Copepod reproductive effort and oxidative status as responses to warming in the marine environment

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Abstract
The marine ecosystems are under severe climate change-induced stress globally. The Baltic Sea is especially vulnerable to ongoing changes, such as warming. The aim of this study was to measure eco-physiological responses of a key copepod species to elevated temperature in an experiment, and by collecting field samples in the western Gulf of Finland. The potential trade-off between reproductive output and oxidative balance in copepods during thermal stress was studied by incubating female Acartia sp. for reproduction rate and oxidative stress measurements in ambient and elevated temperatures. Our field observations show that the glutathione cycle had a clear response in increasing stress and possibly had an important role in preventing oxidative damage: Lipid peroxidation and ratio of reduced and oxidized glutathione were negatively correlated throughout the study. Moreover, glutathione-s-transferase activated in late July when the sea water temperature was exceptionally high and Acartia sp. experienced high oxidative stress. The combined effect of a heatwave, increased cyanobacteria, and decreased dinoflagellate abundance may have caused larger variability in reproductive output in the field. An increase of 7°C had a negative effect on egg production rate in the experiment. However, the effect on reproduction was relatively small, implying that Acartia sp. can tolerate warming at least within the temperature range of 9–16°C. However, our data from the experiment suggest a link between reproductive success and oxidative stress during warming, shown as a significant combined effect of temperature and catalase on egg production rate.

KEYWORDS
climate change, marine ecology, oxidative stress, trade-off, zooplankton

TAXONOMY CLASSIFICATION
Community ecology; Ecophysiology; Ecosystem ecology
Oxidative stress is a condition, which occurs when the production of reactive oxygen species (ROS) in cells exceed the antioxidant defense and repair capacity, causing serious damage to DNA, lipids, and proteins (Costantini, 2008; Hulbert et al., 2007). ROS production is tightly linked with aging and lifespan, as oxidative damage tends to accumulate in aging animals (Finkel & Holbrook, 2000). Furthermore, antioxidant defense and repair mechanisms have been considered to have a trade-off with reproduction effort: Reproduction may increase oxidative stress, and the stress levels tend to increase with higher effort in offspring quantity and quality (Metcalfe & Alonso-Alvarez, 2010). Aging has also been shown to decrease egg quality and production in invertebrates (Giron & Casas, 2003; Powers et al., 2020; Rodríguez-Graña et al., 2010). ROS production can increase due to external stress, such as temperature and salinity change, intense UV light, acidification, and toxins (Lesser, 2006; Lushchak, 2011). Elevated temperature in particular is a relevant environmental factor affecting oxidative status, as it stimulates metabolism and may enhance ROS production via increased oxygen consumption. Especially, ectotherms are influenced by temperature changes in the environment (Lushchak, 2011).

Copepods are an important link between primary producers and higher trophic levels. They experience strong environmental variability on daily, seasonal, and annual scale, as their diel vertical migration behavior exposes them to a large gradient of physiological conditions, such as temperature, salinity, and pH (Almén et al., 2014; Engström-Öst et al., 2019; Lewis et al., 2013). Thus, copepods have traditionally been considered fairly robust to environmental changes, but can be quite sensitive to thermal changes (Garzke et al., 2020; Vehmaa et al., 2013), whereas are less sensitive to pH and slight ocean acidification (Engström-Öst et al., 2019, 2020; Niehoff et al., 2013). UV light can also cause DNA damage and oxidative stress in zooplankton, especially in clear waterbodies (Tartarotti et al., 2014). In the Baltic Sea, harmful UV light is absorbed in the surface layer of the sea. Depending on the chlorophyll concentration, visible light, including UV, is almost completely absorbed between 0.7 and 3 m depth (Dera & Wozniak, 2010). UV light can therefore be an important factor causing eco-physiological changes in copepods, but potential damage is highest in the sea surface of the Baltic Sea.

Elevated temperature tends to favor smaller plankton over large ones, a phenomenon that has ecological consequences in the whole marine ecosystem: The whole community is shifting to smaller size starting from primary producers, and the mean body sizes are decreasing on species level (Daufresne et al., 2009). Elevated temperature causes northward shift of copepod species in the Northern Hemisphere (Beaugrand et al., 2002). Furthermore, associated increase in warmer water species and decrease in coldwater species were recorded already 20 years ago in the North Atlantic (Beaugrand & Reid, 2003). Mäkinen et al. (2017) analyzed long-term data from 1967 to 2013 in a coastal area in southwestern Finland and demonstrated a temperature- and salinity-related decline of large calanoid copepods and increase of smaller-sized brackish taxa. Adult copepods have also reduced in size and abundance due to warming (Garzke et al., 2016). Copepods are expected to grow faster but mature at smaller size in higher temperature due to temperature-size rule (Atkinson, 1994; Bergmann, 1847), which may have negative consequences on egg production. On the other hand, increase in temperature is shown to increase egg production rate and egg hatching success in Acartia copepods (Peck & Holste, 2006). Also an experimental study of Vehmaa et al. (2012) indicated that Acartia sp. females were able to match the phenotype of their eggs to the new environment.

The sea surface temperature (SST) in the Baltic Sea is predicted to increase by almost 2°C during the 21st century (Graham, 2004; HELCOM, 2021; Meier et al., 2012), and within 85 years, the SST has already increased by 1°C in the western Gulf of Finland (Merkouriadi & Leppärenta, 2014). In order to reveal temperature effects on oxidative stress, either antioxidant or oxidative stress biomarkers can be used as physiological measures of oxidative status. Glutathione (GSH) is an antioxidant that has an important role in preventing cell damage by ROS; GSH reduces peroxides during acute oxidative stress and oxidizes and the glutathione cycle reduces GSSG back into GSH. Thus, the ratio of reduced (GSH) and oxidized (GSSG) glutathione can be calculated and used as an indicator of oxidative stress (Lesser, 2006). Glutathione s-transferase (GST) contributes to catalyze reactions between GSH and peroxides, and may even help in protection against lipid peroxidation. Catalase (CAT) is an antioxidant that scavenges hydrogen peroxide (H₂O₂) and catalyzes its conversion to O₂ and water (Halliwell & Gutteridge, 2015). Oxygen radical absorbance capacity (ORAC) is used as a measure of antioxidant capacity (Prior et al., 2003). Oxidative stress can also be interpreted from oxidative damage on biomolecules, such as lipids, proteins, and DNA. Lipids are susceptible to damage caused by peroxides (such as H₂O₂), a condition called lipid peroxidation (LPX) (Halliwell & Gutteridge, 2015).

The biomarkers described above (LPX, CAT, GSH;GSSG, GST, and ORAC) are commonly used for studying oxidative stress in zooplankton (Cailletaud et al., 2007; Souza et al., 2010; Vehmaa et al., 2013). Copepods have an effective glutathione metabolism, which makes them more capable of dealing with excess ROS production (Glippa et al., 2018; Vuori et al., 2015). Nevertheless, previous studies have shown that oxidative stress has a negative effect on viable egg production in calanoid copepods (Garzke et al., 2016), whereas warming has a negative effect on oxidative status in copepods (Glippa et al., 2018; Kim et al., 2015; Won et al., 2015).

