





# Physiological responses to live air transport of red king crab (*Paralithodes camtschaticus*)

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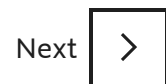
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## Highlights

- Red king crabs were air transported live, mimicking commercial practices.
- Crab haemolymph was collected before and after the transportation.
- $\text{Cl}^-$ ,  $\text{Na}^+$ , lactate and total protein increased in the transported crabs.
- Free [Ala](#), [Arg](#), [Thr](#), [Val](#) and Tau were elevated in the transported crabs.
- Crabs exhibited the typical [physiological responses](#) associated to [hypoxia](#) stress.

## Abstract

Live transport of red king crab from the fishing grounds to the market is an activity that is increasing and the future sustainability of this practice depends on maintaining optimal animal welfare during transport. The current study evaluated the physiological response of red king crab (*Paralithodes camtschaticus*) to live air transport, mimicking current commercial practices. Specifically, this study assessed stress and osmoregulatory haemolymph metabolite variation, together with free amino acids (FAAs) pool variation in the haemolymph of red king crab. Forty male crabs ( $2136 \pm 800$  g) were caught in the North Cape area in the Barents Sea during April 2019. They were transported by the fishing boat directly to a land-based live holding facility in Honningsvåg, Norway where they were held for < 10 days. They were then divided in two groups: i) the first group of 20 crabs underwent immediate haemolymph sampling, and ii) the second group of 20 crabs was divided in subgroups of 5 crabs, placed in a standard air freight Styrofoam boxes with freezer packs and transported by road and air to the Nofima facility, in Tromsø, Norway. The time from the packing until when the haemolymph sampling occurred was 20 h. All samples of haemolymph were taken from the coxa membrane of the third right side walking leg and were analysed for sodium ( $\text{Na}^+$ ), chloride ( $\text{Cl}^-$ ), carbon dioxide ( $\text{CO}_2$ ), lactate, total protein, and FAAs. The survival of the transported crabs was 100 %, and after transport the measured indicators showed a significantly positive (lactate and total protein) or a negative ( $\text{Na}^+$ ,  $\text{Cl}^-$ ) variation in relation to the air transport. From the measured FAAs, five, Alanine (Ala), Arginine (Arg), Threonine (Thr), Valine (Val) and Taurine (Tau), were significantly higher for the group after transport when compared to the group before transport. No FAAs were significantly lower as a result of transport. In conclusion, it was apparent that red king crabs exposed to air during a 20 -h transport period exhibited the typical physiological responses associated to hypoxia stress. Moreover, in this study we report for the first time a change in the FAA pool results due to transport where five out of the twenty-one measured FAA were significantly elevated. Specifically, Ala, an important osmolyte during salinity stress. These results bring new insights into how red king crabs cope with live air transport and offer new evidence for the need to assess the crabs' health and welfare during live holding and transport. Moreover, the results obtained from this study can also contribute to the development of an industry friendly methodology to assess the stress and health status of crabs during and after live transport.



## Keywords

Red king crab; *Paralithodes camtschaticus*; Haemolymph; Live transport; Ions; Free amino acids

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## 1. Introduction

Red king crab (*Paralithodes camtschaticus*) fisheries occur in its native Northern Pacific Ocean, Sea of Japan and Bering Sea regions, and in the area that the species has been introduced into in the Barents Sea. These fisheries are an important resource for the countries bordering these regions, such as the USA, Russia, and Norway, with a combined economic value of 300–350 million US dollars (Dvoretzky and Dvoretzky, 2018; Garber-Yonts and Lee, 2016; Lorentzen et al., 2018).

Typically in Norway, red king crab are captured with vessels operating pots near coastal waters, which is followed by immediate processing, i.e., cooking and freezing, or by live holding for limited periods followed by transport by air to international wholesale markets. Live overseas transport accounts for over seventy percent of harvested Norwegian king crab and these are mainly exported to markets such as South Korea (Voldnes et al., 2020). This strategy is highly lucrative due to the increased value of live red king crab in seafood markets, supermarkets and restaurants. However, the future sustainability of live transport lies in optimising animal welfare during live holding and transport to the destination market (Lorentzen et al., 2018).

