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

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ABSTRACT

Red king crab (*Paralithodes camtschaticus*) has become a valuable resource in the Norwegian fishery. After landing, the crab is exported either as live or as processed into two cooked-frozen sections (i.e., clusters) to high-end market segments in Europe, Asia and in the USA. Live holding can be an alternative to processing right after landing, offering a new possibility to control the time before processing or live export. The live holding period can last, in absence of feeding, from some days up to as long as three months. The present study aimed to evaluate the effect of time and temperature on a series of quality parameters in red king crabs kept live without feeding at 5 and 10 °C up to 92 days. At day 0, 41, 62, and 92, the crabs were processed into cooked clusters followed by analyses. Meat content and cluster yield decreased significantly ($p < 0.05$) with live holding time and temperature as well as occurrence of moulting. Furthermore, the water content and pH of the cooked meat showed a significant increase as a function of live holding time, especially after 62 days of live holding at 10 °C. The live holding time and temperature have substantial negative effects on the product quality, likely related to the deterioration of muscle structure, that occur more markedly and earlier in the crabs kept at 10 °C (between 41 and 62 days) compared to their counterparts at 5 °C (between 62 and 92 days). The effects of live holding conditions appear more evident once the clusters are cooked compared to their raw counterparts. The results show that live holding time and temperature highly influence the quality of both live crabs and processed clusters. Thereby, a detailed knowledge and high control of live holding conditions are required to obtain an optimum quality of red king crabs.

Key-words: Red king crab; Live holding time; Live holding temperature; Processing; Meat; content; Yield.

1. Introduction

Red king crab (*Paralithodes camtschaticus*) have become important for the fish industries located in the northern parts of Norway (Lorentzen et al., 2018). In 2017, a total of 2131 metric tons of red king crab, live and processed, was exported from Norway amounting to NOK 509 million (Norwegian Seafood Council, 2018). Due to the appealing sensory properties of the meat, the product has attracted increasing interest among consumers, especially in high-end market segments in Europe, Asia and in the USA (Voldnes, 2017).

Nowadays, fishing of red king crab is a year-round activity in Norway, enabling the industry to be supplied with crabs irrespective of seasons (Lorentzen et al., 2018). After harvesting and landing, the red king crab is either kept live or processed into clusters. A cluster includes three walking legs and a claw assembled in a shoulder joint. The live holding of red king crab occurs either in containers on shore or in net pens close to the processing facilities and thus, enabling a flexible management of this resource. Also, live holding enables delivery to markets that require stability in terms of volume and quality. The duration of the live holding period depends on the welfare status of the crab, the market requirements to minimum meat content, and of course the fluctuations in the market price (Norwegian Seafood Council, 2018). Live holding after catch improves the welfare of the animal, and this is beneficial, especially in the case of long-distance transport (Siikavuopio & James, 2015). In fact, live holding may actually be required to facilitate recovery after a rough handling related to harvest and transport. This is particularly important for the crabs harvested from May. This will be after the moulting period that typically starts in March and ends in April. Recently-moulted crabs have a low meat content and are more fragile and vulnerable due to a thin and a soft shell (James et al., 2013).

The red king crab is a cold water adapted species normally found between 1 and 10 °C (Christiansen, Sparboe, Saether, & Siikavuopio, 2015). Due to elevated seawater temperatures

69 in the summertime, a higher mortality rate typically occurs during live holding and export
70 (Siikavuopio, James, Olsen, Evensen, & Mortensen, 2016). Consequently, processing to
71 clusters is preferred at this time of the year.

72 Processing starts with the slaughtering, i.e., splitting the crab into two clusters and removal
73 of the carapace, stomach, and hepatopancreas. Afterwards, the clusters are drained, cleaned,
74 and cooked. Methods for heat treatment of the clusters include boiling or steaming (Flick,
75 Granata, & Marsh, 2009; Manuel, 2017; Siikavuopio et al., 2011). After the heat treatment
76 and subsequent cooling, the clusters are subjected to freezing, either in a tunnel freezer or in a
77 brine saturated with NaCl and tempered to −18 °C (Lorentzen et al., 2018). Nowadays, the
78 majority of the red king crab clusters processed in Norway are exported as frozen (Norwegian
79 Seafood Council, 2018). However, the clusters can alternatively be exported as fresh,
80 preferably to markets close to Norway. In both fresh and frozen clusters, besides the sensory
81 properties (e.g., odour, taste, texture and juiciness), the absence of double shell and the
82 presence of a high meat content are also very important for the consumer.

83 The double shell refers to the extra inner membrane between the muscle and the
84 exoskeleton that the crabs tend to develop about 1-2 months before the moulting. The
85 presence of double shell is associated with a firm and tough texture of the meat (Stevens,
86 2014) which is considered as unacceptable by the consumer (Lorentzen, Skuland, Sone,
87 Johansen, & Rotabakk, 2014).

88 The meat content refers to the spatial portion occupied by muscle in the cluster claw and
89 legs. Factors influencing the meat content include season and location of harvest and also the
90 physiological condition of the crab (e.g., the moulting stage) (Hjelset & Sundet, 2004; James
91 et al., 2013; Siikavuopio & James, 2015; Siikavuopio et al., 2016, 2011; Stevens, 2014).
92 Moreover, a lower meat content has often been observed in the clusters obtained from crabs
93 with missing legs (S. I. Siikavuopio, personal communication). The meat content can be

94 improved by feeding the crabs during the live holding period (James et al., 2013). Notably,
95 the meat content is related to the yield, which can be defined as the final weight of the raw or
96 cooked clusters relative to the weight of the whole raw crab. For this reason, a low meat
97 content of both live crabs and processed clusters is considered unacceptable in many markets,
98 and it could, therefore, lead to loss of market shares.

99 To our knowledge, the relationship between live holding conditions of adult male red king
100 crab and the quality of processed clusters has not been published. Previously, it has been
101 shown that live holding conditions of cod affect the final product quality (Akse & Midling,
102 1997). Thus, the aim of this study was to evaluate the quality of processed clusters of red king
103 crab as a function of the live holding conditions. In detail, the crabs were kept live at 5 and 10
104 °C for up to 92 days without feeding. Sampling was performed at day 0, 41, 62, and 92, and
105 the crabs were processed into cooked clusters the following day. The quality parameters
106 studied included the meat content, yield, water content, pH and water holding capacity
107 (WHC) of the product.

2. Material and methods

2.1 Harvest and live holding

In December 2016, adult male red king crabs were harvested by local fishermen in the North Cape area in the Barents Sea using square shaped commercial king crab pots. This specific period of the year was chosen as the meat content of the red king crab is high with a low between-exemplars variation. The crabs (N = 78) were transported live in a dry state covered with gel ice (Cold Ice, Inc., Oakland, CA, USA) in polystyrene boxes by air freight in approximately 3 h to the Aquaculture Research Station in Tromsø, Norway (~70°N). Upon arrival, the crabs were immediately placed in 6 m^3 tanks supplied with natural seawater (4 °C, 34‰ salinity) which was continuously UV-treated, filtered through a 150 µm sand filter and circulating at a flowing rate of 4 L min^{-1} (kg crab)$^{-1}$.

After an observation and acclimatization period of seven days, the first sampling was performed (n = 18, live holding time day 0) (Fig. 1). At the same time, the remaining crabs (n = 60) were equally distributed into six circular tanks (volume 700 L) supplied with continuously UV-treated, filtered, and circulating seawater as previously described. The water temperature was set at 5 °C (± 0.2) in three tanks whereas in the other three tanks the temperature was set at 10 °C (± 0.2). A temperature of 5 °C represents the recommended temperature for the animal, while 10 °C represents the maximum proposed temperature that the crab can be exposed to (Christiansen et al., 2015; Siikavuopio & James, 2015). During the experiment, no cannibalism was observed, and the crabs did not have mutilated or missing legs. Crabs from both temperature groups were sampled at day 41, 62, and 92 during the live holding period.

On each sampling day, 18 crabs were sampled from the tanks, transferred into polystyrene boxes and then covered with gel ice. The boxes were transported in 1 h from the Aquaculture

Research Station to Nofima in Tromsø. The crabs were kept in the boxes in a dry state and processed the following day, within 15 h of their arrival. No mortality was observed upon arrival.

In total, 18 crabs (processed into 36 clusters) were sampled at day 0, while nine crabs (i.e., 18 clusters) from each live holding temperature were sampled after 41 and 62 days of live holding (Fig. 1). At day 92, 11 (i.e., 22 clusters) crabs held at 5 °C and seven crabs (i.e., 14 clusters) held at 10 °C were sampled. In each sampling, a balanced number of crabs was collected from each tank across the live holding temperature groups. By this, moulted exemplars were given priority, if present. In this way, it was possible to evaluate the influence of the live holding conditions to the moulting.

The weight of the total number of crabs eventually sampled and processed in this study (N = 72) ranged between 2158 and 2790 g, with an average weight (± standard deviation) equal to 2379 g (± 273).

2.2 Processing and sample preparation

The procedure for processing the red king crabs into clusters reflected the industrial processing and was maintained consistent throughout the entire experiment (Fig. 1). The processing started with registering the weight of the whole raw crabs, followed by labelling the right and left clusters using T-bar tags (Floy tag, Inc., Seattle, WA, USA). The crabs were split into two separate clusters using a butchering iron. After splitting, the claws were removed from the clusters. Afterwards, remaining gills and viscera were removed from the shoulder joint of the cluster using a knife (Fig. 1, step B). During this operation, the cluster was kept in a vertical position with the shoulder pointing downwards to facilitate drainage of free body fluid (FBF), which is the liquid, predominantly composed of haemolymph, flowing

156 out from the appendages when the exoskeleton is cut (Mizuta, Kobayashi, & Yoshinaka,
157 2001).

158 The weight of the cleaned and drained clusters was registered, and they were placed into
159 wire cages. The clusters were oriented vertically with the shoulder pointing downwards to
160 facilitate further drainage. Afterwards, the cages with the clusters were soaked in a tank
161 containing fresh water (85 L) at 1-2 °C for 30 min for further removal of FBF (Fig. 1, step C).
162 This operation is hereafter referred to as "de-bleeding". Afterwards, the cages were removed
163 from the water, and the clusters were drained for at least 15 min. The weight of each de-bled
164 and drained cluster was registered.

165 The clusters were cooked by soaking the cages into boiling water (Fig. 1, step D). The
166 target of the cooking process was to reach a core temperature of 92 °C in the most proximal
167 article (i.e., *merus*) of the largest walking leg of the cluster. This temperature was achieved
168 after 16 min. In each cooking session, the core temperature of the leg meat was logged every
169 3 s using K-type thermocouples connected to data loggers (model 175H1, Testo, Ltd.,
170 Hampshire, UK) placed in the centre of the *merus* in four of the largest clusters. After
171 cooking, the clusters were cooled down in ice water with 3.5% NaCl (w/v) for 21 min until
172 the core temperature was below 4 °C. Afterwards, the clusters were drained for at least 15
173 min, and the weight of each cooked, cooled, and drained cluster was registered. Clusters from
174 each combination of live holding time and temperature were analysed for meat content and
175 yield. The cooked meat extracted from the *merus* of the cluster legs was analysed for water
176 content, pH and WHC.

177

178 *2.3 Meat content*

179 The meat content was measured on cooked clusters by digital analysis of images of cross-
180 sections of the middle of the *merus* (Fig. 2, upper section). More specifically, the meat content
181 was expressed as the portion of cooked muscle in the cross-section, and it was calculated as:

182 Meat content (%) = [Area occupied by meat / Total inner area] × 100 (1)

183 The cross-sections were obtained by applying a transverse cut across the middle point of the
184 *merus*. Each resulting pair of halves of *merus* was photographed in a light cabinet using a
185 digital camera (model RX 100 III, Sony, Tokyo, Japan) as described by Lian et al. (2018).
186 The sample images were processed using software for digital image analysis (Image Pro Plus,
187 version 6.0, Media Cybernetics, Inc., Bethesda, MD, USA) which allowed to select and
188 quantify the area occupied by the meat and the overall area of the *merus* cross-section
189 delimited by the inner margin of the exoskeleton. The meat content was calculated for the
190 *merus* of up to three parallel legs from the same cluster and in at least seven clusters generated
191 from different crabs for each combination of live holding time and temperature.

192

193 *2.4 Cluster yield and relative weight changes in clusters during processing*

194 To obtain an overview of the cluster yield and the relative cluster weight changes as a
195 response to live holding conditions and processing, four separate calculations were performed.
196 In detail, the yield of raw clusters after splitting was calculated as:

197 $\text{Cluster yield}_{raw}$ (%) = (2B / A) × 100 (2)

198 where 2B is the sum of the weight of the right and left cluster from the same crab after
199 splitting and drainage (Fig. 1, step B), and A is the weight of the corresponding whole raw
200 crab (Fig. 1, step A).

201 Furthermore, the yield of cooked clusters was estimated by:

$$\text{Cluster yield}_{\text{cooked}}\ (\%) = (2D / A) \times 100 \quad (3)$$

where 2D is the weight of the two cooked, cooled, and drained clusters from the same crab (Fig. 1, step D), and A is the weight of the corresponding whole raw crab (Fig. 1, step A).

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

$$\Delta\text{Cluster}_{\text{de-bled}}\ (\%) = [(C - B) / B] \times 100 \quad (4)$$

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

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

$$\Delta\text{Cluster}_{\text{cooked}}\ (\%) = [(D - B) / B] \times 100 \quad (5)$$

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

2.5 Water content, pH and WHC

Analyses of water content, pH and WHC were conducted on samples of cooked meat extracted from the *merus*. More specifically, for each meat sample, the water content was determined in quadruplicate by oven drying at 103 °C for 24 h, whereas the pH was measured in duplicate as described by Lorentzen, Rotabakk, Olsen, Skuland, and Siikavuopio (2016). The analysis of WHC was performed in quadruplicate as described by Skipnes, Østby, and Hendrickx (2007) with some modifications. Briefly, an aliquot of about 2 g of meat was placed on a disk of filter paper (Grade 3, Whatman, Maidstone, UK) supported by a stainless

steel wire mesh located within a flat-bottom round (30 mm diameter) polyethene 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 estimated by:

$$WHC\ (\%) = [(W_0 - \Delta C) / W_0] \times 100 \quad (6)$$

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

2.6 Statistical analyses

The values of the response variables (i.e., meat content, cluster yields, relative cluster weight changes, water content, pH and WHC) were grouped by crab and expressed as the mean (± standard deviation) of three to 18 determinations. Statistical analyses were performed considering each crab as an independent biological replicate.

The effects of live holding time, live holding temperature and moulting on the response variables were investigated by carrying out a factorial analysis of covariance (ANCOVA) or variance (ANOVA) using the general linear model (GLM) procedure in the software Statistica™ (version 8.0, StatSoft, Inc., Tulsa, OK, USA). The normality of residuals and homogeneity of data variance were evaluated by means of normal probability and standardised residuals plots.

For meat content, cluster $yield_{raw}$, cluster $yield_{cooked}$, $\Delta cluster_{de\text{-}bled}$, and $\Delta cluster_{cooked}$, the ANCOVA model included all main effects and one two-way interaction (live holding time × live holding temperature). The factor moulting was included as a covariate whereas live holding time and temperature were considered categorical factors. For water content, pH and WHC, the samples set was too small to evaluate the effect of moulting; therefore, an ANOVA

248 model was used. This included the main effects of live holding time and temperature and their
249 two-way interaction. Significant differences between groups were assessed by *post-hoc*
250 multiple comparisons (Tukey's HSD test).

251 In addition, for all response variables, a one-way ANOVA followed by *post-hoc* pairwise
252 comparisons (Dunnett's test) was carried out on data grouped by the combination of live
253 holding time, live holding temperature and the presence of moulting in order to assess
254 differences between each group and the initial conditions (live holding time day 0).

255 Standard *t*-test for comparison of independent sample means was carried out to assess
256 differences between the weight change of live crabs held at 5 and 10 °C for 92 days. The
257 same *t*-test was used for the response variables meat content, cluster yields, and relative
258 cluster weight changes to assess differences between moulted and non-moulted crabs within
259 each live holding time-temperature group.

260 All statistical analyses were tested at 5% probability level (*p*-value).

3. Results and discussion

3.1 Live holding and processing

The weight of the live crabs decreased slightly during the live holding period of 92 days. The average weight loss in crabs kept at 5 °C was 3.60% (± 1.66; n = 4), while the average weight loss was 2.86% (± 0.45; n = 3) in crabs kept at 10 °C. The observed difference was not statistically significant between the two temperature groups (t-test, p = 0.497).

Despite the fact that moulting usually starts in March for red king crab in the Barents Sea (James et al., 2013), the moulting process unexpectedly occurred in some of the crabs at the end of January and in February. After 62 days of live holding, no moulting was observed in crabs kept at 5 °C, while moulting was observed in six out of the nine crabs sampled from the group kept at 10 °C. After 92 days of live holding, the moulting process had been completed also in three out of eleven crabs at 5 °C and in further four out of seven crabs kept at 10 °C. This shows that an increase in the water temperature during live holding in early winter time may promote the moulting process. This is in accordance with previous findings describing the effect of temperature on moulting of red king crab (Stoner, Ottmar, & Copeman, 2010). An increase in temperature normally increases the metabolic rate for crustaceans (Wickins & Lee, 2002). This is also illustrated in temperature studies performed on red king crab for which a significantly higher metabolic rate was evidenced by higher oxygen consumption during live holding at 10 °C compared to 5 °C (Siikavuopio & James, 2015). The temperature of the water was considered the main factor affecting the energetic balance of red king crab, which, in turn, controlled the moulting (Nilssen & Sundet, 2006; Shirley, Shirley, & Korn, 1990).

During sampling at day 62 and 92, the moulted and non-moulted crabs were identified and subsequently processed. It should be noted that the processing of the crabs sampled at day 62

and 92 involved challenges in performing de-bleeding, cooking, and cooling, as some of the clusters were floating due to their low meat content. To obtain a uniform procedure of processing, lids of wire were placed on the top of the cages during these operations.

The meat content, cluster yields, and relative cluster weight changes are presented and discussed as a function of the main and interaction effects of live holding time and temperature as well as the effect of moulting (Table 1).

3.2. Meat content

The meat content of the crabs decreased during the live holding period (Fig. 2) and was significantly affected by both time ($p < 0.001$) and temperature ($p < 0.001$) (Table 1). More specifically, the meat content of the non-moulted crabs decreased from 87.0% at day 0 to 53.7% and 48.5% at day 92 for crabs kept at 5 and 10 °C, respectively. After as early as 41 days of live holding, the meat content values for both temperatures were significantly different ($p < 0.03$) from the initial value (day 0). After 62 days of live holding, a drop in the meat content from 66.1% to 44.7% was observed in the crabs held at 10 °C. The corresponding meat content of the crabs kept at 5 °C decreased sharply from 68.0% to 51.1% only between 62 and 92 days of live holding. This earlier reduction in the muscle size (i.e., meat content) observed in the crabs kept at 10 °C can be explained by assuming a higher metabolic activity (Wickins & Lee, 2002), but also by the presence of moulted crabs in the 10 °C group at day 62.

The meat content was, in fact, significantly affected by the moulting ($p < 0.001$) during the live holding period (Table 1). In the moulted crabs kept at 10 °C sampled at day 62, the meat content was 39.2%. At day 92, the meat content in the moulted crabs held at 5 and 10 °C was 44.2% and 35.5%, respectively. Furthermore, by comparing the meat content of the moulted

309 and non-moulted crabs within the same time-temperature group, statistically significant
310 differences were registered for the crabs held at 10 °C and sampled at day 62 (p = 0.030) and
311 day 92 (p = 0.033). A similar comparison carried out for the moulted and non-moulted crabs
312 kept at 5 °C and sampled at day 92 did not reveal a significant difference (p = 0.12). This
313 suggests that live holding at 10 °C may make the negative effect of moulting on meat content
314 more evident. The observed differences in meat content between the moulted and non-
315 moulted crabs are in accordance with earlier studies (Hjelset & Sundet, 2004; James et al.,
316 2013; Stevens, 2014).

317 The reduction in meat content observed during the live holding period is assumed to be
318 compensated with FBF as a weight compensation that most probably serves the purpose of
319 securing the biological function of the animal (Mayrand, Guderley, & Dutil, 2000).

320 High variability in the meat content was observed between different crabs within each live
321 holding time-temperature group, resulting in a high standard deviation (Fig. 2). This shows
322 the individual differences between the crabs in terms of their biological response to live
323 holding, but it may, at least in part, also be due to an uneven spatial distribution of the muscle
324 in the *merus*. Although all the cross-sections for meat content measurement were obtained at
325 the middle point of the *merus*, the uneven spatial distribution of the muscle between sample
326 replicates may have contributed to the observed variability. Despite this potential
327 methodology limitation, it can be concluded that the meat content, on average, decreased with
328 longer live holding time and higher temperature in red king crabs without feeding.

329

330 *3.3 Cluster yield*

331 Parallel to the decrease in meat content, the cluster yield also decreased with live holding
332 time (Fig. 3). Both raw and cooked cluster yield were significantly affected by live holding

time and temperature as well as by moulting ($p < 0.003$) (Table 1). In particular, the effects of time and temperature were inter-dependent as indicated by the significant ($p < 0.002$) interaction between these two factors.

In detail, the raw cluster yield (Eq. (2)) was 55.0% at day 0 and decreased to about 44% at day 92 (Fig. 3A), irrespective of the live holding temperature. Similarly to the trend observed for meat content, the largest drop in cluster yield was observed at day 62 and 92 for crabs kept at 10 and 5 °C, respectively. In the case of the moulted crabs, the raw cluster yield was about 40% at day 62 and 92. The yield values for moulted and non-moulted crabs differed significantly ($p = 0.017$) only for the crabs kept at 10 °C sampled at day 92.

During the live holding period, the overall decrease in the raw cluster yield (around 11%) was comparatively higher than the weight loss of the live crabs (2.9–3.6%). This clearly shows that the crab compensates the muscle reduction with FBF which flows out and is drained away from the clusters during the splitting of the crab (Fig. 1, step B).

The yield calculated on cooked clusters (Eq. (3)) was 54.8% at day 0 and decreased to 34.0% and 30.4% at day 92 for crabs kept at 5 and 10 °C, respectively (Fig. 3B). After 41 days of live holding, the yield was 50.9% for the crabs kept at 5 °C, whereas it decreased significantly ($p < 0.05$) to 48.4% for the crabs kept at 10 °C. At day 62, the yield for the crabs kept at 5 °C (48.5%) was not significantly different from day 41 values, whereas the yield for the crabs kept at 10 °C dropped to 29.0%. This clearly shows the effect of live holding at different temperatures, also in relation to moulting. In fact, the values of cooked cluster yield differed significantly ($p < 0.029$) in relation to the presence of moulting within the live holding time-temperature groups at day 62 at 10 °C and at day 92 at 10 °C. This indicates that the negative effect of moulting is exacerbated by high live holding temperature.

356 The yields for the cooked clusters were generally lower than the yields obtained for the
357 corresponding raw clusters. Most probably, during the de-bleeding and the cooking process,
358 any residual FBF, still present after splitting and drainage, was expelled from the cluster.
359 Furthermore, it should be noted that the largest difference between raw and cooked cluster
360 yield was observed after 62 and 92 days of live holding for crabs kept at 10 and 5 °C,
361 respectively. This might be explained by assuming changes in the microstructure of crab
362 muscle during live holding. Modifications of the muscle structure may, in fact, promote
363 higher loss of any liquid loosely bound or physically entrapped in the muscle structure as a
364 response to processing steps such as de-bleeding or cooking.

365 For the moulted crabs, the raw and cooked cluster yields were lower compared to the ones
366 for the non-moulted counterparts. This might be explained by the higher initial content of FBF
367 which was subsequently lost during splitting, de-bleeding, and cooking (Mizuta et al., 2001).
368 These yield differences clearly show the risk for the crab industry of incurring economic
369 losses when processing red king crabs that have recently completed the moulting process.

370

371 *3.4 Relative weight change in clusters*

372 During processing, the relative weight change of de-bled and drained clusters (Eq. (4)), as
373 well as the relative weight change of de-bled, drained, cooked, cooled, and drained clusters
374 (Eq. (5)), was expressed in relation to the weight of corresponding raw clusters (Fig. 4). These
375 calculations were performed in order to elucidate the specific contributions of de-bleeding and
376 cooking to the observed differences between raw and cooked cluster yield.

377 The relative weight change of de-bled clusters was significantly affected by live holding
378 time, interaction time × temperature, and moulting ($p < 0.002$) (Table 1). Nonetheless, these
379 weight changes were generally small as illustrated by the fact that the percentage values of

380 $\Delta cluster_{de\text{-}bled}$ were lingering around the zero line during live holding (Fig. 4A) and were not
381 significantly different ($p \geq 0.05$) from the day 0 value, except for crabs held at 5 °C sampled
382 at day 62 and crabs held at 10 °C sampled at day 92.

383 By contrast, the relative weight change after cooking (i.e., related to the combined effect of
384 de-bleeding and cooking) was more marked, with percentage values of $\Delta cluster_{cooked}$ differing
385 significantly ($p < 0.05$) from the day 0 value for crabs sampled after 62 days of live holding,
386 irrespective of the temperature (Fig. 4B). Moreover, the relative weight change for cooked
387 clusters was significantly affected by the main effect of all factors ($p < 0.001$) and by the
388 interaction live holding time × temperature ($p = 0.001$) (Table 1).

389 In detail, for crabs processed at day 62, the relative cluster weight change after de-bleeding
390 was −5.4% and −2.9% for the crabs kept at 5 and 10 °C, respectively (Fig. 4A). The
391 corresponding relative cluster weight change after cooking was −7.6% and −31.9% for the
392 crabs kept at 5 and 10 °C, respectively (Fig. 4B). For crabs sampled at day 92, the relative
393 cluster weight change after de-bleeding was −3.8% and −12.5% for the crabs at 5 and 10 °C,
394 respectively (Fig. 4A), while the relative cluster weight change after cooking was −22.2% and
395 −33.4% for the crabs kept at 5 and 10 °C, respectively (Fig. 4B).

396 Next to the live holding temperature, also the effect of moulting had an influence on the
397 large difference between the relative weight change after de-bleeding (Fig. 4A) and the one
398 after cooking (Fig. 4B) which was observed at day 62 for crabs kept at 10 °C and at day 92
399 for crabs from both temperature groups. In fact, a significant ($p = 0.029$) difference was
400 observed between the $\Delta cluster_{cooked}$ values of moulted and non-moulted crabs kept at 10 °C
401 sampled at day 92.

