., 2017. Effect of starvation and refeeding on the hepatopancreas of whiteleg shrimp *Penaeus vannamei* (Boone) using computer-assisted image analysis. J. Fish Dis. 40 (11), 1707–1715. https://doi.org/10.1111/jfd.12639.

Cherif, S., Frikha, F., Gargouri, Y., Miled, N., 2008. Fatty acid composition of green crab (*Carcinus mediterraneus*) from the Tunisian mediterranean coasts. Food Chem. 111 (4), 930–933. https://doi.org/10.1016/j.foodchem.2008.05.007.

Christiansen, J.S., Sparboe, M., Saether, B.-S., Siikavuopio, S.I., 2015. Thermal behaviour and the prospect spread of an invasive benthic top predator onto the Euro-Arctic shelves. Divers. Distrib. 21 (9), 1004–1013. https://doi.org/10.1111/ddi.12321.

Coates, C.J., Söderhäll, K., 2020. The stress–immunity axis in shellfish. J. Invertebr. Pathol., 107492 https://doi.org/10.1016/j.jip.2020.107492.

Commission Regulation (EU) No. 116/2010 of 9 February 2010 amending Regulation (EC) No. 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims. Off. J. Eur. Union, L37, 16–18.

Comoglio, L., Smolko, L., Amin, O., 2005. Effects of starvation on oxygen consumption, ammonia excretion and biochemical composition of the hepatopancreas on adult males of the False Southern King crab *Paralomis granulosa* (Crustacea, Decapoda). Comp. Biochem. Physiol. B, Biochem. Mol. Biol. 140 (3), 411–416. https://doi.org/10.1016/j.cbpc.2004.11.003.

Comoglio, L., Goldsmit, J., Amin, O., 2008. Starvation effects on physiological parameters and biochemical composition of the hepatopancreas of the southern king crab *Lithodes santolla* (Molina, 1782). Revista de Biología Marina y Oceanografía 43 (2). https://doi.org/10.4067/s0718-19572008000200012.

Copeman, L.A., Stoner, A.W., Ottmar, M.L., Daly, B., Parrish, C.C., Eckert, G.L., 2012. Total lipids, lipid classes, and fatty acids of newly settled red king crab (*Paralithodes camtschaticus*): comparison of hatchery-cultured and wild crabs. J. Shellfish Res. 31 (1), 153–165. https://doi.org/10.2983/035.031.0119.

D'Abramo, L.R., 1997. Triacylglycerols and fatty acids. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition. Advances in World Aquaculture. The World Aquaculture Society, pp. 71–84.

Ding, L., Fu, H., Hou, Y., Jin, M., Sun, P., Zhou, Q., 2017. Effects of starvation and feeding on blood chemistry, fatty acid composition and expression of vitellogenin and fatty acid-binding protein genes in female swimming crab *Portunus trituberculatus* broodstock. Fish. Sci. 83 (3), 455–464. https://doi.org/10.1007/s12562-017-1075-3.

Dvoretsky, A.G., Bichkaeva, F.A., Baranova, N.F., Dvoretsky, V.G., 2020. Fatty acid composition in the hepatopancreas of the Barents Sea red king crab. Biol. Bull. 47 (4), 332–338. https://doi.org/10.1134/s1062359020040044.

Dvoretsky, A.G., Bichkaeva, F.A., Baranova, N.F., Dvoretsky, V.G., 2021. Fatty acid composition of the Barents Sea red king crab (*Paralithodes camtschaticus*) leg meat. J. Food Compos. Anal. 98, 103826 https://doi.org/10.1016/j.jfca.2021.103826.

Falk-Petersen, J., Renaud, P., Anisimova, N., 2011. Establishment and ecosystem effects of the alien invasive red king crab (*Paralithodes camtschaticus*) in the Barents Sea–a review. ICES J. Mar. Sci. 68 (3), 479–488. https://doi.org/10.1093/icesjms/fsq192.

Gangopadhyay, N., Rai, D.K., Brunton, N.P., Gallagher, E., Harrison, S.M., 2017. Fatty acids, sterols and tocols in Irish barley varieties: profiling and correlation analysis. Eur. J. Lipid Sci. Technol. 119 (4), 1600213 https://doi.org/10.1002/ejlt.201600213.

Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquac. 1 (2), 71–124. https://doi.org/10.1111/j.1753-5131.2009.01006.x.

Gökoğlu, N., 2021. Crustacean shellfish. Shellfish Processing and Preservation. Springer, pp. 7–127. https://doi.org/10.1007/978-3-030-60303-8_2.