Live transport is preceded by live holding, the latter normally consists of keeping the crabs in land-based flow-through tanks supplied with natural seawater until transport. Studies on the capture-based aquaculture of red king crab have aimed to improve crab welfare during this period. For example, it was shown that mortality and occurrence of injuries were related to high stocking density (James et al., 2013; Siikavuopio et al., 2016), and feed intake was negatively related with increasing water temperature (Siikavuopio and James, 2015). However, the impact of live transport on red king crab is largely unknown, with the exception of an abstract by Zagorsky et al. (2014), where two transport methods were compared. Commercially, there are essentially two live transport methods: i) in water, using transport tanks, i.e., vivier-truck tanks, equipped with aeration or oxygenation and, ii) in air, using boxes with chiller packs (packs with frozen filler). The latter is the most common practice for the red king crab, particularly when the markets are beyond the range of road transport. Several studies on the transport of crustaceans have shown that several physiological parameters, health and welfare during live transport can be severely impacted (see review by (Fotedar and Evans, 2011)).

Physiological responses to air transport stressors can be related to air exposure, temperature variations and handling factors. It has been previously described that exposure to air disrupted the oxygen consumption of crustacean and led to an accumulation of metabolites in haemolymph associated with anaerobic metabolism ([Fotedar and Evans, 2011](#)). For example, spiny lobster (*Jasus edwardsii*) emersed during simulated live transport showed elevated haemolymph glucose, lactate, and ammonia ([Speed et al., 2001](#)). Likewise, snow crabs (*Chionoecetes opilio*) exposed to increasing periods of air, mimicking a live transport, showed a spike of lactate in the haemolymph ([James et al., 2019](#)). Temperature variation, another stressor of transport, was shown to cause haemolymph acid-base imbalances in European lobster (*Homarus gammarus*) during live transport ([Whiteley and Taylor, 1992](#)) and oxidative stress in southern king crab (*Lithodes santolla*) during emersion ([Schvezov et al., 2019](#)). Stressors in general, seem to lead to alterations of haemolymph metabolites, and some of these can be used as biomarkers for determining crustaceans' physical condition or vitality.

Haemolymph composition of free amino acids (FAAs) can also be a useful indicator of crustacean's welfare and health. In many crustacean species, amino acids have been found to play an important role in the osmoregulation of intracellular fluid via the increase or decrease of amino acid pool in response to hypo/hyper-osmotic environments. For example, FAAs in the haemolymph of the giant freshwater prawn, (*Macrobrachium rosenbergii*) exposed to high salinities led to an overall increase of amino acids ([Huong et al., 2001](#)). Also, FAAs in the muscle and haemolymph concentrations vary during moulting, i.e., the periodic shedding of the exoskeleton. [Dooley et al. \(2002\)](#) found that the concentration of proline (Pro) in haemolymph was lower in the Australian freshwater crayfish (*Cherax destructor*) in premoult and postmoult compared to intermoult. This FAA plasticity in crustaceans' haemolymph may indicate that some amino acids have an important adaptative role in stress conditions. Whether the transport of red king crab would lead to an alteration of haemolymph FAA pool is unknown.

The objective of the present study was to evaluate the physiological response of red king crab (*Paralithodes camtschaticus*) to live air transport, mimicking current commercial practices. Specifically, to evaluate stress and osmoregulatory haemolymph metabolite variation, as well as FAAs pool variation, in the haemolymph of red king crab.

