Marine and Freshwater Behaviour and Physiology
July 2022, Volume 55, Issue 5-6, Pages 107-119
https://doi.org/10.1080/10236244.2022.2104720
https://archimer.ifremer.fr/doc/00786/89757/

Copepod oxygen consumption along a salinity gradient

Soulié Tanguy [1, 2, *], Engström-Öst Jonna [3], Glippa Olivier [3]

[1] Département de Génie Biologique, Institut National des Sciences Appliquées de Toulouse, Toulouse, France
[2] MARBEC (MARine Biodiversity, Exploitation and Conservation), Univ Montpellier, CNRS, Ifremer, IRD, Montpellier, France
[3] Bioeconomy and Sustainable use of Natural Ressources, Novia University of Applied Sciences, Ekenäs, Finland

* Corresponding author : Tanguy Soulié, email address : tanguy.soulie@gmail.com

Abstract :

Climate change-induced salinity decrease is currently occurring in many estuarine coastal zones, due to increased outflow of freshwater. This freshening can be a problem for brackish-water animals, already living on the edge of their salinity tolerance. We measured oxygen consumption of common copepod Eurytemora affinis along a natural salinity gradient in the western Gulf of Finland. The salinity varied between 3 in the inner bay and 7 in the offshore area along the gradient, pH varied between 7.05 and 7.86. Our results show that respiration increased with decreasing salinity, as expected for a genus more commonly found in estuarine/saline waters, even if it has colonised brackish waters. Our results suggest that future decreasing salinity could enhance respiration rate, and so energy requirements, of large-bodied zooplankton in estuarine areas such as the Baltic Sea and could lead to lower food quality availability for coastal planktivorous fish, such as herring and sprat.

Keywords : Oxygen consumption, coastal zone, salinity, zooplankton, climate change

1. Introduction

Surface water salinity is slowly decreasing in many coastal estuarine areas due to climate change related processes (Kuosa et al., 2017; Nielsen et al. 2020), such as increased precipitation causing more freshwater run-off (Graham, 2004). Certain basins, such as the Gulf of Finland and the Gulf of Bothnia in the brackish Baltic Sea could therefore be subjected to profound changes in hydrography (Meier et al., 2012; Almén et al. 2017), with resulting consequences to biodiversity and plankton community composition (Ojaveer et al., 2010; Kuosa et al. 2017; Mäkinen et al. 2017). Salinity changes can have great impact on aquatic invertebrates especially in brackish-water areas. According to Mäkinen et al. (2017), the freshening of the seawater is expected to benefit small-bodied brackish species at the expense of marine large-bodied copepods.

Already in 1934, Remane (cited in Whitfield et al., 2012) showed that the largest abundance of brackish-water species was found in areas with salinity level between 5 and 7. *Eurytemora affinis*, the species in focus in the current work, has been thoroughly studied in relation to salinity. Invasion ecological studies and biogeographical work using *E. affinis* as a test animal demonstrate its high ability to invade freshwater systems from a saline environment (Lee 1999, Lee et al. 2012, 2015). Notably, high food concentration significantly raised low-salinity tolerance in *E. affinis* (Lee et al. 2016). Embryonic development time, inter clutch time and

clutch size were fairly stable in salinities ranging from 5 to 15 (Devreker et al., 2009), and egg hatching success was highest between salinities 5 and 20 (Kuismanen et al. 2020). However, limited knowledge is available on animal respiration in the brackish-water environment, and how oxygen consumption will change with fluctuating salinity (Feely et al. 2010).

Seasonal hydrography can show large fluctuations, especially in eutrophicated coastal zones, due to primary production, respiration, oxygen deficiency, ocean acidification and precipitation (Waldbusser and Salisbury, 2014, Humborg et al. 2019). Community respiration (i.e., carbon dioxide release) is an important factor that will contribute to coastal hydrography variations (Sunda and Cai, 2012). The model by Sunda and Cai (2012) predicts a larger effect of respiration on the carbonate chemistry processes in the Baltic Sea than in other areas (here: the Gulf of Mexico) due to lower average salinity and temperature. They also show that the greatest respiratory increases in pCO_2, associated with parallel decreases in pH, occurred in the lowest salinity (here 4), consistently over a large range of temperatures. The model was run at several temperatures and showed similar effects for all.

E. affinis is a frequently studied calanoid copepod. It is considered euryhaline, as it can tolerate and live in a wide range of salinities (Lee et al., 2003). *E. affinis* is an important grazer on microalgae in the planktonic food-web and constitutes a central food source for planktivores, such as herring, sprat, three-spined sticklebacks, and mysid shrimps in the Baltic Sea (Viherluoto et al., 2000; Peltonen et al., 2004).

E. affinis populations are decreasing in the Baltic Sea, which is suggested to be a combined consequence of eutrophication, climate change and over-fishing (Suikkanen et al., 2013; Mäkinen et al., 2017). In this regard, the aim of the current study was to measure respiration rates, i.e., oxygen consumption (Gyllenberg & Lundqvist 1979, Li & Gao 2012) in the common crustacean copepod *E. affinis*. The study was performed along a salinity gradient in a brackish-

water area. We sampled copepods at four different sites of different salinity in the western Gulf of Finland and transferred them to respiration chambers in the laboratory. Our main hypothesis was that salinity affects the respiration rate of *E. affinis* (here female egg-carrying individuals) negatively, because of several vital rates being negatively affected by salinity <5 (Devreker et al. 2009, Kuismanen et al. 2020). The salinity gradient used in the present study stretched from 3 in an inner coastal bay to 7 in a pelagic offshore area. The salinity gradient is located at the entrance to the Gulf of Finland, which is known to be a site of strong hydrographic variability affected by strong south-westerly winds, upwellings from the deep Baltic proper, and freshwater inputs from River Svartån (Alenius et al., 1998).