Graeve, M., Kattner, G., Piepenburg, D., 1997. Lipids in Arctic benthos: Does the fatty acid and alcohol composition reflect feeding and trophic interactions? Polar Biol. 18 (1), 53–61. https://doi.org/10.1007/s003000050158.

Hardy, D., Dutil, J.-D., Godbout, G., Munro, J., 2000. Survival and condition of hard shell male adult snow crabs (*Chionoecetes opilio*) during fasting at different temperatures. Aquaculture 189 (3-4), 259–275. https://doi.org/10.1016/s0044-8486(00)00377-x.

ISO, 1998. Meat and Meat Products — Determination of Total Ash. ISO Standard No. 936:1998. https://www.iso.org/standard/24783.html.

Jørgensen, L.L., Nilssen, E.M., 2011. The invasive history, impact and management of the red king crab *paralithodes camtschaticus* off the coast of Norway. In: Galil, B.S., Clark, P.F., Carlton, J.T. (Eds.), In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts. Springer, Netherlands, pp. 521–536. https://doi.org/10.1007/978-94-007-0591-3_18.

Krzeczkowski, R.A., Tenney, R.D., Kelley, C., 1971. Alaska king crab: fatty acid composition, carotenoid index and proximate analysis. J. Food Sci. 36 (4), 604–606. https://doi.org/10.1111/j.1365-2621.1971.tb15139.x.

Larsen, R., Eilertsen, K.-E., Elvevoll, E.O., 2011. Health benefits of marine foods and ingredients. Biotechnol. Adv. 29 (5), 508–518. https://doi.org/10.1016/j.biotechadv.2011.05.017.

Latyshev, N.A., Kasyanov, S.P., Kharlamenko, V.I., Svetashev, V.I., 2009. Lipids and of fatty acids of edible crabs of the north-western Pacific. Food Chem. 116 (3), 657–661. https://doi.org/10.1016/j.foodchem.2009.02.085.

Lian, F., De Conto, E., Del Grippo, V., Harrison, S.M., Fagan, J., Lyng, J.G., Brunton, N.P., 2021a. High-pressure processing for the production of added-value claw meat from edible crab (*Cancer pagurus*). Foods 10 (5), 955. https://doi.org/10.3390/foods10050955.

Lian, F., Siikavuopio, S.I., Hustad, A., Thesslund, T., Lindberg, S.-K., Lorentzen, G., 2021b. Comparative quality evaluation of processed clusters obtained from red king crab (*Paralithodes camtschaticus*) typical of spring and autumn harvests in the Barents Sea. Food Control 123, 107826. https://doi.org/10.1016/j.foodcont.2020.107826.

Loher, T., Armstrong, D.A., 2005. Historical changes in the abundance and distribution of ovigerous red king crabs (*Paralithodes camtschaticus*) in Bristol Bay (Alaska), and potential relationship with bottom temperature. Fish. Oceanogr. 14 (4), 292–306. https://doi.org/10.1111/j.1365-2419.2005.00337.x.

Lorente-Cebrián, S., Costa, A.G.V., Navas-Carretero, S., Zabala, M., Laiglesia, L.M., Martínez, J.A., Moreno-Aliaga, M.J., 2015. An update on the role of omega-3 fatty acids on inflammatory and degenerative diseases. J. Physiol. Biochem. 71 (2), 341–349. https://doi.org/10.1007/s13105-015-0395-y.

Lorentzen, G., Voldnes, G., Whitaker, R.D., Kvalvik, I., Vang, B., Gjerp Solstad, R., Thomassen, M.R., Siikavuopio, S.I., 2018. Current status of the red king crab (*Paralithodes camtschaticus*) and snow crab (*Chionoecetes opilio*) industries in Norway. Rev. Fish. Sci. Aquac. 26 (1), 42–54. https://doi.org/10.1080/23308249.2017.1335284.

Lorentzen, G., Lian, F., Siikavuopio, S.I., 2019. Quality parameters of processed clusters of red king crab (*Paralithodes camtschaticus*) - Effects of live holding at 5 and 10 °C up to 92 days without feeding. Food Control 95, 142–149. https://doi.org/10.1016/j.foodcont.2018.07.041.

Lorentzen, G., Lian, F., Siikavuopio, S.I., 2020. Live holding of snow crab (*Chionoecetes opilio*) at 1 and 5 °C without feeding — quality of processed clusters. Food Control 114, 107221. https://doi.org/10.1016/j.foodcont.2020.107221.

Lorenzo, R.A., Tomac, A., Tapella, F., Yeannes, M.I., Romero, M.C., 2021. Biochemical and quality parameters of southern king crab meat after transport simulation and re-immersion. Food Control 119, 107480. https://doi.org/10.1016/j.foodcont.2020.107480.

