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Copepod oxygen consumption along a salinity gradient

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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. In the current study, we measured oxygen consumption of common copepod *Eurytemora affinis* (hereafter *E. 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, and 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.

22 Our results suggest that future decreasing salinity could enhance respiration rate, and so energy
23 requirements, of large-bodied zooplankton in estuarine areas such as the Baltic Sea and could
24 lead to lower food quality availability for coastal planktivorous fish, such as herring and sprat.

25 **Key words:** oxygen consumption, coastal zone, salinity, zooplankton, climate change

26

27 **1. Introduction**

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

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

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

49 Seasonal hydrography can show large fluctuations, especially in eutrophicated coastal zones,
50 due to primary production, respiration, oxygen deficiency, ocean acidification and precipitation
51 (Waldbusser and Salisbury, 2014, Humborg et al. 2019). Community respiration (i.e., carbon
52 dioxide release) is an important factor that will contribute to coastal hydrography variations
53 (Sunda and Cai, 2012). The model by Sunda and Cai (2012) predicts a larger effect of
54 respiration on the carbonate chemistry processes in the Baltic Sea than in other areas (here: the
55 Gulf of Mexico) due to lower average salinity and temperature. They also show that the greatest
56 respiratory increases in $p\text{CO}_2$, associated with parallel decreases in pH, occurred in the lowest
57 salinity (here 4), consistently over a large range of temperatures. The model was run at several
58 temperatures and showed similar effects for all.

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

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

69 water area. We sampled copepods at four different sites of different salinity in the western Gulf
70 of Finland and transferred them to respiration chambers in the laboratory. Our main hypothesis
71 was that salinity affects the respiration rate of *E. affinis* (here female egg-carrying individuals)
72 negatively, because of several vital rates being negatively affected by salinity <5 (Devreker et
73 al. 2009, Kuismanen et al. 2020). The salinity gradient used in the present study stretched from
74 3 in an inner coastal bay to 7 in a pelagic offshore area. The salinity gradient is located at the
75 entrance to the Gulf of Finland, which is known to be a site of strong hydrographic variability
76 affected by strong south-westerly winds, upwellings from the deep Baltic proper, and
77 freshwater inputs from River Svartån (Alenius et al., 1998).

