

## Research Article

# Evaluation of Morphological and Quality Parameters in Adult Male Red King Crabs (*Paralithodes camtschaticus*) Raised to Commercial Weight from Juveniles

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Red king crab (RKC) has become a valuable resource, and most of this fishery operates in a quota-regulated area (QRA) east of 26°E. West of the QRA, a free fishery area (FFA) is established to limit further migration. Today, juvenile RKC from the FFA are not utilized. This study investigated morphological and quality parameters in adult RKC ( $\geq 800$  g) after live holding (LH) of 23 months starting from juveniles (on average 500 g). During the LH, the RKC were kept in two separate tanks, one at ambient seawater temperature (AST) and one at elevated seawater temperature (AST-E). The RKC were fed *ad libitum*. Both tanks were divided into two sections, one with a sand tray. After 23 months, the RKC were processed and analyzed. The hepatosomatic index, abdomen index, meat content, cluster yield, moisture, whiteness index, and instrumental chewiness differed significantly ( $P < 0.05$ ) between the wild RKC and the RKC from one or both LH groups. The availability of sand indicated improved habitat conditions as several parameters were positively affected. Also, lower variation in morphology and quality parameters of the processed muscle was observed in RKC from the subgroups with access to sand than those without sand.

## 1. Introduction

Red king crab (RKC, *Paralithodes camtschaticus*) is an exclusive and highly valued decapod species that appeal to consumers worldwide for the delicate properties of its meat. In 2021 and 2022, 2261 and 1375 tonnes of RKC were exported from Norway, amounting to \$102 and 83 million, respectively [1]. About 60% of this volume was traded live, whereas the remaining share was traded as fresh or frozen-cooked clusters (three legs and one claw attached in a shoulder joint). Despite the relatively low volumes, this fishery has substantial economic importance in several coastal communities in Northern Norway [2].

In the 1960s, the RKC was introduced on the Murmansk coast (Russia) of the Barents Sea to develop a new commercial fishery [3]. Since then, the RKC has become abundant in the large fjords and coastal areas of Northern Norway. In 2002,

a regular commercial RKC fishery was initiated in coastal areas of northeast Norway. The Norwegian RKC fishery is managed by area, a quota-regulated area (QRA) east of North Cape (26°E and south of 71°30'N) and a free fishery area (FFA) to the west. The management goal for the fishery is to maintain a long-term fishery in the QRA while limiting further migration of RKC westward [4, 5]. Despite efforts to reduce further migration, there have been increasing catches of all RKC sizes in the last few years [6]. According to the regulation in FFA, it is not allowed to return the captured RKC to the sea [7]. From 2010 to 2019, the fishermen received economic compensation for bringing RKC below commercial weight class ( $< 800$  g) and female RKC ( $\geq 800$  g) from the FFA onshore [8, 9]. Nowadays, there is limited profitability in fishing the small RKC, and, therefore, it can be hypothesized a high valorization potential to feed these RKC to commercial weight classes in captivity.

The RKC is a cold-water-adapted species typically found within a temperature span from 2 to 7°C [10] but tolerant to a more comprehensive temperature range during seasonal migrations [11]. Growth of RKC occurs by molting. A 1-year-old RKC molts about six times during its second year, then twice a year until age 5, then annually from 6 to 8, and less frequently when older [12]. The estimated size at maturity for RKC from the Barents Sea (Norway) is at a carapace length (CL) of 104.3 mm [13], which corresponds to an age of approximately six years [12]. This CL threshold corresponds to a carapace width (CW) of 118.5 mm [14].

Distribution studies of RKC in the west Russian waters revealed preferences for soft silt and sand seabed [15]. The RKC is omnivorous, and up to 100 prey species have been observed in their stomachs [3]. These include polychaetes, molluscs, crustaceans, echinoderms, fish, and algae [16, 17].

After bringing the RKC of commercial weight classes, i.e., adult RKC onshore, a short period of live holding (LH) is commonly applied. LH enables flexible management by facilitating commitments through long-term contracts with stakeholders requiring stability in supplied volumes. Previous studies have proven the feasibility of short-term LH of adult RKC of commercial weight classes with feeding [18, 19] and without feeding [20]. From a marketing point of view, it is essential that key quality attributes, such as the meat content of the legs, are maintained during the LH [2, 20].

The group of Sotelano [21] performed a study with southern king crab (SKC) (*Lithodes santolla*) larvae in a high-density culture for 5 months. To our knowledge, similar long-term LH studies dealing with juvenile RKC have not been published. Hence, this study aimed to evaluate morphological and quality parameters in adult male red king crabs raised to commercial weight ( $\geq 800$  g) [6] from juveniles (on average 500 g) under different LH conditions.

## 2. Materials and Methods

**2.1. Capture and Live Holding.** Between September and the end of October 2019, juvenile RKC were captured in the FFA, in more detail, in Eidkjosen, nearby Tromsø (~70°N, Norway). The RKC were transported to the Aquaculture Research Station (ARS) in Kårvik, nearby Tromsø, and placed in 6 m<sup>3</sup> tanks with seawater, where they were kept for observation and acclimatization for five weeks. The RKC were provided with feed and a hiding place during the acclimatization period, after which a total of 131 immature RKC (average weight and carapace width of 483 ± 106 g and 93 ± 7 mm) were selected and used in the LH experiment. The weight and the carapace data were obtained using an electronic weight (Sartorius, Data Weighing Systems, Inc., IL, USA) and a caliper (Teco's, AJ Produkter, Kløfta, Norway), respectively.

