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Live holding of snow crab (*Chionoecetes opilio)* at 1 and 5 °C without feeding — Quality of processed clusters

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ABSTRACT

Snow crab (*Chionoecetes opilio*) has become an essential resource in the Norwegian fishery. Today, the snow crabs are processed on-board into two cooked-frozen sections (i.e., clusters). However, there has been increasing interest in live holding (LH) in on-shore facilities, which enables more flexible management. This study aimed to evaluate the effects of time and temperature during LH without feeding in terms of mortality, limb loss, and the quality of the cooked clusters. Snow crabs ($N = 150$) were held without feeding during both an acclimatization period of 21 days at 4 °C and a subsequent temperature study at 1 and 5 °C for additional 90 days, with a total LH time of 111 days. At the start of the temperature study (LH day 21) and at LH day 49, 89, and 111, the snow crabs ($N = 28$) were sampled and processed, followed by analytical determinations. The hepatosomatic index decreased significantly ($p < 0.05$) with increasing LH time. Both cluster yield and meat content decreased during the LH time, but the effect was significant ($p < 0.05$) only at LH day 89. Except for the water content, the quality parameters of the processed clusters were not significantly affected by the LH temperature ($p > 0.05$). The results indicate that LH up to 89 days in absence of feed can be a possible practice for the business operators, with minimal adverse impact on the commercial value of the product. Although no mortality and a low limb loss rate were observed during the holding period, the marked mobilization of nutrients from the hepatopancreas raises a concern about the physiological condition of the crab during long-term LH without feeding. Thus, further investigation of the potential distress and nutritional status of the snow crab during LH without feeding is required.

1. Introduction

The snow crab *Chionoecetes opilio* (O. Fabricius, 1788) has become valuable for the Norwegian fish industry (Lorentzen et al., 2018). In 2018 and 2019, a total of, respectively, 2803 and 4049 metric tons of snow crab was captured by Norwegian vessels in the Barents Sea (Norwegian Fishermen's Sales Organization, 2020). The captured volume was entirely processed into cooked-frozen clusters. A cluster comprises four walking legs and a cheliped bearing the claw, assembled in a shoulder joint. Cooked-frozen clusters amounted to NOK 165 and 312 million in 2018 and 2019, respectively, and they were exported mainly to the USA, Japan, Denmark, Indonesia, the Netherlands, and South Korea (Norwegian Seafood Council, 2020).

In Norway, it is common practice to process the snow crab on-board immediately after capture. Processing starts with splitting the two clusters from the carapace. The clusters are then cooked, cooled, and frozen on-board. The reason for on-board processing is partly due to the long distance between catching areas and on-shore facilities, and partly due to the lack of an established live holding (LH) technology for snow crab either on-board or on-shore, resulting in high mortality rates (Lorentzen et al., 2018). Nonetheless, it may be foreseen an increasing interest in trading of live snow crab in the near future, following the trend observed in recent years for red king crab (*Paralithodes camtschaticus*) (Lorentzen et al., 2018). In this scenario, LH of snow crab before processing or live export would constitute a valuable supplement to the existing practices (Siikavuopio, James, et al., 2017). The LH may enable flexible management of this resource, especially in the periods of the year with high product demand (Siikavuopio, James, Olsen, Evensen, & Mortensen, 2016). Also, the LH allows stocking of snow crab both on-board and in coastal facilities, resulting in more cost-efficient processing, primarily if it is conducted in processing plants close to the LH location. Furthermore, the operative costs related to LH of snow crab may be reduced when it is carried out without feeding. However, in several crustacean species, the absence of feed can lead to cannibalism and aggressiveness, which in turn affects animal welfare and quality (Wickins & Lee, 2002). Nevertheless, it has been shown that

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adult snow crab can be kept at a temperature ranging from 4.4 to 5.4 °C for up to 100 days in the absence of feed without significant risk of injury or mortality (Siikavuopio, Johansson, James, & Lorentzen, 2019). Still, snow crab may be sensitive to high temperatures during LH (Hardy, Dutil, Godbout, & Munro, 2000; James et al., 2013; Siikavuopio, Whitaker, et al., 2017) as a temperature *preferendum* of 1.0–1.6 °C has been reported for this crab species (Siikavuopio, Bakke, Sæther, Thesslund, & Christiansen, 2019).

In decapod crustaceans, the hepatopancreas, also referred to as midgut gland or perigastric organ, is a crucial organ responsible for the digestion and absorption of nutrients, storage of reserves, excretion of waste metabolites, and production of immune factors like hemocyanin (Cervellione, McGurk, & Van den Broeck, 2017; Rőszer, 2014). Therefore, the hepatopancreas proportion of the total body weight (i.e., the hepatosomatic index) can be considered as a valuable indicator of the nutritional status (Jones & Obst, 2000).

Both the cluster yield and the meat content are important quality parameters for the crab industry and, more in general, for crab business operators and retailers along the supply chain. For the consumers, especially in Asian markets, the meat content of the leg is a decisive quality parameter (Voldnes, Kvalvik, & Nøstvold, 2019). In a previous study on the LH of red king crab without feeding, the yield and meat content decreased substantially with increasing LH time and temperature (Lorentzen, Lian, & Siikavuopio, 2019). In the case of snow crab, however, there is limited knowledge on how cluster yield and meat content are influenced during LH. Hardy et al. (2000) performed LH studies on snow crab without feeding at 1, 5, and 10 °C for up to five months, although with the focus on the survival and physiological conditions of the crabs, rather than on the quality aspects of the processed clusters.

Therefore, the present study aimed to evaluate the effect of time and temperature during LH without feeding on the mortality and limb loss of snow crab as well as on the quality of the processed clusters. In detail, snow crabs were held without feeding both during an acclimatization period of 21 days at 4 °C and a subsequent temperature study at 1 and 5 °C for additional 90 days, with a total LH time of 111 days. The nutritional status of the snow crabs was evaluated on the basis of the hepatosomatic and the cheliped index. The studied quality parameters included the cluster yield and leg meat content as well as the water content, pH, and water holding capacity of the cooked meat extracted from the walking legs.

2. Material and methods

2.1. Harvest and live holding

In October 2016, adult male snow crab were caught by the vessel Northeastern (Opilio AS), with snow crab pots in the NEAFC area (74.58 latitude and 38.49 longitude) at 150 m depth and 2 °C. The crab (N = 150) were transported in containers with natural seawater to the Aquaculture Research Station in Kårvik (Troms, Norway) and immediately placed in two 6 m^3 indoor holding tanks supplied with running natural seawater with a salinity of 33‰ (w/v) at 4 °C. The acclimatization lasted for 21 days, and the crabs were not fed in this period (Fig. 1).

At the end of the acclimatization, the first sampling was performed (N = 28, LH time day 21), and, at the same time, the temperature study started. This was conducted on the remaining snow crabs (N = 122) which were first individually weighed and inspected for injuries, with the focus on limb loss, and then randomly and equally distributed into eight circular indoor tanks (500 L) supplied with seawater at a constant temperature of either 1 or 5 °C (four replicates per LH temperature). The seawater was continuously UV-treated and filtered through a sand filter (150 μm nominal cut-off) at a flow rate of 10 L min^{-1} (kg crab)$^{-1}$. The temperature and the oxygen level of the seawater were monitored daily using appropriate data loggers (HOBO Water Temperature Pro v2, Onset Computer Corp., Bourne, MA, USA; Handy Polaris, OxyGuard International A/S, Farum, Denmark).

In each LH tank, there were at least eight crabs that were marked with vinyl numbered tags (FTF-69, Floy® tag & mfg. Inc., Seattle, WA, USA) glued on the carapace in order to keep them traceable throughout the temperature study.

The average snow crab weight and carapace lengths were 722 g (± 185) and 113 mm (± 7), respectively. The average stocking density in the LH tanks was 14.5 and 9.6 kg m^{-2}, in the beginning and in the end, respectively, of the temperature study. The crabs were kept in the dark, except for daily inspection using a headlight.

The temperature study started on the November 11, 2016 and lasted until the February 9, 2017. Sampling was performed at day 28, 68, and 90 during the temperature study (corresponding to 21, 49, 89, and 111 days of total LH time) by withdrawing a balanced number of snow crabs from each tank across the two LH temperature groups (Fig. 1). At each sampling time, 14 snow crabs were sampled for each LH temperature. The sampled crabs were transferred to polystyrene boxes in a dry state and covered with gel ice (Cold Inc., Oakland, CA, USA), and then transported to Nofima, Tromsø. The maximum time between the collection of the crabs from the tank and the start of processing was 15 h. The snow crabs sampled at LH day 89 and 111, which were bearing the marking tag on the carapace, were inspected for potential limb loss of walking legs and chelipeds.

2.2. Processing

The processing was performed as described in Fig. 1 and started with registering the weight of the live snow crab, which were then split into two clusters using a butchering iron. Each cluster was labeled using a T-bar tag (FD-94, Floy® tag & mfg) in order to keep them traceable throughout the processing.

After splitting, the hepatopancreas was immediately collected from the carapace and weighed. Afterward, the chelipeds were removed from the cluster and weighed. Before the weight was registered, the chelipeds were placed in a vertical position aiming drainage of the free body fluid (FBF) which is the liquid, containing hemolymph and water, dripping freely from the appendages when the exoskeleton is cut (Mizuta, Kobayashi, & Yoshinaka, 2001).

The clusters were cleaned from remaining gills while being kept in a vertical position with the shoulder joint pointing downwards to facilitate drainage of the FBF. The cleaned clusters were then weighed and subsequently soaked into fresh water with salt (3.5% w/v) (The Norwegian Salt Company Ltd., Bergen, Norway) at a temperature of 1–2 °C for 1 h. This operation, which aimed to achieve further removal of the FBF, is hereinafter referred to as de-bleeding (Lorentzen et al., 2019). The clusters were then drained for at least 10 min before their weight was registered.

Subsequently, the clusters were distributed into meshed cages for further processing. Cooking was performed by immersion of the cages into fresh water at 92.2–92.4 °C, targeting a core temperature of 80 °C in the most proximal article (i.e., *merus*) of the second walking leg. This temperature was achieved after about 260 s. The core temperature was logged using K-type thermocouples connected to data loggers (model 175H1, Testo Ltd., Hampshire, UK). Afterward, the clusters were cooled by immersion of the cages into fresh water with ice until the core temperature was below 4 °C. The clusters were then drained for at least 10 min before their weight was registered.

2.3. Hepatosomatic index and cheliped index

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

$$HSI = [(C_H / A) \times 100] \quad (1)$$

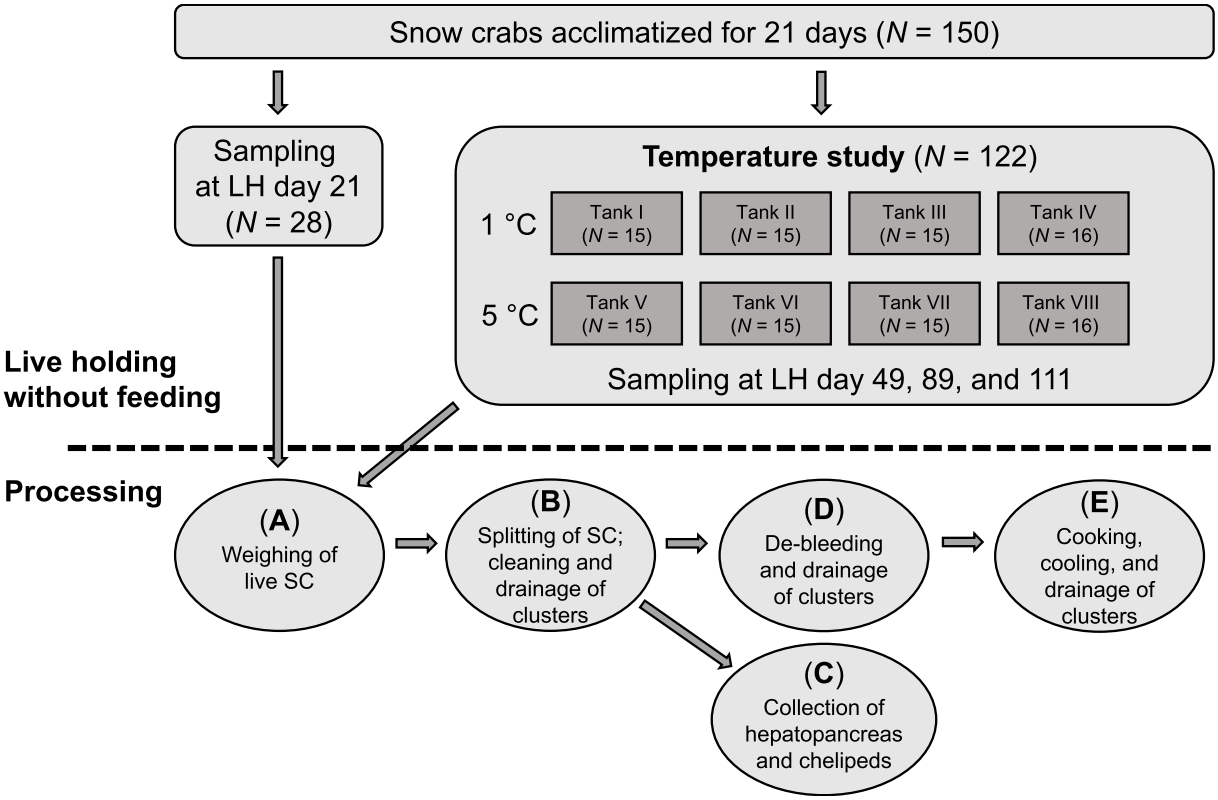

Fig. 1. Experimental set up for the live holding (LH) of snow crab (SC) without feeding at 1 and 5 °C followed by processing. During the temperature study, a balanced number of snow crabs was sampled from each tank across the two temperature groups (n = 14 for each combination of LH time and temperature). Capital letters in brackets correspond to the steps of weight registration.

$$CI = [(C_C / A) \times 100] \quad (2)$$

where C_H and C_C were the weight of the hepatopancreas and of the two chelipeds, respectively, and A was the live snow crab weight.

2.4. Meat content

The meat content of the cooked clusters was measured by digital analysis of images of two parallel longitudinal sections of the *merus*. The digital images were acquired in a lightbox as previously described (Lian et al., 2018). The meat content was calculated as:

$$\text{Meat content (\%)} = [(\text{Area occupied by meat} / \text{Total inner area}) \times 100] \quad (3)$$

The meat content was measured in clusters from the snow crab sampled at the start of the temperature study (LH day 21), and then at LH day 89 and 111. The meat content was analyzed in the *merus* of up to three legs from the same cluster and in at least five clusters generated from different crabs for each of the combinations of LH time and temperature considered.

2.5. Cluster yield and relative weight changes during processing

To obtain an overview of the effect of LH conditions and processing on the cluster yield and the relative cluster weight changes, four separate calculations were performed (Lorentzen et al., 2019).

In detail, the cluster yield (CY) of the raw clusters was calculated as:

$$CY_{raw} (\%) = [(2D / A) \times 100] \quad (4)$$

where 2D was the sum of the weight of the right and left cluster from the same crab after splitting, cleaning, de-bleeding, and drainage (Fig. 1, step D), and A was the live weight of the corresponding crab (Fig. 1, step A).

Furthermore, the yield of the cooked clusters was calculated as:

$$CY_{cooked} (\%) = [(2E / A) \times 100] \quad (5)$$

where 2E was the sum of the weight of the two cooked, cooled, and drained clusters obtained from the same crab (Fig. 1, step E), and A was the live weight of the corresponding crab (Fig. 1, step A).

The cluster weight change relative to the effect of the de-bleeding step was calculated as:

$$\Delta Cluster_{de\text{-}bled} (\%) = [((D - B) / B) \times 100] \quad (6)$$

where D was the weight of a single cluster after de-bleeding and drainage (Fig. 1, step D), and B was the weight of the corresponding single raw cluster after splitting and drainage before the de-bleeding (Fig. 1, step B).

The cluster weight change relative to the combined effect of the de-bleeding and cooking was calculated as:

$$\Delta Cluster_{cooked} (\%) = [((E - B) / B) \times 100] \quad (7)$$

where E was the weight of a single cluster after cooking, cooling, and drainage (Fig. 1, step E), and B was the weight of the corresponding single raw cluster as described above.

2.6. Water content, pH, and water holding capacity of cooked meat

The water content, pH, and water holding capacity (WHC) were analyzed on the meat extracted from the *merus* of the cooked clusters.

The analysis of the water content (n = 9 for LH day 21; n = 4 for each of the other combinations of LH time and temperature) was performed as described by Lorentzen et al. (2019). The pH (n = 4 for LH day 21; n = 2 for the other time and temperature combinations) was measured as described by Bendall (1973).

The analysis of the WHC (n = 12 for LH day 21; n = 4 for the other

Table 1
Values (mean ± standard deviation) of the response variables obtained during live holding (LH) without feeding for the snow crabs sampled at the start of the temperature study (LH day 21), and then at LH day 49, 89, and 111. From LH day 21 until LH day 111, the snow crabs were held at two temperatures, either 1 or 5 °C.