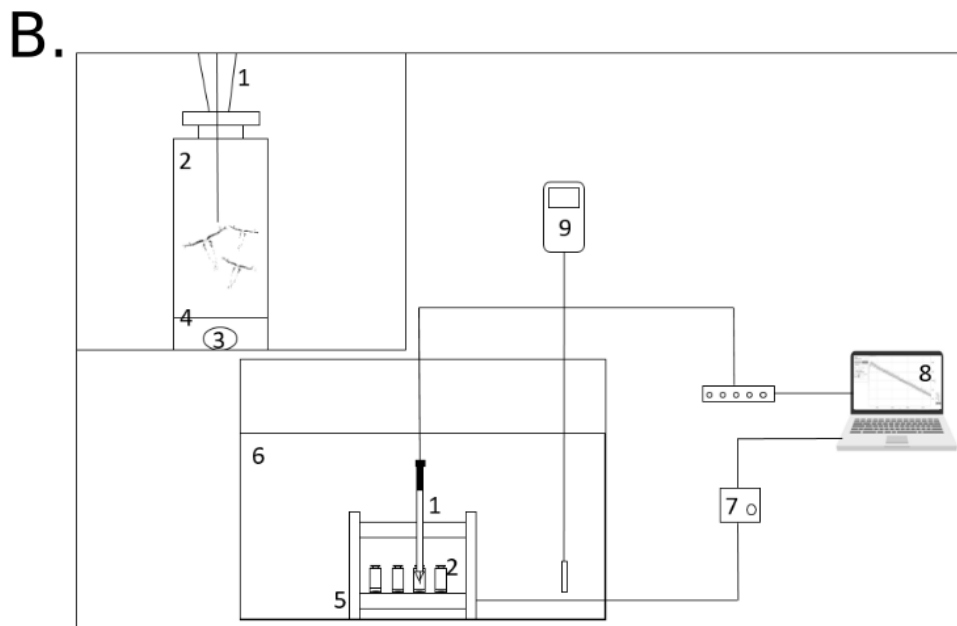
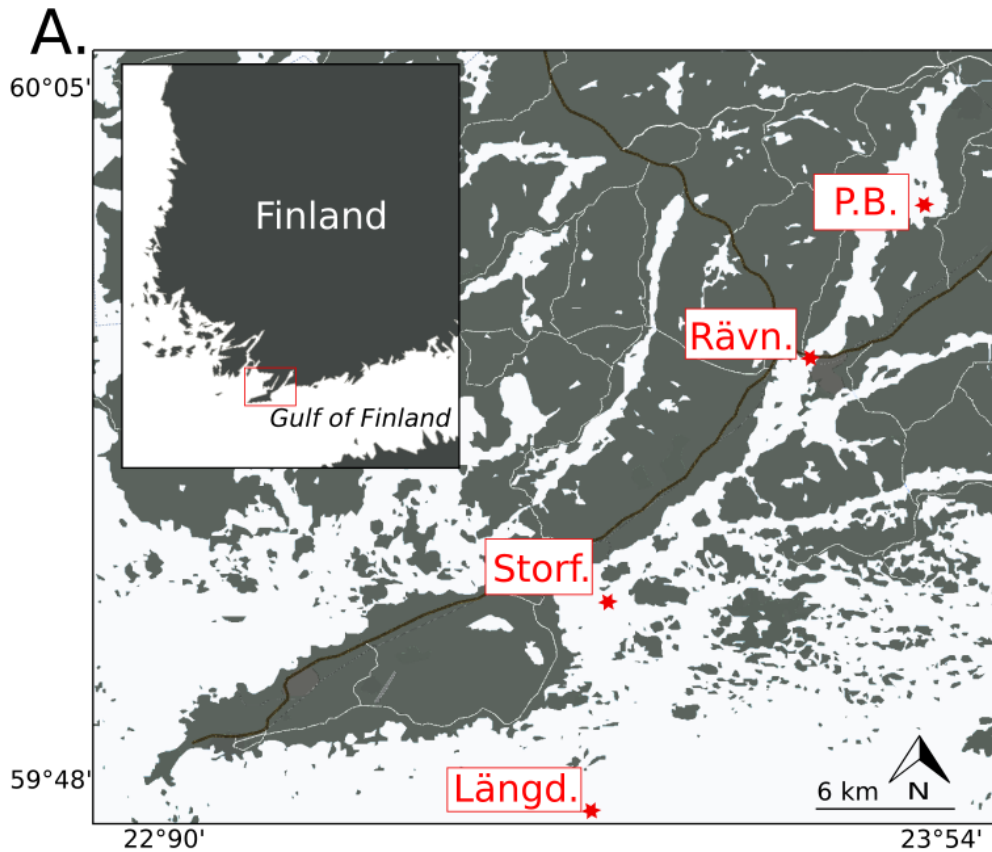
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79 **2. Material and methods**

80 2.1 Sampling

81 We collected copepods along a brackish salinity gradient to reveal differences in oxygen
82 consumption of the euryhaline species *Eurytemora affinis*. Sampling of seawater and
83 zooplankton took place at four sites along the gradient, from June 19th, 2017, to August 15th,
84 2017. The sampling sites were Pojoviken Bay (60°02'7.10''N, 23°31'6.50''E), Rävsnäs
85 (60°0'6.238''N, 23°27'4.826''E), Storfjärden (59°52'5.6''N, 23°15'1.4''E) and Längden
86 (59°46'4.64'' N, 23°15'6.76''E), reaching from a shallow (10 m) less-saline site (Pojoviken
87 Bay) to a deep (60 m) more-saline pelagic site Längden off Tvärminne archipelago (**Fig. 1A.**).
88 Those sites were chosen as they differ mostly by their salinity due to their different distance to
89 the shore, even if factors other than salinity could greatly differ sporadically over a year. The
90 water from these sites was used for the respiration experiments. Sampling took place once or
91 twice per week during the summer growth season. During each sampling occasion we took a
92 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**.



98

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

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

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

129

130 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 $12^{\circ} \pm 0.1^{\circ}\text{C}$ (mean \pm 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 $\pm 0.1^{\circ}\text{C}$). 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**.

155 Depending on the abundance of *E. affinis* adult female specimens found in the collected
156 samples, between 2 and 4 replicates (10 animals repl⁻¹) were done for each sampling time. For
157 each sample, one or two control runs without animals were conducted with filtered seawater to
158 compensate for potential microbial respiration, and to ensure that the measured respiration was
159 due to the incubated animals. A non-parametric Kruskal-Wallis test was performed between O₂
160 concentration at start and after 2h of incubation to make sure that O₂ concentration showed no
161 significant decrease over the allotted time period. Filtered seawater was produced from field-
162 collected water using 200 µm plankton mesh, and then vacuum-filtered using GF/C glass
163 microfibre filters 1.2 µm (Ø 47 mm Whatman®) and stored at +12°C.