## 2. Material and methods

### 2.1. Experimental design

The current study consisted of evaluating the effect of live air transport of red king crab on several haemolymph parameters. For this purpose, two groups of 20 crabs were randomly sampled from a wild captured stock of crabs maintained at a live holding facility. The first group (from now on “before transport”) underwent immediate haemolymph sampling at the beginning of the trial. The second group (from now on “after transport”) of 20 crabs were divided in subgroups of 5 crabs, placed in standard air freight Styrofoam boxes (dimensions 78 cm × 39 cm × 23 cm) with freeze packs (0.5 L) and transported by road (approx. 4 h) and air (approx. 3 h) to Nofima AS experimental facility (Tromsø, Norway). After arrival at Nofima AS crabs were stored overnight (13 h) in a cooling room (2 °C) in their boxes. The time from the packing in the holding facility until the haemolymph sampling occurred was 20 h.

## 2.2. Animals

Red king crabs were captured in crab pots by commercial fishery vessels in the North Cape area in the Barents Sea during April 2019. Immediately after, the crabs were transported to a live-holding facility (Capefish AS), in Honningsvåg, Norway, where they were kept in indoor flow-through tanks (0.7 m<sup>3</sup>) for < 10 days using ambient seawater (salinity 33 ppt, temperature 3–5 °C). During this period crabs were starved as is standard industry practice. Forty male (2136 ± 800 g) crabs that were post-moult and did not have damaged legs were selected and used in the present study. The individuals that met the above criteria were randomly picked from a larger population kept at the live-holding facility that were captured on different days within a period of 10-days.

## 2.3. Sampling and sample analysis

Samples of haemolymph (2 ml) were taken from the coxa membrane of the third right side walking leg (4<sup>th</sup> pereopod) using a Vacuette® needle (20 G x 1.5') and a clot activator vacuum tube (Greiner Bio-One, Kremsmunster, Austria). After 15 min. resting in ice, samples were centrifuged for 10 min. at 2500 G at room temperature (approx. 22 °C) and immediately after, the supernatant was transferred to an Eppendorf tube freeze and stored at - 80 °C for further analysis.

Haemolymph sodium (Na<sup>+</sup>), chloride (Cl<sup>-</sup>), carbon dioxide (CO<sub>2</sub>), lactate and total protein were determined from the crab serum using an automated clinical chemistry system (Pentra C400, Horiba, CA, USA). The diagnostic reagents were supplied by Horiba ABX (Montpellier, France) and its determination used the following methods: Na<sup>+</sup> and Cl<sup>-</sup>, ion selective electrode; CO<sub>2</sub>, enzymatic test using phosphoenolpyruvate carboxylase and a

stable NADH analog; lactate, Trinder method and; total protein, Biuret reaction/colorimetric method.

Extraction of FAA from haemolymph samples protocol was adapted from [Dooley et al. \(2000\)](#). Briefly, 0.5 ml of the samples was added in glass vials together with 0.05 ml (20 mM) of DL-Norleucine (Sigma-Aldrich; internal standard) and 2 ml of 80 % ethanol. Then, the glass vials were placed in a shaking water bath at 70 °C for 20 min. Later, the samples were centrifugated at 4000 G for 10 min at room temperature (approx. 22 °C), the supernatant removed, and the pellet resuspended in 2 ml of 80 % ethanol. The process was repeated three times and the samples were stored at 4 °C until further analysis.

Amino acid analysis consisted of a chromatographic separation on an ion exchange column, as previously described by [Mæhre et al. \(2013\)](#). Prior to the load of samples into the analyser, the samples were evaporated, and the pellet resuspended in 1 ml of the lithium citrate loading buffer. The analysis was performed using a Biochrom 30 amino acid analyser (Biochrom Co, Cambridge, UK) and the UV signals were analysed by Chromeleon software (Dionex, Sunnyvale, CA, USA) and compared with A9906 physiological amino acids (Sigma Chemicals Co, St. Louis, MO, USA).

