



HAL
open science

Responses of the copepod *Eurytemora affinis* to trace metal exposure: A candidate for sentinel to marine sediment resuspension effects

Shagnika Das, Baghdad Ouddane, Sami Souissi

► To cite this version:

Shagnika Das, Baghdad Ouddane, Sami Souissi. Responses of the copepod *Eurytemora affinis* to trace metal exposure: A candidate for sentinel to marine sediment resuspension effects. *Marine Pollution Bulletin*, 2022, 181, pp.113854. 10.1016/j.marpolbul.2022.113854 . hal-03922260

HAL Id: hal-03922260

<https://hal.science/hal-03922260v1>

Submitted on 22 Jul 2024

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.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Responses of the copepod *Eurytemora affinis* to trace metal exposure: a**
2 **candidate for sentinel to marine sediment resuspension effects**

3 Shagnika Das ¹, Baghdad Ouddane ², Sami Souissi^{1*}

4 ¹ Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, IRD, UMR 8187 – LOG - Laboratoire
5 d'Océanologie et de Géosciences, Station marine de Wimereux, F-59000 Lille, France.

6 ² Univ. Lille, CNRS, UMR 8516 – LASIRE - Equipe Physico-chimie de l'Environnement,
7 Bâtiment C8, F-59000 Lille, France.

8

9 **Highlights-**

10 1. *E. affinis* can be used as a sentinel to sediment resuspension from pelagic community.

11 2. In 96 h, 38 % higher mortality was observed from polluted sediment (PS).

12 3. *E. affinis* bioaccumulated higher concentration of trace metals from PS.

13 4. Dissolved trace metal caused higher mortality than sediment resuspension.

14 5. Specific responses to polluted sediment resuspension was noted in *E. affinis*.

15

16 **Abstract-**

17 Our study reports the ability of *Eurytemora affinis* to indicate certain responses in 96h when
18 exposed to resuspended sediment from a polluted site (PS, Seine estuary, France), less
19 polluted site (LPS, Canche estuary, France) and dissolved trace metals. Mortality from
20 dissolved trace metal was highest (57.5%) followed by PS (38.59%) > LPS (24.04%). The
21 exposure to PS sediment resulted in significantly lower no. of early larval stage (nauplii < 2),
22 sex-ratio (39.24% of males) and higher ovigerous female (>10). *Eurytemora affinis*
23 bioaccumulated high concentrations of Copper (27.3 mg/kg), Nickel (12.8 mg/kg), Lead
24 (21.8 mg/kg) and Arsenic (13.7 mg/kg) from PS exposure with significantly lower

25 bioaccumulation of metals from LPS. The bioconcentration factor (BCF) was highest from
26 dissolved toxicity (>2000) followed by PS that showed significantly higher BCF for Nickel
27 and Copper, compared to LPS. The responses of *E. affinis* to different matrices exemplify its
28 role as a sentinel.

29

30 **Keywords-** *Eurytemora affinis*, sediment resuspension, sex-ratio (% of males), Seine estuary
31 (France), Canche estuary (France).

32

33

34 **1. Introduction**

35 The effect of sediment resuspension on pelagic and epibenthic species has been
36 explored in previous investigations on fishes, amphipods, copepods, and other
37 invertebrates (Henry et al., 2004; Charry et al., 2018; Borcier et al., 2019, 2020;
38 Das et al., 2020a). The resuspension of sediments usually occurs in areas that are
39 often characterized by strong hydrodynamic forces and previous studies have
40 illustrated that the possible causes of sediment suspension in macrotidal estuaries
41 are the tidal cycles, currents, waves, including man-made factors like dredging,
42 construction of jetties and dykes (Deloffre et al., 2005, 2006, 2007; Mitchell and
43 Uncles, 2013; Wang et al., 2013; Grasso et al., 2018). Sediment resuspension
44 affects aquatic organisms through turbidity, suspended particulate matters (SPM),
45 and also through the exposure to a varied range of contaminants that are often
46 embedded into the sediment column especially near the anthropogenic areas
47 (Kerambrun et al., 2013; Lesueur et al., 2015; Buttino et al., 2018; Charry et al.,
48 2018; Das et al., 2020a,b). The concern on the effect of sediment resuspension is
49 increasing with the escalating industries coupled with the extensive dredging and
50 coastal constructions built for human exigencies. In such domains, collectively

51 both the SPM and toxic substances have been reported to exhibit high mortality,
52 reduced body size, and affect the egg production in many pelagic to epi-benthic
53 species in the ecosystem (Sew et al., 2018; Das et al., 2020a). Although such
54 toxicological surveys are high on demand and are important, it is equally very
55 challenging to assess the toxicity of sediment resuspension to nearby organisms
56 and as a consequence only few literatures are available on this issue.

57 The Seine estuary is one such tidal flat that has been categorized as a zone with a
58 high sediment resuspension due to fabrication of dykes on both sides of the
59 channel aiding the local residents (Lafite and Romaña, 2001). Previous works
60 described how such constructions reduced the tidal flat area and how its distinct
61 morphology in the turbidity zone is impacted by huge resuspension from rivers,
62 semidiurnal tides, flood and ebb tide current along with waves approaching from
63 the Bay of Seine; highlighting the typical characteristics of this estuary (Allen et
64 al., 1980; Avoine et al., 1981; Le Hir et al., 2001; Deloffre et al., 2005, 2006). On
65 the other hand, Seine estuary is also an industrial site with loads of contaminants
66 prevailing in that area, practicing commercial pursuits for over decades (Cailleaud
67 et al., 2007; Minier et al., 2006; Dauvin et al., 2008; Ouddane et al., 2018, Zidour
68 et al., 2019; Das et al., 2020a). Such toxic exposures embedded in the sediment
69 are often bioaccumulated by a range of pelagic and benthic organisms which gets
70 biomagnified into tertiary organisms posing serious threats to human life
71 (Kerambrun et al., 2013, Kadiene et al., 2019 a, b; Zidour et al., 2019; Das et al.,
72 2020a; Dayras et al., 2020). Hence, in such domains with strong resuspension of
73 sediments and prevalence of contaminants, new tools and indicators should be
74 explored to assess the health of the area (Cailleaud et al., 2009, 2010; Kerambrun
75 et al., 2013; Sew et al., 2018; Das et al., 2020a).

76 In ecotoxicology, the use of a dominant species in the form of a biological tool
77 known as bio-indicator species has often proved to be an advantage to examine
78 the health of the ecosystem (Parmar et al., 2016; Farias et al., 2018; Chiarelli et al.,
79 2019; