



**HAL**  
open science

## Copepod oxygen consumption along a salinity gradient

Tanguy Soulié, Jonna Engström-Öst, Olivier Glippa

► **To cite this version:**

Tanguy Soulié, Jonna Engström-Öst, Olivier Glippa. Copepod oxygen consumption along a salinity gradient. *Marine and Freshwater Behaviour and Physiology*, 2022, pp.1-13. 10.1080/10236244.2022.2104720 . hal-03755529

**HAL Id: hal-03755529**

**<https://hal.science/hal-03755529v1>**

Submitted on 22 Aug 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Copepod oxygen consumption along a salinity gradient

Tanguy Soulié<sup>1,2,\*</sup>, Jonna Engström-Öst<sup>3,\*</sup>, Olivier Glippa<sup>3</sup>

<sup>1</sup>Département de Génie Biologique, Institut National des Sciences Appliquées de Toulouse, Toulouse, France

<sup>2</sup>MARBEC (MARine Biodiversity, Exploitation and Conservation), Univ Montpellier, CNRS, Ifremer, IRD, Montpellier, France

<sup>3</sup>Novia University of Applied Sciences, Raseborgsvägen 9, FI-10600 Ekenäs, Finland

\*Corresponding authors: Tanguy Soulié, tanguy.soulie@gmail.com

Jonna Engström-Öst, jonna.engstrom-ost@novia.fi

## 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).

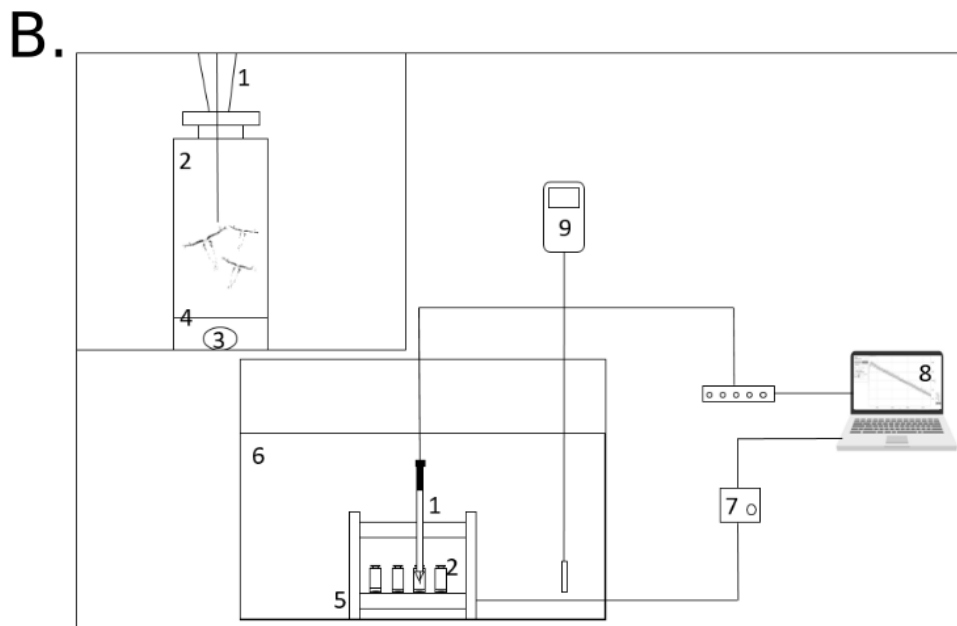
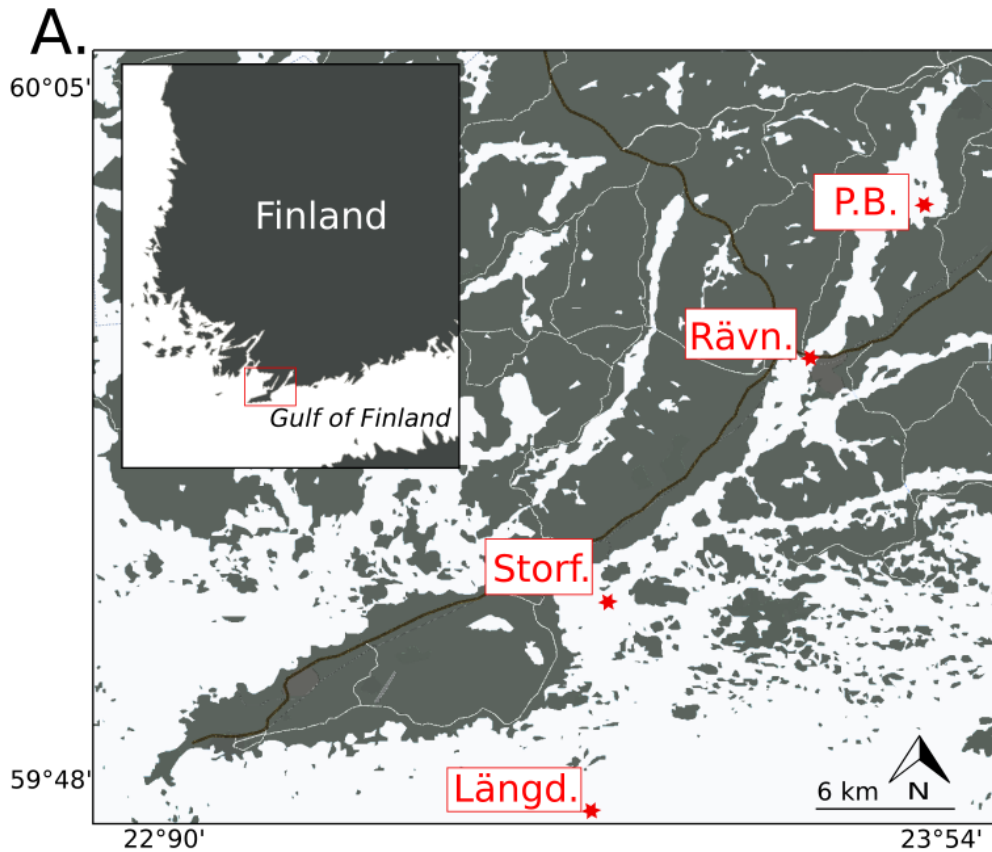
78

