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ARTICLE

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Salinity–temperature interaction drives metabolic and energetic changes in an Arctic crustacean

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Abstract

The Arctic is shifting towards a prevalence of warm and more saline Atlantic-like waters. These changes in the marine environment pose significant challenges for the ecophysiology of marine invertebrates. Here, we measured the metabolic enzyme activity of citrate synthase and lactate dehydrogenase, as well as the energy content and level of oxidative damage in 71 individuals (~10–14 individuals/station) of *Thysanoessa inermis* collected in six fjords in Svalbard that were characterized by different levels of influence of Atlantic water and, thus, temperature and salinity variability in the water column. *T. inermis* inhabiting fjords with strong influence of Atlantic water masses had lower lipid and protein content, and higher anaerobic metabolism compared to those from more Arctic fjord types, with Isfjorden driving mostly such difference. Moreover, *T. inermis* collected in fjords with high variability in both temperature and salinity had lower lipid content than that in stations with more stable temperature and salinity. Our results suggest that *T. inermis* in fjords influenced by Atlantic waters is possibly under stress leading to increased metabolism, consequently enhancing energy consumption. If the energy consumption is not compensated for, by an uptake, it could result in a decrease in the total biomass of *T. inermis* with possible consequences for the entire Arctic food web.

KEYWORDS
aerobic metabolism, anaerobic metabolism, Atlantification, lipids, oxidative damage, Svalbard, *Thysanoessa inermis*

INTRODUCTION

The current IPCC report (Calvin et al., 2023) indicates that global sea surface temperatures (SSTs) have increased significantly since the 1900s and that extreme weather events have become more frequent. Furthermore, sea levels have risen significantly, and global oceanic circulation patterns are changing. The consequences of climate warming are especially pronounced in the Arctic, with warming and sea ice loss being particularly pronounced in the eastern Fram Strait

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and the northern Barents Sea, where the inflow of warm saline Atlantic water ("Atlantification") has increased over the past decades (Dörr et al., 2021; Lind et al., 2018; Smedsrud et al., 2013).

Atlantification affects the biogeochemistry, ice cover, atmospheric climate, and marine animal ecology and ecophysiology (Lundesgaard et al., 2022). It also alters ecosystem structure, with some species increasing while others decline (Ramondenc et al., 2023). In the pelagic ecosystem, shifts of community structure at lower trophic levels can affect higher trophic level planktivores, such as fish, invertebrates, marine mammals, and seabirds (Huenerlage, Cascella, et al., 2016; Huenerlage, Graeve, & Buchholz, 2016; Kohlbach et al., 2023). In addition to Atlantification, glacier runoff impacts Arctic ecosystems by increased turbidity that affects light conditions in the water column, with repercussion on the vertical distribution of zooplankton (Szeligowska et al., 2021, 2022).

Sea ice loss and increased freshwater inflow will lower sea surface salinity and expose organisms that perform diel vertical migration to significant variability in both salinity and temperature during migration. This could have a significant influence on the physiology of organisms and especially their energy metabolism. The metabolism of ectothermic organisms increases exponentially with environmental temperature (Fry et al., 1947). Increased mitochondrial metabolism during rising environmental temperature leads to higher reactive oxygen species (ROS) production, which causes damage to biomolecules and tissues if an effective defense system against ROS is lacking (Banh et al., 2016). For example, the mitochondrial respiration and ROS production of the Antarctic polar bivalve *Laternula elliptica* increased significantly with temperature when exposing gills to temperature between 1 and 7°C (Heise et al., 2003). Similar changes in increased ROS production and damage to biomolecules during heat waves have been observed in other invertebrates (e.g., Abele et al., 2002; Paital & Chainy, 2014). Furthermore, if the temperature optimum of a species is exceeded, it can lead to a mismatch between the capacity of O_2 supply and demand. This limits the amount of O_2 available to maintain essential biological processes and can, for example, reduce growth and energy reserves of animals (Kaiser et al., 2022; Pörtner et al., 2017; Verberk et al., 2016).

Changes in salinity might have similar effects on the metabolism of organisms. Stenohaline species, in particular, need to increase their energy metabolism to keep homeostasis when environmental salinity changes significantly. This increased metabolism for osmoregulation might lead to both increased ROS production and reduction in energy reserves (Bal et al., 2021; Guerin & Stickle, 1997; Intanai et al., 2009; Whiteley et al., 2001). Shen et al. (2023) showed that salinity changes increased oxidative stress, and impaired osmoregulation, leading to disturbed energy metabolism in crab larvae (*Eriocheir sinensis*). While these studies are extremely important to assess the effect of temperature and salinity separately, there are still only a limited number of studies that addressed the combined effects of daily salinity and temperature changes on the energy metabolism and reserves of marine invertebrates, such as zooplankton. Combining two environmentally stressful conditions helps us to identify whether the interactive effects of two or more environmental stressors could be synergistic, and worsen the ones observed under single-stressor exposure. If the energy reserves of zooplankton decline and energy metabolism increases in response to a synergistic effect between increased temperature and decreasing salinities, it might have significant consequences on higher trophic levels as the food quality of the whole food web is at risk. The aim of our work was to evaluate how variation in salinity and temperature throughout the water column affects the energy content (in terms of lipid, protein, glycogen, and glucose), the aerobic (through citrate synthase activity, CS) and anaerobic (through lactate dehydrogenase activity, LDH) energy metabolism enzyme activities (as a proxy for metabolic activity), as well as cellular damage (measured using lipid peroxidation, LPX and protein carbonylation, Carb) of krill species *Thysanoessa inermis*. CS and LDH are enzymes involved in the aerobic and anaerobic metabolism of organisms, respectively. By measuring the activity of CS, we can evaluate whether the level of aerobic metabolism increases or decreases in response to thermal and salinity variation. However, if there is a stressful condition due to exacerbation of those environmental conditions, the organism could suppress aerobic metabolism in favor of the anaerobic metabolism, like glycolysis, where LDH activity is involved with. Cellular damage, like LPX and Carb, is a response of the cells to the negative effect of ROS produced by mitochondria respiration and that are not counteracted by antioxidant enzymes. If the production of ROS exceeds the antioxidant counteractive capacity, the biomolecules of the cell are depleted, resulting in membrane destruction and protein denaturation. Therefore, studying these damages would give a measure of stress of the cell in response to environmental stress that is not well counteracted. Finally, measurement of the energy content could provide information about the energy reserves that the organism is consuming in response to environmental variation. While glycogen and glucose measurement could provide information relative to the fast response to an environmental stressor, the lipid and protein content would give more insights into the quantity of energy

storage depletion in response to a prolonged stressful condition. Here, we compare these variables in *T. inermis* from six different fjords that vary in their influence by Atlantic water and, thus, have dissimilar variation in salinity and temperature throughout the water columns. The arcto-boreal euphausiid *T. inermis* (Buchholz et al., 2012) is one of the most abundant krill in Arctic and sub-Arctic marine ecosystems (Dalpadado et al., 2008; Dalpadado & Skjoldal, 1996). *T. inermis* is an important prey for many fish and marine mammals (Savenkoff et al., 2013). Based on the fatty acid composition, *T. inermis* is considered primarily herbivorous (Falk-Petersen et al., 2000) and it performs diel vertical migration from 250 m depth to surface for foraging (Sourisseau et al., 2008). Thus, it can experience significant variations in both salinity and temperature in strongly stratified fjords in Svalbard and less so in well-mixed locations. We hypothesized that (1) *T. inermis* from fjords having significant differences in salinity and temperature in the water column will show an increase in metabolism and (2) higher variability in temperature and salinity will exacerbate oxidative damages and (3) cause a reduction in energy reserves.