Response variable	Start of temperature study (LH day 21)	LH day 49		LH day 89		LH day 111		Significance (*p*-value)
		1 °C	5 °C	1 °C	5 °C	1 °C	5 °C	
ΔLive crab weight (%)	0.00	NA	NA	−1.94 ± 0.67	−2.02 ± 0.86	−1.65 ± 0.59	−2.09 ± 0.45	0.667
HSI (%)	6.5 ± 0.9^{a}	5.4 ± 0.9^{ab}	5.3 ± 0.8^{b}	5.0 ± 1.1^{b}	4.7 ± 0.6^{b}	4.8 ± 1.2^{b}	4.5 ± 0.6^{b}	< **0.001**
CI (%)	22.3 ± 2.7	21.7 ± 1.4	21.7 ± 2.2	23.2 ± 1.8	23.5 ± 1.6	21.3 ± 2.8	22.4 ± 1.3	0.109
Meat content (%)	87.6 ± 7.7^{a}	NA	NA	83.2 ± 5.2^{ab}	79.9 ± 8.2^{ab}	77.3 ± 7.0^{b}	75.9 ± 8.4^{b}	**0.023**
CY_{raw} (%)	50.1 ± 3.2^{a}	47.6 ± 2.5^{ab}	47.8 ± 2.6^{ab}	47.4 ± 2.3^{ab}	46.1 ± 3.0^{b}	46.2 ± 1.6^{b}	47.1 ± 2.1^{ab}	< **0.001**
CY_{cooked} (%)	49.6 ± 3.3^{a}	47.7 ± 2.7^{ab}	47.8 ± 2.6^{ab}	46.0 ± 3.3^{ab}	44.1 ± 4.6^{b}	45.3 ± 2.1^{b}	46.0 ± 2.8^{ab}	< **0.001**
$\Delta Cluster_{de\text{-}bled}$ (%)	−1.91 ± 2.42^{ab}	−0.59 ± 1.00^{a}	−0.64 ± 0.84^{a}	−1.70 ± 1.73^{ab}	−0.55 ± 1.46^{a}	−3.58 ± 1.85^{b}	−2.74 ± 1.41^{ab}	< **0.001**
$\Delta Cluster_{cooked}$ (%)	−1.02 ± 2.61^{a}	−0.02 ± 1.75^{a}	0.01 ± 1.25^{a}	−3.08 ± 2.89^{ab}	−4.71 ± 5.20^{ab}	−2.02 ± 2.28^{ab}	−2.30 ± 2.46^{b}	< **0.001**
Water content (%)	80.5 ± 1.8^{a}	82.5 ± 0.7^{a}	81.5 ± 0.4^{a}	81.7 ± 1.6^{a}	81.4 ± 0.8^{a}	82.6 ± 0.7^{a}	80.1 ± 0.4^{a}	**0.042**
pH	7.71 ± 0.34	7.91 ± 0.07	7.81 ± 0.17	7.58 ± 0.24	7.74 ± 0.02	7.94 ± 0.09	7.62 ± 0.25	0.845
WHC (%)	73.5 ± 5.8	73.9 ± 5.1	67.8 ± 2.3	77.4 ± 2.7	76.3 ± 1.9	67.9 ± 1.8	71.6 ± 6.9	0.061

Note. Different superscript letters within a row indicate significantly different mean values ($p < 0.05$, one-way ANOVA followed by Scheffe's *post-hoc* test). Significant probability values ($p < 0.05$) are highlighted in bold.
Abbreviations. NA, data not available; HSI, hepatosomatic index; CI, cheliped index; CY_{raw}, cluster yield raw; CY_{cooked}, cluster yield cooked; $\Delta Cluster_{de\text{-}bled}$, cluster weight change relative to the effect of the de-bleeding; $\Delta Cluster_{cooked}$, cluster weight change relative to the combined effect of the de-bleeding and cooking; WHC, water holding capacity.

time and temperature combinations) was performed as described by Skipnes, Østby, and Hendrickx (2007) with some modifications. Briefly, about 2 g of meat were placed on a disk of filter paper (Grade 3, Whatman™, Maidstone, UK) supported by a stainless-steel wire mesh located within a round (30 mm diameter) flat-bottom polyethylene tube. The tubes with the samples were centrifuged (Sorvall RC-5C, GMI Inc., Ramsey, MN, USA) at 1200 rpm for 15 min at 4 °C. The WHC was then calculated as:

$$WHC\ (\%) = [((W_0 - \Delta C) / W_0) \times 100] \quad (8)$$

where W_0 was the initial water content of the sample, and ΔC was the difference in sample weight before and after centrifugation.

2.7. Statistical analyses

The values of each response variable were expressed as mean (± standard deviation) and, unless otherwise specified, they were the result of determinations conducted on at least 24 snow crabs at the start of the temperature study (LH day 21) and on at least 11 snow crabs for each of the other combinations of LH time and temperature. The statistical analyses were performed considering each crab as a biological replicate.

The general effects of LH time and temperature on the response variables were investigated carrying out a full factorial analysis of variance (ANOVA) using the software Statistica™ (vers. 13.5, TIBCO Software Inc., Palo Alto, CA, USA). The explanatory variables LH time and temperature were handled as categorical factors, and the data obtained from the snow crabs sampled at the start of the temperature study (i.e., LH day 21, before distributing the 122 crabs into two temperature groups) were not considered for the full factorial ANOVA. In addition, for each response variable, a one-way ANOVA followed by *post-hoc* multiple comparisons (Scheffe's test) was conducted on data grouped by the combination of LH time and temperature in order to highlight significant differences between groups, including also the data at the start of the temperature study.

To elucidate the structure of data interdependency, correlation analysis and principal component analysis (PCA) were carried out using the package *FactoMineR* in the software R (vers. 3.6.2) (R Core Team, 2020). In detail, the Pearson product-moment correlation matrix of the response variables (HSI, CI, CY_{raw}, CY_{cooked}, meat content, $\Delta Cluster_{de\text{-}bled}$, and $\Delta Cluster_{cooked}$) was computed based on pairwise-complete observations ($n = 30$ for meat content, $n = 99$ for the other response variables considered) followed by a pairwise two-sided Pearson correlation significance test. The PCA was performed on the same response variables but considering "meat content" as a supplementary quantitative variable as these data were not available for one of the levels (i.e., LH day 49) of the factor LH time. Also, the data obtained from the snow crabs sampled at the start of the temperature study (LH day 21) were handled as supplementary observations.

All statistical analyses were carried out at 5% probability level ($p = 0.05$).