402 More in general, the comparison between the relative cluster weight change after de-
403 bleeding (Fig. 4A) and after cooking (Fig. 4B) clearly shows that cooking has the highest

404 impact on the final cluster yield. Furthermore, the extent of weight loss due to cooking is
405 highly dependent on the live holding conditions and moulting. More specifically, the impact
406 of cooking on cluster weight loss becomes substantial during live holding in the temporal
407 ranges 41–62 days and 62–92 days for crab kept at 10 and 5 °C, respectively.

408 It can be postulated that, under these live holding conditions, the crab muscle may undergo
409 structural modifications such as weakening of muscle fibres or connective tissue. These
410 changes might be accelerated at the high live holding temperature or by physiological
411 phenomena related to moulting, similarly to what observed for soft shell mud crab (Mizuta et
412 al., 2001). The microstructural changes may make the muscle more susceptible to thermal
413 denaturation, hence to weight loss during cooking (Benjakul & Sutthipan, 2009). In crab, the
414 muscle fibres in the legs are anatomically organised in sheets or bundles attached to the shell
415 on either side of the joints (Venugopal, 2006). During cooking, the muscle shrinks and
416 loosens the connections to the shell and the joints, and this phenomenon results in a release of
417 liquid which is pressed out of the muscle cells (Niamnuy, Devahastin, & Soponronnarit,
418 2008). In similar cooking studies of cod, the cooking process is considered to be the main
419 reason for both water loss and texture changes due to the unfolding of proteins that leads to
420 the release of water from the sarcoplasm and myofibrils (Skipnes, Van der Plancken, Van
421 Loey, & Hendrickx, 2008). In previous studies, it is shown that physical changes during
422 processing of seafood have been related to the microstructure of the muscle (Benjakul,
423 Visessanguan, Kijroongrojana, & Sriket, 2008; Bhat, Chouksey, Balange, & Nayak, 2017). In
424 this regard, differences in the microstructure of crab muscle due to different live holding
425 conditions may exacerbate the cook loss.

426 The relative cluster weight changes depicted in Fig. 4 clearly indicate that the crab industry
427 should avoid the processing into cooked clusters of not only recently-moulted crabs but also
428 non-moulted crabs kept live without feeding at 10 °C for more than 41 days or at 5 °C for

429 more than 62 days. However, it has to be noted that also the commercialisation of such crabs
430 either as live crabs or in the form of ready-to-cook raw or de-bled clusters may cause
431 substantial economic losses in the long term due to final consumer dissatisfaction.

432

433 *3.5 Water content, pH and WHC*

434 During live holding, the water content, pH and WHC were analysed on the cooked meat
435 extracted from the *merus* of cluster legs (Table 2). At both live holding temperatures, a slight
436 increase in water content was observed when compared to day 0. The live holding time
437 significantly affected the water content of the cooked meat (p = 0.036), while the factor
438 temperature and the interaction time × temperature were not significant (Table 3). A similar
439 increase of water content of the leg meat in relation to the absence of feeding was observed
440 for snow crabs (*Chionoecetes opilio*) during fasting at different temperatures (Hardy, Dutil,
441 Godbout, & Munro, 2000).

442 The pH value registered for the crabs at day 0 was in line with the pH previously reported
443 for freshly-cooked leg meat of red king crab (Table 2) (Lorentzen et al., 2014). The pH values
444 showed the tendency to increase during live holding especially after 62 days and for the crabs
445 held at 10 °C. In these crabs, the pH value of the cooked meat was significantly different ($p <$
446 0.05) from the pH value for crabs at day 0. Both live holding time and temperature
447 significantly affected ($p < 0.045$) the pH values of the cooked meat (Table 3). The increased
448 pH might be explained by assuming a higher presence of basic nitrogen compounds in the
449 crab muscle, which, in turn, might be caused by higher levels of proteinase activity (Benjakul
450 & Sutthipan, 2009) for longer live holding time and higher temperature.

451 The WHC of the cooked meat was in the range of 67.2 (± 1.2) to 78.7% (± 5.9) (Table 2).
452 Live holding time and temperature affected significantly the WHC ($p < 0.016$) (Table 3). The

453 values of WHC showed the tendency to increase during live holding especially for crabs kept
454 at 10 °C, although the observed pattern was not strictly ordinal with live holding time. The
455 trend for WHC values (i.e., lower WHC for longer live holding time and higher temperature)
456 was in contrast with what could be expected on the basis of the results of cooked cluster yield
457 (Fig. 3B) and relative cluster weight change after cooking (Fig. 4B).

4. Conclusion

Live holding conditions affected significantly ($p < 0.05$) the meat content, yield and relative weight changes of the clusters as well as the water content, pH and water holding capacity of the cooked meat. More specifically, during the live holding period of 92 days at 5 and 10 °C, the meat content and the cluster yield decreased significantly ($p < 0.05$) with longer live holding time and higher temperature. In contrast, only a marginal loss of wet weight was observed in the live crabs. This phenomenon may be due to the acquisition of free body fluid as a compensation of a decreased muscle volume.

Unexpectedly, the moulting occurred in some crabs during the live holding period. This was observed during sampling at day 62 in the crabs kept at 10 °C, and at day 92 in the crabs kept at 5 and 10 °C. This shows that the time of moulting can be influenced by live holding conditions. In our study, the early moulting is assumed to be related to the temperature. In the moulted crabs, the meat content and yield were lower compared to their non-moulted counterparts. This difference was particularly evident in the last part of the live holding period.

Based on the results obtained, live holding of red king crabs without feeding is not recommended for more than 41 days at 10 °C or for more than 62 days at 5 °C. As a follow-up, a corresponding study at temperatures close to 0 °C may be performed to reveal if this could delay the quality deterioration observed in this study. Such a low live holding temperature would however imply additional costs due to water cooling systems.

For the red king crab industry, it is essential to gain detailed knowledge on how the live holding conditions influence the moulting as this can highly impair the product quality and lead to economic losses. Furthermore, it is important to acquire accurate information regarding the maximum potential live holding period at specified conditions that allow

maintaining satisfactory meat content and yield of live crabs and processed clusters. Such knowledge is of vital importance for optimal live holding management.

Declaration of interest

The authors declare no conflicts of interest.

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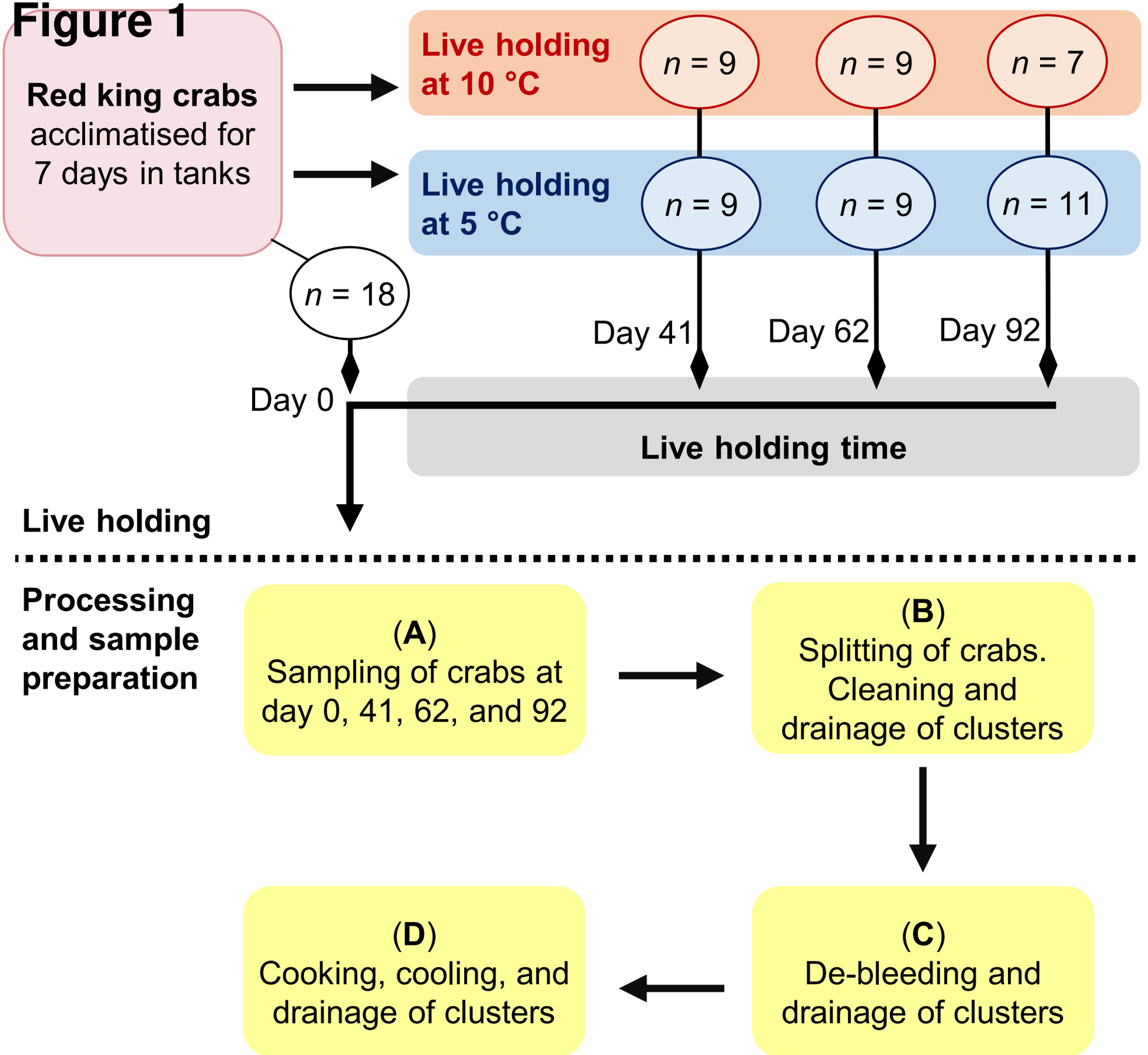
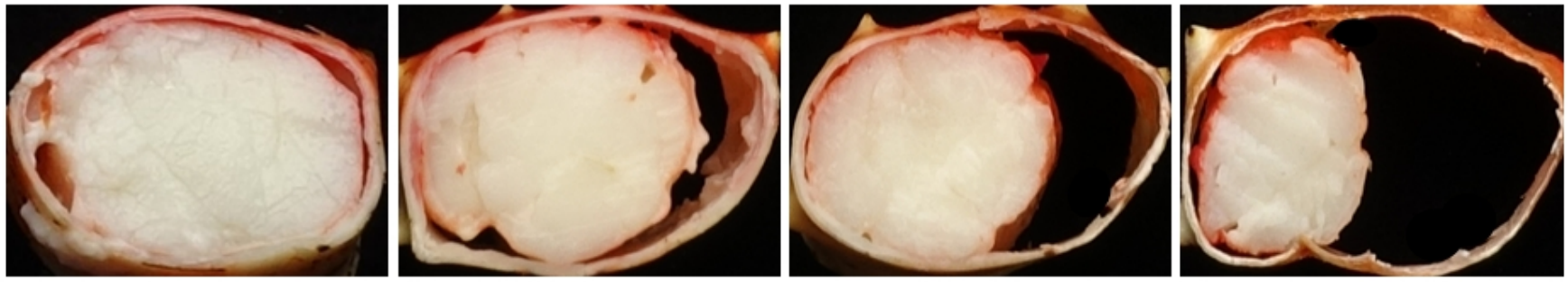
***Highlights (for review)**