Ma, Q.-Q., Chen, Q., Shen, Z.-H., Li, D.-L., Han, T., Qin, J.-G., Chen, L.-Q., Du, Z.-Y., 2017. The metabolomics responses of Chinese mitten-hand crab (*Eriocheir sinensis*) to different dietary oils. Aquaculture 479, 188–199. https://doi.org/10.1016/j.aquaculture.2017.05.032.

Marques, A., Teixeira, B., Barrento, S., Anacleto, P., Carvalho, M.L., Nunes, M.L., 2010. Chemical composition of Atlantic spider crab *Maja brachydactyla*: human health implications. J. Food Compos. Anal. 23 (3), 230–237. https://doi.org/10.1016/j.jfca.2009.10.007.

Mcleod, L.E., Carter, C.G., Johnston, D.J., 2004. Changes in the body composition of adult male southern rock lobster, *Jasus edwardsii*, during starvation. J. Shellfish Res. 23 (1), 257+.

Mercier, L., Racotta, I.S., Yepiz-Plascencia, G., Muhlia-Almazán, A., Civera, R., Quiñones-Arreola, M.F., Wille, M., Sorgeloos, P., Palacios, E., 2009. Effect of diets containing different levels of highly unsaturated fatty acids on physiological and immune responses in Pacific whiteleg shrimp *Litopenaeus vannamei* (Boone) exposed to handling stress. Aquac. Res. 40 (16), 1849–1863. https://doi.org/10.1111/j.1365-2109.2009.02291.x.

Munian, A., Cockcroft, A., Hoffman, L.C., Auerswald, L., 2020. Depot lipids in mature palinurid decapods (Crustacea). Invertebr. Reprod. Dev. 1–11. https://doi.org/10.1080/07924259.2020.1846631.

Nilssen, E.M., Sundet, J.H., 2006. The introduced species red king crab (*Paralithodes camtschaticus*) in the Barents Sea: II. Growth increments and moulting probability. Fish. Res. 82 (1–3), 319–326. https://doi.org/10.1016/j.fishres.2006.05.008.

Oug, E., Cochrane, S.K.J., Sundet, J.H., Norling, K., Nilsson, H.C., 2011. Effects of the invasive red king crab (*Paralithodes camtschaticus*) on soft-bottom fauna in Varangerfjorden, northern Norway. Mar. Biodivers. 41 (3), 467–479. https://doi.org/10.1007/s12526-010-0068-6.

Ozhigin, V.K., Ingvaldsen, R.B., Loeng, H., Boitsov, V.D., Karsakov, A.L., 2011. The barents Sea. In: Jakobsen, T., Ozhigin, V.K. (Eds.), The Barents Sea Ecosystem, Resources, Management. Half a Century of Russian - Norwegian Cooperation. Tapir Academic Press, pp. 39–76. Retrieved April 01, 2021 from. https://imr.brage.unit.no/imr-xmlui/handle/11250/109444.

Parolini, C., 2019. Effects of fish *n*-3 PUFAs on intestinal microbiota and immune system. Mar. Drugs 17 (6), 374. https://doi.org/10.3390/md17060374.

Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. In: Kainz, M., Brett, M., Arts, M. (Eds.), Lipids in Aquatic Ecosystems. Springer, pp. 309–326. https://doi.org/10.1007/978-0-387-89366-2_13.

Pavlova, L.V., 2009. Estimation of foraging on the sea urchin *Strongylocentrotus droebachiensis* (Echinoidea: echinoida) by the red king crab *Paralithodes camtschaticus* (Malacostraca: decapoda) in coastal waters of the Barents Sea. Russ. J. Mar. Biol. 35 (4), 288–295. https://doi.org/10.1134/s1063074009040038.

Pinchukov, M.A., Sundet, J.H., 2011. Red king crab. In: Jakobsen, T., Ozhigin, V.K. (Eds.), The Barents Sea Ecosystem, Resources, Management. Half a Century of Russian - Norwegian Cooperation. Tapir Academic Press, pp. 160–167. Retrieved April 01, 2021 from: https://imr.brage.unit.no/imr-xmlui/handle/11250/109444.

Ponomareva, T., Timchenko, M., Filippov, M., Lapaev, S., Sogorin, E., 2021. Prospects of red king crab hepatopancreas processing: fundamental and applied biochemistry. Recycling 6 (1), 3. https://doi.org/10.3390/recycling6010003.

R Core Team, 2020. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reppond, K.D., 2009. Biochemistry of Red King Crab (*Paralithodes Camtschaticus*) From Different Locations in Alaskan Waters. NMFS-NWFSC-102. Retrieved April 01, 2021 from:. U.S. Dept. Commer., NOAA Tech. Memo https://repository.library.noaa.gov/view/noaa/3668.