3. Results and discussion

3.1. Limb loss and mortality

During the temperature study (LH day 21–111), the oxygen level was above 95% in all eight tanks. No mortality nor molting was observed, supporting the findings reported by Siikavuopio, Bakke, et al. (2019) and Siikavuopio, Johansson, et al. (2019), and showing that the snow crab has the ability to endure prolonged periods without feed. In the LH interval day 21–89, 11 and nine crabs held at 1 and 5 °C, respectively, were inspected for potential limb loss. One walking leg was lost by one crab at 1 and by two crabs at 5 °C. In the LH interval day 21–111, none of the seven crabs inspected in the 1 °C group presented any limb loss, whereas one out of the 11 crabs inspected in the 5 °C group missed one leg. No clear differences in limb loss were observed between the two LH temperatures. Overall, a low limb loss rate shows a low territorial or aggressive behavior, and our results are in accordance with previous LH studies of snow crab held without feeding (Siikavuopio, Johansson, et al., 2019) where a low limb loss rate was observed even after 100 days of LH at a temperature ranging from 4.4 to 5.4 °C.

3.2. Changes in the live crab weight

Although the snow crabs were not fed during the LH, only a minor weight loss was observed. In the first 68 days of the temperature study

Table 2
Full factorial ANOVA for the response variables of the study. Live holding time and temperature were considered categorical factors in the ANOVA model.

Response variable	Factor	*df*	*SS*	*p*-value
ΔLive crab weight	Time	1	0.08	0.663
	Temperature	1	0.51	0.287
	Time × Temperature	1	0.24	0.461
	Error	28	12.10	
HSI	Time	2	6.8	**0.018**
	Temperature	1	1.1	0.242
	Time × Temperature	2	0.1	0.963
	Error	69	55.2	
CI	Time	2	40.3	**0.006**
	Temperature	1	3.8	0.315
	Time × Temperature	2	4.1	0.575
	Error	69	253.4	
Meat content	Time	1	132.5	0.123
	Temperature	1	29.2	0.459
	Time × Temperature	1	4.3	0.776
	Error	21	1079.7	
CY_{raw}	Time	2	17.9	0.218
	Temperature	1	0.1	0.901
	Time × Temperature	2	14.1	0.300
	Error	69	395.9	
CY_{cooked}	Time	2	101.1	**0.007**
	Temperature	1	1.9	0.656
	Time × Temperature	2	22.0	0.321
	Error	69	657.0	
$\Delta Cluster_{de\text{-}bled}$	Time	2	91.8	< **0.001**
	Temperature	1	7.8	0.053
	Time × Temperature	2	5.0	0.294
	Error	69	137.6	
$\Delta Cluster_{cooked}$	Time	2	190.4	< **0.001**
	Temperature	1	7.3	0.349
	Time × Temperature	2	9.4	0.567
	Error	69	568.3	
Water content	Time	2	1.9	0.327
	Temperature	1	9.5	**0.003**
	Time × Temperature	2	4.9	0.070
	Error	18	14.2	
pH	Time	2	0.08	0.508
	Temperature	1	0.02	0.544
	Time × Temperature	2	0.11	0.417
	Error	6	0.33	
WHC	Time	2	228.6	**0.006**
	Temperature	1	8.2	0.486
	Time × Temperature	2	87.8	0.094
	Error	17	274.2	

Note. Significant probability values ($p < 0.05$) are highlighted in bold.
Abbreviations. *df*, degrees of freedom; *SS*, sum-of-squares; HSI, hepatosomatic index; CI, cheliped index; CY_{raw}, cluster yield raw; CY_{cooked}, cluster yield cooked; $\Delta Cluster_{de\text{-}bled}$, cluster weight change relative to the effect of the de-bleeding; $\Delta Cluster_{cooked,}$ cluster weight change relative to the combined effect of the de-bleeding and cooking; WHC, water holding capacity.

(LH day 21–89), the average weight loss at 1 and 5 °C was 1.94% (± 0.67) and 2.02% (± 0.86), respectively (Table 1). At the end of the experiment, after 111 days of LH, the corresponding values for weight loss were 1.65% (± 0.59) and 2.09% (± 0.45). The differences in live crab weight were not significantly ($p > 0.05$) affected by either the LH time or temperature (Table 2). The minor variation in the live snow crab weight, observed during the LH period, shows the resilience on how unfed snow crabs can maintain their weight even up to 111 days of LH without feeding.

Our results are in agreement with previous LH studies on snow crab (Siikavuopio, Johansson, et al., 2019), European lobster (*Homarus gammarus*) (Albalat et al., 2019), southern king crab (*Lithodes santolla*) (Sacristán, Di Salvatore, Fernández-Gimenez, & Lovrich, 2019), and American lobster (*Homarus americanus*) (Stewart, Horner, & Arie, 1972) where also minor weight changes were observed. The absence of feed affects the metabolic activities of the crab and essential processes are maintained at the expense of accumulated endogenous energy reserves, which may result in weight loss (Comoglio, Smolko, & Amin, 2005). It has, however, been suggested that the volume of lost tissue mass is replaced by seawater to maintain the necessary body volume and internal turgidity (Comoglio, Goldsmit, & Amin, 2008; Dall, 1974). This may explain the relatively low live crab weight loss obtained in the present study.

3.3. Hepatosomatic index, cheliped index, and meat content

On day 21, at the start of the temperature study, the hepatosomatic index (HSI) was 6.5% (± 0.9). During LH, the HSI decreased with the time to values of 4.8% (± 1.2) and 4.5% (± 0.6) at the end of the experiment (LH day 111) for the snow crabs held at 1 and 5 °C, respectively (Table 1). The HSI of the snow crabs sampled during the temperature study was significantly lower ($p < 0.05$) than the initial HSI, except for the snow crabs sampled at LH day 49 from the 1 °C group. Although the earlier decrease in HSI observed in the snow crabs held at 5 °C may suggest a higher metabolic rate at this temperature than at 1 °C, overall only the LH time had a significant ($p = 0.018$) effect on the HSI values (Table 2). The trend observed for HSI values in the present study has also been reported by Siikavuopio, Bakke, et al. (2019) and Siikavuopio, Johansson, et al. (2019) for snow crabs held for 100 days without feeding at a temperature ranging from 4.4 to 5.4 °C, where the HSI decreased from 6.1% to 3.1%.