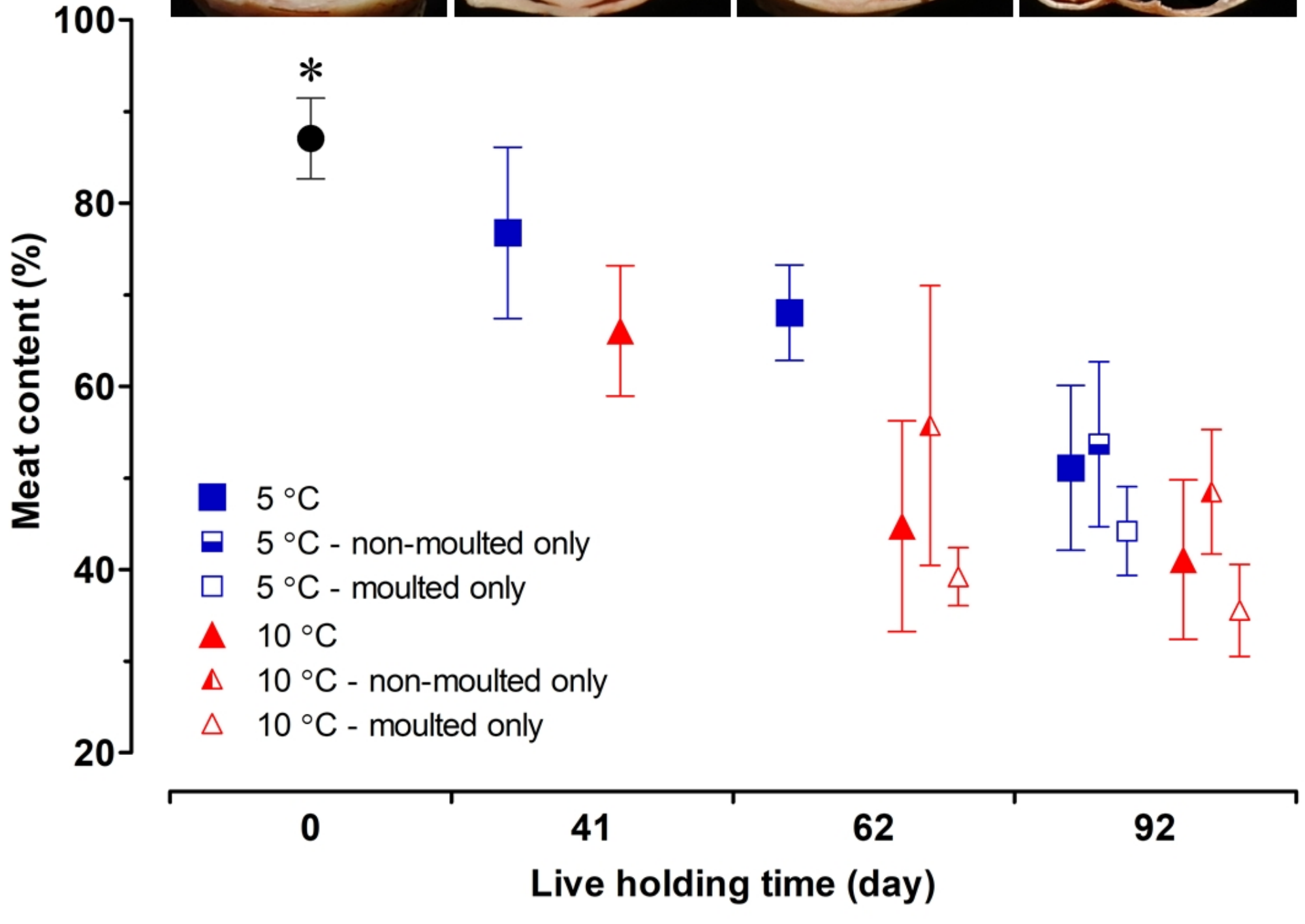
Highlights

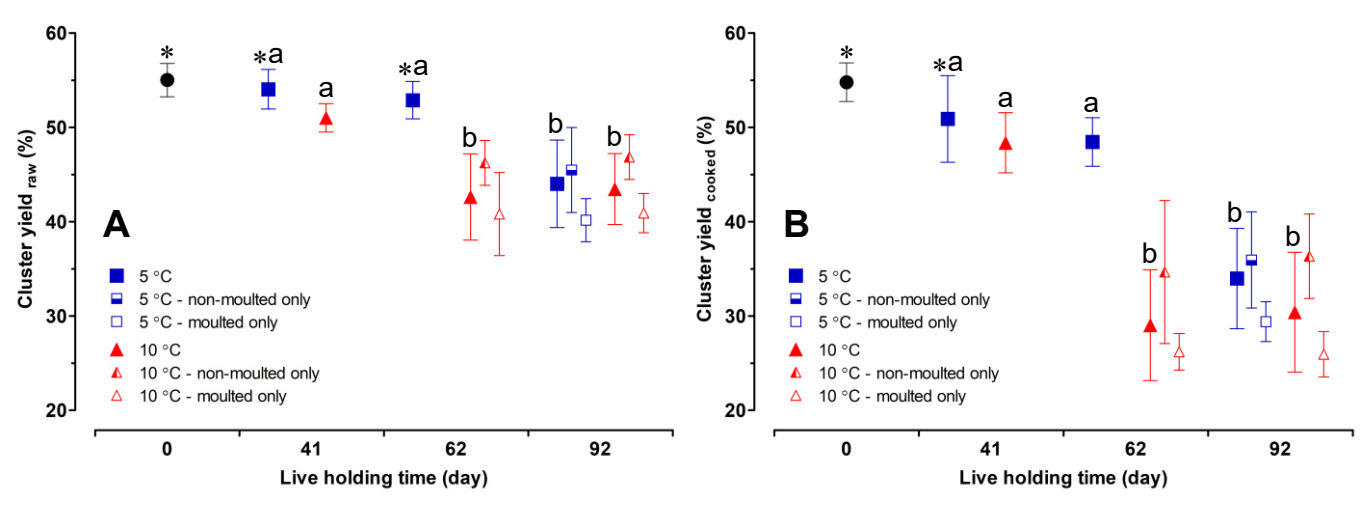
- Live holding of red king crabs up to 92 days at 5 and 10 C without feeding
- Sampling and processing of cooked clusters at day 0, 41, 62 and 92.
- The live holding conditions resulted in reduced meat content and a lower yield.

Figure 1


Red king crabs
acclimatised for
7 days in tanks
Live holding
at 10 °C
Live holding
at 5 °C
n = 9
n = 9
n = 7
n = 9
n = 9
n = 11
n = 18
Day 41
Day 62
Day 92
Day 0
Live holding time
Live holding
Processing
and sample
preparation
(A)
Sampling of crabs at
day 0, 41, 62, and 92
(B)
Splitting of crabs.
Cleaning and
drainage of clusters
(C)
De-bleeding and
drainage of clusters
(D)
Cooking, cooling, and
drainage of clusters




100
80
60
40
20
Meat content (%)
*
5 °C
5 °C - non-moulted only
5 °C - moulted only
10 °C
10 °C - non-moulted only
10 °C - moulted only
0
41
62
92
Live holding time (day)


A
B
Cluster yield raw (%)
Cluster yield cooked (%)
60
50
40
30
20
*
*a
a
b
5 °C
5 °C - non-moulted only
5 °C - moulted only
10 °C
10 °C - non-moulted only
10 °C - moulted only
0
41
62
92
Live holding time (day)