MATERIALS AND METHODS

Study area

Svalbard is located at the entrance to the Arctic Ocean at the transition between Atlantic and Arctic climate and biogeographic zones. The waters around Svalbard can be divided into a warm, well-mixed and ice-free Atlantic domain along the western coast, and a cold, stratified, and seasonally ice-covered Arctic domain in the north and east (Cottier et al., 2007; Loeng, 1991; Skogseth et al., 2020). Over recent decades, climate warming has resulted in an expansion of the Atlantic domain, exacerbating sea ice loss, ocean warming, and increased influx of Atlantic waters, and substantial changes have been documented across the Svalbard ecosystems involving both physical and biogeochemical processes (Asbjørnsen et al., 2020; Csapó et al., 2021; Lundesgaard et al., 2022). For our study, euphausiids were collected in six fjords of different hydrographic characteristics in the Svalbard archipelago during a cruise with R/V *Helmer Hanssen* in September 2022 (Figure 1, Table 1). Based on the geographic position, the water type influx prevalence and other physicochemical variables monitored through the years and during the current study (conductivity, temperature, and depth [CTD] data), the fjords were divided into three different group types: Atlantic, Arctic, and Mixed types.

Rijpfjorden is located on the northern side of Nord-Austlandet. While Atlantic waters prevail north of Svalbard as the Atlantic water boundary current turns eastwards and flows along the shelf break, the wide and shallow shelf limits the contact between Rijpfjorden and the core of the Atlantic water. Influx of Atlantic water into Rijpfjorden can occur mainly in autumn, but the fjord overall maintains a more Arctic signature, with water temperatures down to −1.8°C for most parts of the year (January–July) and an ice cover lasting 6–8 months (Hop et al., 2019). The oxygen level varied between 83 and 108 O_2sat% from bottom to 5 m depth.

Storfjorden is an open fjord situated between the east coast of Spitsbergen and the west coasts of Edgeøya and Barentsøya. Storfjorden is influenced by both Arctic waters from the eastern Barents Sea and Atlantic waters entering from the south (Skogseth et al., 2005). Sea ice is forming during winter and spring, and Storfjorden is an important area for formation of cold, saline deep water that transfers into the Barents Sea. The oxygen levels varied between 79 and 114 O_2sat%.

Wahlenbergfjorden is a seasonally ice-covered fjord located on the western side of Nord-Austlandet. The deepest basin (290 m) in the outer part of Wahlenbergfjorden is openly connected to Hinlopen, an up to 400-m-deep strait between Spitsbergen and Nord-Austlandet.

Hinlopen is a strait between Spitsbergen and Nord-Austlandet. The northern part of Hinlopen is affected by inflow of Atlantic water, while Arctic waters prevail in the south. Water of Atlantic origin flowing along the shelf edge north of Svalbard enters Hinlopen Strait through the Hinlopen Trough and can reach Wahlenbergfjorden. The oxygen saturations varied between 94 and 109 and between 96 and 102 O_2sat% in Wahlenbergfjorden and Hinlopen, respectively.

Kongsfjorden is a deep and open fjord located at the west coast of Svalbard that is strongly influenced by Atlantic water from the West Spitsbergen Current carrying organisms of a more boreal origin (Tverberg et al., 2019). While it can receive an inflow of Arctic water masses through the Coastal Current, it has remained largely ice-free since 2005. The oxygen saturation in this location varied between 80 and 104 O_2sat% from bottom until 5 m depth.

Isfjorden is the largest fjord on the west coast of Svalbard. While the inner fjords of the Isfjorden system are influenced by glacial and fluvial inputs, the central part of Isfjorden is strongly affected by inflowing Atlantic water and annual variation in the inflow of water masses has been observed. The central part of Isfjorden has largely been ice-free since 2006 (Søreide et al., 2022). The water oxygen saturation varied between 89 and 102 O_2sat%.

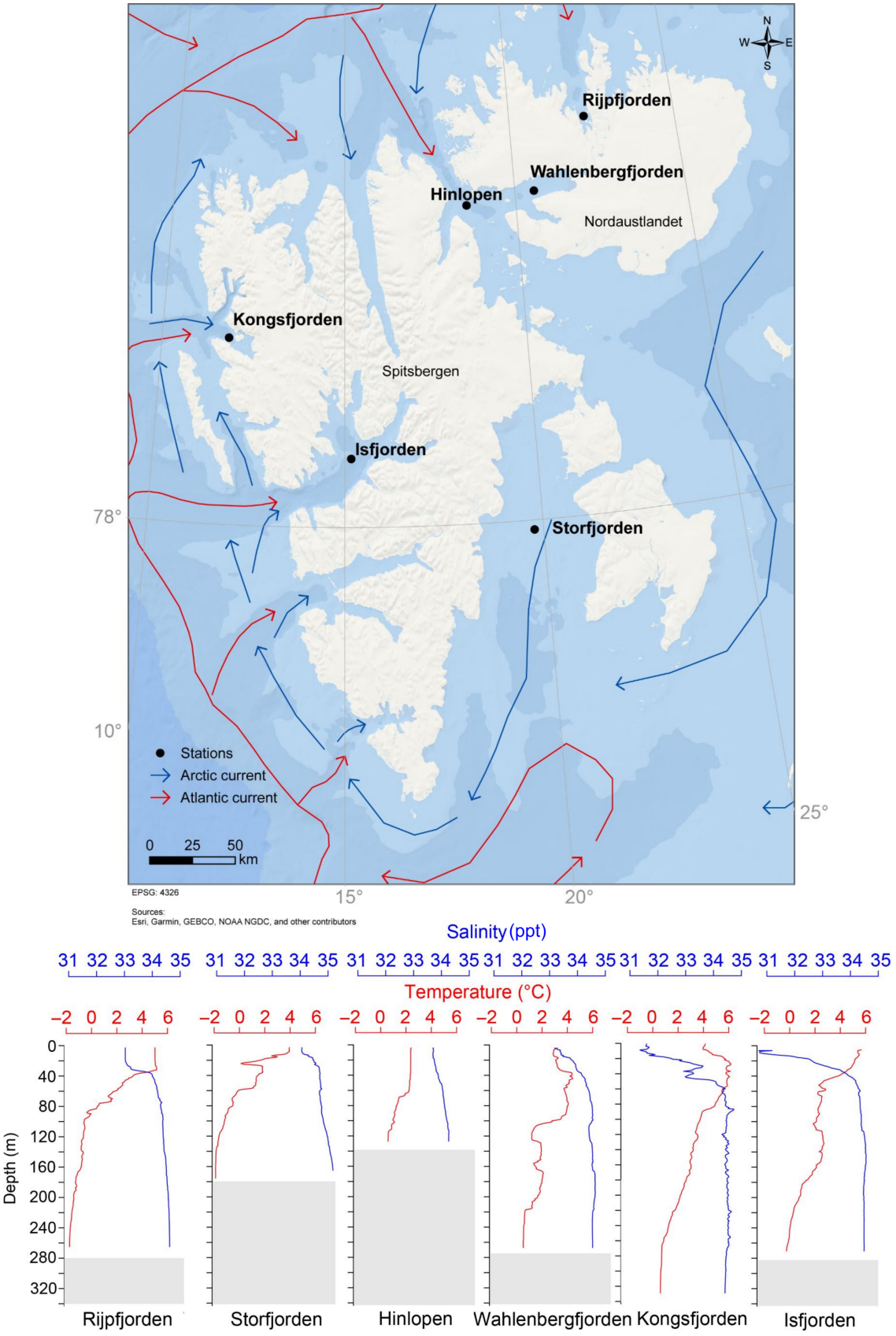

FIGURE 1 Map of the study area and vertical profiles of salinity and temperature at sampling locations in the six fjords in September 2022.

TABLE 1 Overview of fjords' sampling locations, and average ± SD and delta (=maximum − minimum value recorded) values of temperature and salinity of the whole water column until 5 m depth.

Fjord	Current	*N*	Date (d/m/y)	Lat (N)	Long (E)	Depth (m)	Temp (°C)	Delta temp (°C)	Salinity (ppt)	Delta salinity (ppt)	Chl (μg L^{-1})
Rijpfjorden	Arctic	12	4/9/22	80°08′	22°28′	260	0.27 ± 2.23	6.89	34.31 ± 0.47	1.60	0.347
Storfjorden	Arctic	11	7/9/22	77°95′	19°77′	190	−0.35 ± 1.70	5.83	34.80 ± 0.28	1.14	0.351
Hinlopen	Mixed	10	6/9/22	79°67′	18°56′	130	1.70 ± 0.70	1.79	34.03 ± 0.19	0.58	0.245
Wahlenbergfjorden	Mixed	13	5/9/22	79°72′	20°56′	270	2.20 ± 1.25	3.91	34.41 ± 0.30	1.40	2.942
Kongsfjorden	Atlantic	11	1/9/22	78°98′	11°83′	330	3.84 ± 1.80	5.58	34.63 ± 0.73	3.38	0.469
Isfjorden	Atlantic	14	10/9/22	78°36′	15°17′	270	2.37 ± 1.54	5.75	34.39 ± 1.03	3.82	0.691

Note: Chlorophyll *a* samples were collected at 5 m depth.

Abbreviations: Arctic, prevalence of Arctic water, low temperature; Atlantic, prevalence of Atlantic waters; Chl, Chlorophyll; Ind, individuals; Lat, latitude; Long, longitude; Mixed, mixed Arctic and Atlantic waters; Temp, temperature.

Sampling

At each station, measurements of temperature, salinity, and fluorescence were obtained by a ship-board CTD profiler (SBE911plus, SeaBird Electronics) with a SeaPoint fluorometer attached. The latitude and longitude of sampling locations are given in Table 1 as well as the average temperature and salinity of the sampling location from the bottom to 5 m depth. We have also calculated the variation (i.e., delta = maximum value − minimum value recorded) of both salinity and temperature from the bottom to 5 m depth to estimate how much variation in these variables euphausiids will encounter during their diel vertical migrations. These delta values were used in the statistical analyses.

To estimate chlorophyll *a* concentration at surface (5 m), seawater samples of 100 mL were filtered through 25 mm GF/F filters (Whatman) in triplicates, extracted in 100% methanol for 24 h at 4°C and measured fluorometrically with an AU10 Turner Fluorometer (Turner Design) according to the method by Parsons et al. (1984).

Euphausiids were sampled using a large Nansen closing net (Macrozooplankton net, Hydro-Bios, mouth opening of 2.01 m^2, 7.0-m-long net bag, 1.55-mm mesh) especially designed to ensure a sufficient volume of filtered water to catch large and fast-swimming krill and amphipods (Søreide et al., 2003). The net was deployed oblique from the surface to 20 m over the sea floor while the R/V *Helmer Hanssen* moved at 3.7 km h^{-1} (2 knots).

Upon retrieval, the cod-end was immediately emptied into a container filled with ambient sea water, and 10–14 live and actively swimming krill were collected with forceps and placed in petri dishes filled with sea water (see sample size per station; Table 1). The species was identified under a Leica M50 stereo microscope, and an image of each individual was taken when possible. The length of each individual was measured from the tip of the rostrum to the end of the telson at an accuracy of 1 mm. The individuals were dried with tissue paper and stored in 1.5-mL plastic Eppendorf tubes, and frozen in −80°C while live. Samples were stored at −80°C until molecular analyses.

Biomarker methods

The frozen krill were weighed to get the wet weight of each individual and then crushed to powder in a tissue lyser (Qiagen) (2 times for 1 min at 30 shakes per second). Thereafter, the frozen powder was divided into two subsamples, one for the energy content measurements (lipids, proteins, glycogen, and glucose) and one for the enzymatic (CS and LDH) and oxidative stress (LPX and Carb) measurements. For energy content measurements, samples were homogenized in 1:5 mg μL^{-1} 0.1 M citrate buffer (pH 5.0) using a Bullet Blender (Next Advance, speed 8, 5 min). The samples were aliquoted for protein, lipid, glucose, and glycogen measurements. The aliquots for glucose and glycogen were boiled for 2.5 min.

For the enzymatic activity and oxidative stress measurements, samples were homogenized in 1:5 mg μL^{-1} of 100 mM K-phosphate buffer containing 150 mM KCl (pH 7.4) using a Bullet Blender (speed 8, 5 min). Aliquots were taken first for LDH activity measurements that were further diluted with 50 mM Tris solution (pH 7.4). Second aliquots were taken for CS activity measurements. The remaining samples were further diluted to reach 1:10 mg of tissue μL^{-1} of buffer and a third aliquot was taken for LPX analyses. Thereafter, the leftovers of the samples were centrifuged at +4°C, 10,000*g* for 15 min and aliquoted for Carb and protein measurements. All the samples were flash-frozen in liquid nitrogen and stored at −80°C until further analyses.

The CS enzyme activity measurements were done according to Anttila et al. (2013), having the following substrate concentrations: DTNB 0.17 mM, oxaloacetate 1.9 mM, and acetyl CoA 0.14 mM. In the protocol, the background signal was determined by measuring the absorbance without the addition of oxaloacetate. The LDH enzyme activity was also measured according to Anttila et al. (2013) having the following substrate concentrations: NADH 0.25 mM and pyruvate-Na 25 mM. In the protocol, the background signal was determined by measuring the absorbance without the addition of pyruvate-Na. Both enzyme activities were done using the color formation and activity was measured for 3 min at room temperature at a wavelength of 412 nm for CS and 340 nm for LDH. After the measurements, the protein concentrations were determined for each sample using a PierceTM BCA Protein Assay kit (ThermoFisher), which were used to normalize the enzyme activity. The activities were calculated per mg of protein in samples. LPX was determined based on the protocol from Vuori and Kanerva (2018), using as reagent 2.5 mM ammonium iron (II) sulfate in 0.25 M sulfuric acid and 0.111 mM xylenol orange in methanol. After 2 h of incubation, the absorbance of the sample was measured at a wavelength of 570 nm and the LPX was calculated per mg of protein. Carb was conducted following the instructions of the Protein Carbonyl Content Assay Kit from Sigma-Aldrich (MAK094-1KT). The kit is based on the principle that carbonyl content is determined by the derivatization of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) leading to the formation of stable dinitrophenyl (DNP) hydrazone adducts. It was measured by spectrophotometer at 375 nm and the amount of carbonylation (in nanomoles) was expressed per amount of protein (in milligrams) in the samples (measured with BCA kit). The total lipid content was measured using the phospho-vanillin method (Frings et al., 1972) with vanillin reagent and phosphoric acid, and the absorbance was measured at 540 nm. Total protein content was measured using a protein dye binding method (Protein Assay kit, Sigma–Aldrich) and the absorbance was measured at 562 nm. Total glycogen and glucose contents were measured using the amyloglucosidase method (Carr & Neff, 1984) based on a glucose standard curve and as a reagent an O-toluidine mix with acetic acid, measured at 650 nm. The energy contents were calculated per gram of tissue.