The aim of this work was to study the relationship between offspring production rate and oxidative stress response of a key copepod species Acartia sp., both in the field over the whole productive season, and in an experimental setup using different temperatures. We hypothesized that the production of ROS exceeds the antioxidant defense in higher temperatures, causing oxidative stress. Furthermore, we expected to find a trade-off between oxidative status and offspring production at elevated temperatures,
suggesting increasing costs of reproduction due to warming (Vehmaa et al., 2013).

2 | MATERIALS AND METHODS

2.1 | Field sampling

Water samples and zooplankton were collected bimonthly, in total six times between May and August 2018. Additional sampling was conducted three times in June for the experiment. Sampling took place in a pelagic area Storfjärden (59°52′56″N, 23°15′14″E), close to the Tvärminne Zoological Station in the southwestern Gulf of Finland (Figure 1). CTD (conductivity, temperature and depth) data were obtained from Tvärminne Zoological Station monitoring series. Oxygen and temperature were measured at every five m until 30 m depth during each sampling occasion, using YSI pro ODO oxygen sensor. One water sample was collected at 5, 10, and 15 m depth using a 5 L Limnos water sampler. From each depth, samples for pH were carefully collected in 250-ml glass bottles without airspace. Chlorophyll $a$ (Chl $a$) water samples were collected from the same depths. One 40 ml phytoplankton sample was obtained by mixing 5 L Limnos samples collected at 5, 10, and 15 m depth and by filtering the water through a 10 m plankton net.

In the laboratory, pH was measured using a WTW inoLab series pH meter. Chl $a$ samples were processed by filtering 100 ml of seawater using 25-mm glass fiber filters (Whatman GF/C). The filters were submerged in 10 ml of ethanol (96%) and stored at −20°C, and determined by fluorometry (Varian Cary Eclipse Fluorescence Spectrophotometer), using a 96-well microplate reader. Each 40 ml phytoplankton sample was treated with acid Lugol’s solution and stored at 3°C. The samples were analyzed semi-quantiatively by counting the individuals and using an Utermöhl sedimentation chamber. Corresponding phytoplankton groups were identified and their size measured using a 40x and 20x magnification with a microscope (Leica). Ten phytoplankton groups were monitored, of which diatoms, chlorophytes, chrysophytes, cyanobacteria, dinoflagellates, and prasinophytes were most common. As the phytoplankton sample was run through a 10 m net, microalgae <10 m are missing.

2.1.1 | Zooplankton

In order to collect copepods for the in situ egg production and oxidative stress analyses, three zooplankton samples were taken between 30 m depth and the surface using a 200 m plankton net with cod-end, and emptied into a cooler with seawater from 10 m (Engström-Öst et al., 2015). Zooplankton was kept in a climate chamber at ambient sea water temperature until sorting commenced. The animals were always used during day of sampling, usually within a few hours. During each sampling occasion, 50 adult female Acartia sp. copepods were sorted using glass Pasteur pipettes and incubated in 250-ml false bottom chambers ($N = 5, 10$ females/chamber, mesh size: 120 m) containing 1.2 m filtered seawater (FSW) at ambient temperature (approximately 10 m depth) in a climate controlled room. The main Acartia species occurring in the area is Acartia bifilosa, but A. tonsa is present especially in late summer (Almén et al., 2014; Engström-Öst et al., 2015; Katajisto & Viitasalo, 1998; Katajisto & Viitasalo, 1998). Acartia females were unfed to obtain egg numbers produced from past resources. In Finiguerra et al. (2013), total egg production and survivorship during starvation were uncorrelated in Acartia tonsa. After 24 h, females, eggs, and hatched nauplii were separated by sieving and counted, and the females were conserved with acid Lugol’s solution for body size analysis. Acid Lugol’s solution can affect body size to some extent (up to 17% in copepods) (Jaspers & Carstensen, 2009), which needs to be considered when comparing the body sizes in this study to those reported in studies using other conservation methods.

The number of eggs, nauplii, and live females was used for calculating in situ egg production rate (eggs female$^{-1}$ d$^{-1}$). Additionally, at each sampling occasion, 30 females ($N = 5$) were picked by forceps into 1.5-ml Eppendorf tubes, snap-frozen in liquid $N$, and stored at −80°C for biomarker analysis.

2.1.2 | Measurements

In the laboratory, pH was measured using a WTW inoLab series pH meter. Chl $a$ samples were processed by filtering 100 ml of seawater using 25-mm glass fiber filters (Whatman GF/C). The filters were submerged in 10 ml of ethanol (96%) and stored at −20°C, and determined by fluorometry (Varian Cary Eclipse Fluorescence Spectrophotometer), using a 96-well microplate reader. Each 40 ml phytoplankton sample was treated with acid Lugol’s solution and stored at 3°C. The samples were analyzed semi-quantitatively by counting the individuals and using an Utermöhl sedimentation chamber. Corresponding phytoplankton groups were identified and their size measured using a 40x and 20x magnification with a microscope (Leica). Ten phytoplankton groups were monitored, of which diatoms, chlorophytes, chrysophytes, cyanobacteria, dinoflagellates, and prasinophytes were most common. As the phytoplankton sample was run through a 10 m net, microalgae <10 m are missing.
2.2 | Experimental setup

In order to evaluate copepod reproductive output and oxidative stress, female copepod *Acartia* sp. were incubated for egg production, hatching, survival, and oxidative stress measurements in three temperatures: control temperature 9°C, and two elevated temperatures 13°C and 16°C (Figure 2). The 9°C treatment represents the seawater temperature at 10 m depth prior to the experiment. The two other temperatures represent a projected increase by 2100 according to IPCC RCP 8.5 (current emission trajectory) model prediction (IPCC, 2014). Copepods for the experiment were collected in similar manner as described in Section 2.1, and sampling was conducted on three occasions: 14, 16, and 21 June 2018. Female *Acartia* sp. were gently sorted with glass pipette and transferred to 2.2-L bottles (N = 3) containing 1.2 m FSW for each temperature treatment (ca. 50 ind. Bottle −1). A few males (5–6 ind.) were added to each bottle (Engström-Öst et al., 2015; Vehmaa et al., 2012, 2013). Nevertheless, there may be potential male bias in the data, as we were not able to check the male reproductive stage during sorting. FSW was produced by filtering seawater through a 10-m plankton net, and subsequently through GF/C glass fiber filters (47 mm, Whatman). The conditions in the field during the experiment were monitored as mentioned above (see Field sampling). The bottles were incubated in three different climate chambers, set at 9°C, 13°C, and 16°C. To keep the water temperature as stable as possible, the bottles were incubated in water baths. The copepods were fed once daily with a commercial high-quality solution consisting of *Isochrysis*, *Pavlova*, *Tetraselmis*, *Thalassiosira pseudonana*, and *T. weisflogii* (Shellfish diet 1800, Reed Mariculture) with a final concentration of 10,000 mmol L−1, which corresponds to a subsaturated food concentration for copepods (Klein Breteler and Gonzalez, 1982).