In crustaceans, the hepatopancreas is the primary organ for accumulation and storage of lipids and, to a lesser degree, of glycogen (Comoglio et al., 2008). In particular, lipids play a fundamental role in the physiology of cold-water marine organisms, where they provide both a high-density source of energy and are vital components for the functionality of cellular membranes (Stoner, Ottmar, & Copeman, 2010). In the absence of feed, the crab must utilize endogenous resources and tissues through catabolic activities to survive. In this regard, lipids are the first class of compounds being mobilized and metabolized (Jones & Obst, 2000; Watts, McGill, Albalat, & Neil, 2014; Wen, Chen, Ku, & Zhou, 2006).

The average values of the cheliped index (CI) were in the range of 21.3–23.5% during the temperature study (LH day 21–111) (Table 1) with no significant differences ($p > 0.05$) between snow crabs from different combinations of LH time and temperature. This indicates that, in the absence of feed, the snow crab tends to preserve the muscle tissue in the chelipeds in the effort to maintain their functionality and, therefore, the ability for foraging and feeding.

The average meat content of the leg of cooked clusters decreased significantly ($p < 0.05$) during the temperature study from the initial value of 87.6% (± 7.7) to 77.3% (± 7.0) and 75.9% (± 8.4) at the end of the experiment (LH day 111) for the snow crabs held at 1 and 5 °C, respectively (Table 1).

As illustrated in Fig. 2 with images of the *merus* of cooked snow crab clusters, the decrease in meat content is linked to the shrinkage of the muscle. The cooked muscle of the crabs appears remarkably shrunk along both the longitudinal and horizontal (i.e., axial) plane of the *merus*. It is interesting to note that, by contrast, the cooked muscle of red king crab appeared to be shrinking predominantly along the horizontal plane (Lorentzen et al., 2019). This shows the possible presence of differences between these two species in the degradation patterns of the muscle fibers in compensating for the absence of feed resources.

Irrespective of the degradation pattern, in crustaceans, a reduction in meat content during LH without feeding has often been associated

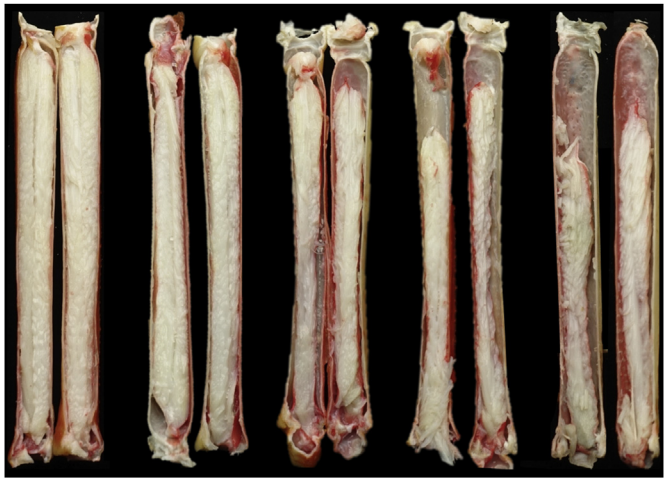

Fig. 2. Longitudinal cross-sections of *merus* of legs of cooked clusters of snow crab showing the meat content during live holding without feeding.

with protein catabolism (Sánchez-Paz et al., 2006). Moreover, the holding temperature has been described as a crucial parameter when combined with the absence of feed, determining the degradation rate of specific tissues (Albalat et al., 2019). Nonetheless, in the present study, even though the lowest meat content was observed in the snow crabs held at high temperature (5 °C), the meat content appeared to be mostly dependent on the LH time ($p = 0.123$), with a more limited influence of the LH temperature ($p = 0.459$) (Table 2). Hardy et al. (2000) reported that the protein content of the *merus* varied significantly with time, although not across temperatures (1–10 °C) during the fasting of snow crab. It can be hypothesized that the decrease in meat content observed in our study might be linked to a corresponding decrease in the protein content.

It should be highlighted that the extent of the reduction in muscle mass due to the LH time and temperature without feeding appears to be highly species-specific. Differently than the case of snow crab, in red king crabs held without feeding at 5 and 10 °C, the leg meat content decreased significantly as early as after 41 days with a significant influence of the temperature (Lorentzen et al., 2019). By contrast, the cluster yield of southern red king crab (*Lithodes santolla*) during LH without feeding for up to 60 days at 6–8 °C, remained in the range of 52–54%, and the level of proteins in the pereopod muscle remained unchanged (Sacristán et al., 2019).

Our results show that the priority of the snow crab is to compensate for the absence of feed with the mobilization of reserves in the hepatopancreas, as indicated by the rapid decrease in HSI. Secondly, the crab utilizes the muscle, as evidenced by the fact that the meat content was significantly reduced only at the end of the temperature study (LH day 111) (Table 1). Overall, this indicates that during LH without feeding, the snow crabs had the tendency to limit the mobilization of reserves in the muscle of the walking legs and chelipeds, in the effort to preserve the functionality of this tissue for motility, defense, and foraging purposes (Sacristán et al., 2017; Sánchez-Paz et al., 2006).

3.4. Cooking, cluster yield, and relative weight changes during processing

Although a core temperature of 80 °C was targeted in the cooking process, the temperature observed varied in the range of 80–86 °C. After cooking, the clusters were cooled for up to 15 min, resulting in a core temperature ranging from 0.6 to 3.2 °C. The variations in the core temperature in the cooking and cooling steps were most likely linked to differences in cluster size.

The cluster yield (CY) of raw and cooked snow crabs were calculated throughout the temperature study (LH day 21–111) (Table 1). The yield of raw clusters (CY_{raw}) varied in the range of 50.1 (± 3.2) to 46.1 (± 3.0) %, whereas the yield of cooked clusters (CY_{cooked}) varied in the range of 49.6 (± 3.3) to 44.1 (± 4.6) % (Table 1). For both CY_{raw} and CY_{cooked}, the lowest average values were registered for the snow crabs held for 68 days at 5 °C (total LH time 89 days). The LH time significantly affected ($p = 0.007$) the CY_{cooked}. In general, the effect of LH time on both CY_{raw} and CY_{cooked} was larger than that of LH temperature (Table 2).