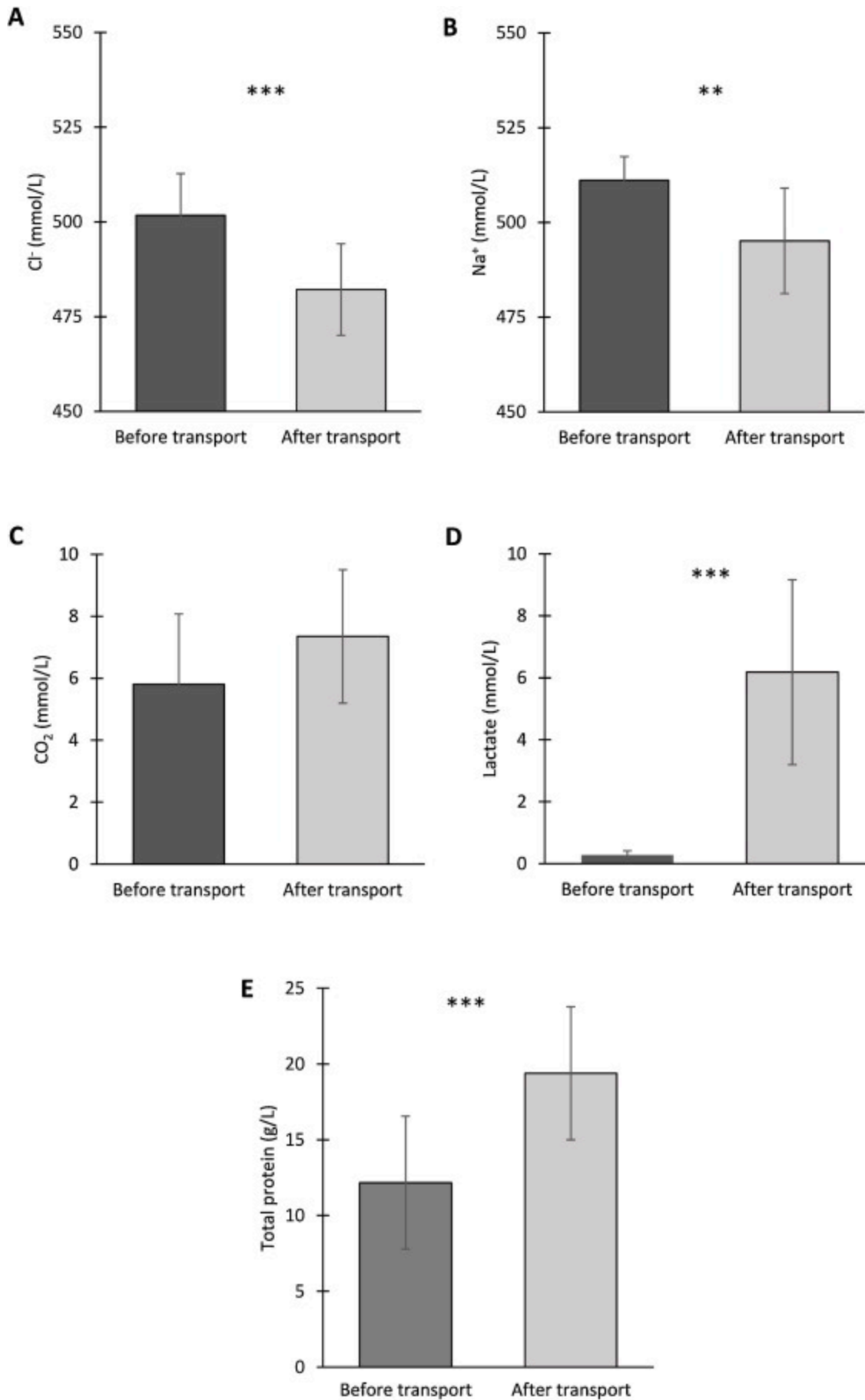
## 2.4. Statistics

Statistical analyses were performed with IBM SPSS Statistics V26 (IBM, Corp., USA). Free amino acid results that were below the amino acid analyser detection limit were given the minimal value, i.e. 0.5 nmol. Individual crabs are considered replicates. Individuals with insufficient serum for analysis were removed; N = 17 (before transport) and N = 14 (after transport) for automated clinical chemistry system analysis; N = 13 (before and after transport) for FAA analysis. Homogeneity of variance was assessed using Levene's test and normality using the Shapiro-Wilk test. Independent samples *t*-test was used to compare differences between the two groups. A significant level ( $\alpha$ ) of 0.05 was used for all analyses. All data are presented as mean  $\pm$  standard deviation (S.D.).