The experiment lasted 72 h in total: 24-h acclimation (Dutz & Christensen, 2018; Vehmaa et al., 2013) and 48-h experiment. The bottles were slowly stirred a few times per day. Dissolved oxygen (mg L−1) was measured daily, and copepod condition monitored. After the experiment, the bottle was emptied through a 200-m mesh, and copepod survival, number, and condition were checked under a stereo microscope (Leica). The water was rerun through a 48-m mesh to collect eggs that were transferred to a petri dish. The eggs were counted and set to hatch in the same temperature as they were produced. Thirty females in good condition were transferred to Eppendorf tubes for biomarker analysis (similarly as mentioned above). All remaining live females in each replicate were preserved in an Eppendorf tube with acid Lugol’s solution for body size measurements. Prosome length \( P_L \) was measured under a microscope Leica MZ12 attached to Nikon DS-L3 camera.

2.3 | Biomarker analyses

The *Acartia* samples (field and experiment) were analyzed for oxidative status biomarkers (Glippa et al., 2018; Vuori et al., 2015) in the Laboratory of Animal Physiology, University of Turku, Finland. The analyses were carried out according to protocols in Vuori and Kanerva (2018a, 2018b, 2018c, 2018d, 2018e). Concerning Oxygen Radical Antioxidant Capacity ORAC Activity Assay (Cell Biolabs), we used the assay kit protocol. Zooplankton samples were entirely homogenized in 100 L of 0.1 M K2HPO4 + 0.15 M KCl buffer (pH 7.4) using a Tissue Lyser II bead mill (Qiagen). An aliquot of raw homogenate (25 L) was immediately frozen in liquid \( N \) and stored at −80°C for lipid peroxide determination (LPX). Then, the sample homogenate was centrifuged at 10,000 g for 15 min at 4°C and the supernatant was divided into aliquots for glutathione-s-transferase (GST), catalase (CAT), and ORAC assay and for glutathione sample preparation. The glutathione sample was deproteinized by adding 5% sulfosalicylic acid (SSA) and subsequently incubated on ice for 10 min., and centrifuged for 10 min. at 10,000 g at 4°C. The supernatant was divided into two different tubes for reduced (GSH) and oxidized glutathione (GSSG), and 33 mM M2VP (1-methyl-2-vinylpyridinium trifluoromethanesulfonate, Sigma Chemicals) in 0.1 M HCl, a scavenger of GSH, was added to the GSSG sample. The sample homogenate...
2.4 | Statistical analyses

Statistical analyses were conducted using free software R, version 3.6.1, R Core Team (2019). Differences in the mean \( P_L \) between treatments of the experiment and the sampling days were tested using a Kruskal–Wallis rank sum test. A Spearman correlation test was used when testing any correlations. Linear mixed models were carried out by using the lmer function in the lmerTest package in R (Kuznetsova et al., 2017). The assumption of LMM, that is, the normality of model residuals, was assessed by use of the Shapiro–Wilk test. Log transformation was made for ORAC and in situ GST activity to gain better fit for the data and linear response. In all models, temperature treatment (experiment) or in situ temperature from 10 m depth (field) was used as a fixed effect and sampling date as a random effect. Additionally, temperature effects on biomarkers were tested; in these models, biomarkers were used (separately) as response variables. The Akaike Information Criterion (AIC) was used for model selection; models having the lowest AIC were selected. Interaction (x) between treatments and biomarkers was used only when the model had a smaller AIC value than the model without interaction. All biomarkers were also separately used as a response variable. One incubation bottle was excluded from the data as an outlier due to low egg production rates.

3 | RESULTS

3.1 | Environmental conditions

The thermocline at Storfjärden occurred below 5 m in June but descended to approximately 25 m in mid-July. The surface water temperatures were between 8 and 11°C in May–June, while the bottom temperatures remained below 5°C (Figure 3a). The temperature increased steeply between July 9 and 30; it remained between 22 and 25°C down to the thermocline, and the bottom temperature was as high as 12°C. The salinity within the water column varied between 5.1 and 7.2 throughout the season (Figure 3b). Temperature correlated negatively with salinity (−0.71, \( p < .01 \)) and oxygen concentration (−0.68, \( p < .01 \)), and positively with pH (0.74, \( p < .01 \)) at 10 m depth. The temperature and salinity data are missing for June 14 and 16.

In general, pH at 10 m depth was higher in May than in August (Figure 3c). However, the peak in pH (8.7) was reached on July 30, and the lowest measurement (7.5) was recorded August 13. The oxygen concentration at 10 m depth was highest in May (13.2 mg L\(^{-1}\)) and decreased toward the end of the sampling season, the minimum being 6.5 mg L\(^{-1}\) on August 6 (Figure 3d). Average dissolved oxygen was 10.1 ± 2.01 g L\(^{-1}\) over the season.

3.2 | Food conditions—Phytoplankton community structure (>10 m) and Chl \( a \)

The May–June phytoplankton community (>10 m) was dominated by chrysophytes, which decreased in abundance after mid-July.
(Figure 4a,b). Dinoflagellates were abundant from May to mid-June and were few in August. Phytoplankton community was rich in diatoms throughout the sampling period (20–25% of the total phytoplankton between May 22 and June 21), except for July, when they formed 2% of the total phytoplankton (in cell numbers). Cyanobacteria abundance was low in the beginning of the season (1–7%) until the bloom started in late June. The peak in abundance was in mid-August, when the proportion of cyanobacteria of the whole phytoplankton community reached 42%. Other taxa abundant throughout the summer were Chlorophyta and Prasinophyta. Overall food availability, measured as Chl a concentrations, peaked in mid-July (5.3 g L⁻¹), and the lowest concentration was detected 2 weeks earlier, on June 25 (1.5 g L⁻¹, Figure 4c). Chl a concentration correlated positively with both temperature at 10 m (0.64, df = 28, p < .01) and proportion of cyanobacteria (0.68, df = 55, p < .01) (Figure 5).

3.3 | Reproduction and female body size

The prosome length of adult females varied between 602 and 889 m during the experiment (Table 1). Mean P₂ did not differ between treatments or between sampling dates (Kruskal–Wallis rank sum test, p > .05). Also, offspring production rate did not correlate with P₂ of Acartia sp., indicating that neither egg production nor the biomarkers were affected by female body size. Egg production varied between 1.7 and 17.2 eggs female⁻¹ d⁻¹ in the experiment. As a comparison, the in situ egg production varied between 0.01 and 8.4 eggs female⁻¹ d⁻¹ (Figure 5). On average, egg production rate was nearly four times as high in the experiment than in situ. The egg hatching rate in the experiment varied between 0.1 and 87.7%, and the mean in control treatment was 43±13%, which is slightly less than in warmer temperature treatments: 48±14% in 13°C and 46±10% in 16°C (Figure 6). However, the difference between treatments was not significant. In situ egg production did not correlate with Chl a concentration (0.14, p > .05, df = 27) or temperature (−1.2, p > .05, df = 25).

Copepod female survival during in situ egg incubations was usually high. Occasionally, one individual was found dead throughout the sampling season, except for 30 June when mortality was slightly higher (1–3 individuals out of 10). During experiments, around 1–4 out of 50 were dead after the experiments.

3.4 | Oxidative stress in experimental setup

CAT activity ranged from 5.1 to 10.8 mol min⁻¹ mg⁻¹ during the experiment with an overall mean of 8.3 mol min⁻¹ mg⁻¹ (Figure 7). The range of ORAC readings observed during the experiment was 18–104 M trolox equivalents mg⁻¹. The smallest variability of ORAC between replicates was observed in the 16°C treatment, which also had the lowest mean within treatments, whereas the highest measurements were in the control treatment (9°C). GST activity ranged from 0.06 to 0.2 mol min⁻¹ mg⁻¹ during the experiment, the overall mean being 0.14 mol min⁻¹ mg⁻¹. GST varied little between treatments. GSH/GSSG ratio was in general much lower in the experiment than in the field, varying from only 1.7 to 2.8. LPX showed the lowest measures in the control treatment and the highest in 16°C (mean 56–76 M cumene hydroperoxide equivalents mg⁻¹ mg protein⁻¹).

3.5 | Effects of warming on reproduction and oxidative status

Warming had a negative effect on GST and ORAC in the experimental setup: GST activity differed between both treatments (13°C and 16°C) and the control (linear mixed models), whereas the change in ORAC was significant only in the 16°C treatment. Also, +7°C increase in temperature had a negative effect on offspring production rate (Table 2). The interaction of the CAT activity and the 13°C treatment had a significant effect on offspring production rate, whereas the treatment × GST activity interaction showed almost the same effect on both 13°C and 16°C treatments, shown as a statistical trend (Figure 8, p < .09).

3.6 | Oxidative status in the field

The activity of CAT varied between 2.6 and 10 mol min⁻¹ mg⁻¹, except for August 6, when the average activity peaked and the highest activities were recorded (14.3 mol min⁻¹ mg⁻¹, Figure 9). The average GST activity was the lowest in May and the highest on July 30, when GSH:GSSG ratio was also relatively high. In August, GST activity lowered considerably, while LPX was the highest and the GSH:GSSG ratio was the lowest. Overall, the GSH:GSSG ratio and LPX were negatively correlated during the field period (−0.7, p < .001). ORAC showed high variation between replicates; it varied between 0 and 104 M trolox equivalents mg⁻¹, whereas the average was never higher than 67. We found no ORAC activity in weeks 22 and 23 (below detection level). In contrast to the experiment, temperature in 10 m had a positive effect on in situ GST (Table 2).

4 | DISCUSSION

This study focused on the effects of warming on oxidative stress and reproduction of Acartia sp. in the Gulf of Finland. The work consisted of two parts: monitoring of in situ egg production and oxidative stress in May–August, and an experimental incubation, using three different temperature scenarios. The main finding in the experimental part was that temperature had negative effects on reproduction, GST and ORAC. In field monitoring, we saw that a strong heat wave in late July–August coincided with increasing oxidative stress.
4.1 Seasonality affecting reproduction in the field

The annual average seawater surface temperature (SST) at Storfjärden has increased by 1°C during 1927–2012 (Merkouriadi & Leppäranta, 2014). Previously, it has been reported that temperature influences the egg production rate (EPR) of Acartia sp. in the Baltic Sea (Diekmann et al., 2012; Koski & Kuosa, 1999; Peck & Holste, 2006; Vehmaa et al., 2012). In the current work, we did not detect direct temperature effects on reproduction in the field.

However, the in situ copepod EPR or eco-physiological responses such as oxidative stress and AOX could still have been indirectly affected by food quality as chlorophyll a correlated significantly with the seasonal cyanobacteria abundance (Figure 4a). Toxic cyanobacteria are known to either cause oxidative stress in many organs of various species or alter the antioxidant system (Martins et al., 2017). Copepod Acartia spp. can feed on toxic cyanobacteria Nodularia (Engström-Öst et al., 2015), and the toxin nodularin can cause increased antioxidant defenses (e.g., GST) in Gammarus (Turja et al., 2014).

The copepods were not provided food during the 24-h incubations, and this may have increased the variability between in situ and experimental EPR. On the other hand, comparison of EPR between field and laboratory was not the main aim of this paper. Tester and Turner (1990) have demonstrated that it takes 24 h for Acartia copepods to make eggs. Koski and Kuosa (1999), on the other hand, used 48 h as the length of experimental acclimation. In situ EPR was
low throughout the season, despite available dinoflagellates, which are a high-quality food source for *Acartia* sp. reproduction (Vehmaa et al., 2011). In July and August, accelerated warming, cyanobacteria blooms, and decreasing dinoflagellate abundance may have caused larger variability in *in situ* egg production between sampling occasions.

### 4.2 | Glutathione cycle responds to increasing stress in the field

How temperature will affect mechanisms in the cell is still not well known, especially concerning processes associated with redox chemistry during natural conditions (Reviewed by Birnie-Gauvin et al., 2017). Changes in oxidative status in adult *Acartia* sp. females were detected from several biomarkers in field-collected animals. GSH:GSSG ratio and LPX correlated negatively, which was expected as high LPX and low GSH:GSSG ratio, indicate oxidative stress (Lesser, 2006; Lushchak, 2011). Interestingly, our GSH:GSSG ratios

![Figure 5](image-url)

**Figure 5** *In situ* egg production rate during May–August 2018. Mean values (X) are shown above each boxplot. The boxplots show the median (vertical line), interquartile range (IQR, the box), and minimum and maximum within 1.5 × IQR (“whiskers”) and outliers (circle).

![Figure 6](image-url)

**Figure 6** Egg production rate (a) and hatching success (b) in the experiment. Mean values (X) are shown above each boxplot. The boxplots show the median (vertical line), interquartile range (IQR, the box), and minimum and maximum within 1.5 × IQR (“whiskers”) and outliers (circle).

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**Table 1** Body sizes (prosome length P₇) of adult *Acartia* sp. copepods after egg incubation in the experiment, and egg production rates (EPR) with standard deviations.
in the field were low compared to previous studies on *Acartia* sp.: 
Glippa et al. (2018) reported ratios reaching 14, which is nearly three 
times higher than the highest mean ratio in this study (5.4 in June 
25, see Figure 9). They also reported in general higher ORAC, more 
than ten times higher, considering that the animals were residing in 
approximately the same temperature conditions.

We found a positive response of increasing temperature on in situ GST, which is logic as the GST activity peaked in high temperatures (22–25°C from 0 to 20 m in July 30). GST is an enzyme that metabolizes organic hydroperoxides, which partially explains lower LPX during GST peak (Halliwell & Gutteridge, 2015). In fish, it has been shown that more acute exposure to warming caused higher antioxidant (AOX) levels, whereas when fish were exposed to more chronic type of temperature rise, AOX were close to baseline (Carney Almroth et al., 2015). Simultaneously with the peak in GST was a relatively high GSH:GSSG ratio in our data. Likely, this shows an effective response of glutathione cycle during temperature-driven stress. This is in accordance with a previous suggestion that glutathione metabolism is efficient in copepods (Sokolova, 2013; Vuori et al., 2015).