Statistical analyses

All statistical analyses were conducted in RStudio using the R version 4.2.2 (RStudio Team, 2021). To see whether mass, length, energy content, energy metabolism and oxidative damage of krill differed among stations, we ran a one-way ANOVA test. Thereafter, we investigated in more depth whether the type of water mass (Arctic, Mixed, or Atlantic) shaped the biological responses. The fjords were divided into different types as indicated in Table 1. The definitions were based on the water temperatures and salinities during the sampling time; see *Results*. Thereafter, a one-way ANOVA test was run using water type as the categorical variable (Atlantic, Arctic, and Mixed). If there were significant differences among stations and water types, the pairwise contrasts were analyzed using Tukey's test.

A general linear model was used to analyze how *T. inermis* was influenced by variation in temperature (delta, i.e., difference between highest and lowest observed value within station) and variation in salinity that they encounter during diel vertical migration. The models were run for each biological response (lipid, protein, glycogen, and glucose content, as well as CS and LDH activity, and LPX and Carb amount) using environmental variables (delta salinity and delta temperature; see Table 1) as the fixed effect, against each biological response as the dependent variable. The models included the depth of the station, chlorophyll *a* concentration and the mass of individuals as cofactors. The depth was included in the model due to the high variability in the depth between the stations (123–326 m). Furthermore, since the mass of krill was found to be significantly different among stations and water masses, and because mass also could influence the energetics of krill, it was included in the model as covariate. Similarly, chlorophyll *a* concentration varied among the stations; thus, it was included in the model since food availability could influence the measured biological variables. We performed a model selection followed by a step-by-step approach based on the corrected Akaike information criterion (AIC_c), which was obtained by running the function *compare_performance* in package performance (Lüdecke et al., 2021). First, we tested which combination of the cofactors in the model (having delta temperature and salinity as fixed effects in interaction) had the lowest AIC_c values. The best model was kept for the next step where it was evaluated whether the interaction effect or the additive model (i.e., without interaction effect) of delta salinity and temperature resulted in a lower AIC_c value. The final model for each biological variable was determined by the lowest AIC_c value. Each model residual distribution was visually assessed using the function *simulateResiduals* in DHARMa R package (Hartig, 2018). The model selection for each biological variable is shown in Appendix S1. The final models are shown in Table 2. In all statistical tests, significance was considered at

TABLE 2 The final models for each biological variable, based on the lowest corrected Akaike information criterion (AIC_c).

Variable	Final model
Lipid content	Lipids ~ ΔTemperature × ΔSalinity + Chl *a* + mass
Protein content	Proteins ~ ΔTemperature × ΔSalinity + Chl *a*
Glycogen content	Glycogen ~ ΔTemperature + ΔSalinity + depth
Glucose content	Glucose ~ ΔTemperature + ΔSalinity + Chl *a*
Citrate synthase activity (CS)	CS ~ ΔTemperature + ΔSalinity + mass
Lactate dehydrogenase activity (LDH)	LDH ~ ΔTemperature + ΔSalinity + mass
Lipid peroxidation (LPX)	LPX ~ ΔTemperature + ΔSalinity + depth + mass
Protein carbonylation (Carb)	Carb ~ ΔTemperature + ΔSalinity + depth

Note: See Appendix S1 for more information about models. Chl *a* is the surface chlorophyll *a* concentration (5 m), depth is the bottom depth at the station. "×" sign stands for interaction, whereas "+" sign is for additional.

$p < 0.05$. The model outputs were plotted using the *ggplot2* package (Wickham, 2016).

RESULTS

Hydrography and fjord types

The water columns in Isfjorden and Kongsfjorden were stratified with a layer of fresh (salinity < 32.5 ppt), but with warm (>4.5°C) water in the upper 20–30 m (Figure 1). Following water mass definitions by Skogseth et al. (2020), the main water body was characterized by Atlantic water in Kongsfjorden (temperature > 3°C, salinity > 34.9 ppt; Skogseth et al., 2020) and Transformed Atlantic Water (temperature = 1–3°C, salinity = 34.7–34.9 ppt) in Isfjorden. Rijpfjorden was also characterized by a warmer and less saline surface layer in the upper 40 m (Figure 1). However, temperatures were <0°C below 80 m and reached −1.8°C at depth. In Storfjorden, salinity was relatively high already at the surface layer, but the temperature dropped similarly to that in Rijpfjorden and reached −2°C at depth. In Rijpfjorden, Arctic waters (temperature < 0°C, salinity = 34.3–34.8 ppt) prevailed, and in Storfjorden, water masses had the characteristics of winter-cooled water (temperature < −0.5°C, salinity > 34.4 ppt).

Hinlopen and Wahlenbergfjorden did not show a strong surface stratification, and the water column was well mixed (Figure 1). Temperatures varied between 0 and 2°C, and water masses can be defined as intermediate (temperature > 1°C, salinity = 34–34.7 ppt; Skogseth et al., 2020). Based on these observations, we defined Isfjorden and Kongsfjorden as "Atlantic" fjords, Rijpfjorden and Storfjorden as "Arctic" fjords, and Hinlopen and Wahlenbergfjorden as "mixed" fjord types. In the following analyses, we will use these groupings to compare the eco-physiological variables among these three fjord types.

Biomass and size

There was a significant difference in body mass of *T. inermis* collected for analyses in the different fjord types ($F_{2,68} = 6.6$, $p = 0.002$), with larger organisms collected in Arctic fjords, compared to the ones collected in mixed ($p = 0.01$) and Atlantic ($p = 0.004$) fjord types (Figure 2A). The same pattern was observed for collected individuals in length ($F_{2,68} = 7.2$, $p = 0.001$), where those individuals collected in Arctic fjord types were longer than the ones collected in mixed ($p = 0.02$) and Atlantic ($p = 0.001$) fjord types (Figure 2B).

Biomass and length of *T. inermis* differed significantly among the stations (mass: $F_{5,65} = 8.5$, $p < 0.001$; length: $F_{5,65} = 11.4$, $p < 0.001$) (Appendix S1: Figure S1), with *T. inermis* collected in Rijpfjorden being significantly larger than those from Isfjorden and Wahlenbergfjorden.

Energy content

Lipid content of *T. inermis* differed significantly based on fjord type ($F_{2,68} = 3.3$, $p = 0.04$), with Atlantic ones having lower lipid content than individuals collected in mixed fjord types ($p = 0.04$) (Figure 3A). There was no significant difference in either protein ($F_{2,68} = 2.5$, $p = 0.09$), glycogen ($F_{2,64} = 0.9$, $p = 0.38$) or glucose ($F_{2,65} = 1.7$, $p = 0.17$) content of *T. inermis* from the different fjord types even though numerically *T. inermis* from Atlantic fjord types had the lowest energy content values reaching almost statistical significance for protein (Figure 3B–D).