## 3. Results

The survival of the transported crabs was 100 %.

The haemolymph of crabs transported had significantly lower Na<sup>+</sup> ( $\downarrow$  3.1 %) and Cl<sup>-</sup> ( $\downarrow$  3.9 %) ions when compared to levels before transport ([Fig. 1A, B](#)).



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Fig. 1. Concentration of  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{CO}_2$ , lactate and total protein in haemolymph (mean  $\pm$  S.D.).  $N = 17$  (before transport) and  $N = 14$  (after transport).  $P$ -value  $< 0.010$  (\*\*) and  $< 0.001$  (\*\*\*)

The haemolymph  $\text{CO}_2$  did not differ between the two treatments (Fig. 1C), whereas the haemolymph lactate was significantly higher ( $\uparrow 2134\%$ ) in the transported crabs (Fig. 1D).

The haemolymph total protein concentration of the transported group presented a significantly higher ( $\uparrow 59.3\%$ ) value when compared to levels before transport (Fig. 1E).

The average concentrations of haemolymph FAA from the two groups are shown in Table 1. Among all measured FAAs, Alanine (Ala), Arginine (Arg), Glutamine (Gln), Glycine (Gly), Leucine (Leu), Proline (Pro) and Taurine (Tau) were the most abundant, all with concentrations above  $20\ \mu\text{g/mL}$ . From these measured FAA, five - Ala, Arg, Threonine (Thr), Valine (Val) and Tau - were significantly higher for the group after transport when compared to the group before the transport. No FAAs were significantly lower as a result of transport.

Table 1. Mean ( $\pm$  S.D.) concentrations of free amino acids ( $\mu\text{g/mL}$ ) before and after the transport ( $N = 13$ ).

Amino acid	Before transport	After transport	$P$ -value <sup>a</sup>
<b>Ala</b>	12.3 $\pm$ 4.4	32.3 $\pm$ 12.3	<b>&lt; 0.001</b>
<b>Arg</b>	33.1 $\pm$ 18.6	61.1 $\pm$ 21.6	<b>0.002</b>
<b>Asn</b>	13.0 $\pm$ 6.2	12.9 $\pm$ 10.3	0.990
<b>Asp</b>	13.1 $\pm$ 6.3	11.4 $\pm$ 10.2	0.629
<b>Cys</b>	11.9 $\pm$ 5.7	10.4 $\pm$ 9.3	0.630
<b>Glu</b>	14.4 $\pm$ 6.9	12.6 $\pm$ 11.2	0.630
<b>Gln</b>	28.0 $\pm$ 11.1	20.8 $\pm$ 14.9	0.176
<b>Gly</b>	31.5 $\pm$ 16.4	45.5 $\pm$ 27.2	0.124
<b>His</b>	15.2 $\pm$ 7.3	13.4 $\pm$ 11.8	0.646
<b>Ile</b>	12.9 $\pm$ 6.2	16.5 $\pm$ 12.1	0.344
<b>Leu</b>	15.1 $\pm$ 10.6	29.3 $\pm$ 24.8	0.075
<b>Lys</b>	14.3 $\pm$ 6.9	21.7 $\pm$ 16.6	0.157