The temperatures were higher than the average temperatures recorded in the past 85 years during the same month (Merkouriadi & Leppäraanta, 2015). High temperature in the deeper parts of the water column is also highly relevant, since *Acartia* sp. are known to dwell close to the bottom during the day and near the surface at midnight in the study site (Almén et al., 2014). It has been observed that during moderate stress, AOX levels are overexpressed, and the redox balance can be maintained, thereby avoiding oxidative stress (Reviewed by Sokolova, 2013). It is possible that this phenomenon occurred in the beginning of the heatwave. Prolonged heatwave could have induced oxidative stress (Glippa et al., 2018; Kim et al., 2015; Vehmaa et al., 2013; Won et al., 2015), affecting *Acartia* sp. in the following
week, when LPX values tripled, GSH:GSSG ratio declined from 4.8 to 1.4, and GST level lowered, too. Simultaneous to the highest LPX, CAT activity peaked. The peak of CAT suggests accumulated H$_2$O$_2$ and thus oxidative stress; glutathione peroxidase typically activates in first line to remove smaller amounts of H$_2$O$_2$ (Costantini, 2014).

To conclude, proposed environmental stress caused by temperature increase, accompanying changes in oxygen, pH, and cyanobacteria, led to oxidative stress in Acartia copepods. Furthermore, our data show that the glutathione cycle of Acartia sp. (including GSH and GST) responds strongly to increasing stress. This is in accordance with 

| Response variable | Fixed effects          | Estimate ± SE | df  | t-value | p-value |
|-------------------|------------------------|---------------|-----|---------|---------|
| EPR               | (Intercept)            | 11.8 ± 1.61   | 2   | 7.33    | .02*    |
|                   | (Intercept)            | 12.7 ± 1.41   | 2.93| 9.01    | .00**   |
| 13°C              | 0.91 ± 1.08            | 21.01         | 0.85| .41     |
| 16°C              | −2.51 ± 1.04           | 20.99         | −2.41| .03*    |
|                   | (Intercept)            | 12.43 ± 2.04  | 7.46| 6.09    | .00***  |
| LPX               | 0.01 ± 0.02            | 16.06         | 0.45| .66     |
| 13°C              | 0.43 ± 1.2             | 15.99         | 0.36| .73     |
| 16°C              | −3.18 ± 1.29           | 16            | −2.45| .03*    |
|                   | (Intercept)            | 20.8 ± 5.62   | 18.46| 3.7     | .00**   |
| CAT               | −0.95 ± 0.67           | 17.22         | −1.42| .17     |
| 13°C              | −20.16 ± 8.94          | 17.28         | −2.25| .04*    |
| 16°C              | −10.58 ± 6.47          | 17.14         | −1.64| .12     |
| CAT × 13°C        | 2.43 ± 1.04            | 17.31         | 2.33| .03*    |
| CAT × 16°C        | 0.95 ± 0.79            | 17.17         | 1.19| .25     |
|                   | (Intercept)            | 13.71 ± 1.84  | 6.38| 7.46    | 0***    |
| ORAC              | −0.02 ± 0.02           | 20.2          | −0.92| .37     |
| 13°C              | 0.27 ± 1.28            | 20.11         | 0.21| .84     |
| 16°C              | −3.09 ± 1.22           | 20.04         | −2.54| .02*    |
|                   | (Intercept)            | 4.13 ± 8.82   | 20.62| 0.47    | .64     |
| GST               | 53.08 ± 53.33          | 19.5          | 1.19| .33     |
| 13°C              | 16.06 ± 9.62           | 19.49         | 1.67| .11     |
| 16°C              | 3.82 ± 9.64            | 19.59         | 0.4 | .7      |
| GST × 13°C        | −110.16 ± 61.29        | 19.49         | −1.8 | .09    |
| GST × 16°C        | −35.82 ± 61.96         | 19.6          | −0.58| .57     |
|                   | (Intercept)            | 11.93 ± 3.38  | 19  | 3.53    | 0**     |
| GSH:GSSG          | 0.42 ± 1.73            | 17.79         | 0.24| .81     |
| 13°C              | −4.92 ± 5.84           | 17.14         | −0.84| .41     |
| 16°C              | −4.32 ± 5.56           | 18.38         | −0.78| .45     |
| GSH:GSSG × 13°C   | 3.81 ± 3.5             | 17.19         | 1.09| .29     |
| GSH:GSSG × 16°C   | 1.39 ± 3.1             | 18.49         | 0.45| .66     |
| ORAC              | (Intercept)            | 3.83 ± 0.18   | 22  | 20.78   | >.01*   |
| 13°C              | −0.36 ± 0.28           | 22            | −1.30| .2      |
| 16°C              | −0.60 ± 0.26           | 22            | −2.31| .03*    |
| GST               | (Intercept)            | 0.16 ± 0.01   | 23  | 17.87   | 0***    |
| 13°C              | −0.03 ± 0.01           | 23            | −2.62| .02*    |
| 16°C              | −0.03 ± 0.01           | 23            | −2.5 | .02*    |
| in situ GST       | (Intercept)            | −3.19 ± 0.4   | 5.02| −7.87   | .00***  |
| in situ temperature| 0.10 ± 0.03            | 5.05          | 3.38| .02*    |

Note: Egg production rate (EPR) or biomarkers were used as response variables, while treatments (13 or 16°C), or in situ temperature from 10 m depth, were used as fixed effects. Interaction between fixed effects marked with ×. Sampling date was used as a random effect in all models. The p-values: ***<.001, **<.01, *<.05, <.1.
FIGURE 8 The interaction of temperature and (a) GST activity (b) CAT activity on offspring production rate in the experiment. The p-values: *<.05, <.1

with a previous suggestion that glutathione metabolism is efficient in copepods (Vuori et al., 2015).

4.3 | Negative temperature effect on reproductive rate, ORAC, and GST in the experiment

We found a significant negative effect of 16°C treatment on egg production rate. Vehmaa et al. (2013) found that a 3°C temperature increase (from 17 to 20°C) had a negative effect on egg viability and hatching rate, but not on egg production. Despite negative effects on reproduction, increased temperature has positively affected the abundance of *Acartia* sp., observed from a long-term monitoring data in a southwest coast of Finland in 1967–2013 (Mäkinen et al., 2017).

Our work shows that a temperature increase of 4–7°C is tolerable to *Acartia* sp., proving its robustness and that it does not cease to reproduce in higher temperatures. The optimal temperature for *A. bifilosa* in light of reproduction is approximately 13–18°C (Koski & Kuosa, 1999), implying that our study was conducted within the tolerance range of *A. bifilosa*. A small decrease (~2.5 eggs female\(^{-1} \text{d}^{-1}\)) in modeled reproduction rate in +7°C temperature treatment is important to note, but the fact that it is a relatively small decrease in EPR proves that *Acartia* may be a good survivor in the warmer future seas. The effect of temperature on oxidative stress was detected in GST, which was negatively affected by 13°C and 16°C treatments. Another biomarker for antioxidant defense, ORAC, had a negative response in 16°C treatment. This is partly in contrast with results by Vehmaa et al. (2013) whose experiment showed that temperature increase of 3°C had a positive effect on ORAC and oxidative damage in *Acartia* sp. It is possible that the generally high oxidative stress levels observed in all treatments (including the control) may have hindered the differences between treatments. Unknown factors in the experiment, in addition to elevated temperatures, may have induced stress, too. However, we may exclude shortage of food and oxygen depletion from these factors because copepods were well fed during the incubation, and a normoxic level of dissolved oxygen was recorded throughout the experiment. Normoxia is here >6 g L\(^{-1}\), according to Diaz (2016) in freshwater environments. The mortality during the experiment was relatively low (max. 8%), but there are several possible reasons for mortality, such as during the incubation ending process, where they were sieved and separated from the eggs, or pipetting.

Egg production rate was significantly affected by the interaction of the 13°C treatment and CAT activity, and also, the similar result for GST was almost significant. Interestingly, at 9°C both CAT and GST showed (nonsignificant) negative trends with egg production rate, while in 13°C (significant) and in 16°C (nonsignificant), the trends were positive. This suggests that temperature has affected the relationship between reproduction and antioxidants, and that there is a possible trade-off between reproduction and oxidative stress in higher temperatures. A trade-off between these two traits has been emphasized multiple times in the literature in copepods (Garzke et al., 2016; Rodríguez-Graña et al., 2010; Vehmaa et al., 2013), and also widely in the animal kingdom (Metcalfe & Alonso-Alvarez, 2010). However, it has to be kept in mind that animals have mechanisms in coping with stress: Repeated (but not continuous) stress may protect animals from further damage by ROS due to hormetic effects (Hood et al., 2018). Furthermore, female copepods have been suggested to be able to transfer part of the accumulated oxidative damage into offspring (Rodríguez-Graña et al., 2010).

5 | CONCLUSIONS

We showed that *Acartia* sp. females can tolerate an increase of 4–7°C to ambient temperature (9°C) and were able to reproduce in the experimental conditions with only a small decrease of egg production rate observed in the warmest treatment. Biomarkers of oxidative stress and antioxidant defense showed clear progression during the productive season in summer 2018 when oxidative
stress increased in August, possibly due to seasonal effects, such as cyanobacteria blooms, and temperatures that increased above the optimum. Glutathione cycle had a clear response to increasing stress and possibly had an important role in preventing oxidative damage: lipid peroxidation and ratio of reduced and oxidized glutathione were negatively related throughout field season and in the experiment. The role of glutathione-s-transferase in antioxidant defense was shown as increased activity in the field when stress was introduced, and catalase activity peaked when the stress level was at its highest. In addition to temperature, food quality at the sampling site Storfjärden was probably an important factor affecting in situ egg production rate at least in May–June, when the water column was rich in dinoflagellates. Possibly, the combined effect of increased temperatures, high abundance of cyanobacteria, and low abundance of dinoflagellates caused higher variability in in situ egg production rate in July–August. Our data suggest a possible trade-off between antioxidant defense and reproduction.

Finally, the interaction of temperature and salinity on reproduction still needs further studies. *Acartia* sp. are both eurythermal and euryhaline, but their tolerance to osmotic stress depends on temperature (Diekmann et al., 2012). The freshwater input to the Baltic Sea is increasing in future (HELCOM, 2021; Meier et al., 2012), and the typical salinity in our study area is often below the optimum salinity range 7–16 of *Acartia* sp. (Dutz & Christensen, 2018). Salinity is an important abiotic factor for brackish-water copepods, and even small salinity changes may have surprising effects on oxidative status in copepods (Cailleaud et al., 2007; Martínez et al., 2020).
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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT
Data used in this study were deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.3bk3j9km3.

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REFERENCES
Almén, A. K., Vehmaa, A., Brutemark, A., & Engström-Öst, J. (2014). Coping with climate change? Copepods experience drastic variations in their physicochemical environment on a diurnal basis. Journal of Experimental Marine Biology and Ecology, 460, 120–128. https://doi.org/10.1016/j.jembe.2014.07.001
Atkinson, D. (1994). Temperature and organism size: A biological law for ectotherms? Advances in Ecological Research, 25, 1–58.
Beaugrand, G., & Reid, P. C. (2003). Long-term changes in phytoplankton, zooplankton and salmon related to climate. Global Change Biology, 9(6), 801–817. https://doi.org/10.1046/j.1365-2486.2003.00632.x
Beaugrand, G., Reid, P. C., Frédéric Ilbaze, J., Lindley, A., & Edwards, M. (2002). Reorganization of North Atlantic marine copepod biodiversity and climate. Science, 296(5573), 1692–1694. https://doi.org/10.1126/science.1071329
Bergmann, C. (1847). About the relationships between heat conservation and body size of animals. Goett Stud, 1, 595–708.
Birnie-Gauvin, K., Costantini, D., Cooke, S. J., & Willmore, W. G. (2017). A comparative and evolutionary approach to oxidative stress in fish: A review. Fish and Fisheries, 18(5), 928–942. https://doi.org/10.1111/faf.12215
Cailleau, K., Maillet, G., Budzinski, H., Souissi, S., & Forget-Leray, C. (2007). Effects of salinity and temperature on the expression of enzymatic biomarkers in Eurytemora affinis (Calanoida, Copepoda). Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 147(4), 841–849. https://doi.org/10.1016/j.cbpa.2006.09.012
Carney Almroth, B., Asker, N., Wassmurf, B., Rosengren, M., Jutfelt, F., Gråns, A., Sundell, K., Axelsson, M., & Sturve, J. (2015). Warmer water temperature results in oxidative damage in an Antarctic fish, the bald notothen. Journal of Experimental Marine Biology and Ecology, 468, 130–137. https://doi.org/10.1016/j.jembe.2015.02.018
Costantini, D. (2008). Oxidative stress in ecology and evolution: Lessons from avian studies. Ecology Letters, 11(11), 1238–1251. https://doi.org/10.1111/j.1461-0248.2008.01246.x
Costantini, D. (2014). Oxidative stress and hormesis in evolutionary ecology and physiology. A marriage between mechanistic and evolutionary approaches (p. 362).
Daufresne, M., Lengfellner, K., & Sommer, U. (2009). Globalwarming benefits the small in aquatic ecosystems. Proceedings of the National Academy of Sciences, 106(31), 12788–12793. https://doi.org/10.1073/pnas.0902080106
Dera, J., & Wóźniak, B. (2010). Solar radiation in the Baltic Sea. Oceanologia, 52(4), 533–582. https://doi.org/10.5697/oc.52-4.533
Diaz, R. J. (2016). Anoxia, Hypoxia, And Dead Zones BT - Encyclopedia of Estuaries (pp. 19–29). Springer. https://doi.org/10.1007/978-94-017-8801-4_82
Diekmann, A. B. S., Clemmesen, C., Michael, A. S., John, M. P., & Peck, M. A. (2012). Environmental cues and constraints affecting the seasonality of dominant calanoid copepods in brackish, coastal waters: A case study of Acartia, Temora and Eurytemora species in the southwest Baltic. Marine Biology, 159(11), 2399–2414. https://doi.org/10.1007/s00227-012-1955-0
Dutz, J., & Christensen, A. M. (2018). Broad plasticity in the salinity tolerance of a marine copepod species, Acartia longiremis, in the Baltic Sea. Journal of Plankton Research, 40(3), 342–355. https://doi.org/10.1093/plankt/fby013
Engström-Öst, J., Brutemark, A., Vehmaa, A., Motwani, N. H., & Katja Jost, T. (2015). Consequences of a cyanobacteria bloom for copepod re- production, mortality and sex ratio. Journal of Plankton Research, 37(2), 388–398. https://doi.org/10.1093/plankt/fbv004
Engström-Öst, J., Glippa, O., Feely, R. A., Kanerva, M., Keister, J. E., Alin, S. R., Carter, B. R., McLaskey, A. K., Vuori, K. A., & Bednaršek, N. (2019). Eco-physiological responses of copepods and pteropods to ocean warming and acidification. Scientific Reports, 9(1), 1–13. https://doi.org/10.1038/s41598-019-41213-1
Engström-Öst, J., Kanerva, M., Vuori, K., Riebesell, U., Spilsa, C., & Glippa, O. (2020). Oxidative stress and antioxidant defence responses in two marine copepods in a high CO2 experiment. Science of the Total Environment, 745, 140600. https://doi.org/10.1016/j.scitotenv.2020.140600
Finiguerra, M. B., Dam, H. G., Avery, D. E., & Burris, Z. (2013). Sex-specific tolerance to starvation in the copepod Acartia tonsa. Journal of Experimental Marine Biology and Ecology, 446, 17–21. https://doi.org/10.1016/j.jembe.2013.04.018
Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. Nature, 408(6809), 239–247. https://doi.org/10.1038/35041687
Garzke, J., Hansen, T., Ismar, S. M. H., & Sommer, U. (2016). Combined effects of ocean warming and acidification on copepod abundance,
body size and fatty acid content. PLoS One, 11(5), 1–22. https://doi.org/10.1371/journal.pone.0155952