2. Material and methods

2.1 Sampling

We collected copepods along a brackish salinity gradient to reveal differences in oxygen consumption of the euryhaline species *Eurytemora affinis*. Sampling of seawater and zooplankton took place at four sites along the gradient, from June 19th, 2017, to August 15th, 2017. The sampling sites were Pojoviken Bay (60°02’7.10’’N, 23°31’6.50’’E), Rävnäs (60°0’6.238’’N, 23°27’4.826’’E), Storfjärden (59°52’5.6’’N, 23°15’1.4’’E) and Längden (59°46’4.64” N, 23°15’6.76”E), reaching from a shallow (10 m) less-saline site (Pojoviken Bay) to a deep (60 m) more-saline pelagic site Längden off Tvärminne archipelago (**Fig. 1A**.). Those sites were chosen as they differ mostly by their salinity due to their different distance to the shore, even if factors other than salinity could greatly differ sporadically over a year. The water from these sites was used for the respiration experiments. Sampling took place once or twice per week during the summer growth season. During each sampling occasion we took a Conductivity, Temperature, Density (CTD) profile (Mini CTD, www.valeport.co.uk) for

93 salinity and temperature between the surface and the depth (**Table 1**). Note that at Längden,
94 CTD was not deployed to the bottom (60 m depth) but only at 53m due to technical constraints.
95 As copepods migrate from being in the bottom during the day to being at the surface at night,
96 average salinity and temperature from bottom to surface were calculated and used in the
97 analyses, and are presented in **Table 1**.

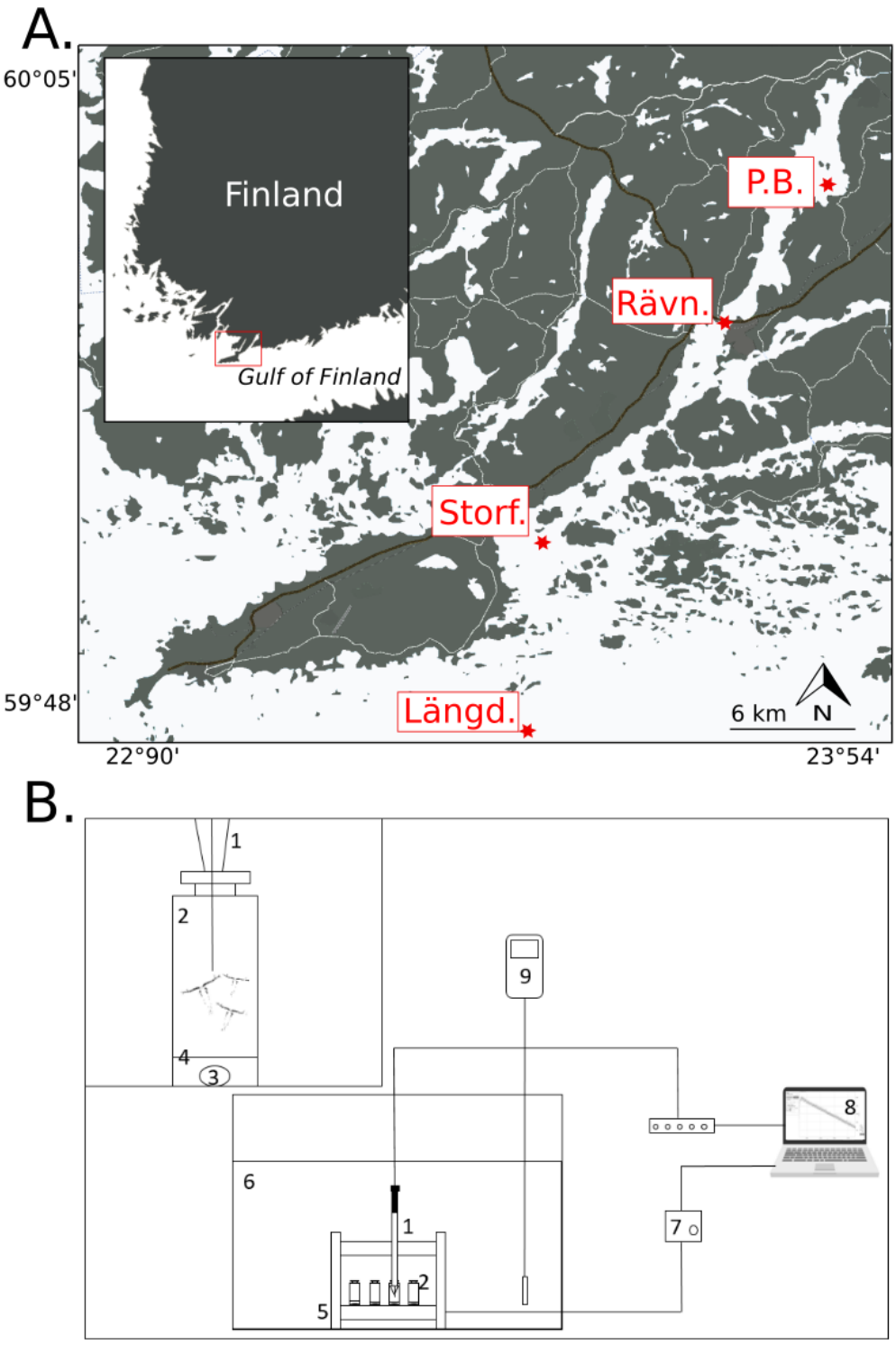

Figure 1. Location of sampling sites (**A.**) and experimental set-up (**B.**) In **A.**, P. B.: Pojoviken Bay, Rävn.: Rävnäs, Storf.: Storfjärden, and Längd: Längden. In **B.**, an oxygen micro-optode (1) was put in 4mL glass chambers (2) equipped with a magnet (3) and a mesh (4). The respiration chambers were placed onto a rack (5) immersed in a temperature-constant water bath (6), which was connected to the stirring controller device (7), and to the laptop (8). The bath water temperature was continuously monitored (9). The oxygen micro-optode was connected to the laptop, which recorded the measurements every second and displayed the respiration rate in real-time.