Delta salinity ($F_{5,65} = 8.2$, $p = 0.006$) and temperature ($F_{5,65} = 5.3$, $p = 0.02$) had a significant influence on the lipid content. However, there was also a significant interaction between temperature and salinity ($F_{5,65} = 8.5$, $p = 0.005$) (Figure 4A). The model showed that the lipid content was highest if *T. inermis* inhabited areas with high delta salinity and low delta temperature, whereas their lipid content reduced drastically when the delta temperature was high if the habitat also had high delta salinity (mainly Isfjorden station). However, according to the model, *T. inermis* from habitats with low

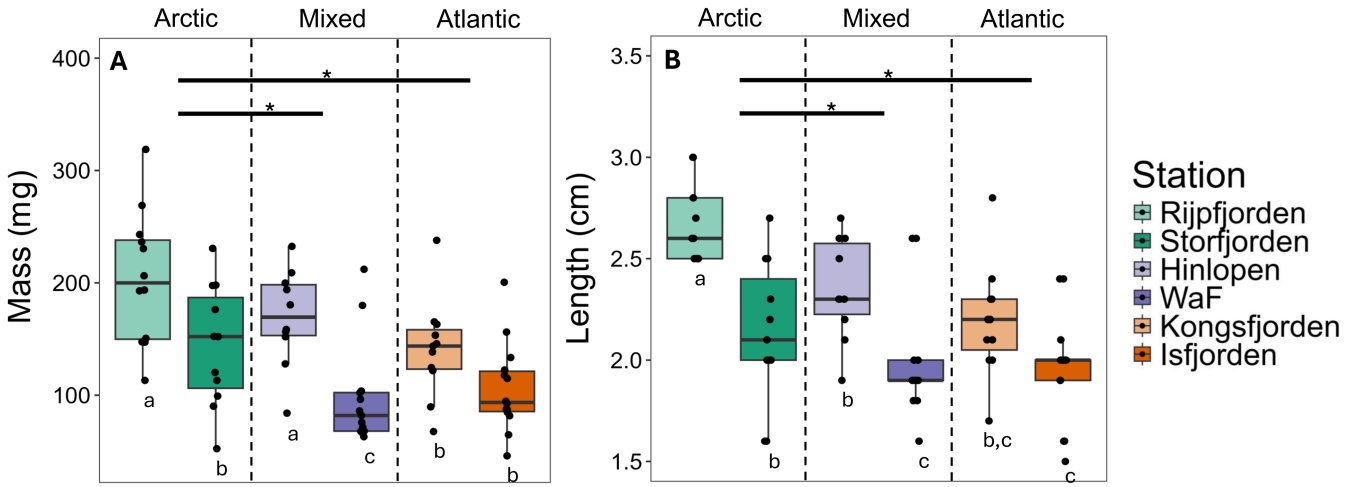

FIGURE 2 Boxplots showing the (A) mass and (B) length of *Thysanoessa inermis* individuals collected from different fjords ($N = 10$–14 individuals/station) and divided also by fjord types. Midline in each box represents the median while the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The upper and lower whiskers extend from the hinge to the largest and smallest values no further than 1.5 × IQR from the hinge (where IQR is the interquartile range, or distance between the first and third quartiles). Each dot corresponds to an individual. Asterisks identify statistically significant differences among fjord types. Lowercase letters identify statistically significant differences among stations. WaF, Wahlenbergfjorden.

delta salinity (mainly Hinlopen station) did not respond to the change of delta temperature. The *T. inermis* from habitats having medium delta salinity responded to the increase of delta temperature intermediately. The mass also had a significant effect on the lipid content ($F_{5,65} = 18.3$, $p < 0.001$).

The delta temperature ($F_{4,66} = 7.7$, $p = 0.007$) and delta salinity ($F_{4,66} = 6.1$, $p = 0.02$) in the water column affected the protein content of *T. inermis* similarly to the lipid content. There was also a significant interaction between temperature and salinity ($F_{4,66} = 6.9$, $p = 0.01$) (Figure 4B). The directions in the model were the same as with lipid content.

According to the model, the glycogen content of the *T. inermis* is low in locations with high delta temperature, but this effect was only marginally significant ($F_{3,64} = 3.9$, $p = 0.05$). The delta salinity did not influence glycogen content ($F_{3,64} = 1.7$, $p = 0.2$) (Figure 4C). In the model, the delta temperature ($F_{3,64} = 2.3$, $p = 0.1$) did not affect the glucose content of *T. inermis*. However, in *T. inermis* from habitats with high delta salinity, the glucose content was slightly reduced ($F_{3,64} = 4.1$, $p = 0.047$) (Figure 4D).

When the differences in energy content were assessed at the station level, we found that the lipid content was also different between some stations ($F_{5,65} = 3.2$, $p = 0.01$), with *T. inermis* from Hinlopen showing higher lipid content compared to those from Isfjorden ($p = 0.021$). The protein content was also found to be different among stations ($F_{5,65} = 3.0$, $p = 0.02$). Organisms from Isfjorden showed lower amounts of protein compared to Storfjorden ($p = 0.02$) and Wahlenbergfjorden ($p = 0.04$). Glucose and glycogen content were not significantly different between the stations. We also observed a high variability in energy content values between Storfjorden and Wahlenbergfjorden.

Energy metabolism

The activity of CS measured from each individual was not affected by the fjord types ($F_{2,66} = 1.2$, $p = 0.3$) (Figure 3E). On the other hand, LDH activity was highly influenced by fjord types ($F_{2,67} = 9.3$, $p < 0.001$) with individuals from Atlantic fjords showing higher activity than the ones found in Arctic fjords ($p < 0.001$) (Figure 3F).

Neither delta salinity ($F_{3,65} = 0.5$, $p = 0.5$) nor temperature ($F_{3,65} = 0.2$, $p = 0.6$) influenced the CS activity (Figure 4E), nor did the mass of the *T. inermis* ($F_{3,65} = 0.1$, $p = 0.7$). However, according to the model, LDH activity increased significantly with high delta salinity ($F_{3,66} = 16.2$, $p < 0.001$), while high delta temperature decreased LDH activity ($F_{3,66} = 5.24$, $p = 0.03$) (Figure 4F). The mass of the *T. inermis* did not affect the LDH activity ($F_{3,66} = 0.1$, $p = 0.8$).

CS activity was not significantly different between the stations ($F_{5,63} = 1.3$, $p = 0.3$), whereas LDH activity varied significantly among stations ($p = 0.004$). The activity of LDH was especially different between Isfjorden and Rijpfjorden ($p = 0.03$) and Isfjorden and Storfjorden, respectively ($p = 0.04$), with Isfjorden