## 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 19<sup>th</sup>, 2017, to August 15<sup>th</sup>,  
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](http://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](http://www.ysi.com)).

119 Zooplankton were collected using a 200  $\mu\text{m}$  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](http://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  $\text{s}^{-1}$ ). 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<sup>-1</sup>) 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 O<sub>2</sub>  
160 concentration at start and after 2h of incubation to make sure that O<sub>2</sub> 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).

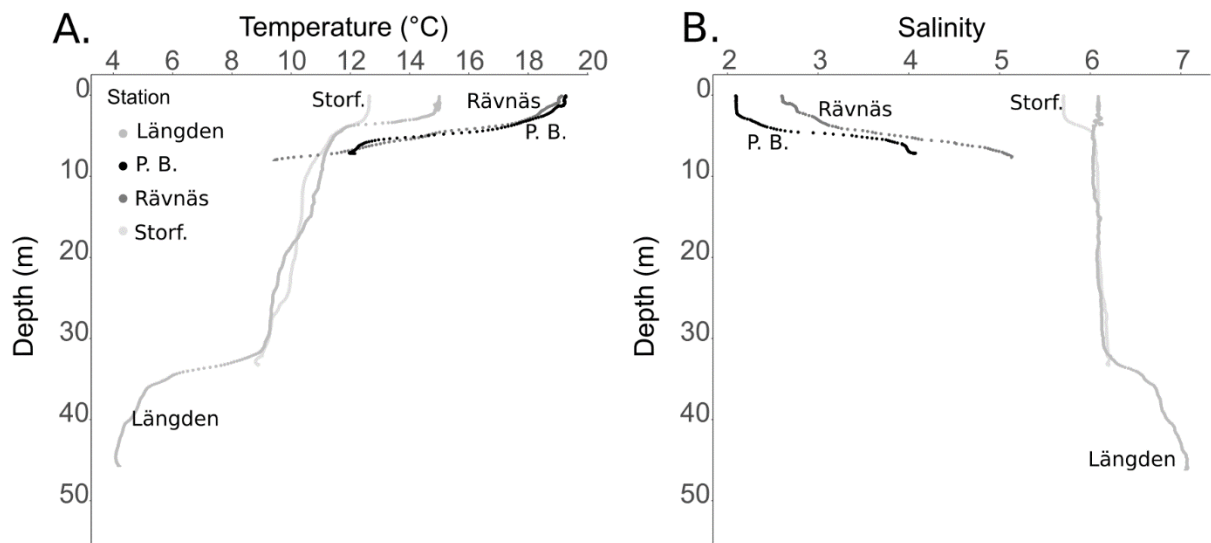
191

## 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<sup>-1</sup> in the inshore areas in the middle of the summer to 11 mg l<sup>-1</sup> 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 10<sup>th</sup>), Pojoviken  
 211 Bay (P. B., July 24<sup>th</sup>), Rävsnäs (July 31<sup>st</sup>), Storfjärden (Storf., July 3<sup>rd</sup>). Only one profile per site is  
 212 represented in the figure.

213

214

215

216

217

218

219

220

221

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.