Seawater was sampled from the targeted depth (bottom water) using a 2 L Limnos water sampler. We sampled water in 250 mL airtight glass bottles in triplicate for pH (**Table 1**), to be determined at room temperature in the laboratory later the same day. Field samples were adjusted to room temperature and measured with a WTW Inolab 720 pH-Meter, calibrated with buffers pH 7 and 10. For chlorophyll *a* analysis, seawater (200 mL) was filtered on a 25 mm glass-fibre filter (GF/F Whatman). Subsequent to 5 mL ethanol extraction overnight, samples were measured in triplicate in a 96-well plate, using a spectrophotometer (Varian Cary Eclipse Fluorescence Spectrophotometer) at 664 nm incident ray. For the calibration of the spectrophotometer, three standards (1.04, 10.4 and 104 µg L^{-1}) were used. The blank was calibrated with pure ethanol (96%). Dissolved oxygen (DO) concentration and saturation were monitored at the depth where zooplankton was collected using an YSI DO meter (www.ysi.com).

Zooplankton were collected using a 200 µm plankton net with cod end, with tows from the bottom to the surface. This was done as zooplankton are distributed deeper in the water column during the day. At the deep site Längden, while the maximum depth is around 60m, the net was lowered to 30 m only due to technical constraints. After each tow, zooplankton was transferred to a cooler containing seawater from bottom water from each site and icepacks. The animals were transported to the laboratory where they were stored and acclimated at 12°C for up to 4 hours until further experiments. The laboratory temperature was set as 12°C on the first sampling day and kept constant for all the experiments (Rumed P530 Climatic Chamber, www.rumed.de). The salinity and the oxygen content in the cooler were measured prior to experiments.

2.2 Copepod handling and respiration measurements

131 The water used for the respiration experiment was collected in 8 L containers, transported in
132 coolers and transferred to a climate chamber at arrival. To measure the oxygen consumption
133 rates from four different sites along the salinity gradient (salinities 3-7), two oxygen
134 microsensors (optodes) were used (**Fig. 1B**.). Approximately 12 female copepods were
135 randomly selected, sorted on ice and acclimated for ~1-2 h prior to the start of the measurement
136 incubation in a 400 ml glass beaker in 12°C. Ten ovigerous females (egg numbers not recorded)
137 in good condition were transferred from the beaker to the measurement unit (= 4 ml glass
138 cylinder chamber) filled with filtered seawater (see above) and acclimated for another 15 min,
139 before respiration rates were monitored for 2h. The chamber was equipped with a glass ring
140 and a 200 μm mesh circle and a glass-coated magnet, to keep the water properly circulated in
141 the chamber. The mesh was used to prevent the copepods from being injured by the magnet.

142 Respiration rates were monitored at 12° ± 0.1°C (mean ± SD) using two micro-respiration
143 multimeters (four-channel multimeter, Unisense A/S). The setup enables a continuous and
144 precise follow-up of dissolved oxygen concentration in the chamber (1 measurement s^{-1}). The
145 0% DO concentration calibration was done using a 0.1M NaOH solution (50 mL) in which 1 g
146 sodium ascorbate was added. The solution was left to incubate for 2h in order to reach the 0%
147 saturation point. The 100% DO calibration was achieved by vigorous oxygenation of the water.
148 The micro-chambers were placed on a plastic rack in a temperature-controlled water bath (12
149 ± 0.1°C). Stirring (500 RPM) was achieved using a glass-coated magnet placed in the chamber,
150 used to prevent the formation of an oxygen gradient in the chamber. The experiments were run
151 in a dark climate chamber, for at least 2h. Real-time data were recorded on a laptop via the
152 Unisense® rate software. The connection between the oxygen sensors and the laptop was done
153 with a Unisense® MicroOptode Meter. A precise description of the experimental device is
154 presented in **Fig. 1B**.

Depending on the abundance of *E. affinis* adult female specimens found in the collected samples, between 2 and 4 replicates (10 animals $repl^{-1}$) were done for each sampling time. For each sample, one or two control runs without animals were conducted with filtered seawater to compensate for potential microbial respiration, and to ensure that the measured respiration was due to the incubated animals. A non-parametric Kruskal-Wallis test was performed between O_2 concentration at start and after 2h of incubation to make sure that O_2 concentration showed no significant decrease over the allotted time period. Filtered seawater was produced from field-collected water using 200 µm plankton mesh, and then vacuum-filtered using GF/C glass microfibre filters 1.2 µm (⌀ 47 mm Whatman®) and stored at +12°C.

Individual dry weight was measured for each respiration measurement. To do so, after each respiration experiment, copepods were extracted from the respiration chamber using a Pasteur pipette and put on a Petri dish. Then, animals (10) were picked using forceps and put in foil cups, which were beforehand weighed using a precision scale (Metler Toledo). Foil cups were then put to dry at 60°C in a oven for 24 hours. Then, foil cups were weighed again, and individual dry weight was calculated as the difference of weight between the foil cup when it was empty and with copepods, divided by the number of weighed animals (10). Respiration rates were standardized by individual dry weights.