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

172

173 2.3. Statistical analysis

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

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

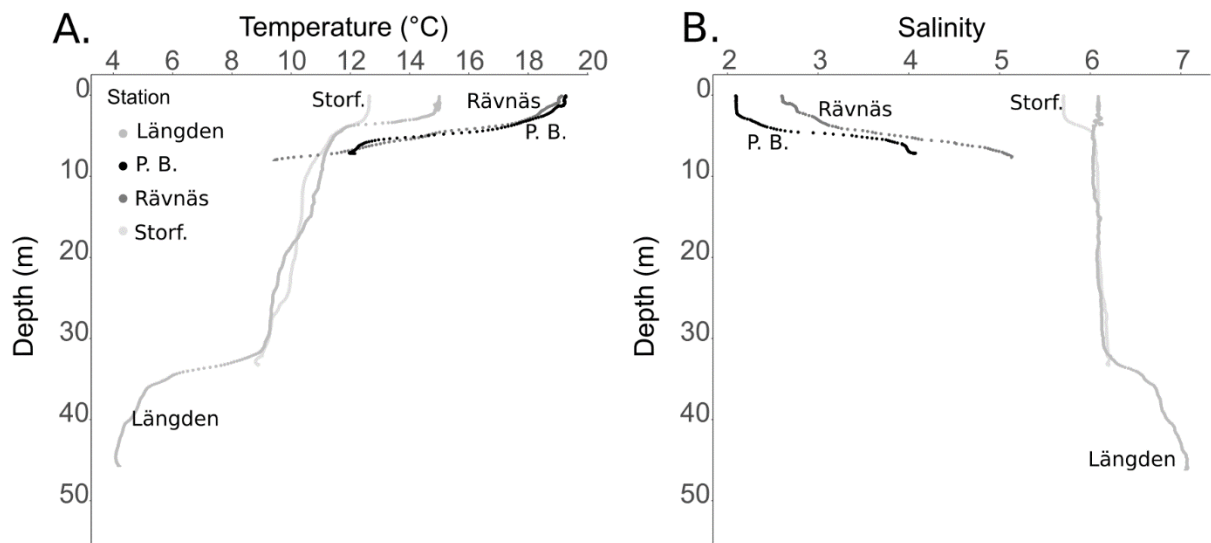
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192 **3. Results**

193 3.1. Seasonal environmental conditions

194 The average salinity from bottom to surface ranged from 3 in the inner archipelago (Pojoiviken
195 Bay) to 7.1 in the deep offshore areas (Längden) (**Table 1, Fig. 2**). The average temperature
196 from bottom to surface ranged from 4.1°C to 11.2°C, of which the higher temperatures were
197 recorded in inshore areas (Pojoiviken Bay and Rävsnäs) during late summer (July - August).
198 Dissolved oxygen (DO) concentration below the thermocline (10 m) was monitored to ensure
199 that the zooplankton in the sampling area were not suffering from hypoxia. DO varied between
200 4.6 mg l⁻¹ in the inshore areas in the middle of the summer to 11 mg l⁻¹ at Storfjärden. DO was
201 in general highest in early summer and decreased towards the end of the summer. The pH below
202 the thermocline (10 m) peaked at the Storfjärden monitoring station, and the lowest recording