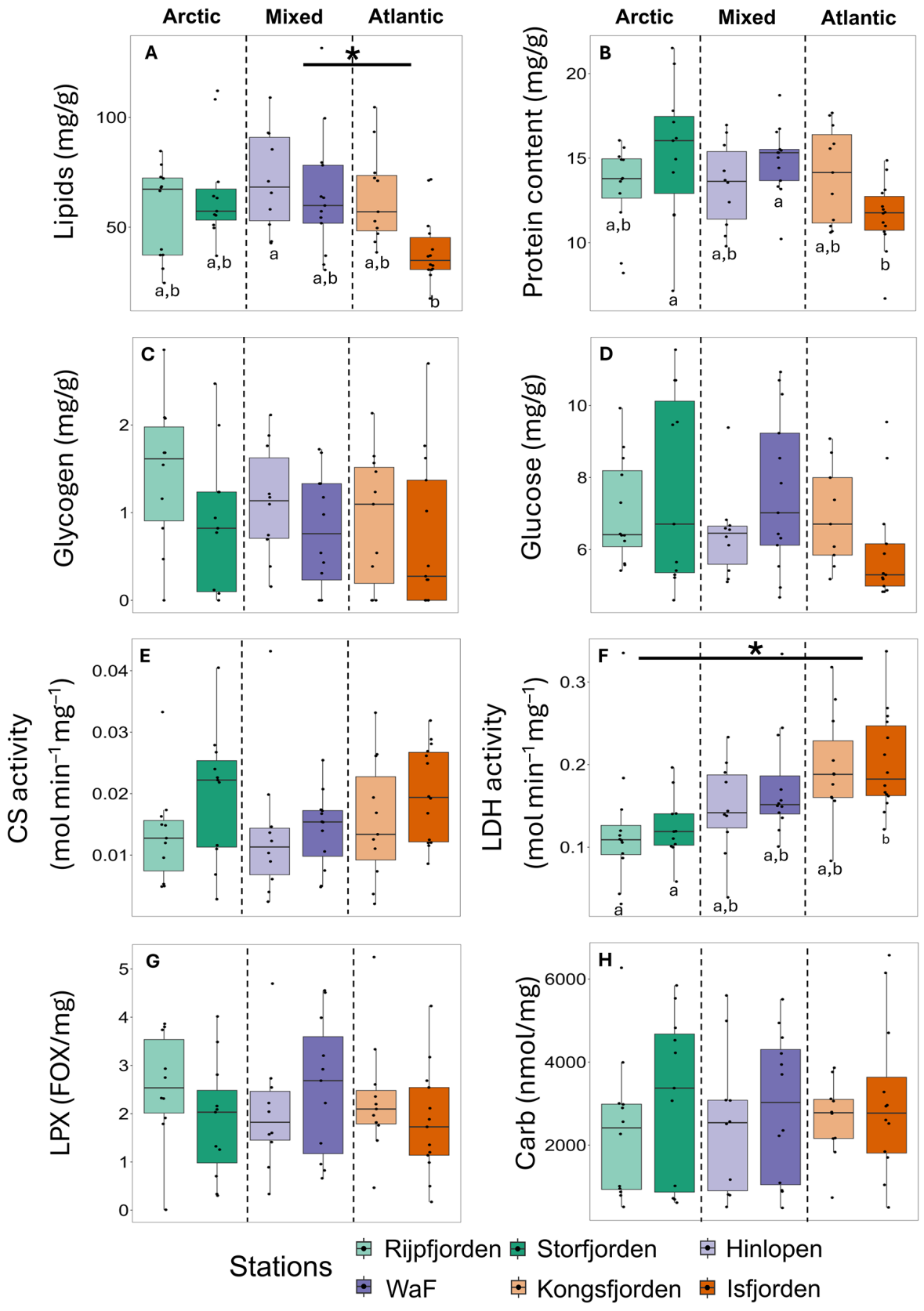

FIGURE 3 Legend on next page.

showing higher LDH activity than Rijpfjorden and Storfjorden.

Oxidative damage

The amount of oxidative damage, assessed as LPX and Carb, was not found to be different regardless of fjord type (LPX: $F_{2,63} = 0.2$, $p = 0.8$; Carb: $F_{2,64} = 0.05$, $p = 0.9$) (Figure 3G,H).

According to the model, the LPX was not influenced by the delta in salinity and temperature or their interaction in the water column ($F_{5,60} = 2.8$, $p = 0.09$; $F_{5,60} = 1.8$, $p = 0.2$; $F_{5,60} = 2.2$, $p = 0.1$; respectively) (Figure 4G). However, the mass of the *T. inermis* affected LPX ($F_{5,60} = 4.8$, $p = 0.03$) as did the depth of the sampling location ($F_{5,60} = 5.2$, $p = 0.02$). Similarly, Carb was not influenced by the delta in temperature ($F_{3,63} = 0.01$, $p = 0.9$) or salinity ($F_{3,63} = 0.4$, $p = 0.6$) (Figure 4H). The depth of the sampling location did not affect Carb either ($F_{3,63} = 0.3$, $p = 0.6$).

Likewise, LPX and Carb did not differ significantly between stations (LPX: $F_{5,60} = 0.7$, $p = 0.6$; Carb: $F_{5,61} = 0.4$, $p = 0.8$), but there was a high variation in these variables between individuals from Storfjorden and Wahlenbergfjorden.

DISCUSSION

Svalbard has experienced increased Atlantification over the last decades. This phenomenon will not only bring warmer and saltier waters into the Arctic regions but will advance melting of sea ice (Asbjørnsen et al., 2020; Lind et al., 2018; Polyakov et al., 2023; Stroeve & Notz, 2018). Moreover, warming in the Arctic regions will likely accelerate melting of inland ice (Tepes et al., 2021), which, in turn, will result in increased freshwater runoff from land, resulting in a diverse and varying distribution of water masses and significant variation in temperature and salinity within the water column. In this scenario, it becomes fundamental to assess the potential effects of temperature and salinity on zooplankton ecophysiology to improve our ability to predict changes in zooplankton abundance and distribution in a changing Arctic. In the present study, we assessed the energy content, as well as the metabolic performance and the oxidative damage of individuals of *T. inermis* inhabiting areas with different degrees of influence of Atlantic water, and, thus, temperature and salinity variability in the water column in the Svalbard Archipelago.

In accordance with our predictions, we found that a euphausiid species inhabiting more Atlantic fjord types had the lowest energy content and the highest anaerobic metabolism compared to those from other fjord types. Especially *T. inermis* from Isfjorden, belonging to Atlantic fjord type and having high variability in both salinity and temperature, had low energy content. Indeed, our models also showed that high variability in the temperature and salinity within the water column had a significant effect on the energy metabolism and content of *T. inermis*.

Energy content

According to our model predictions, it is seemingly costly and energy-consuming for *T. inermis* to inhabit areas of high variability in both salinity and temperature (Buchholz et al., 2010). In our models, although they were not taking into account the maturity stage and the sex of each individual even though counting for size, we found that the lowest lipid and protein contents were found when both delta salinity and delta temperature were high, suggesting a strong multiple-stressor effect. However, models predicted that both lipid and protein content are high in environments where delta salinity is high, but delta temperature is low. Plourde et al. (2014) reported that surface salinity was a strong predictor for daytime weighted mean depth (WMD) of *Thysanoessa raschii*, that is, the surface salinity, as such, explaining the main part of the variance in WMD distribution. Our result showing that energy content was the highest in *T. inermis* when exposed to high salinity variability indicates that the species probably benefits from habitats where the surface salinity is relatively low. This result is nicely supported by the finding in Plourde et al. (2014) showing that salinity influences the WMD of *T. raschii*,

FIGURE 3 Boxplot showing the (A) lipid, (B) protein, (C) glycogen, (D) glucose, (E) citrate synthase (CS), (F) lactate dehydrogenase (LDH), (G) lipid peroxidation (LPX), and (H) protein carbonylation (Carb) of *Thysanoessa inermis* collected from different fjords ($N = 10$–14 individuals/station) and divided also by fjord types. Midline in each box represents the median while the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The upper and lower whiskers extend from the hinge to the largest and smallest values no further than 1.5 × IQR from the hinge (where IQR is the interquartile range, or distance between the first and third quartiles). Each dot corresponds to an individual. Asterisks identify statistically significant differences among fjord types. Lowercase letters identify statistically significant differences among stations. WaF, Wahlenbergfjorden.