2.3. Statistical analysis

All data residuals were checked for deviations in variances and normal distribution using the Shapiro-Wilk and Levene tests, and analysed using common least square linear regression. The environmental data were checked for collinearity using the Variance inflation factor (VIF), and for autocorrelation using the Durbin-Watson estimate. The VIF represents the ratio of the variance of a model including multiple factors to the variance of the model if it only includes a

single independent parameter. Hence, a high value for the VIF indicates that the tested parameter is highly collinear with the other factors included in the model. VIF was calculated with salinity as the single independent variable and environmental parameters (pH, salinity, water temperature, Chl *a*) for the multifactor model, and a value of 1.823 was obtained, suggesting that salinity is poorly collinear with the other tested environmental factors. The Durbin-Watson test was performed for the residuals of a linear regression model with respiration rate against salinity, and a value of 1.076 was obtained, which is indicative of low positive linear autocorrelation. The dependent response variable oxygen consumption was analysed against the environmental variable in focus (salinity). In addition, an analysis of variance (ANOVA) followed by a post-hoc Tukey Honest Significant Difference (Tukey HSD) were used to assess differences between respiration rates among sampling stations. All the analyses were performed using the free software R, version 3.4.3 (R Core Team, 2013).

3. Results

3.1. Seasonal environmental conditions

The average salinity from bottom to surface ranged from 3 in the inner archipelago (Pojoviken Bay) to 7.1 in the deep offshore areas (Längden) (**Table 1**, **Fig. 2**). The average temperature from bottom to surface ranged from 4.1°C to 11.2°C, of which the higher temperatures were recorded in inshore areas (Pojoviken Bay and Rävnäs) during late summer (July - August). Dissolved oxygen (DO) concentration below the thermocline (10 m) was monitored to ensure that the zooplankton in the sampling area were not suffering from hypoxia. DO varied between 4.6 mg l^{-1} in the inshore areas in the middle of the summer to 11 mg l^{-1} at Storfjärden. DO was in general highest in early summer and decreased towards the end of the summer. The pH below the thermocline (10 m) peaked at the Storfjärden monitoring station, and the lowest recording

occurred in the inner archipelago at Rävnäs and Pojoviken Bay (**Table 1**), varying between 7.1 and 7.8, and no general trend was detected in the data. Chlorophyll *a* (Chl *a*) concentration varied from 1.3 µg l^{-1} in July at Storfjärden to 5.2 µg l^{-1} at Rävnäs in August, and was generally higher in Rävnäs compared to the other sampling sites (**Table 1**). Finally, the individual dry weight of the sampled copepods varied between 0.001 mg (Längden, Storfjärden) to 0.004 mg (Rävnäs, Storfjärden), with generally higher values in Rävnäs than in other stations (**Table 1**).

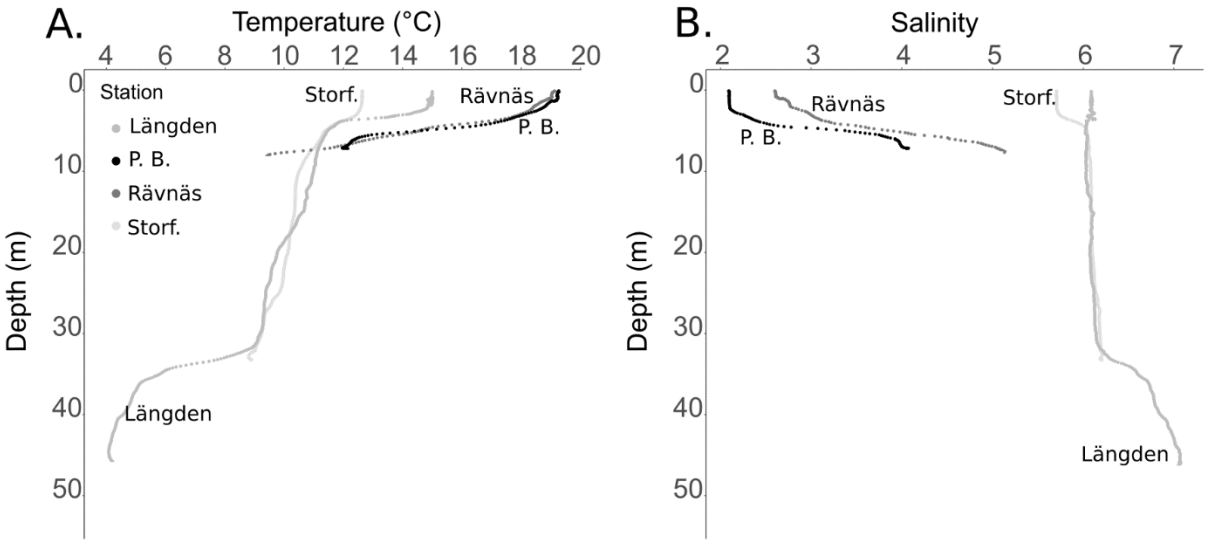

Fig. 2. Temperature (**A**) and salinity (**B**) profiles of the sampling sites Längden (July 10th), Pojoviken Bay (P. B., July 24th), Rävnäs (July 31st), Storfjärden (Storf., July 3rd). Only one profile per site is represented in the figure.

Table 1. Hydrographical and biological conditions measured between June and August at the sampling sites along the environmental gradient used for respiration measurements. Water temperature, salinity and DO correspond to average values from bottom to surface.