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



209

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

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

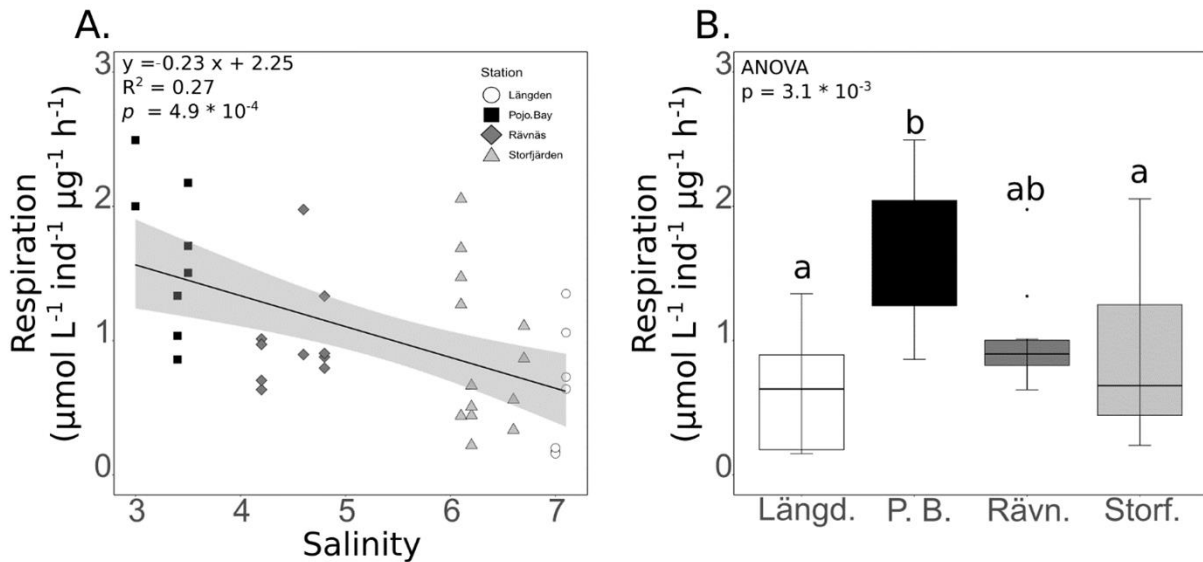
Sampling station	Depth (m)	Date (first to last sample)	Water temperature (°C)	pH	Salinity	Chl <i>a</i> (µg L ⁻¹)	DO saturation (%)	Copepod dry weight (mg)
Längden	59 – 60	July 10 th – August 10 th	4.1 – 5.3	7.3 – 7.36	7 – 7.1	1.9 – 2.8	58.5 – 65	0.001-0.002
Pojoviken Bay	12 - 14	June 29 th – July 24 th	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 31 st – August 15 th	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 19 th – August 3 rd	5.4 – 9.3	7.46 – 7.86	6.1 – 6.7	1.3 – 2.7	69.8 – 88.7	0.001-0.004

225

226

227 3.2. Respiration rates

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



237
238

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

249

250

251 4. Discussion

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

260 in which *Eurytemora* respiration rate was assessed. Indeed, Barthel (1983) obtained a mean
261 respiration rate of $10.8 \mu\text{mol L}^{-1} \text{ind}^{-1} \text{h}^{-1}$ for *E. affinis* at 15°C and a salinity of 12, while Roddie
262 et al. (1984) found respiration rates ranging from 0.5 to $2.4 \mu\text{mol L}^{-1} \text{ind}^{-1} \text{h}^{-1}$ at 10°C and a
263 salinity ranging from 3 to 10. In addition, Raymont (1959) found a mean respiration rate of 4.4
264 $\mu\text{mol L}^{-1} \text{ind}^{-1} \text{h}^{-1}$ for *E. herdmani* at 15°C . Conversely, Gyllenberg and Lundqvist (1978) found
265 lower respiration rates for *E. hirundoides*, ranging from 0.15 to $0.99 \mu\text{mol L}^{-1} \text{ind}^{-1} \text{h}^{-1}$, at a
266 temperature of 4°C . This discrepancy could be due to differences in temperature between
267 experiments, as copepods respiration rate increases with temperature (Heine et al. 2019). This
268 could also be due to methodological reasons, such as the acclimation and exposure time during
269 the experiments. Finally, physiological reasons might explain these discrepancies, either due to
270 the initial physiological state of the organisms or to different physiological responses to stress
271 factors.

272 *Eurytemora* is an estuarine genus that has colonized the freshwater environment repeatedly over
273 time, and notably through locally adapted populations (Lee, 1999). The genus inhabits presently
274 a wide range of salinities, being most common in salinities between 5 and 15 (Devreker et al.,
275 2009), and which acclimation in different salinities is widely studied (Lee 1999; Lee and
276 Petersen, 2003, Xuereb et al., 2012). The Baltic *Eurytemora* clade originally arrived from the
277 Caspian Sea (Sukhikh et al., 2013). In our study, the fact that the respiration rate of *E. affinis*
278 was lower in higher salinity conditions might be related to this saline origin, even if it is known
279 that *Eurytemora* has colonized brackish waters and has adapted to low saline waters by
280 increasing its metabolism (Lee 1999). *Eurytemora* can tolerate freshwater due to the activity of
281 specialised organs called ‘*Crusalis organs*’, which handle ion transport and are structures found
282 for the first time in crustaceans (Johnson et al., 2014). However, this ability of *Eurytemora* to
283 tolerate low salinities leads to an increased requirement in energy, which results in an
284 enhancement of the respiration rate. Consequently, several published works show that low

285 salinity can be stressful for *Eurytemora* (Lee, 1999, Xuereb et al., 2012, Kuismanen et al.,
286 2020).

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

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

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

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

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

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336 **6. Declaration of interest statement**

337 The authors declare no conflict of interest.

338 **7. References**

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