Overall, at each sampling point, CY_{raw} and CY_{cooked} were within the same value range. By contrast, Lorentzen et al. (2019) reported marked differences between the yield of raw and cooked clusters of red king crab held for two months or longer without feeding. The difference was explained by a higher loss of water and water-soluble proteins due to changes in the microstructure of the crab muscle during the LH, which became apparent after the cooking process. The negligible difference observed between raw and cooked cluster yield in the present study suggests that the LH in the absence of feeding might have a lower impact on the muscle microstructure of the walking legs of the snow crab when compared to the red king crab. Further investigation, however, is warranted in order to elucidate any differences between the striated skeletal muscle of these two crab species, possibly linked to a characteristic sarcomere length (Claxton, Govind, & Elner, 1994) or distribution of intramuscular collagen fibers (Mizuta, Yoshinaka, Sato, & Sakaguchi, 1994).

The relative cluster weight changes during de-bleeding and cooking were calculated to clarify the dynamics of mass transfer in these processing steps. The weight changes due to de-bleeding ($\Delta Cluster_{de\text{-}bled}$) were generally small, ranging on average from −0.55 to −3.58%, with the lowest values observed for the snow crabs sampled at the end of the temperature study (LH day 111) (Table 1). The corresponding weight changes due to de-bleeding and cooking ($\Delta Cluster_{cooked}$) were also small, ranging on average from 0.01 to −4.71%, with the lowest values obtained for the snow crabs sampled at LH day 89 and at the end of the temperature study (LH day 111). In general, the proportion of weight loss occurring during cooking became significantly ($p < 0.001$) larger with the LH time (Table 2), and this, in turn, may indicate increased sensitivity of the myowater to the effect of heat with longer LH time in the absence of feed.

3.5. Water content, pH, and water holding capacity of cooked meat

The average water content of the cooked snow crab muscle ranged from 80.1% (± 0.4) to 82.6% (± 0.7) throughout the temperature study (LH day 21–111) (Table 1). The average values of pH ranged from 7.58 (± 0.24) to 7.94 (± 0.09), and no clear pattern emerged along the LH period. The pH range is comparable to the ones previously reported for cooked snow crab clusters (Lorentzen, Rotabakk, Olsen, Skuland, & Siikavuopio, 2016). The water holding capacity (WHC) of the cooked meat was in the range of 67.8% (± 2.3) to 77.4% (± 2.7). In general, during the temperature study, the water content and the WHC were significantly affected by the LH temperature ($p = 0.003$) and LH time ($p = 0.006$), respectively (Table 2). Nevertheless, no significant differences ($p > 0.05$) were found for the average values of water content, pH, or WHC between snow crabs from different combinations of LH time and temperature (Table 1).

3.6. Interdependency of response variables

The results of the correlation analysis are depicted in the correlogram (Fig. 3). The highest positive correlation ($r = 0.93$) was found between the yield of raw (CY_{raw}) and cooked (CY_{cooked}) clusters. Meat content was also positively correlated with the relative cluster weight changes during de-bleeding ($\Delta Cluster_{de\text{-}bled}$, $r = 0.68$) and cooking ($\Delta Cluster_{cooked}$, $r = 0.67$). This not only indicates that the cluster weight variation during processing is closely linked with the leg meat content, but also that the leg muscle fibers of snow crab may be less sensitive to liquid loss during thermal processing as compared to the

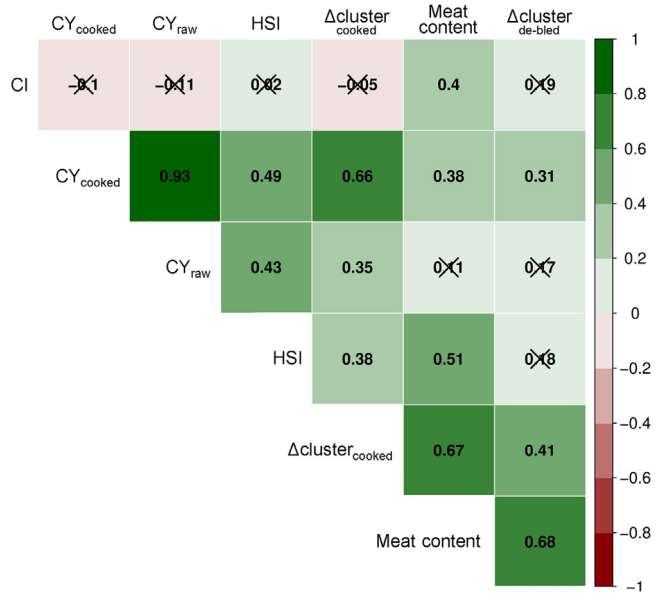

Fig. 3. Correlogram illustrating the Pearson correlation coefficient (r) between the response variables hepatosomatic index (HSI), cheliped index (CI), meat content, raw cluster yield (CY_{raw}), cooked cluster yield (CY_{cooked}), cluster weight change relative to the effect of the de-bleeding ($\Delta Cluster_{de\text{-}bled}$), and cluster weight change relative to the combined effect of the de-bleeding and cooking ($\Delta Cluster_{cooked}$). Insignificant ($p > 0.05$) correlations are marked by crosses.

case of red king crab (Lorentzen et al., 2019).

Furthermore, to obtain an overview of the quality parameters in relation to each individual snow crab in the temperature study, the data were subjected to principal component analysis (PCA) (Fig. 4). The loading plot (Fig. 4A) illustrates the first and second principal components (PC-1 and PC-2), which cumulatively accounted for 69.9% of the data variance. It can be noticed that among the quality parameters measured, HSI, CY_{raw}, $\Delta Cluster_{cooked}$, and CY_{cooked} were well described by PC-1 with standardized loadings ranging 0.64–0.95, while, on the contrary, CI was highly positively correlated with PC-2 (standardized loading 0.85).