Amino acid	Before transport	After transport	P-value <sup>a</sup>
Met	14.6 ± 7.0	16.6 ± 12.2	0.625
Phe	16.2 ± 7.8	15.5 ± 12.7	0.865
Pro	50.3 ± 24.6	41.0 ± 17.0	0.276
Ser	11.8 ± 5.0	13.6 ± 8.2	0.512
Thr	11.7 ± 5.6	21.6 ± 12.1	<b>0.016</b>
Trp	16.6 ± 8.0	14.5 ± 12.9	0.629
Tyr	17.8 ± 8.5	16.0 ± 13.8	0.693
Val	12.4 ± 5.7	21.3 ± 12.2	<b>0.028</b>
Tau	20.8 ± 10.9	40.8 ± 30.2	<b>0.040</b>

a

Values in bold indicate significant differences,  $P < 0.05$ .

## 4. Discussion

In the current study an array of haemolymph biomarkers was measured to assess red king crabs' condition after air transport. The survival rate of the transport was 100 %. Osmoregulatory and stress haemolymph indicators showed a positive (lactate and total protein), a negative ( $\text{Na}^+$ ,  $\text{Cl}^-$ ) or a neutral ( $\text{CO}_2$ ) variation in relation to the 20 -h air transport. The current study also showed that the haemolymph FAA pool changes in response to transport, namely by the increase in concentration of Ala, Arg, Thr, Val and Tau.

Haemolymph metabolites have been investigated as a tool for monitoring the physiological condition in wild and cultured crustaceans exposed to different environmental conditions, including the impact of live transport in commercially important marine crustaceans, such as shrimp, lobster and crab. Apart from some species-specific and transport-specific variations, some common responses are usually described. For instance, air exposure during transport usually results in anoxic stress to the animals. A physiological compensation mechanism to anoxic condition consists of an increase in haemocyanins, which are a class of proteins responsible for transporting oxygen between the tissues in crustaceans. Total protein measurement in the haemolymph is a proxy parameter to capture this response. For example, [Lorenzon et al. \(2008\)](#) found an increase of total protein in the brown crab (*Cancer pagurus*) transported in boxes with high air humidity. However, this compensation

mechanism also seems to be condition-specific dependent, as no changes were found in total protein in emersed snow crab (James et al., 2019). In the current study, total protein had a 59.3 % increase in the transported crab, which contrasts with the previous work from (Zagorsky et al., 2014), where total protein remained unchanged in crabs transported in boxes with wet material. However, the research by Zagorsky et al. (2014) is only available as a conference abstract so it is difficult to compare the results to assess the possible cause for this difference. The results from the current study indicate that this total protein haemolymph increase could be due to the increment of circulating hemocyanin induced by hypoxia as part of an ongoing physiological compensation process (Cheng et al., 2003).

The anoxic stress usually leads to haemolymph acid-base imbalances, namely acidosis and ion variations. Crustacean haemolymph lactate is a major end-product of anaerobic metabolism and several studies have shown its accumulation due to anoxic stress or emersion. For instance, this response was observed in emersed or air transported brown crab (Lorenzon et al., 2008), snow crab (James et al., 2019), spiny lobster (Speed et al., 2001) and Norwegian lobster (*Nephrops norvegicus*) (Spicer et al., 1990). In the current study, the animals transported showed a significant increase of lactate from close to 0 to over 6 mmol/L. Previously, Zagorsky et al. (2014) described an increase of red king crab haemolymph lactate from 0.5 to 1.0 mmol/L to 2.4–3.4 mmol/L during air transport. The elevation of lactate levels may suggest an increase in the anaerobic metabolism and presumably result from an inability to maintain an adequate supply of oxygen to tissues. Pre-cooling to ~2 °C before air exposure and thus reducing the metabolic stress was shown to lower lactate levels in southern king crab (*Lithodes santolla*) (Lorenzo et al., 2020). Nevertheless, the effect of air exposure seems to surpass the effect of lowering the temperature on lactate levels as shown by several studies (Lorenzon et al., 2008, 2007; Zagorsky et al., 2014). Moreover, lactate may have an adaptative role during air exposure by increasing hemocyanin oxygen affinity (Bridges, 2001). This may suggest that anoxic stress is likely the main factor during transport for the elevated lactate.