Sampling station	Depth (m)	Date (first to last sample)	Water temperature (°C)	pH	Salinity	Chl *a* (µg L^{-1})	DO saturation (%)	Copepod dry weight (mg)
Längden	59 – 60	July 10th – August 10th	4.1 – 5.3	7.3 – 7.36	7 – 7.1	1.9 – 2.8	58.5 – 65	0.001-0.002
Pojoviken Bay	12 - 14	June 29th – July 24th	5.4 – 7.9	7.12 – 7.23	3 – 3.5	2 – 3.4	49.5 – 50.8	0.002-0.003
Rävnäs	9 - 10	July 31st – August 15th	9.8 – 11.2	7.05 – 7.2	4.2 – 4.6	4.9 – 5.2	38.2 – 54.5	0.002-0.004
Storfjärden	32 - 33	June 19th – August 3rd	5.4 – 9.3	7.46 – 7.86	6.1 – 6.7	1.3 – 2.7	69.8 – 88.7	0.001-0.004

3.2. Respiration rates

Respiration rates of *E. affinis* varied between 0.3 and 5.9 µmol L^{-1} ind^{-1} h^{-1}. When normalized by body weight, they varied from 0.1 to 2.4 µmol L^{-1} ind^{-1} $µg^{-1}$ h^{-1}, and rates increased significantly with decreasing salinity (**Fig 3A**). They were significantly different depending on the sampling station (ANOVA, $p = 3.1 \times 10^{-3}$, **Fig. 3B**.). More precisely, respiration rates were significantly higher at Pojoviken Bay (salinity 3-3.5) compared to Längden (salinity 7-7.1) and Storfjärden (salinity 6.1-6.7), while they were not significantly different at Längden, Rävnäs (salinity 4.2-4.6) and Storfjarden (Tukey HSD, **Fig 3B**.). The controls were performed similarly as the treatment, but without animals, and showed no significant changes in oxygen concentration (Kruskal-Wallis, p = 0.7453).

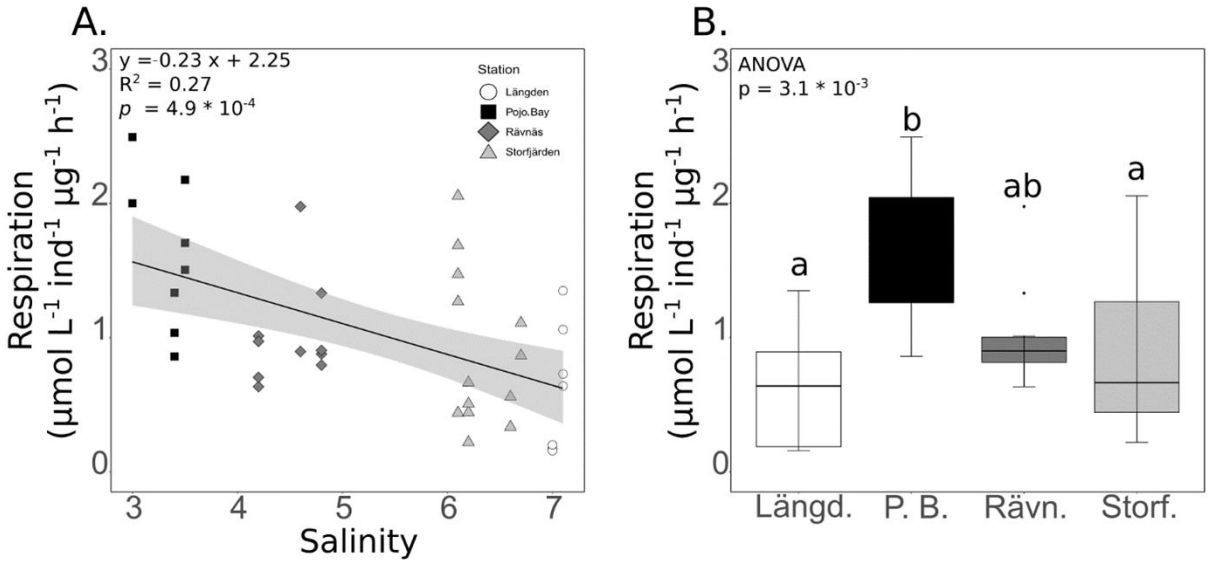

Fig 3. **A**. Respiration rates standardized by body weight against salinity and **B.** Respiration rates standardized by body weight of Eurytemora affinis ovigerous females at the four different sampling stations (Längd. : Längden, P. B.: Pojoviken Bay, Rävn.: Rävnäs, Storf.: Storfjärden). In **A**., the line and shaded area represent the least square linear relationship and its 95% confidence interval, respectively. The equation, R^2 and p-value of the linear relationship are indicated on the upper left corner of the panel. In **B.**, for each box, the lower quartile, median and upper quartile values are displayed with horizontal lines. The result of an ANOVA test comparing respiration rates among stations is indicated on the upper left corner of the panel. Letters represent significance groups obtained with a Tukey HSD post-hoc test comparing respiration rates among sampling stations.

4. Discussion

The goal of the present work was to experimentally assess how the respiration rate of *E. affinis* changes along a natural salinity gradient in order to better our understanding about the potential effects of future climate-change induced salinity decrease on a key copepod species of the Baltic Sea. We hypothesized that, due to the fact that *E. affinis* is more commonly found in brackish or saline waters, its respiration rate would be higher in low salinities. We incubated *E. affinis* copepods from the Baltic Sea in water collected along a brackish-water salinity gradient and indeed found that their oxygen consumption increased significantly with decreasing salinity. Respiration rates ranged from 0.3 to 5.9 µmol L^{-1} ind^{-1} h^{-1}, which is consistent with other studies

in which *Eurytemora* respiration rate was assessed. Indeed, Barthel (1983) obtained a mean respiration rate of 10.8 µmol L^{-1} ind^{-1} h^{-1} for *E. affinis* at 15°C and a salinity of 12, while Roddie et al. (1984) found respiration rates ranging from 0.5 to 2.4 µmol L^{-1} ind^{-1} h^{-1} at 10°C and a salinity ranging from 3 to 10. In addition, Raymont (1959) found a mean respiration rate of 4.4 µmol L^{-1} ind^{-1} h^{-1} for *E. herdmani* at 15°C. Conversely, Gyllenberg and Lundqvist (1978) found lower respiration rates for *E. hirundoides*, ranging from 0.15 to 0.99 µmol L^{-1} ind^{-1} h^{-1}, at a temperature of 4°C. This discrepancy could be due to differences in temperature between experiments, as copepods respiration rate increases with temperature (Heine et al. 2019). This could also be due to methodological reasons, such as the acclimation and exposure time during the experiments. Finally, physiological reasons might explain these discrepancies, either due to the initial physiological state of the organisms or to different physiological responses to stress factors.