Risso, S.J., Carelli, A.A., 2012. Nutrient composition of raw and cooked meat of male southern king crab (*Lithodes santolla* Molina, 1782). J. Aquat. Food Prod. Technol. 21 (5), 433–444. https://doi.org/10.1080/10498850.2011.608482.

Ritar, A.J., Dunstan, G.A., Crear, B.J., Brown, M.R., 2003. Biochemical composition during growth and starvation of early larval stages of cultured spiny lobster (*Jasus edwardsii*) phyllosoma. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. 136 (2), 353–370. https://doi.org/10.1016/s1095-6433(03)00167-3.

Rocha, F., Rocha, A.C., Baião, L.F., Gadelha, J., Camacho, C., Carvalho, M.L., Arenas, F., Oliveira, A., Maia, M.R.G., Cabrita, A.R., Pintado, M., Nunes, M.L., Almeida, C.M.R., Valente, L.M.P., 2019. Seasonal effect in nutritional quality and safety of the wild sea urchin *Paracentrotus lividus* harvested in the European Atlantic shores. Food Chem. 282, 84–94. https://doi.org/10.1016/j.foodchem.2018.12.097.

Rőszer, T., 2014. The invertebrate midintestinal gland ("hepatopancreas") is an evolutionary forerunner in the integration of immunity and metabolism. Cell Tissue Res. 358 (3), 685–695. https://doi.org/10.1007/s00441-014-1985-7.

Sacristán, H.J., Rodríguez, Y.E., De los Angeles Pereira, N., López Greco, L.S., Lovrich, G.A., Fernández Gimenez, A.V., 2017. Energy reserves mobilization: strategies of three decapod species. PLoS One 12 (9), e0184060. https://doi.org/10.1371/journal.pone.0184060.

Sacristán, H.J., Di Salvatore, P., Fernández-Gimenez, A.V., Lovrich, G.A., 2019. Effects of starvation and stocking density on the physiology of the male of the southern king crab *Lithodes santolla*. Fish. Res. 218, 83–93. https://doi.org/10.1016/j.fishres.2019.05.006.

Sacristán, H.J., Mufari, J.R., Lorenzo, R.A., Boy, C.C., Lovrich, G.A., 2020. Ontogenetic changes in energetic reserves, digestive enzymes, amino acid and energy content of *Lithodes santolla* (Anomura: lithodidae): baseline for culture. PLoS One 15 (5), e0232880. https://doi.org/10.1371/journal.pone.0232880.

Saini, R.K., Keum, Y.-S., 2018. Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance — a review. Life Sci. 203, 255–267. https://doi.org/10.1016/j.lfs.2018.04.049.

Sánchez-Paz, A., García-Carreño, F., Muhlia-Almazán, A., Peregrino-Uriarte, A.B., Hernández-López, J., Yepiz-Plascencia, G., 2006. Usage of energy reserves in crustaceans during starvation: status and future directions. Insect Biochem. Mol. Biol. 36 (4), 241–249. https://doi.org/10.1016/j.ibmb.2006.01.002.

Siikavuopio, S.I., James, P., 2015. Effects of temperature on feed intake, growth and oxygen consumption in adult male king crab *Paralithodes camtschaticus* held in captivity and fed manufactured diets. Aquac. Res. 46 (3), 602–608. https://doi.org/10.1111/are.12207.

Siikavuopio, S.I., James, P., Olsen, B.R., Evensen, T., Mortensen, A., 2016. Holding wild caught red king crab, *Paralithodes camtschaticus*: effects of stocking density and feeding on survival and meat content. Aquac. Res. 47 (3), 870–874. https://doi.org/10.1111/are.12546.

Siikavuopio, S.I., Johansson, G.S., James, P., Lorentzen, G., 2019.). Effect of starvation on the survival, injury, and weight of adult snow crab, *Chionoecetes opilio*. Aquac. Res. 50 (2), 550–556. https://doi.org/10.1111/are.13926.

Solstad, R.G., Descomps, A., Siikavuopio, S.I., Karstad, R., Vang, B., Dragøy Whitaker, R., 2021. First observation of seasonal variations in the meat and co-products of the snow crab (*Chionoecetes opilio*) in the Barents Sea. Sci. Rep. 11 (1) https://doi.org/10.1038/s41598-021-85101-z.

Sprecher, H., 2000. Metabolism of highly unsaturated *n*-3 and *n*-6 fatty acids. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1486 (2-3), 219–231. https://doi.org/10.1016/s1388-1981(00)00077-9.

Stevens, B., Jewett, S., 2014. Growth, molting, and feeding of king crabs. In: Stevens, B. G. (Ed.), King Crabs of the World. CRC Press, pp. 315–362. https://doi.org/10.1201/b16664-11.

Sundet, J.H., 2014. Red king crab in the barents Sea. In: Stevens, B.G. (Ed.), King Crabs of the World. CRC Press, pp. 485–500. https://doi.org/10.1201/b16664-15.

Thériault, I., Pernet, F., 2007. Lipid nutrition and settlement behaviour in American lobster *Homarus americanus*. Aquat. Biol. 1, 121–133. https://doi.org/10.3354/ab00015.

Thomas, M.D., Schram, J.B., Clark-Henry, Z.F., Yednock, B.K., Shanks, A.L., Galloway, A. W.E., 2020. Juvenile Dungeness crabs (*Metacarcinus magister*) selectively integrate and modify the fatty acids of their experimental diets. Philos. Trans. Biol. Sci. 375 (1804), 20200038 https://doi.org/10.1098/rstb.2020.0038.

Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquac. Res. 41 (5), 717–732. https://doi.org/10.1111/j.1365-2109.2008.02150.x.

Voldnes, G., Kvalvik, I., Nøstvold, B., 2016. Levende krabbe i Sør-Korea [Live crab in South-Korea]. Nørsk Sjømat 5, 8–10.

Voldnes, G., Kvalvik, I., Nøstvold, B., 2020. Taking care of a highly valuable resource throughout the value chain - Lack of market orientation in red king crab export? Mar. Policy 117, 103965. https://doi.org/10.1016/j.marpol.2020.103965.

Wang, X., Jin, M., Cheng, X., Hu, X., Zhao, M., Yuan, Y., Sun, P., Jiao, L., Betancor, M.B., Tocher, D.R., Zhou, Q., 2021. Dietary DHA/EPA ratio affects growth, tissue fatty acid profiles and expression of genes involved in lipid metabolism in mud crab *Scylla paramamosain* supplied with appropriate *n*-3 LC-PUFA at two lipid levels. Aquaculture 532, 736028. https://doi.org/10.1016/j.aquaculture.2020.736028.

Watts, A.J.R., McGill, R.A.R., Albalat, A., Neil, D.M., 2014. Biophysical and biochemical changes occur in *Nephrops norvegicus* during starvation. J. Exp. Mar. Biol. Ecol. 457, 81–89. https://doi.org/10.1016/j.jembe.2014.03.020.

Wen, X., Chen, L., Ku, Y., Zhou, K., 2006. Effect of feeding and lack of food on the growth, gross biochemical and fatty acid composition of juvenile crab, *Eriocheir sinensis*. Aquaculture 252 (2–4), 598–607. https://doi.org/10.1016/j.aquaculture.2005.07.027.

WHO/FAO (World Health Organization & Food and Agriculture Organization of the United Nations), 2011. Report of the Joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish Consumption. January 2010 Retrieved April 01, 2021 from:. World Health Organization, Rome, Italy, pp. 25–29 https://apps.who.int/iris/handle/10665/44666.

Woll, A.K., Berge, G.M., 2007. Feeding and management practices affect quality improvement in wild-caught edible crab (*Cancer pagurus*). Aquaculture 269 (1–4), 328–338. https://doi.org/10.1016/j.aquaculture.2007.04.022.

Xu, C., Ma, Q., Li, E., Liu, Y., Wang, X., Du, Z., Qin, J.G., Chen, L., 2021. Response of lipid molecular structure to dietary lipid type in Chinese mitten crab *Eriocheir sinensis*: a deep lipidomics analysis. Aquac. Rep. 19, 100596 https://doi.org/10.1016/j.aqrep.2021.100596.

Yuan, Y., Xu, F., Jin, M., Wang, X., Hu, X., Zhao, M., Cheng, X., Luo, J., Jiao, L., Betancor, M.B., Tocher, D.R., Zhou, Q., 2021. Untargeted lipidomics reveals metabolic responses to different dietary *n*-3 PUFA in juvenile swimming crab (*Portunus trituberculatus*). Food Chem. 354, 129570 https://doi.org/10.1016/j.foodchem.2021.129570.

Zhuang, K., Wu, N., Wang, X., Wu, X., Wang, S., Long, X., Wei, X., 2016. Effects of 3 feeding modes on the volatile and nonvolatile compounds in the edible tissues of female chinese mitten crab (*Eriocheir sinensis*). J. Food Sci. 81 (4), S968–S981. https://doi.org/10.1111/1750-3841.13229.