Haemolymph acid-base imbalances are common in crab transport and are usually represented by an increase in pH resulting from lactate and CO<sub>2</sub> accumulation due to the reduction in respiratory capacity. A sharp rise in haemolymph CO<sub>2</sub> could have been expected, nevertheless, in the current study this was not observed. This effect may have been masked by the variation on serum CO<sub>2</sub> levels after transport. An increase in CO<sub>2</sub> levels is accompanied by an elevation of haemolymph bicarbonate levels (Mota et al., 2020). An elevation of haemolymph bicarbonate was observed in several crustaceans as a response to hypoxia caused acidosis which was moderated by a Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange system (Cheng et al., 2003). The freshwater giant prawn (Cheng et al., 2003) and American lobster

(*Homarus americanus*) (Lorenzon et al., 2007) exposed to reduced dissolved oxygen, i.e. hypoxia, showed a reduction of haemolymph  $\text{Cl}^-$  concentration. In the present study, the haemolymph  $\text{Cl}^-$  ions (and  $\text{Na}^+$ ) had a significant but minor reduction (between 3–4 %), which may indicate an ongoing acid-base compensation which would be worth testing in the future by measuring more indicators, including serum bicarbonate. Moreover, this observation adds to the evidence that red king crab is an osmoregulator, as previously suggested in other studies (Ilyushchenko and Zenzerov, 2012; Stesko and Manushin, 2017). Moreover, Ilyushchenko and Zenzerov (2012) showed that crabs can survive in a salinity as low as 8 ppt, therefore showing the capability to actively exchange ions between the internal and external environment.

Haemolymph FAA changes during the transport of a red king crab or other crustacean species is reported for the first time in this study. Apart from a fundamental role in muscle and other tissue composition, amino acids have important physiological roles in crustaceans, especially in their free form, i.e. FAA. Maintaining haemolymph osmolality is one of these roles (Romano and Zeng, 2012). Hyperosmotic stress was shown to cause changes in haemolymph FAA pool in giant freshwater prawn, particularly by elevating the concentration of the non-essential amino acids Ala, Pro, Gly and the essential amino acids, Arg and lysine (Lys), after a 6-h transfer to seawater (Cheng et al., 2003). Similarly, results suggest that FAA, mainly Gly, Pro, and Ala, are involved in intracellular isosmotic regulation in the muscle of European lobster acclimatised to fresh water (Haond et al., 1999). In contrast, Australian freshwater crayfish acclimated to different salinities did not show differences in the FAA from muscle or haemolymph (Dooley et al., 2000). The latter authors suggested that the role of FAA on osmoregulation varies if the animal is an osmoconformer (weak or no regulatory capacity) or osmoregulator (active regulation of the internal fluids). In the current study, five FAA increased Ala, Arg, Thr, Val and Tau in response to the air transport. From these, FAA, Ala and Tau have been previously reported with an important osmoregulation role. Namely, Ala had the most significant difference ( $p$ -value < 0.001) and relative increase (+ 162 %). Free Ala has been previously proposed to be one of the most abundant and potent osmolytes for intracellular isosmotic regulation in crustaceans (Emiko and Hiroki, 1994; Okuma et al., 1995). Abe et al. (1999) also observed an increase of Ala in Japanese mitten crabs (*Eriocheir japonicus*) exposed to salinity stress. Whether the increase of Ala and the other amino acids in this study reveals a coping mechanism to transport stress is unknown and should be the focus of future studies. Alternatively, one may also consider that the increase of amino acid could be a result from haemoconcentration, i.e. the increase of haemolymph metabolites resulting from the loss of fluid in the tissues during an air exposure transport.