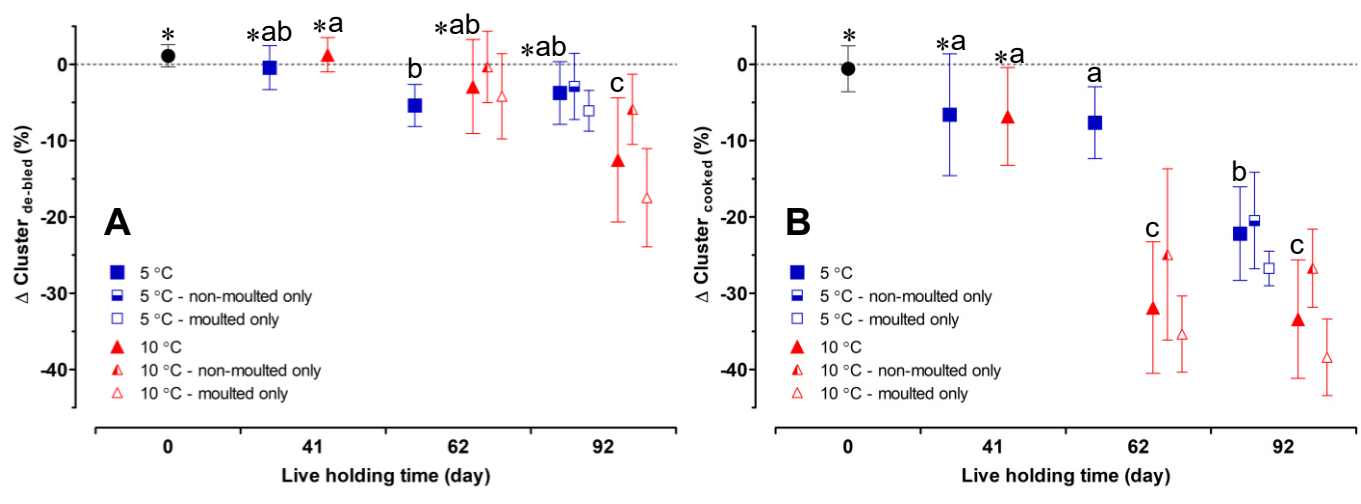

A
B
Δ Cluster de-bled (%)
Δ Cluster cooked (%)
0
-10
-20
-30
-40
*
*ab
*a
b
c
5 °C
5 °C - non-moulted only
5 °C - moulted only
10 °C
10 °C - non-moulted only
10 °C - moulted only
0
41
62
92
Live holding time (day)

Figure captions

Fig. 1. Flowchart illustrating the experimental set-up for the live holding and the sequence of activities for crab processing and sample preparation. Processing steps are indicated in brackets with capital letters corresponding to steps of weight registration.

Fig. 2. Meat content (%) in the *merus* of legs of cooked clusters of red king crabs sampled during the live holding period at 5 and 10 °C up to 92 days. Above, images of cross-sections of *merus* with 89.5% (left), 72.3%, 64.6%, and 38.5% meat content. Below, the meat content is expressed as mean values ± standard deviation indicated with vertical bars. The symbol (*) indicates the mean values which are not significantly different ($p \geq 0.05$) from the mean value observed at day 0.

Fig. 3. Cluster yield (%) of raw (A) and cooked clusters (B) of red king crabs sampled during the live holding period at 5 and 10 °C up to 92 days. Results are expressed as mean values ± standard deviation which is indicated with vertical bars. Mean values accompanied by different lowercase letters are significantly different ($p < 0.05$). The symbol (*) indicates the mean values which are not significantly different ($p \geq 0.05$) from the mean value observed at day 0.

Fig. 4. Relative weight change (%) of processed clusters after de-bleeding (A) and after de-bleeding, cooking, and cooling (B). The clusters were obtained from red king crabs sampled during the live holding period at 5 and 10 °C up to 92 days. Results are expressed as mean values ± standard deviation which is indicated with vertical bars. Mean values accompanied by different lowercase letters are significantly different ($p < 0.05$). The symbol (*) indicates the mean values which are not significantly different ($p \geq 0.05$) from the mean value observed at day 0.

Table 1

Factorial ANCOVA for the response variables meat content, cluster $yield_{raw}$, cluster $yield_{cooked}$, $\Delta cluster_{de\text{-}bled}$, and $\Delta cluster_{cooked}$. In the ANCOVA model, the factor moulting was included as a covariate whereas live holding time and temperature were considered categorical factors.

Response variable	Equation	Factor	*df*	*SS*	*p*-value
Meat content	1	Time	2	2681.0	< 0.001
		Temperature	1	1172.7	< 0.001
		Time × Temperature	2	154.3	0.268
		Moulting	1	975.8	< 0.001
		Error	46	2620.5	
		R^2 | R^2_{adj}	0.79 | 0.76		
Cluster $yield_{raw}$	2	Time	2	280.9	< 0.001
		Temperature	1	82.4	0.003
		Time × Temperature	2	120.6	0.002
		Moulting	1	181.1	< 0.001
		Error	44	359.3	
		R^2 | R^2_{adj}	0.79 | 0.76		
Cluster $yield_{cooked}$	3	Time	2	1082.1	< 0.001
		Temperature	1	312.6	< 0.001
		Time × Temperature	2	319.6	< 0.001
		Moulting	1	403.8	< 0.001
		Error	37	560.0	
		R^2 | R^2_{adj}	0.88 | 0.86		
$\Delta Cluster_{de\text{-}bled}$	4	Time	2	262.5	0.002
		Temperature	1	1.4	0.777
		Time × Temperature	2	394.2	< 0.001
		Moulting	1	202.6	0.001
		Error	47	834.3	
		R^2 | R^2_{adj}	0.57 | 0.51		
$\Delta Cluster_{cooked}$	5	Time	2	2047.3	< 0.001
		Temperature	1	852.9	< 0.001
		Time × Temperature	2	612.9	0.001
		Moulting	1	505.6	< 0.001
		Error	47	1842.3	
		R^2 | R^2_{adj}	0.80 | 0.77		

Note. *df*, degrees of freedom; *SS*, sum-of-squares; R^2, coefficient of determination; R^2_{adj}, adjusted coefficient of determination.

Table 2

Water content, pH and water holding capacity (WHC) values (mean ± standard deviation) for the meat of the *merus* of cooked clusters of red king crabs sampled during the live holding period at 5 and 10 °C up to 92 days.

Live holding time (day)	Live holding temperature (°C)	Water content (%)	pH	WHC (%)
0		78.5 ± 0.8*	7.19 ± 0.01*	67.8 ± 1.3*
41	5	81.0 ± 1.9	7.02 ± 0.08*	74.4 ± 1.0
	10	80.5 ± 0.9*	7.07 ± 0.05*	75.7 ± 0.9
62	5	82.8 ± 1.1	7.19 ± 0.01*	72.5 ± 2.7*
	10	82.3 ± 1.2	7.60 ± 0.21	78.7 ± 5.9
92	5	80.7 ± 1.4	7.52 ± 0.10*	67.2 ± 1.2*
	10	82.8 ± 0.7	7.66 ± 0.22	75.5 ± 1.1

Note. The symbol (*) within a column indicates the mean values which are not significantly different ($p \geq 0.05$) from the mean value observed at day 0.

Table 3

Factorial ANOVA for the response variables water content, pH, and water holding capacity (WHC). Live holding time and temperature were considered categorical factors in the ANOVA model.

Response variable	Factor	*df*	*SS*	*p*-value
Water content	Time	2	12.90	0.036
	Temperature	1	0.97	0.447
	Time × Temperature	2	8.66	0.094
	Error	18	28.82	
	R^2 | R^2_{adj}	0.44 | 0.28		
pH	Time	2	0.62	0.004
	Temperature	1	0.12	0.045
	Time × Temperature	2	0.07	0.228
	Error	6	0.11	
	R^2 | R^2_{adj}	0.88 | 0.78		
WHC	Time	2	82.75	0.016
	Temperature	1	157.61	< 0.001
	Time × Temperature	2	51.85	0.060
	Error	17	131.84	
	R^2 | R^2_{adj}	0.70 | 0.61		

Note. *df*, degrees of freedom; *SS*, sum-of-squares; R^2, coefficient of determination; R^2_{adj}, adjusted coefficient of determination.