At the start of the LH experiment (December 3, 2019), male RKC juveniles were selected and evenly distributed into two tanks, one at ambient seawater temperature (AST), and one at elevated seawater temperature (AST-E). The tanks were square-shaped with rounded corners (bottom area of 4 m<sup>2</sup> and water depth of 42 cm) with a rubber-painted bottom to improve gripping. Both tanks were divided into two sections (i.e., subgroups), applying a grid. In one of

the sections, the RKC had the availability of sand in trays (WS), while in the other section, no sand was available (NS). The sand trays (height 10 cm) covered 6.6% of the bottom area of the tank bottoms, and the sand was collected from the shore in Kvalsundet (nearby the ARS). The sand contained debris of blue mussels, barnacles, sea urchins, seaweed, and kelp, and it was used to explore potential habitat improvements for the juveniles. The sand was exchanged every 4–8 weeks.

Both tanks were supplied with filtered (60 µm drum filter) and UV-treated seawater (salinity of 34‰) at 55 L·min<sup>-1</sup>. The LH study included four subgroups (AST/NS, AST/WS, AST-E/NS, and AST-E/WS) with RKC numerosity ( $n$ ) of 32, 33, 33, and 33, respectively. During the LH period, the mortality in all groups was examined. The RKC were defined as dead when no movement of the extremities was observed. Often, these RKC were observed upside down in the tank as well.

In the AST tank, the water temperature fluctuated according to the ambient seawater temperature, whereas in the AST-E tank, the water was warmed up from 0.1 to 2.0°C above the AST. The temperatures were logged continuously (HOBO TidbiT v2-UTBI-001, Onset®, MA, USA), while dissolved oxygen was measured weekly (YSI, ProSolo ODO/CT, Yellow Springs, OH, USA).

The light conditions were regulated daily according to the natural photoperiod for Tromsø (70°N). The color temperature of the lamps was 6500 K, which appears as pure white.

According to data for RKC growth [12, 22], two molts were assumed necessary to ensure a weight increase of at least 800 g. Irrespective of LH group, the RKC molted two times during the LH time of 23 months.

The RKC were fed *ad libitum* three times weekly throughout the LH period. The feed given ranged from 2.5 to 11.1 g (kg<sup>-1</sup> crab) day<sup>-1</sup>. Before the molting, the feed requirement was in the lower range, while after the molting, the need for feed was correspondingly higher due to increased appetite. The diet's main component (95%) was shrimp, while the RKC were occasionally offered herring, blue mussels, sea urchins, and capelin. In the last year of the LH, the diet was only shrimp.

**2.2. Sampling and Processing.** On November 2, 2021, a selection of the RKC from the two tanks ( $n=6$  per subgroup) was sampled, with an average weight and CW of 1313 ± 226 g and 138 ± 8 mm, respectively. Criteria for the selection were that the RKC were of good vitality. In addition, wild RKC ( $n=5$ ), with an average weight and CW of 1402 ± 243 g and 1411 ± 7 mm, respectively, were captured in late October 2021 in Eidkjosen, and they were used as controls. In the autumn, the quality of wild RKC is at their best regarding meat content; thus, these RKC were used for this purpose.

The selected RKC and the RKC controls were transferred into polystyrene boxes in a dry state and covered with a moist paper towel and gel ice (Cold Inc., Oakland, CA, USA). Next, the RKC were transported to Nofima (Tromsø)

by car (30 min), stored overnight at 1-2°C, and processed the following day, within 17 h of their arrival.

All the RKC's were processed as described by Lian et al. [23]. In brief, one leg on each side of the crab was labeled using T-bar tags (Floy Tag Inc., Seattle, WA, USA). Next, the crab was split into two clusters and drained from free body fluid (FBF). The drained cluster weight was then registered before soaking them in the water at 1-2°C for approximately 2 h to remove FBF in excess. Afterward, the clusters were withdrawn from the water and drained for at least 15 min before registering their weight.

The clusters were then placed in wire baskets and cooked by soaking in boiling water for 11 min, targeting a core temperature of 92°C in the second walking leg's most proximal article (*merus*). This cooking regimen applied reflects the industrial procedure. After cooking, the clusters were cooled in ice water with 3.5% NaCl (w/v) for a minimum of 30 min to ensure a leg core temperature below 4°C. The clusters were then drained for at least 15 min before weight registration. The clusters were then air-packed into plastic bags (thickness: 80 µm; dimensions: 220 × 600 mm, Finnvacuum, Helsinki, Finland), closed with metallic clips, and stored at 4°C in a climate chamber (BINDER, GmbH, Tuttlingen, Germany) for 2 days.

### 2.3. Analyses

**2.3.1. Hepatosomatic, Cheliped, and Abdomen Indexes, Shell Thickness, and Meat Content.** The hepatosomatic index (HSI) and the cheliped index (CI) were calculated as follows:

$$\begin{aligned} \text{HSI} &= (W_H/W_{\text{RKC}}) \times 100, \\ \text{CI} &= (W_C/W_{\text{RKC}}) \times 100, \end{aligned} \quad (1)$$

where  $W_H$ ,  $W_C$ , and  $W_{\text{RKC}}$  are the raw hepatopancreas, chelipeds, and crab weight, respectively.

The abdomen index (AI) was calculated as follows:

$$\text{AI} = (W_A/W_{\text{RKC}}) \times 100, \quad (2)$$

where  $W_A$  and  $W_{\text{RKC}}$  are the raw abdomen muscle and crab weight, respectively.

The meat content (MC) was measured using digital image analysis of cross sections of the middle of the *merus* as previously described [20]. The MC was calculated as follows:

$$\text{MC} = (\text{area occupied by meat}/\text{total inner area}) \times 100. \quad (3)$$

The thickness of the shell was measured in proximity to the middle of the *merus* of the second walking leg by applying a caliper (precision ± 0.05 mm). Before the measurement, the inner side of the shell was wiped with paper tissue to remove eventual meat or cartilage residues.