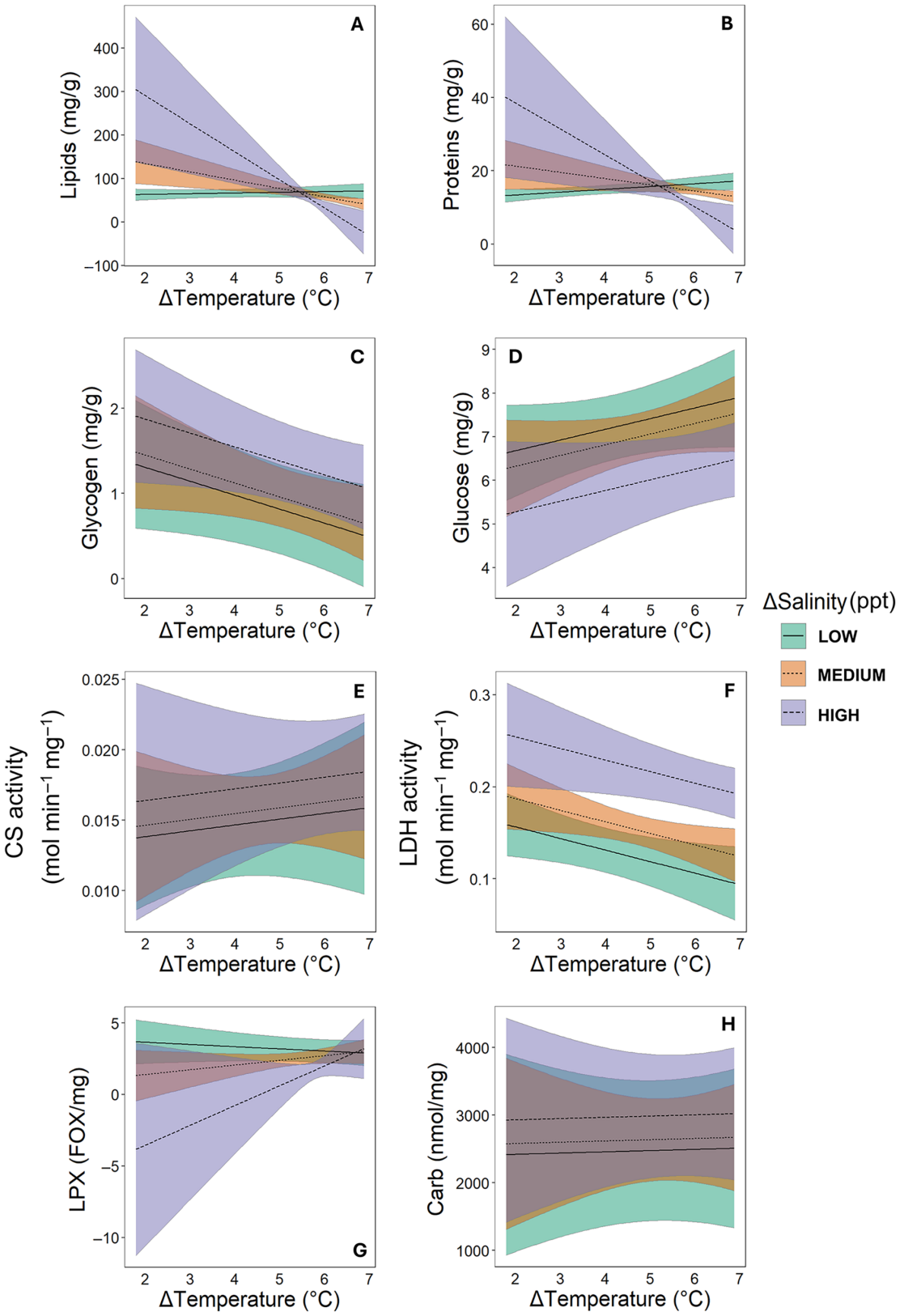

FIGURE 4 Regression plot showing the (A) lipid, (B) protein, (C) glycogen, (D) glucose, (E) citrate synthase (CS), (F) lactate dehydrogenase (LDH), (G) lipid peroxidation (LPX), and (H) protein carbonylation (Carb), of *Thysanoessa inermis* against the variation in temperature. The regression lines have been categorized by the variation in salinity (low, medium, and high variation). Shaded areas around each regression line represent CIs.

often observed in the cold and fresher intermediate layer, which also can have very low temperatures. Buchholz et al. (2010) found that *T. inermis* dominated in a more Arctic influenced fjord, suggesting that the species prefers cold conditions. In fjords influenced more by Atlantic waters, *T. inermis* was still abundant, but the proportion of more boreal species, such as *T. raschii* and *Meganyctiphanes norvegica*, has increased in abundance between the mid-1990s and the early 2000s, *M. norvegica* being the most dominant species in areas such as outer Kongsfjorden. Falk-Petersen et al. (2000) define *T. inermis* as the most northern krill species and as an "Arctic indicator," based on its lipid composition. Our observations that lipid and protein levels of *T. inermis* reduced when they were also exposed to high delta temperature show that while *T. inermis* can tolerate well high variability in salinity, the added variability in temperature goes beyond its capacity and the species prefers stable temperatures. Especially in Isfjorden, having strong Atlantic influence, and high variability in both salinity and temperature, the energy reserves were low, confirming the previous observations of the species preferring more Arctic types of waters.

According to Buchholz et al. (2010), krill in general are euryhaline and osmoconformers, meaning they, indeed, are tolerant to high variability in salinity. They inhabit areas of steep vertical salinity gradients, and they perform diel vertical migration in neritic areas, characterized by tides. Luo et al. (2023) showed that high fat concentrations protected marine euryhaline crustaceans (here: mud crab *Scylla paramamosain*) to cope with decreases in salinity and energy-demanding osmotic pressure. Indeed, in our study as well, *T. inermis* was predicted to have high lipid content when exposed to high variation in salinity, which means low surface salinity. Differences in glucose and glycogen content between fjord types were not significant, but glycogen levels were predicted to be lower in *T. inermis* from habitats with high delta temperature, while high delta salinity had a marginal reducing effect on glucose level. Salinity and temperature have also been shown to have mixed effects on glucose and glycogen contents of juvenile crayfish (*Cherax quadricarinatus*) (Prymaczok et al., 2016).

In our study, the lipid and protein contents were lower in *T. inermis* inhabiting fjords with strong Atlantic influence, particularly Isfjorden. However, our results do not take into account the variability given by each individual's maturity stage and sex. These two parameters are especially important when assessing energy storage in the wild. Life stage was partially controlled by using the size of each individual as a covariate in our models. Nevertheless, these results need to be taken carefully when interpreting the results. Nevertheless, there is a concern that if fjords are having more Atlantic influence in the future and especially if the seawater temperature rises, it might have negative physiological effect on this euphausiid species and its abundance. Reduced lipid and protein levels, when observed as a result of environmental stress or in relation to life stages, after exposure to high variability of water temperatures and salinity, if prolonged through time and without compensation from feeding, are worrying as they imply a decrease in energy content availability for predators at higher levels in the food chain, using krill as prey. Currently, *T. inermis* is an important prey species for a number of predators, such as polar cod, capelin, and other planktivores (Dalpadado et al., 2016; Orlova et al., 2015). Thus, a reduction in the physiological properties of *T. inermis*, such as energy content, might have a cascading effect on the whole food web. Nevertheless, Fossheim et al. (2015) showed that the fish communities of the Barents Sea were strongly responding to borealization, changing the community to a more Atlantic one. The same change is likely also occurring for the zooplankton community (Kaiser et al., 2022).

Thus, it is likely that current predators in Svalbard might need to switch prey species to more Atlantic ones.