Eurytemora is an estuarine genus that has colonized the freshwater environment repeatedly over time, and notably through locally adapted populations (Lee, 1999). The genus inhabits presently a wide range of salinities, being most common in salinities between 5 and 15 (Devreker et al., 2009), and which acclimation in different salinities is widely studied (Lee 1999; Lee and Petersen, 2003, Xuereb et al., 2012). The Baltic *Eurytemora* clade originally arrived from the Caspian Sea (Sukhikh et al., 2013). In our study, the fact that the respiration rate of *E. affinis* was lower in higher salinity conditions might be related to this saline origin, even if it is known that *Eurytemora* has colonized brackish waters and has adapted to low saline waters by increasing its metabolism (Lee 1999). *Eurytemora* can tolerate freshwater due to the activity of specialised organs called '*Crusalis organs*', which handle ion transport and are structures found for the first time in crustaceans (Johnson et al., 2014). However, this ability of *Eurytemora* to tolerate low salinities leads to an increased requirement in energy, which results in an enhancement of the respiration rate. Consequently, several published works show that low

salinity can be stressful for *Eurytemora* (Lee, 1999, Xuereb et al., 2012, Kuismanen et al., 2020).

The salinity gradient studied in the present work is natural and is affected by several environmental factors in the coastal zone, partly by freshwater inflow via River Svartån in the northern part, or by upwelling from the main Baltic basin in the offshore area. Also, winds prevailing in the area affect the strength of the salinity gradient (Vuorinen et al., 1998 and references therein). Many studies predict decreasing salinity in the area due to increasing precipitation and resulting run-off (Meier et al., 2012). In our study, salinity could be one of the main factors explaining the increase in respiration, as a negative relationship was found between salinity and respiration rate. This result is congruent with Kuismanen et al. (2020) who measured egg production, egg hatching success and survival of *Eurytemora* in salinities ranging between 0 and 25 and showed that salinity between 10 and 15 seemed to be the optimal salinity range for Baltic *Eurytemora.* When the need for energy increases, respiration increases, and stressful conditions induce an elevated energy need (Mauchline 1998, Whiteley, 2011; Li and Gao, 2012). Therefore, the enhanced respiration rate of *E. affinis* found in low salinities in the present work suggests an increased food intake of the species to compensate for the extra energy demand, which could have important consequences for the entire food web.

In addition to the salinity decrease, other environmental factors could have explained the reported enhancement of respiration. Temperature plays an important role in metabolism. Hence, as the temperatures varied between 4.1 and 11.9°C over the summer at the different sites, we acclimated the animals to the laboratory temperature (12 °C) in order to mitigate the potential effect of temperature on the respiration rate, even if the temperature difference between the sampling sites and the incubation in the laboratory may have affected the measured respiration rate. Nevertheless, the water temperature is predicted to greatly increase in the next century due to climate change (IPCC 2018). Hence, warming might affect *Eurytemora*

respiration rates in the Baltic Sea, and the effects of warming may play a synergistic role with decreasing salinity, as it is known that it generally increases plankton respiration rate (López-Urrutia et al., 2006; O'Connor et al., 2009). Moreover, Xuereb et al. (2012) found a synergic effect of temperature and salinity on the gene expression of *Eurytemora* originating from the Seine Estuary, suggesting increased stress for the copepods in warming water and decreasing salinity.

The pH is another important factor modulating copepod respiration rate: in the Mediterranean Sea, acidification had a negative effect on some physiological traits of *Acartia clausi* (Zervoudaki et al. 2014). In the present study, low pH was generally associated with low salinity, and it is not possible to disentangle the effects of pH and salinity on the respiration rates. Physiological conditions, age, biomass, UV radiations and food conditions could also play an important role in respiration rate (Ma et al. 2013). In order to mitigate the effect of physiological and food conditions, only ovigerous females were considered, and they were starved shortly before the experiments. It should however be noted that ovigerous females are known to respire more than females without egg sacs (Svetlichny et al., 2017).

To conclude, we showed that the respiration rate of *E. affinis* responded as expected; rates increased in lower salinity along a salinity gradient. Even though it is not possible to identify salinity as the only factor explaining the enhanced respiration rate, our results imply that the predicted freshening of the Baltic Sea with climate change could have important consequences on the metabolism of copepods, and *in fine* on the entire ecosystem. In this context of global change, future studies should investigate the interactive effects of multiple environmental factors, such as temperature and salinity, on common copepod respiration in the coastal zone.

5. Acknowledgements

We thank Ruslan Gunko, Romi Rancken and Markus Öst for discussions. We also would like to thank the personnel of Tvärminne Zoological Station for help with practicalities. The project received funding from the Academy of Finland (project nr. 276947).

6. Declaration of interest statement

The authors declare no conflict of interest.

7. References

Alenius, P., Myrberg, K., & Nekrasov, A. (1998). The physical oceanography of the Gulf of Finland: a review. *Boreal Environment Research*, *3*(2), 97-125.

Almén, A.-K., Glippa, O., Pettersson, H., Alenius, P. & Engström-Öst, J. (2017). Changes in wintertime pH and hydrography of the Gulf of Finland (Baltic Sea) with focus on depth layers. *Environmental Monitoring and Assessment*, pp. 147-189.

Barthel, K.-G. (1983). Food uptake and growth efficiency of Eurytemora affinis (Copepoda: Calanoida). *Marine Biology*, 74, 269-274.