Garzke, J., Sommer, U., & Ismar-Rebitz, S. M. H. (2020). Zooplankton growth and survival differentially respond to interactive warming and acidification effects. Journal of Plankton Research, 42(2), 189–202. https://doi.org/10.1093/plankt/fbaa005

Giron, D., & Casas, J. (2003). Mothers reduce egg provisioning with age. Ecology Letters, 6(4), 273–277. https://doi.org/10.1046/j.1461-0248.2003

Glippa, O., Engström-Öst, J., Kanerva, M., Rein, A., & Vuori, K. (2018). Oxidative stress and antioxidant defense responses in acartia copepods in relation to environmental factors. PLoS One, 13(4), 1–15. https://doi.org/10.1371/journal.pone.0195981

Graham, L. P. Climate change effects on river flow to the Baltic Sea. AMBIO: A Journal of the Human Environment, 33(4), 235–241. https://doi.org/10.1579/0044-7447-33.4.235

Halliwell, B., & Gutteridge, J. M. C. (2015). Free radicals in biology and medicine. Oxford University Press. https://books.google.fi/books?id=3DlKCgAAQBAJ

HELCOM. (2021). Climate Change in the Baltic Sea Area. Technical Report 180, 2021.

Hood, W. R., Zhang, Y., Mowry, A. V., Hyatt, H. W., & Kavazis, A. N. (2018). Life history trade-offs within the context of mitochondrial hormesis. Integrative and Comparative Biology, 58(3), 567–577. https://doi.org/10.1093/icb/icy073

Hulbert, A. J., Pamplona, R., Buffenstein, R., & Buttemer, W. A. (2007). Long-term exposure to salinity variations induces protein carboxylation in the copepod Acartia tonsa. Journal of Experimental Marine Biology and Ecology, 370(1–2), 97–105. https://doi.org/10.1016/j.jembe.2007.01.049

Katajisto, T., Viitasalo, M., & Koski, M. (1998). Seasonal occurrence and hatching of calanoid eggs in sediments of the northern Baltic Sea. Marine Ecology Progress Series, 163, 133–143. https://doi.org/10.3354/meps163313

Kim, B.-M., Rhee, J.-S., Lee, K.-W., Kim, M.-J., Shin, K.-H., Lee, S.-J., Lee, Y.-M., & Lee, J.-S. (2015). UV-B radiation-induced oxidative stress and p38 signaling pathway involvement in the benthic copepod Tigriopus japonicus. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 167, 15–23. https://doi.org/10.1016/j.cbpc.2014.08.003

Klein Breteler, W. C. M., & Gonzalez, S. R. (1982). Influence of cultivation and food concentration on body length of calanoid copepods. Marine Biology, 71(2), 157–161. https://doi.org/10.1007/BF00394624

Koski, M., & Kuosa, H. (1999). The effect of temperature, food concentration and female size on the egg production of the planktonic copepod Acartia biflosa. Journal of Plankton Research, 21(9), 1779–1789. https://doi.org/10.1093/plankt/21.9.1779

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). InterTest package: Tests in linear mixed effects models. Journal of Statistical Software, 82(13):1–26. https://doi.org/10.18637/jss.v082.i13

Lesser, M. P. (2006). Oxidative stress in marine environments: Biochemistry and physiological ecology. Annual Review of Physiology, 68(3), 253–278. https://doi.org/10.1146/annurev.physiol.68.040104.110001

Lewis, C. N., Brown, K. A., Edwards, L. A., Cooper, G., & Findlay, H. S. (2013). Sensitivity to ocean acidification parallels natural pCO2 gradients experienced by Arctic copepods under winter sea ice. Proceedings of the National Academy of Sciences of the United States of America, 110(51), E4960–E4967. https://doi.org/10.1073/pnas.1315162110

Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. Aquatic Toxicology, 101(1), 13–30. https://doi.org/10.1016/j.aquatox.2010.10.006

Mäkinen, K., Vuorinen, I., & Hänninen, J. (2017). Climate-induced hydrography change favours small-bodied zooplankton in a coastal ecosystem. Hydrobiologia, 792(1), 83–96. https://doi.org/10.1007/s10750-016-3046-6

Martínez, M., Rodríguez-Graña, L., Santos, L., Denicolia, A., & Calliari, D. (2020). Long-term exposure to salinity variations induces protein carboxylation in the copepod Acartia tonsa. Journal of Experimental Marine Biology and Ecology, 1016. https://doi.org/10.1016/j.jembe.2020.151337

Martins, N. D., Yunes, J. S., Monteiro, D. A., Rantin, F. T., & Kalinín, A. L. (2017). Microcytin-LR leads to oxidative damage and alterations in antioxidant defense system in liver and gills of Brycon amazonicus (SPIX & AGASSIZ, 1829). Toxicon, 139, 109–116.