**2.3.2. Cluster Yield during Processing.** During the processing steps, the weight of the raw RKC's and the raw and cooked clusters were registered as previously described [20]. To obtain an overview of the yield as a response to the LH

conditions, the raw cluster yield (CY) was calculated as follows:

$$\text{CY}_{\text{raw}} = (W_{2\text{CR}}/W_{\text{RKC}}) \times 100, \quad (4)$$

where  $W_{2\text{CR}}$  is the sum of the weight of the right and left raw clusters from the same RKC after splitting and  $W_{\text{RKC}}$  is the raw crab weight. Furthermore, the yield of cooked clusters was calculated as follows:

$$\text{CY}_{\text{cooked}} = (W_{2\text{CC}}/W_{\text{RKC}}) \times 100, \quad (5)$$

where  $W_{2\text{CC}}$  is the weight of the two cooked, cooled, and drained clusters from the same RKC and  $W_{\text{RKC}}$  is the raw crab weight.

**2.3.3. Moisture, Color, and Instrumental Texture.** Moisture, color, and instrumental texture measurements were carried out on samples of cooked leg meat extracted from the *merus*.

The moisture was measured according to the AOAC [24] method 950.46. The color parameters  $L^*$  (lightness),  $a^*$  (green-red), and  $b^*$  (blue-yellow) were determined using a portable color spectrophotometer (CM-600d, Minolta Ltd., Osaka, Japan). The whiteness index (WI) was then calculated as described by Lian et al. [23].

The instrumental texture was investigated by performing a double-compression test on two chunks of cooked meat (thickness of approximately 15 mm), using a texture profile analyzer (TA-HD plus, Stable Micro System Ltd., Godalming, Surrey, UK) and following the protocol described by Lian et al. [23]. The software Exponent (version 6.1.14.0, Stable Micro System Ltd.) was used for processing the texture data. The calculated texture parameters (i.e., hardness and chewiness) were expressed as values normalized over the surface area of the meat chunk in contact with the compression plate.

**2.3.4. Sensory Evaluation.** After 2 days of storage at 4°C, a sensory evaluation of the cooked leg meat was carried out by comparing the wild RKC's with the two subgroups in the AST group (i.e., with sand (WS) and without sand (NS)). The evaluation was conducted as a triangle test, testing wild against AST/WS, wild against AST/NS, and finally AST/WS against AST/NS in the following pattern: ABB, BAB, BBA, BAA, ABA, and AAB. The samples were coded with random three-digit numbers. The assessors were instructed to correctly point out the odd RKC sample in the triangle test. Each of the 18 assessors evaluated all three pairwise combinations, and the samples were distributed among the assessors ensuring that each assessor evaluated three samples of each group. The assessors were recruited from staff members at Nofima familiar with RKC products. The test room was arranged to host 12 separate test sites, free from extraneous odor and noise and regulated at a comfortable temperature.

**2.4. Statistical Analyses.** Significant differences in the morphological and quality parameters between the wild, AST, and AST-E groups were analyzed by one-way variance analysis (ANOVA) followed by *post hoc* pairwise multiple

comparisons (Tukey's test). Prior to ANOVA, the normality of the residuals and homogeneity of the variances were tested using the Kolmogorov–Smirnov test and the Brown–Forsythe test, respectively. As the data of the parameters CI and  $CY_{\text{cooked}}$  did not pass the Kolmogorov–Smirnov test, a nonparametric test (Kruskal–Wallis) followed by *post hoc* pairwise multiple comparisons test (Dunn's test) was used instead of ANOVA for these two parameters. All tests were performed in the software GraphPad Prism (version 9.5.0, GraphPad Software, Boston, MA, USA).

Partial least squares (PLS) regression was performed using Unscrambler 11.0 (CAMO Analytics AS, Oslo, Norway) to investigate relationships between temperature and the presence of sand upon the biological characteristics of the experimental groups. Significant effects were detected using the Martens uncertainty test (based on the jack-knife principle).

For amplifying data visualization and interpretation, principal component analysis (PCA) was carried out using the package *FactoMineR* in the software R (version 4.0.3) [25]. The PCA was performed considering the response variables HSI, CI, MC, shell thickness,  $CY_{\text{raw}}$ ,  $CY_{\text{cooked}}$ , WI, and chewiness. The observations related to the wild RKC were handled as supplementary data in the computation of the PCA.

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

### 3. Results

**3.1. Temperature and Oxygen Conditions.** During the LH period of 23 months, the temperature of the AST group ranged from 3 (March to April) to 10°C (August to September), while for the AST-E group, the temperature ranged from 5 to 11°C. Irrespective of the temperature group or time of the year, the oxygen levels were above 85%.

**3.2. Weight and Mortality.** The RKC underwent two moltings (from January to February) during the LH period to reach the commercial weight of more than 800 g in both LH groups. In more detail, the average RKC weight, measured immediately before processing, was 1255 ( $\pm 196$ ), 1337 ( $\pm 302$ ), 1263 ( $\pm 266$ ), and 1477 ( $\pm 168$ ) g for the four subgroups AST/NS, AST/WS, AST-E/NS, and AST-E/WS, respectively.

During the LH period, the mortality rate in the four subgroups AST/NS, AST/WS, AST-E/NS, and AST-E/WS was 25, 9, 33, and 15%, respectively, leading to an average mortality rate of 20.6%.

**3.3. Morphology and Quality Parameters.** The HSI, CI, AI, MC, and shell thickness varied among the wild RKC and the two LH groups (Figure 1). Regarding the HSI, no significant ( $P \geq 0.05$ ) differences among the LH groups were detected. At the same time, this parameter was significantly ( $P < 0.05$ ) higher for the wild RKC when compared to both of the LH groups (Figure 1(a)). No significant ( $P \geq 0.05$ ) differences in

CI were detected among the three groups (Figure 1(b)). In contrast, AI in the wild RKC was significantly ( $P < 0.05$ ) higher than AI in RKC kept at elevated temperature (AST-E) (Figure 1(c)). The MC of the wild RKC was significantly ( $P < 0.05$ ) higher than that of the RKC in the AST group (Figure 1(d)). Regarding the shell thickness, no significant differences ( $P \geq 0.05$ ) were detected between the three LH groups (Figure 1(e)). Although not including the effect of the presence of sand in the ANOVA, a PLS analysis revealed that the availability of sand had a significant ( $P < 0.05$ ) and positive effect for the “shell thickness.” Furthermore, it seems that the ambient seawater temperature (AST) positively affected the shell thickness (Figure 2).

Both the raw and cooked cluster yields of the wild RKC were significantly ( $P < 0.05$ ) higher than those of the RKC from the elevated temperature group (AST-E), while the RKC from the AST group did not differ significantly ( $P \geq 0.05$ ) from the wild RKC nor the RKC from the AST-E group.

Afterward, the muscle from the clusters was extracted from the shell and analyzed for moisture, WI, and texture. The moisture content of the wild RKC muscle was significantly ( $P < 0.05$ ) lower than that of the AST group (Figure 3(c)). The moisture of the AST-E group was not analyzed. The WI of the wild RKC was significantly ( $P < 0.05$ ) higher than that of the RKC from both the LH groups (Figure 3(d)). Regarding the texture parameter “hardness,” no significant ( $P \geq 0.05$ ) differences among the three LH groups were detected (Figure 3(e)), while for the parameter “chewiness,” the wild RKC and the RKC at elevated temperature (AST-E) obtained significantly ( $P < 0.05$ ) lower values (i.e., being less chewy) when compared to the RKC kept at ambient temperature (AST) (Figure 3(f)).

Also, a triangle test with sensory evaluation between the wild RKC and the crabs from the two subgroups at AST was performed. In total, 4, 4, and 6 out of the 18 assessors correctly pointed out the odd crab from the three test patterns, respectively. Thus, no significant ( $P \geq 0.05$ ) differences between the RKC from the wild group and the AST/NS and AST/WS subgroups were demonstrated.

**3.4. Principal Component Analysis (PCA).** A PCA was performed to provide a visual overview of the morphological and quality parameters (Figure 2). The first (PC-1) and the second (PC-2) principal components accounted for 41.5 and 23.0% of the variance, respectively. The variance of the yield of the raw ( $CY_{\text{raw}}$ ) and cooked clusters ( $CY_{\text{cooked}}$ ) was well explained along PC-1, while the variance of the cheliped index (CI) and “chewiness” was well explained along PC-2 (Figure 2). The effect of the temperature (standardized loadings: PC-1,  $-0.26$ ; PC-2,  $-0.46$ ) accounted for a larger share of variance compared to the effect of the presence of sand in the LH compartment (standardized loadings: PC-1,  $0.37$ ; PC-2,  $0.16$ ). The vectors for the sand, shell thickness, WI, and  $CY_{\text{raw}}$  point in a similar direction, suggesting a possible interdependency (Figure 2(a)). Moreover, the CI and “chewiness” are orthogonal to  $CY_{\text{raw}}$  and  $CY_{\text{cooked}}$ .

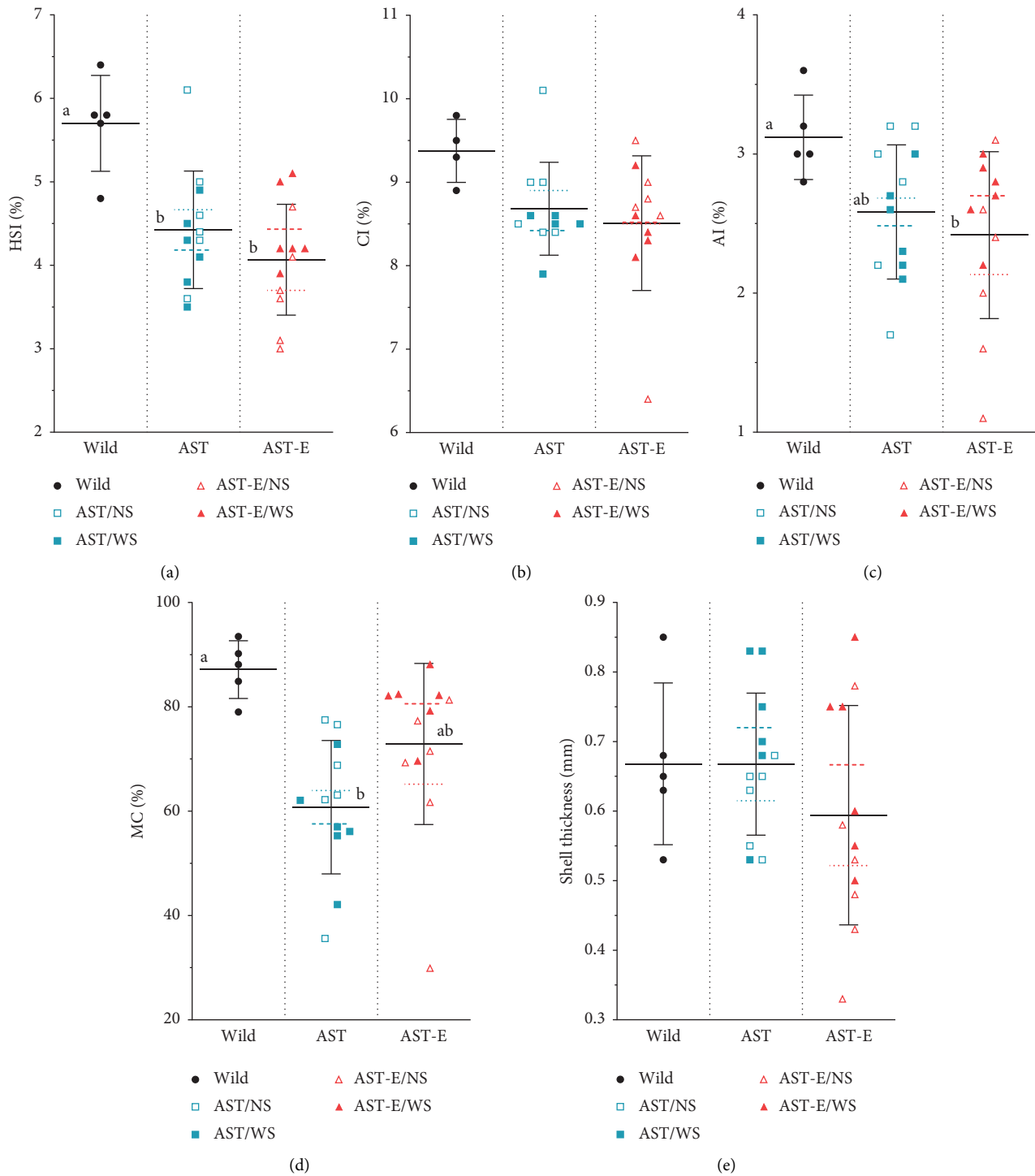


FIGURE 1: Plots illustrating the values (mean ± standard deviation, SD) of hepatosomatic index (HSI) (a), cheliped index (CI) (b), abdomen index (AI) (c), meat content (MC) (d), and shell thickness (e) in red king crabs obtained from wild specimens (control) and specimens kept for 23 months in live holding (LH) at ambient seawater temperature (AST) and elevated seawater temperature (AST-E) either without (NS) or with (WS) accessibility to sand. Long horizontal lines with error bars indicate group (wild/AST/AST-E) means and SD, whereas short dotted and dashed lines indicate, respectively, the NS and WS subgroup means within a LH temperature. Significantly ( $P < 0.05$ ) different mean values between the groups wild/AST/AST-E are indicated with different lowercase letters.

showing a weak interdependency. In the score plot (Figure 2(b)), an apparent clustering of the observations for the wild RKC is observed along the positive quadrants for PC-1

in conjunction with the majority of the observations for the subgroups WS irrespective of the water temperature (Figure 2(a)). In the score plot, a higher level of clustering

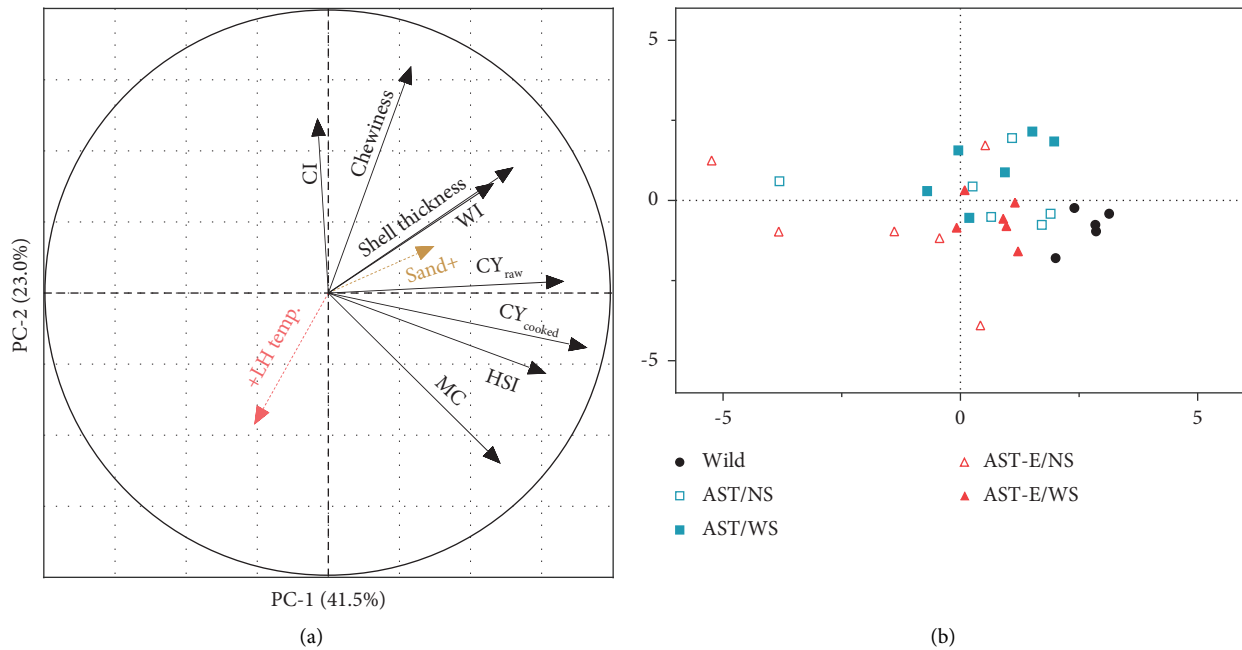


FIGURE 2: Loading (a) and score (b) plot obtained by principal component analysis of morphological and quality parameters in red king crab (RKC) specimens kept for 23 months in live holding (LH) at ambient seawater temperature (AST) and elevated seawater temperature (AST-E) either without (NS) or with (WS) accessibility to sand. The LH temperature and accessibility to sand are represented as supplementary explanatory variables (i.e., factors). The observations related to the wild RKC specimens were handled as supplementary data. HSI, hepatosomatic index; CI, cheliped index; MC, meat content; CY<sub>raw</sub>, raw cluster yield; CY<sub>cooked</sub>, cooked cluster yield; WI, whiteness index.

could also be observed for WS subgroups compared to the NS subgroups (Figure 2(b)).