Lee, D.B., Choi, K.H., Lee, J.S., Lee, S., Park, C. & Shin, H.C. (2014). Use of oxygen microsensors to measure the respiration rates of five dominant copepods and *Euphausia crystallorophias* furcilia from the Amundsen Sea, West Antarctica. *Marine and Freshwater Behaviour and Physiology*, *47*(6), 361-371.

Devreker, D., Souissi, S., Winkler, G., Forget-Leray, J., & Leboulenger, F. (2009). Effects of salinity, temperature and individual variability on the reproduction of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: a laboratory study. *Journal of Experimental Marine Biology and Ecology*, *368*(2), 113-123.

354 Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., Krembs, C. &
355 Maloy, C. (2010). The combined effects of ocean acidification, mixing, and respiration on
356 pH and carbonate saturation in an urbanized estuary. *Estuarine, Coastal and Shelf Science*,
357 *88*(4), 442-449.

358 Graham, L. P. (2004). Climate change effects on river flow to the Baltic Sea. *AMBIO: A*
359 *Journal of the Human Environment*, *33*(4), 235-241.

360 Gyllenberg, G., & Lundqvist, G. (1978). Oxygen consumption of Eurytemora hirundoides
361 nauplii and adults as a function of salinity. In Annales Zoologici Fennici, 15(4), pp 328-
362 330. Finnish Zoological and Botanical Publishing Board.

363 Gyllenberg, G., & Lundqvist, G. (1979). The effects of temperature and salinity on the
364 oxygen consumption of Eurytemora hirundoides (Crustacea, Copepoda). In Annales
365 Zoologici Fennici (pp. 205-208). Finnish Academy of Sciences, Societas Scientiarum
366 Fennica, Societas pro Fauna et Flora Fennica and Societas Biologica Fennica Vanamo.

367 Heine, K. B., Abebe A., Wilson A. E., & Hood W. R. (2019). Copepod respiration increases
368 by 7% per °C increase in temperature: A meta-analysis. Limnology and Oceanography
369 Letters 4(3), 53-61.

370 IPCC, 2018: Summary for Policymakers. In: Global Warming of 1.5°C. An IPCC Special
371 Report on the impacts of global warming of 1.5°C above pre-industrial levels and related
372 global greenhouse gas emission pathways, in the context of strengthening the global
373 response to the threat of climate change, sustainable development, and efforts to
374 eradicate poverty [Masson-Delmotte, V., P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea,
375 P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R.
376 Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T.
377 Waterfield (eds.)]. In Press.

Johnson, K. E., Perreau, L., Charmantier, G., Charmantier-Daures, M., & Lee, C. E. (2013). Without gills: localization of osmoregulatory function in the copepod *Eurytemora affinis*. *Physiological and Biochemical Zoology*, *87*(2), 310-324.

Kuismanen, L., Forsblom, L., Engstöm-Öst, J., Bämstedt, U. & O. Glippa (2020) Salinity effects on egg production, hatching, and survival of *Eurytemora affinis* (Copepoda, Calanoida). *Crustaceana*, *93*(3-5). 429-445.

Kuosa, H., Fleming-Lehtinen, V., Lehtinen, S., Lehtiniemi, M., Nygård, H., Raateoja, M., Raitaniemi, J., Tuimala, J., Uusitalo, L. & Suikkanen, S. (2017). A retrospective view of the development of the Gulf of Bothnia ecosystem. *Journal of Marine Systems*, *167*, 78-92.

Lee, C. E., 1999. Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution*, pp. 1423-1434.

Lee, C. E. (2016). Evolutionary mechanisms of habitat invasions, using the copepod *Eurytemora affinis* as a model system. *Evolutionary Applications*, *9*(1), 248-270.

Lee, C. E., & Petersen, C. H. (2003). Effects of developmental acclimation on adult salinity tolerance in the freshwater-invading copepod *Eurytemora affinis*. *Physiological and Biochemical Zoology*, *76*(3), 296-301.

Lee, C. E., Remfert, J. L., & Gelembiuk, G. W. (2003). Evolution of physiological tolerance and performance during freshwater invasions. *Integrative and Comparative Biology*, *43*(3), 439-449.

Lee, C. E., Moss, W. E., Olson, N., Chau, K. F., Chang, Y. M., & Johnson, K. E. (2013). Feasting in fresh water: impacts of food concentration on freshwater tolerance and the

399 evolution of food × salinity response during the expansion from saline into fresh water
400 habitats. *Evolutionary Applications*, *6*(4), 673-689.

401 Li, W. & Gao, K., 2012. A marine secondary producer respires and feeds more in a high CO_2
402 ocean. *Marine Pollution Bulletin,* pp. 699-703.

403 López-Urrutia, Á., San Martin, E., Harris, R. P., & Irigoien, X. (2006). Scaling the metabolic
404 balance of the oceans. *Proceedings of the National Academy of Sciences*, *103*(23), 8739-
405 8744.

406 Ma, Z., Li, W., & Gao, K. (2013). Impacts of UV radiation on respiration, ammonia
407 excretion, and survival of copepods with different feeding habits. *Hydrobiologia*, 701(1),
408 209-218

409 Mauchline, J., 1998. *The Biology of Calanoid Copepods, Volume 33.* s.l.:Series Editors.

410 Meier, H.M., Andersson, H.C., Arheimer, B., Blenckner, T., Chubarenko, B., Donnelly, C.,
411 Eilola, K., Gustafsson, B.G., Hansson, A., Havenhand, J., Höglund, A., Kuznetsov, I.,
412 MacKenzie, B.R., Müller-Karulis, B., Neumann, T., Niiranen, S., Piwowarczyk, J.,
413 Raudsepp, U., Reckermann, M., Ruoho-Airola, T., Savchuk, O.P., Schenk, F., Schimanke,
414 S., Väli, G., Weslawski, J.-M. Zorita, E. (2012). Comparing reconstructed past variations
415 and future projections of the Baltic Sea ecosystem—first results from multi-model
416 ensemble simulations. *Environmental Research Letters*, *7*(3), 034005.