In conclusion, it was apparent that red king crab exposed to air during a 20-h transport period exhibited the typical physiological responses associated to hypoxia stress by increasing haemolymph lactate, total protein and decreasing  $\text{Cl}^-$ . Moreover, the current study reports for the first time a change in FAA pool due to transport. Five out of the twentyone measured FAA were significantly elevated. Specifically, Ala, an important osmolyte during salinity stress. These results offer new insights into how red king crabs cope with live air transport mimicking commercial conditions. They also support the need to assess the crabs' welfare and health during live holding and transport. Moreover, the results obtained from this study can also contribute to the development of an industry friendly methodology to assess the stress and health status of crabs during and after live transport.

## CRedit authorship contribution statement

**Vasco C. Mota:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft. **Sten I. Siikavuopio:** Conceptualization, Writing - review & editing. **Philip James:** Conceptualization, Visualization, Writing - review & editing.

## Declaration of Competing Interest

The authors report no declarations of interest.

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...The hemolymph of red king crabs is the largest tissue in these animals by volume and consists of water, inorganic salts, organic compounds, and hemocytes (Morado et al., 2014). In the Barents Sea, hemolymph studies are limited to hemocyanin screenings in relation to the meat content of red king crabs (Moiseeva and Moiseev, 2008), research of thyroid hormones in relation to size and sex (Dvoretzky et al., 2022c), assays of circulating ecdysteroids in relation to molting status (Dvoretzky and Dvoretzky, 2010), assessment of free amino acids in relation to stress (Mota et al., 2021), and studies of seasonal variations in levels of vertebrate-like sex hormones in relation to sex and size (Dvoretzky et al., 2021b). Also, there are no data regarding fatty acid profiles in the cardiac muscle of red king crab...

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## [Live holding of red king crab \(\*Paralithodes camtschaticus\*\) and snow crab \(\*Chionoecetes opilio\*\) – Effect on microbial growth in processed leg meat during refrigerated storage](#)

2022, Food Microbiology

### *Citation Excerpt :*

...The mobilization and breakdown of proteic compounds may lead to higher availability of nutrients, especially in the form of NPN, which can act as a readily available substrate for bacteria (Madigan et al., 2018). In this regard, it should be noted that, in RKC, the level of specific free amino acids, which

are a form of NPN, can increase in association with stress conditions as they play an essential physiological role in the adaptive response of crustaceans (Mota et al., 2021). Furthermore, the decreasing leg meat content with longer LH time and higher LH temperature may have indirectly influenced the thermal exposure of the meat during the cooking process at different LH sampling points, potentially with contrasting effects....

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The red king crab (RKC, *Paralithodes camtschaticus*) and snow crab (SC, *Chionoecetes opilio*) are valuable decapods that can undergo live holding (LH) in onshore facilities before either live export or processing into two cooked-frozen sections (i.e., clusters). This study investigated the effect of the LH time (up to two months without feeding) and temperature (5 | 10°C for RKC; 1 | 5°C for SC) on the total viable psychrotrophic count (TVC<sub>p</sub>) and *Pseudomonas* spp. in the leg meat of cooked RKC and SC. The effect of freezing after cooking was also evaluated. The counts were determined during storage at 4°C after cooking on the clusters undergoing either immediate refrigeration (IR) or 24-month frozen storage before refrigeration (FBR). In the RKC cooked leg meat, the LH temperature significantly affected the TVC<sub>p</sub>, with LH at 10°C leading to higher counts, while the *Pseudomonas* spp. levels were mainly influenced by the freezing, with lower levels in FBR samples compared to their IR counterparts. In the SC cooked leg meat, the LH conditions did not significantly affect the counts, which were instead significantly lowered by the freezing. From an industrial and commercial standpoint, it can be concluded that LH of RKC at high temperature (10°C) led to a shorter microbial shelf-life of cooked RKC clusters, with the clusters undergoing FBR showing longer microbial shelf-life than their IR counterparts. By contrast, with the mild cooking regime applied, the LH time and temperature of SC had a lower influence on the microbial shelf-life of cooked SC clusters than that given by the choice of the storage type (IR or FBR) after cooking.



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