## 4. Discussion

**4.1. Morphology.** Feeding of juvenile RKC towards commercial sizes succeeded. When compared to the initial weight of the juveniles, i.e.,  $483 \pm 106$  g, the weight increment varied among the LH groups. Although no significant differences were observed, the RKC with the availability of sand showed a higher average weight increment when compared to the corresponding RKC without sand. Some mortality could be expected during molting, as the RKC are vulnerable to predation by the specimens that are not going through molting then. Although not having data for comparison, an average mortality rate of 20.6% in this study is considered low.

The feed offered was consumed, indicating good live holding conditions. However, the results (Figures 1 and 3) show differences in morphological and quality parameters between the wild RKC and the juvenile RKC after 23 months of LH. Clearly, one or more of the LH conditions were suboptimum. For example, for the wild RKC, the HSI, AI, MC, CY<sub>raw</sub>, CY<sub>cooked</sub>, moisture, WI, and chewiness differed significantly for one or both LH groups.

For the parameters HSI, CI, AI, MC, yield, WI, and texture, an apparent distribution pattern within the three groups appears (Figures 1 and 3). In detail, the distance between maximum and minimum values of the wild RKC group shows less variation when compared to the

corresponding LH groups AST and AST-E. This indicates a higher degree of homogeneity of the wild RKC and a corresponding lower homogeneity, i.e., heterogeneity, of the RKC kept in captivity. This is also supported by the clustering of this group in the score plot (Figure 2(b)).

Moreover, a pattern between the subgroups, i.e., with (WS) and without the availability of sand (NS), is also observed. That is, the WS subgroups showed lower variation than the corresponding NS. This observation is also supported by the higher level of clustering for the RKC with access to sand compared to those without sand (Figure 2(b)).

RKC and other crustaceans need dietary sources of minerals for growth because of repeated molts wherein minerals are lost [26]. The sand contains minerals and debris like kelp, sea urchin, and blue mussels, contributing to a more varied diet. This aligns well with the observations of the RKC in the subgroups with the availability of sand as the animals were observed scooping up the sand by the chela, passing it to the mouth.

In the case of shrimps, which also go through molting cycles, the availability of minerals depends on the aquatic environment, feed offered, the form of the mineral, amount of minerals stored in the body, interaction of other elements present in the gastrointestinal tract, and interactions between body tissues and minerals with other dietary ingredients or metabolites [27]. It can be assumed that these factors are also relevant for the RKC juveniles and that the sand contained dietary ingredients from which the animals