417 Mäkinen, K., Vuorinen, I., & Hänninen, J. (2017). Climate-induced hydrography change
418 favours small-bodied zooplankton in a coastal ecosystem. *Hydrobiologia*, *792*(1), 83-96.

O'Connor, M. I., Piehler, M. F., Leech, D. M., Anton, A., & Bruno, J. F. (2009). Warming and resource availability shift food web structure and metabolism. *PLoS biology*, *7*(8), e1000178.

Ojaveer, H., Jaanus, A., MacKenzie, B. R., Martin, G., Olenin, S., Radziejewska, T., Telesh, I., Zettler, M. & Zaiko, A. (2010). Status of biodiversity in the Baltic Sea. *PLOS ONE*, *5*(9), e12467.

Peltonen, H., Vinni, M., Lappalainen, A., & Pönni, J. (2004). Spatial feeding patterns of herring (*Clupea harengus* L.), sprat (*Sprattus sprattus* L.), and the three-spined stickleback (*Gasterosteus aculeatus* L.) in the Gulf of Finland, Baltic Sea. *ICES Journal of Marine Science*, *61*(6), 966-971.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna Austria. https://www.R-project.org

Raymont, J. E. G. (1959). The respiration of some planktonic copepods. III. The oxygen requirements of some American species. *Limnology and Oceanography* 4(4), 479-491

Remane, A., 1934. Die Brackwasserfauna. Verhandlungen Der Deutschen Zoologischen Gesellschaft 36, 34e74. (in German)

Roddie, B. D., Leakey R.J.G., and Berry A.J. (1984). Salinity-temperature tolerance and osmoregulation in Eurytemora affinis (Pope) (Copepoda: Calanoida) in relation to its distribution in the zooplankton of the upper reaches of the Forth estuary. *Journal of Experimental Marine Biology and Ecology*, 79(2), 191-211.

439 Suikkanen, S., Pulina, S., Engström-Öst, J., Lehtiniemi, M., Lehtinen, S., & Brutemark, A.
440 (2013). Climate change and eutrophication induced shifts in northern summer plankton
441 communities. *PLOS ONE*, *8*(6), e66475.

442 Sukhikh, N., Souissi, A., Souissi, S., & Alekseev, V. (2013). Invasion of *Eurytemora* sibling
443 species (Copepoda: Temoridae) from north America into the Baltic Sea and European
444 Atlantic coast estuaries. *Journal of Natural History*, *47*(5-12), 753-767.

445 Sunda, W. G., & Cai, W. J. (2012). Eutrophication induced CO_2-acidification of subsurface
446 coastal waters: interactive effects of temperature, salinity, and atmospheric pCO_2.
447 *Environmental Science & Technology*, *46*(19), 10651-10659.

448 Svetlichny, L., Hubareva, E., & Isinibilir, M. (2017). Comparative trends in respiration rates,
449 sinking and swimming speeds of copepods *Pseudocalanus elongatus* and *Acartia clausi*
450 with comments on the cost of brooding strategy. *Journal of Experimental Marine Biology*
451 *and Ecology*, *488*, 24-31.

452 Viherluoto, M., Kuosa, H., Flinkman, J., & Viitasalo, M. (2000). Food utilisation of pelagic
453 mysids, *Mysis mixta* and *M. relicta*, during their growing season in the northern Baltic Sea.
454 *Marine Biology*, *136*(3), 553-559.

455 Vuorinen, I., Hänninen, J., Viitasalo, M., Helminen, U., & Kuosa, H. (1998). Proportion of
456 copepod biomass declines with decreasing salinity in the Baltic Sea. *ICES Journal of*
457 *Marine Science*, *55*(4), 767-774.

458 Vuorinen, I., Hänninen, J., Rajasilta, M., Laine, P., Eklund, J., Montesino-Pouzols, F.,
459 Corona, F., Junker, K., Meier, H.E.M. & Dippner, J. W. (2015). Scenario simulations of
460 future salinity and ecological consequences in the Baltic Sea and adjacent North Sea areas–
461 implications for environmental monitoring. *Ecological Indicators*, *50*, 196-205.

462 Waldbusser, G. G., & Salisbury, J. E. (2014). Ocean acidification in the coastal zone from an
463 organism's perspective: multiple system parameters, frequency domains, and habitats.
464 *Annual Review of Marine Science*, *6*, 221-247.

465 Whitfield, A. K., Elliott, M., Basset, A., Blaber, S. J. M., & West, R. J. (2012). Paradigms in
466 estuarine ecology–a review of the Remane diagram with a suggested revised model for
467 estuaries. *Estuarine, Coastal and Shelf Science*, *97*, 78-90.

468 Xuereb, B., Forget-Leray, J., Souissi, S., Glippa, O., Devreker, D., Lesueur, T., Marie, S. &
469 Danger, J.-M. & Boulangé-Lecomte, C. (2012). Molecular characterization and mRNA
470 expression of grp78 and hsp90A in the estuarine copepod *Eurytemora affinis*. *Cell Stress*
471 *and Chaperones*, *17*(4), 457-472.

472 Zervoudaki, S., Frangoulis, C., Giannoudi, L., & Krasakopoulou, E. (2014). Effects of low pH
473 and raised temperature on egg production, hatching and metabolic rates of a Mediterranean
474 copepod species (*Acartia clausi*) under oligotrophic conditions. *Mediterranean Marine*
475 *Science*, *15*(1), 74-83.

476
477