Meier, H. E. M., Andersson, H. C., Arheimer, B., Blencenkter, T., Chubarenko, B., Donnelly, C., Ellola, K., Gustafsson, B. G., Hansson, A., Havenhand, J., Högland, A., Kuznetsov, I., MacKenzie, B. R., Müller-Karulis, B., Neumann, T., Niiranen, S., Piwowarczyk, J., Raudsepp, U., Reckermann, M., ... Zorita, E. (2012). Comparing re-constructed past variations and future projections of the Baltic Sea ecosystem - First results from multi-model ensemble simulations. Environmental Research Letters, 7(3), 34005. https://doi.org/10.1088/1748-9326/7/3/034005

Merkouriadi, I., & Leppäranta, M. (2014). Long-term analysis of hydrography and sea-ice data in Tvärminne, Gulf of Finland, Baltic Sea. Climatic Change, 124(4), 849–859. https://doi.org/10.1007/s10584-014-1130-3

Merkouriadi, I., & Leppäranta, M. (2015). Influence of sea ice on the seasonal variability of hydrography and heat content in Tvärminne, Gulf of Finland. Annals of Glaciology, 56(69), 274–284. https://doi.org/10.3189/2015AoG69A003

Metcalfe, N. B., & Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: The role of reactive oxygen species in shaping phenotypes from conception to death. Functional Ecology, 24(5), 984–996. https://doi.org/10.1111/j.1365-2435.2010.01750.x

Niehoff, B., Schmithüsen, T., Knüppel, N., Daase, M., Czerny, J., & Boxhammer, T. (2013). Mesozooplankton community development at elevated CO2 concentrations: Results from a mesocosm experiment in an Arctic fjord. Biogeosciences, 10(3), 1391–1406. https://doi.org/10.5194/bg-10-1391-2013

Peck, M. A., & Holste, L. (2006). Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in Acartia tonsa (Calanoida: Copepodida): Optimizing intensive cultures. Aquaculture, 255(1–4), 341–350. https://doi.org/10.1016/j.aquaculture.2005.11.055

Powers, M. J., Weaver, R. J., Heine, K. B., & Hill, G. E. (2020). Predicting adult lifespan and lifetime reproductive success from early-life reproductive events. Marine Biology, 167(10), 147. https://doi.org/10.1007/s00227-020-03765-z

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., & Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. Journal of Agricultural and Food Chemistry, 51(11), 3273–3279. https://doi.org/10.1021/jf0262256

R Core Team. (2019). R: A Language and Environment for Statistical Computing.

Rodriguez-Graña, L., Calliari, D., Skółd, H. N., Winding Hansen, B., & Tiselius, P. (2010). Gender-specific ageing and non-Mendelian inheritance of oxidative damage in marine copepods. Marine Ecology Progress Series, 40, 1–13. https://doi.org/10.3354/meps08459
Schlitzer, R. (2016). Ocean Data View. https://odv.awi.de
Sokolova, I. M. (2013). Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integrative and Comparative Biology, 53(4), 597–608. https://doi.org/10.1093/icb/icct028
Souza, M. S., Modenutti, B. E., Carrillo, P., Villar-Argaiz, M., Medina-Sánchez, J. M., Bullejos, F., & Balseiro, E. G. (2010). Stoichiometric dietary constraints influence the response of copepods to ultraviolet radiation-induced oxidative stress. Limnology and Oceanography, 55(3), 1024–1032. https://doi.org/10.4319/lo.2010.55.3.1024
Tartarotti, B., Saul, N., Chakrabarti, S., Trattner, F., Steinberg, C. E. W., & Sommaruga, R. (2014). UV-induced DNA damage in Cyclops abyssorum populations from clear and turbid alpine lakes. Journal of Plankton Research, 36(2), 557–566. https://doi.org/10.1093/plankt/fbt109
Tester, P. A., & Turner, J. T. (1990). How long does it take copepods to make eggs? Journal of Experimental Marine Biology and Ecology, 141(2–3), 169–182. https://doi.org/10.1016/0022-0981(90)90222-X
Turja, R., Guimarães, L., Nevala, A., Kankaanpää, H., Korpinen, S., & Lehtonen, K. K. (2014). Cumulative effects of exposure to cyanobacteria bloom extracts and benzo [a] pyrene on antioxidant defence biomarkers in Gammarus oceanicus (Crustacea: Amphipoda). Toxicicon, 78, 68–77.
Vehmaa, A., Brutemark, A., & Engström-Öst, J. (2012). Maternal effects may act as an adaptation mechanism for copepods facing pH and temperature changes. PLoS One, 7(10), 1–8. https://doi.org/10.1371/journal.pone.0048538
Vehmaa, A., Hogfors, H., Gorokhova, E., Brutemark, A., Holmborn, T., & Engström-Öst, J. (2013). Projected marine climate change: Effects on copepod oxidative status and reproduction. Ecology and Evolution, 3(13), 4548–4557. https://doi.org/10.1002/ece3.839
Vehmaa, A., Larsson, P., Vidoudez, C., Pohnert, G., Reinikainen, M., & Engström-Öst, J. (2011). How will increased dinoflagellate: diatom ratios affect copepod egg production? - A case study from the Baltic Sea. Journal of Experimental Marine Biology and Ecology, 401(1–2), 134–140. https://doi.org/10.1016/j.jembe.2011.01.020
Vuori, K., & Kanerva, M. (2018a). Catalase (CAT) activity assay for zooplankton samples (pp. 4–6). https://doi.org/10.17504/protocols.io.mwsc7ee
Vuori, K., & Kanerva, M. (2018b). Glutathione peroxidase (GP) activity assay for zooplankton samples (pp. 7–8). https://doi.org/10.17504/protocols.io.mv8c69w
Vuori, K., & Kanerva, M. (2018c). Glutathione reductase (GR) activity assay for zooplankton samples (pp. 13–15). https://doi.org/10.17504/protocols.io.mjfc4jn
Vuori, K., & Kanerva, M. (2018d). Lipid peroxidation (LPX) assay for zooplankton homogenates (pp. 6–8). https://doi.org/10.17504/protocols.io.m4vc8w6
Vuori, K. A., & Kanerva, M. (2018e). Glutathione-S-Transferase (GST) activity assay for zooplankton samples (pp. 7–9). https://doi.org/10.17504/protocols.io.mwrc7d6
Vuori, K. A., Lehtonen, K. K., Kanerva, M., Peltonen, H., Nikinmaa, M., Berezina, N. A., & Boikova, E. (2015). Oxidative stress biomarkers in the copepod Limnocalanus macrurus from the northern Baltic Sea: Effects of hydrographic factors and chemical contamination. Marine Ecology Progress Series, 538(December), 131–144. https://doi.org/10.3354/meps11471
Won, E.-J., Han, J., Lee, Y., Kumar, K. S., Shin, K.-H., Lee, S.-J., Park, H. G., & Lee, J.-S. (2015). In vivo effects of UV radiation on multiple endpoints and expression profiles of DNA repair and heat shock protein (Hsp) genes in the cyclid copepod Paracyclopina nana. Aquatic Toxicology, 65, 1–8. https://doi.org/10.1016/j.aquatox.2015.05.002

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