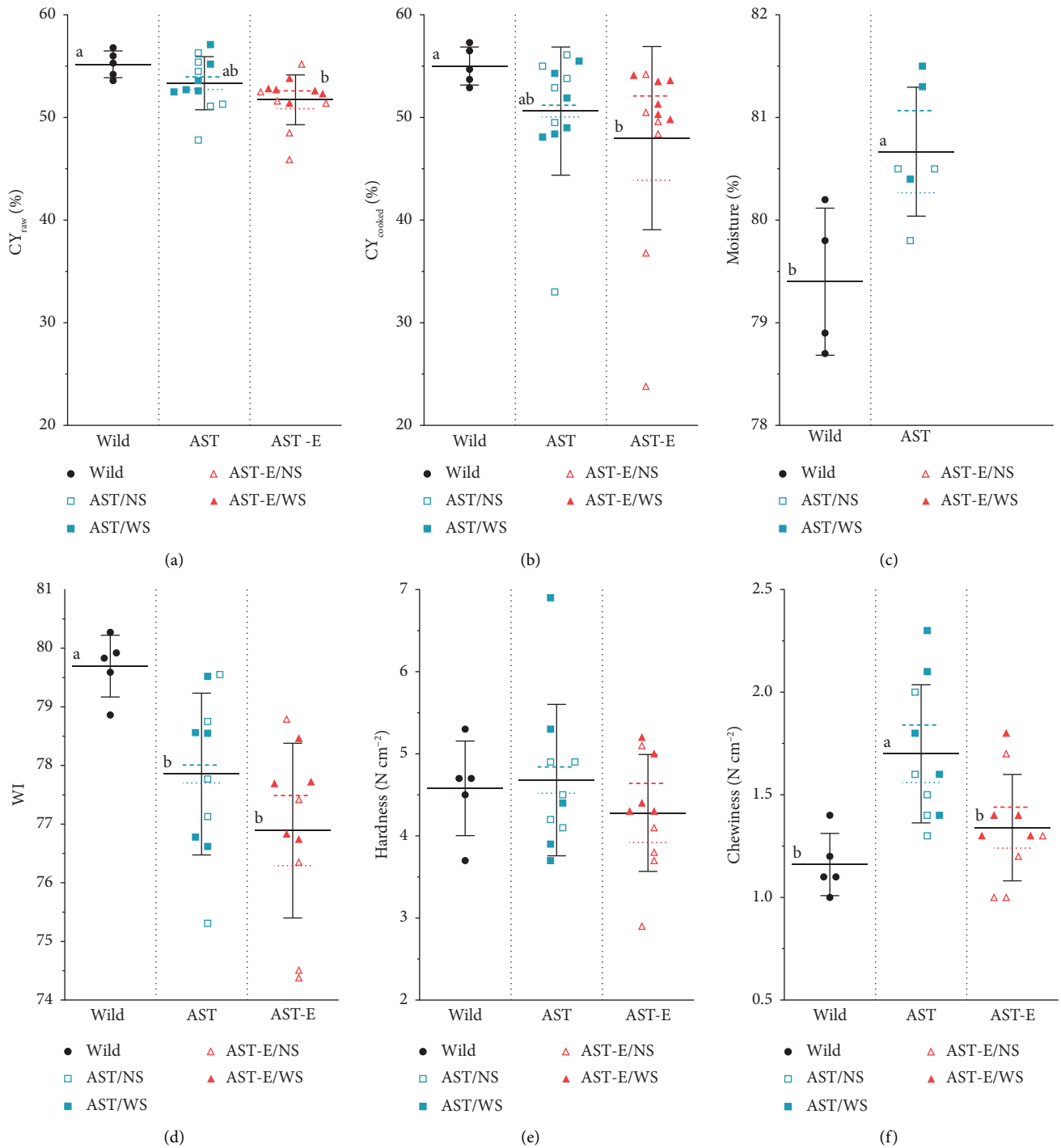


FIGURE 3: Plots illustrating the values (mean  $\pm$  standard deviation, SD) of raw cluster yield (CY<sub>raw</sub>) (a), cooked cluster yield (CY<sub>cooked</sub>) (b), cooked leg meat moisture (c), whiteness index (WI) (d), instrumental hardness (e), and chewiness (f) in red king crabs obtained from wild specimens (control) and specimens kept for 23 months in live holding (LH) at ambient seawater temperature (AST) and elevated seawater temperature (AST-E) either without (NS) or with (WS) accessibility to sand. Long horizontal lines with error bars indicate group (wild/AST/AST-E) means and SD, whereas short dotted and dashed lines indicate, respectively, the NS and WS subgroup means within a LH temperature. Significantly ( $P < 0.05$ ) different mean values between the groups wild/AST/AST-E are indicated with different lowercase letters.

could benefit. Calcium carbonate is the most important inorganic compound in the mineral phase of crustacean shells [26]. Depending on the degree of calcification,

crustaceans lose a significant amount of calcium during molting, and marine species commonly extract calcium from seawater [26].



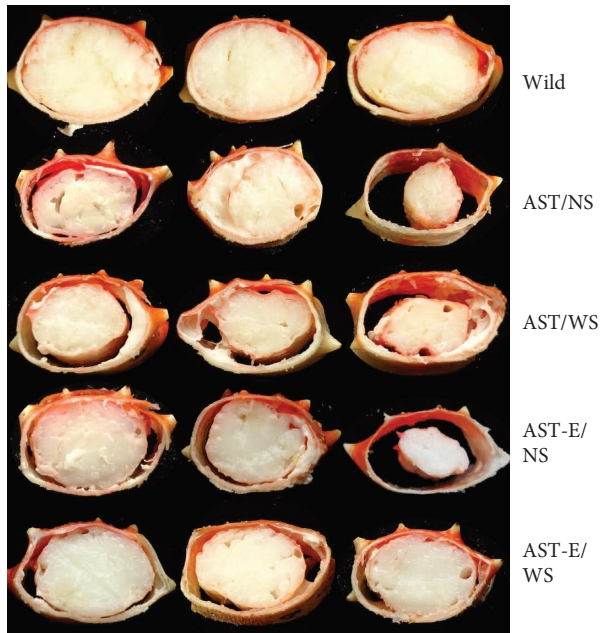


FIGURE 4: Cross sections of the middle of the *merus* of cooked red king crab (RKC) obtained from wild specimens (control) and specimens kept for 23 months in live holding at ambient seawater temperature without sand (AST/NS), ambient seawater temperature with sand (AST/WS), elevated seawater temperature without sand (AST-E/NS), and elevated seawater temperature with sand (AST-E/WS).

The variation in MC values can potentially reflect a hierarchy among the RKC (Figure 1(d)). For instance, the subgroups with sand available, which have a more varied diet, showed less variation compared to the corresponding subgroups without available sand. Thus, it is hypothesized that the absence of sand negatively influenced the LH conditions, which was observed as increased variation in MC values.

Similar assumptions can be made for the HSI as this parameter indicates the biological and nutritional status of the RKC [28]. The RKC kept in captivity showed a significantly ( $P < 0.05$ ) lower HSI than the wild RKC. Hence, the lower HSI values indicate suboptimum LH conditions for these RKC. Differences between wild and the LH RKC in our study may also be related to different temperature regimes. A growth study of RKC showed that adult males fed at and subjected to constant temperatures of 4, 8, and 12°C obtained a better food conversion efficiency at the lowest temperature [19]. Temperature preference for marine animals often reflects optimal temperature in terms of feed utilization. Studies conducted on adult male RKC show that they avoid temperatures above 4°C [29].

Another element that could contribute to suboptimum LH conditions is the natural shift in the conditions for wild RKC from juvenile to adult. During the ontogenetic stages, from juvenile RKC, their natural habitat and, thus, food source may change, and that size gain and habitat shift often occur concomitantly [26, 30]. In our study, the same habitat

and feed (shrimp, herring, sea urchins, and capelin) were used through the entire LH period of 23 months.

In previous studies at Nofima, adult RKC were fed *ad libitum* for up to three months (unpublished data). Interestingly, the MC values correspond to the results in this study (Figure 1(d)), showing that the challenges related to feeding and LH conditions also apply to adult RKC during short-term LH. Furthermore, similar open channels were also observed in the cross sections of the processed clusters from both the LH groups, while this was not the case for the wild RKC (Figure 4). It is assumed that the channels reflect the density of the myofibrillar lattice. In case of more space between myofibrils, the channels become visible after the denaturation and coagulation of the myofibrillar proteins during the cooking process. As previously described, legs with a low meat content commonly compensate with FBF. Hence, the thermal exposure during the cooking process is assumed to be similar irrespective of meat content. Thereby, thermal exposure is not assumed to be causative for the observed differences in the meat content (Figure 4).