Energy metabolism

Similarly to energy content, the energy metabolism of *T. inermis* was also influenced by the fjord type. Individuals from habitats, highly influenced by Atlantic water masses, especially Isfjorden, had significantly higher energy metabolism, especially the anaerobic LDH activity, than those from Arctic fjord types. Since metabolism increases with temperature in ectothermic animals (Fry et al., 1947), this is not unexpected. The anaerobic metabolism (LDH activity) was in general significantly higher than the aerobic CS activity, as previously observed in other euphausiids (Bellucci, 2004; Bucklin et al., 2002). The significant effect of temperature, especially on anaerobic metabolism, suggests that temperature is approaching a critical threshold for *T. inermis* since the anaerobic metabolism is activated in stress situations (Pörtner et al., 2017). Thus, high anaerobic LDH activity in Atlantic fjord types could mean that *T. inermis* either was under significant stress and/or the temperatures were above their optimum. Huenerlage, Cascella, et al. (2016) and Huenerlage, Graeve, and Buchholz (2016) showed that exposure to 6°C can indeed induce heat shock response in *T. inermis* and we observed that SST in the Atlantic fjord types ≥6°C (Figure 1). Our energy content results are also coherent with the metabolism findings, and in combination, suggest that *T. inermis* in the current study was under thermal stress in Atlantic fjords, especially in Isfjorden, where the LDH activity was

found to be significantly different from the Arctic fjords. High energy metabolism can lead to reduction of energy reserves, especially in stressful situations, and when animals cannot restore the energy stores as fast as metabolism uses them, energy reserves may become scarce.

The modeling results of the energy metabolism were also interesting, but somewhat unexpected. The delta salinity and temperature did not have a significant effect on aerobic CS activity, whereas both of these environmental factors influenced LDH activity. The high delta salinity increased the LDH activity in our study. Previously, it has been shown that changes in environmental salinity have a significant effect on LDH activity in a number of different marine invertebrates (*Neanthes arenaceodentata*, Cripps & Reish, 1973; *Crangon crangon* L., Menezes et al., 2006; *Scrobicularia plana*, Fossi et al., 2011; *Carcinus maenas*, Rodrigues et al., 2012; *Litopenaeus vannamei*, Jia et al., 2018; *Pinctada fucata*, Sun et al., 2021), as they need to keep osmotic homeostasis. Indeed, this could be the reason for the increased LDH activity in our study since the activity was highest in *T. inermis* exposed to high variability in salinity on a daily basis when performing diel vertical migration during foraging.

The way delta temperature was predicted in the model to affect the LDH activity was somewhat unexpected since animals from habitats having high delta temperature had the lowest LDH activity. However, we analyzed the effects of the delta and not average temperature in the model, and the high delta temperature implies that *T. inermis* from habitats of high delta temperature is spending part of the day in temperatures as low as −1.8°C (cf. Figure 1). Thus, the low LDH activity of krill from high delta temperature habitat could be due to a compensation of the extremely low temperatures they face on a daily basis. Indeed, *Thysanoessa* spp. can inhabit extremely low temperatures (Plourde et al., 2014), and generally, the metabolism of ectotherms is significantly reduced in low temperatures (Fry et al., 1947), explaining the low LDH activity in *T. inermis* from habitats with low temperatures.

Damages

Contrary to the result of energy content and metabolism, LPX and Carb were not influenced by the variation in temperature and salinity in our study. These observations seem to confirm the lack of response in aerobic CS activity, which was found to be stable, despite high variability in salinity and temperature within the stations. However, the preference toward anaerobic metabolism, as seen by the increase in LDH activity, cannot result in oxidative damage as LDH does not involve oxygen in its reactions and does not, thus, produce molecules that could cause oxidative damage. Our results contrast with Martínez et al. (2020), who recorded high oxidative damage in copepods under long-term salinity exposure. This was likely an effect of increased metabolic demands associated with a significant increase in salinity. Significant changes in salinity are linked to physiological responses potentially involving multiple processes at individual and molecular levels that can cause production of ROS. The variability in salinity across the water column in our study was not that high (only salinity 3.8, whereas in a study by Martínez et al., 2020, the difference in salinities between treatments was fluctuating between 10 and 20), causing the ROS production not to be high either. Moreover, in Martínez et al. (2020), a longer exposure to high salinity variation was used, whereas in our study, we only caught a picture of the salinity range that krill encounter during their diel vertical migration. Therefore, the difference from the previous study could be due to different times of exposures. We cannot, however, rule out that the absence of damage was due to a high antioxidant production. In many cases, organisms respond to stress by increasing their defense mechanisms. However, sometimes those defense mechanisms are not efficient enough to compensate for the stress leading to an increase in oxidative damages instead, for example, in Pacific whiteleg shrimp *Li. vannamei* (Parrilla-Taylor et al., 2013). If the stress is short and minor, it can be controlled by the antioxidant enzymes (Dorts et al., 2009; Wei & Yang, 2016). Furthermore, it is important to note that the individuals we measured in this study were the ones alive when the net came to the surface so it could be that they represented the ones having the best condition and therefore is better to handle the variation in the environment.

It needs to be, however, noted that our measurements were taken only in autumn 2022; thus, in order to draw more robust conclusions, the energy content and its changes should be followed for several years and investigated, for example, if the situation is worse in the years when temperatures are high, and also in relation to other factors, such as food availability fluctuations, and the predation pressure that both could affect the energy content of those organisms. Other environmental factors that we have not measured in this study, could also play a role; thus, this should be also addressed in follow-up studies.

CONCLUSIONS

The aim of this study was to improve our understanding of how variability in temperature and salinity as

encountered in different habitats in Svalbard can affect the energetics of *T. inermis*. Energy reserves, in particular proteins and lipids, of *T. inermis* were significantly predicted to be reduced by an increase in variation in temperature and salinity in the water column and by the degree to which the habitat was affected by Atlantic waters. *T. inermis*, especially from Isfjorden, having significant Atlantic influence and high variability in salinity and temperature, had low energy reserves. We also observed significantly higher anaerobic metabolism in *T. inermis* from habitats with a strong Atlantic influence who at the same time had a lower energy content.

These observations suggest that an increase in water temperatures as currently observed due to ocean warming may potentially be detrimental to *T. inermis* in habitats strongly affected by Atlantification. If these conditions persist over time, we could assist in population decline of those organisms if they fail to adapt to the new condition, or adaptation by selection towards more strong phenotypes. How this may affect the pelagic food web remains unclear. While *T. inermis* is an essential prey for higher trophic levels, other euphausiid species, as well as pelagic amphipods may thrive under warm conditions and become more prevalent in locations strongly affected by Atlantification (Buchholz et al., 2010, 2012; Dalpadado et al., 2016) and could potentially replace *T. inermis* as a food source. Long-term studies from Kongsfjorden have shown that pelagic predators are well adapted to deal with changes in the prey community caused by Atlantification (Vihtakari et al., 2018).

While we here demonstrate the effects of temperature on *T. inermis* energetics, it is also unclear how warming may affect other aspects, such as spawning success of *T. inermis* in the Arctic. Currently, *T. inermis* mainly spawns in the Norwegian Sea (Drobysheva, 1982; Orlova et al., 2015; Skjoldal et al., 2004); and is considered non-fertile in the Arctic due to low temperature constraints (Dalpadado et al., 2008; Timofeev, 2006). However, Buchholz et al. (2012) suggested that spawning of *T. inermis* would be possible in the Arctic systems under warmer conditions. Future studies should address the effects of temperature on development and reproduction. If spawning success changes with warming, a northwards extension in the spawning area may increase population size and counteract temperature-mediated changes in energy content.

AUTHOR CONTRIBUTIONS

Pauline Bourdin: Data curation; formal analysis; investigation; visualization; writing—original draft. **Giovanna Mottola:** Data curation; formal analysis; investigation; visualization; writing—original draft. **Ella von Weissenberg:** Investigation; writing—review and editing. **Malin Daase:** Investigation; project administration; resources; writing—review and editing. **Jonna Engström-Öst:** Conceptualization; funding acquisition; project administration; supervision; writing—original draft. **Katja Anttila:** Conceptualization; funding acquisition; project administration; resources; supervision; writing—original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Mottola et al., 2026) are available from Dryad: https://doi.org/10.5061/dryad.44j0zpcr4.

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