The vectors for LH time and temperature (handled as supplementary variables) were orthogonal in the space delineated by PC-1 and PC-2, suggesting a weak interdependency of the effect of these factors on the quality parameters. Moreover, the effect of LH time (standardized loadings: PC-1 −0.42, PC-2 −0.16) accounted for a larger portion of total data variance compared to the effect of the LH temperature (standardized loadings: PC-1 −0.05, PC-2 0.19) (Fig. 4A).

The score plot given in Fig. 4B highlights the grouping of the observations according to the LH time. It can be noticed a clear clustering of the observations on the basis of different LH time, mainly along the PC-1. On the contrary, as depicted in the score plot in Fig. 4C, it was observed no clustering of the observations according to the LH temperature.

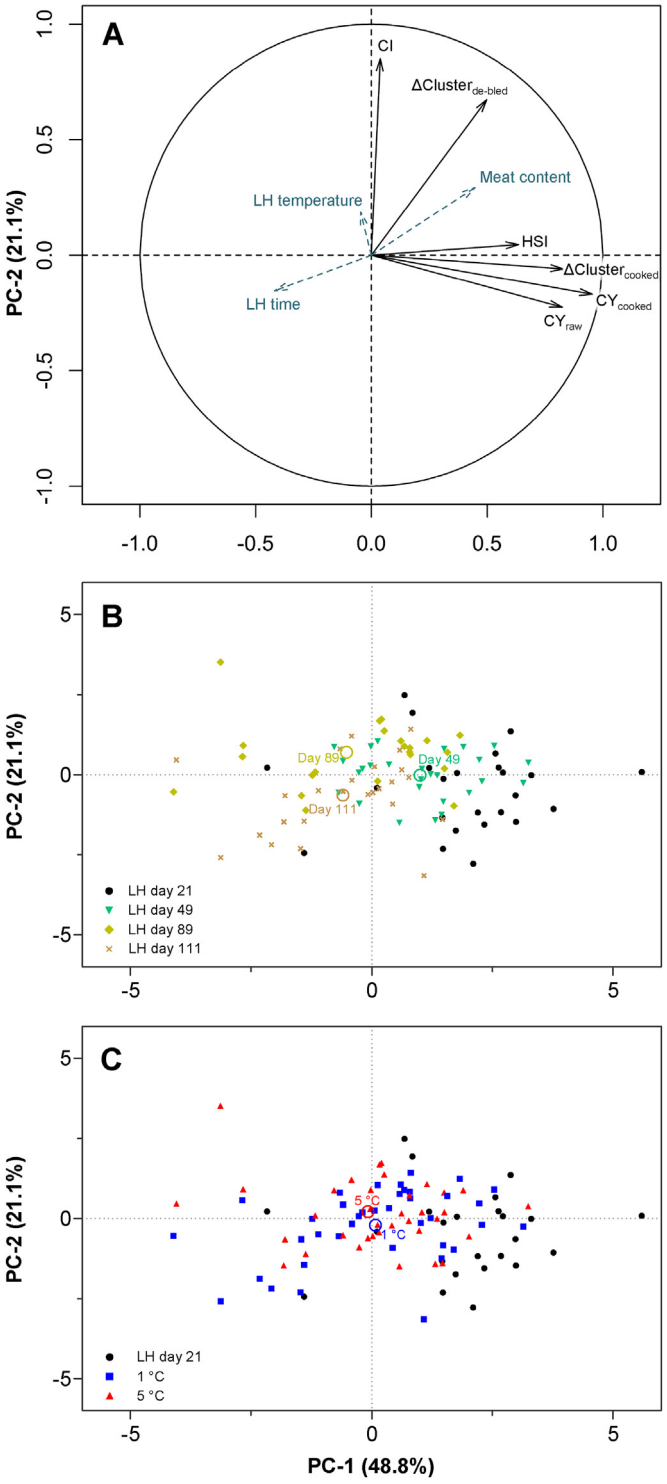

Fig. 4. Loading plot (A) and score plots with observations grouped by live holding (LH) time (B) and LH temperature (C) obtained by principal component analysis. In the score plots, the centroid of each factor level is indicated with (○). The data acquired for the snow crabs sampled at the start of the temperature study (day 21 of LH) were handled as supplementary observations. The response variables hepatosomatic index (HSI), cheliped index (CI), raw cluster yield (CY_{raw}), cooked cluster yield (CY_{cooked}), cluster weight change relative to the effect of the de-bleeding ($\Delta Cluster_{de\text{-}bled}$), and cluster weight change relative to the combined effect of the de-bleeding and cooking ($\Delta Cluster_{cooked}$) were considered as active variables. The meat content was handled as a supplementary quantitative variable. Live holding time and temperature were represented as supplementary explanatory variables (i.e., factors).

4. Conclusion

Live holding of snow crab without feeding for up to 111 days resulted in no mortality and nearly no limb loss. The hepatosomatic index decreased significantly with increasing live holding time, whereas the cheliped index remained unchanged, indicating that, in the absence of feed, the reserves in the hepatopancreas were mobilized before the actual muscle was used, most likely to preserve the functionality of the claws. Both meat content and cluster yield also decreased during the live holding period, but the effect was significant only after 89 days, suggesting that, also in the walking legs, the reserves were mobilized

only at a late stage in the absence of feed.

Compared to other species such as red king crab, the quality parameters of processed snow crab clusters were only marginally affected by the live holding temperature.

Live holding of snow crab in the absence of feed can be a possible practice for crab business operators, with minimal adverse impact on the commercial value of the product. Although no mortality and a low limb loss rate were observed, the severe decrease in the hepatosomatic index raises a concern about the welfare during long-term live holding without feeding. Thus, future work should focus on the physiological conditions of the snow crab to verify their nutritional status and potential distress issues during live holding without feeding.

CRediT authorship contribution statement

Grete Lorentzen: Conceptualization, Methodology, Validation, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization. **Federico Lian:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Sten Ivar Siikavuopio:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare no conflicts of interest.

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