Many authors have reported sand or sediments in the stomachs of king crabs [31–33]. Cunningham [31] also reported that the crabs consumed sand and suggested that this was a method by which crabs obtained small crustaceans when larger prey was unavailable.

**4.2. Processing and Quality Parameters.** During the slaughtering and further processing, the yield for cooked clusters declined for both the LH groups (Figures 3(a) and 3(b)). A decrease in the CY, from raw to cooked, has previously been observed for adult RKC without feeding for up to three months [20]. During the LH, these RKC compensated for a muscle volume reduction with free body fluid (FBF) in the legs. Also, the decline was assumed to be related to structural modifications of the muscle fibers, weakening the fibers and connective tissue. The changes were assumed to make the muscle more susceptible to thermal denaturation, thereby losing weight during cooking [34].

As previously stated, the LH conditions in this study were suboptimum, and despite being fed *ad libitum*, it can be hypothesized that a hierarchy was established (Figures 1(a) and 1(d)). It is assumed that the individuals facing suboptimum LH conditions and thereby a gradually lower MC compensated with FBF. This hypothesis is also supported by the significantly ( $P < 0.05$ ) higher moisture content of the muscle from AST group than for the corresponding wild RKC (Figure 3(c)). Consequently, the observed decline in cooked yield for some individuals could reflect suboptimum LH conditions. For the wild RKC, the CY did not decline, showing that the muscle did not undergo similar modifications during the cooking process.

From an industrial point of view, factors affecting the yield during processing are essential. Depending on the market demand, business operators can alternate between exporting RKC as live or processed into cooked and frozen clusters. Knowledge of the risk of losing yield during the processing should be incorporated to make the optimum



decision. Hence, further knowledge of the LH conditions influencing the muscle microstructure is highly required.

The WI of the cooked leg meat did not differ significantly ( $P \geq 0.05$ ) between the two LH groups. However, the WI values for wild RKC were significantly higher ( $P < 0.05$ ) compared to the LH groups. This might be ascribed to differences in the surface properties of the cooked leg meat, specifically in the ratio between absorbed and reflected light [35, 36]. Hence, a higher light scattering and a whiter muscle can be linked to a structurally denser myofibrillar network. Furthermore, an interdependency between WI and the shell thickness was observed (Figure 2(a)). As the shell thickness depends on the access of calcium and minerals in general, it can be hypothesized that the WI also is influenced by this.

**4.3. Instrumental Texture and Sensorial Evaluation of Cooked Leg Muscle.** According to the binominal law, to claim that RKC from two groups are significantly different, at least 10 out of 18 assessors must correctly point out the odd crab sample in the triangle test [37, 38]. Although significant ( $P < 0.05$ ) differences in instrumental “chewiness” were observed between the wild and AST groups (Figure 3(f)), no significant differences ( $P \geq 0.05$ ) between the wild group and the AST/NS and AST/WS subgroups were demonstrated in the sensory triangle test.

**4.4. General Considerations.** The differences in morphological and quality parameters evidently reflect the effect of the LH conditions for the juveniles resulting in a RKC and a product not identical to the wild RKC. Thus, after the LH, the RKC did not become a strict analog to the wild RKC, i.e., the controls. However, the cooked leg meat from the different groups can be considered similar as no sensorial differences between these groups were detected. Apart from the parameters explored in this study, elements like the nutritional composition of the feed, minerals of the sand, frequency of feeding, feed quality, feed intake, temperature, design of the LH tanks, stocking density, and physical activity are all factors expected to influence the growth, morphology, and the final product quality attributes. For instance, how does the available space in the tanks influence the microstructure of the muscle when considering the species' natural migratory behavior?

The behavior of RKC in farming conditions has been little studied, especially the formation of hierarchy and negative social interaction [39]. Social interactions may explain the variation between individuals in relation to the MC in our experiment.

It is well known that the presence of RKC implies intense predation on benthic biota, thereby severely degrading benthic ecosystems [3]. In the case of a future industry with LH of RKC either from juveniles until commercial sizes or a short-term LH of adult RKC, such degradation could potentially be limited. Besides, a goal-oriented fishery for RKC juveniles in the FFA would also contribute to limiting further expansion along the coast. This aligns well with the governmental aim to limit its distribution as the RKC is defined as an invasive species [4].

The results presented in this paper can serve as the basis for future LH research on juvenile red king crabs. Questions concerning conditions for LH, like changes and adjustments of LH regimen according to the size and age of juveniles, the nutritional composition of the feed, and the potential effect of a varying initial weight of the juveniles, will need to be addressed. These questions clearly illustrate that LH and feeding of juvenile red king crabs are in preliminary phase and that more knowledge in this field is needed.

In conclusion, this study demonstrates that juvenile male red king crabs (RKC), weighing approximately 500 g, can reach commercial weight ( $\geq 800$  g) after 23 months of live holding. The mortality was low, and the feed offered was consumed, indicating good live holding conditions. However, the results show differences in morphological and quality parameters between the wild RKC and the RKC after the live holding. These results show potential for improving the biotic and abiotic factors related to the live holding of juvenile red king crabs to adults. Among the parameters explored, hepatosomatic index (HSI), abdomen index (AI), meat content (MC), cluster yield, moisture, whiteness index (WI), and chewiness differed significantly ( $P < 0.05$ ) between the wild RKC and the RKC from one or both of the live holding groups. The subgroups with sand available indicated an improved habitat as several parameters were affected positively. Also, in these subgroups, a lower variation in morphology and quality parameters of the processed muscle was observed compared to the corresponding subgroups without sand. Further studies are warranted to optimize the live holding conditions of juveniles to achieve morphology and quality parameters closer to the ones of wild red king crabs.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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