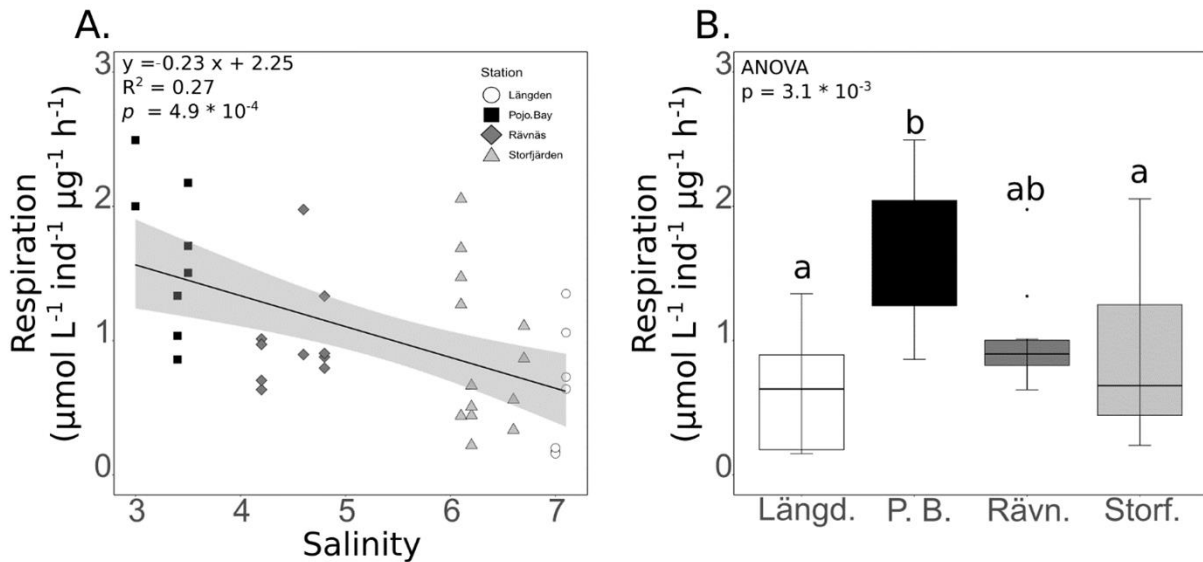
Sampling station	Depth (m)	Date (first to last sample)	Water temperature (°C)	pH	Salinity	Chl <i>a</i> (µg L <sup>-1</sup> )	DO saturation (%)	Copepod dry weight (mg)
Längden	59 – 60	July 10 <sup>th</sup> – August 10 <sup>th</sup>	4.1 – 5.3	7.3 – 7.36	7 – 7.1	1.9 – 2.8	58.5 – 65	0.001-0.002
Pojoviken Bay	12 - 14	June 29 <sup>th</sup> – July 24 <sup>th</sup>	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 <sup>st</sup> – August 15 <sup>th</sup>	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 <sup>th</sup> – August 3 <sup>rd</sup>	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<sup>-1</sup> ind<sup>-1</sup> h<sup>-1</sup>. When normalized  
 229 by body weight, they varied from 0.1 to 2.4 µmol L<sup>-1</sup> ind<sup>-1</sup> µg<sup>-1</sup> h<sup>-1</sup>, 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, Rävsn.: Rävsnäs, 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  $15^\circ\text{C}$  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  $10^\circ\text{C}$  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  $15^\circ\text{C}$ . 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  $4^\circ\text{C}$ . 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.

## 332 **5. Acknowledgements**

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

## 336 **6. Declaration of interest statement**

337 The authors declare no conflict of interest.

## 338 **7. References**

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

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

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

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

350 Devreker, D., Souissi, S., Winkler, G., Forget-Leray, J., & Leboulenger, F. (2009). Effects of  
351 salinity, temperature and individual variability on the reproduction of *Eurytemora affinis*  
352 (Copepoda; Calanoida) from the Seine estuary: a laboratory study. *Journal of Experimental*  
353 *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.

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

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

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

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

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

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

394 Lee, C. E., Remfert, J. L., & Gelembiuk, G. W. (2003). Evolution of physiological tolerance  
395 and performance during freshwater invasions. *Integrative and Comparative Biology*, 43(3),  
396 439-449.

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

399 evolution of food  $\times$  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 respire and feeds more in a high CO<sub>2</sub>  
402 ocean. *Marine Pollution Bulletin*, pp. 699-703.

403 López-Urrutia, Á., San Martín, 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.

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

422 Ojaveer, H., Jaanus, A., MacKenzie, B. R., Martin, G., Olenin, S., Radziejewska, T., Telesh,  
423 I., Zettler, M. & Zaiko, A. (2010). Status of biodiversity in the Baltic Sea. *PLOS ONE*,  
424 5(9), e12467.

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

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

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

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

435 Roddie, B. D., Leakey R.J.G., and Berry A.J. (1984). Salinity-temperature tolerance and  
436 osmoregulation in *Eurytemora affinis* (Pope) (Copepoda: Calanoida) in relation to its  
437 distribution in the zooplankton of the upper reaches of the Forth estuary. *Journal of*  
438 *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<sub>2</sub>-acidification of subsurface  
446 coastal waters: interactive effects of temperature, salinity, and atmospheric pCO<sub>2</sub>.  
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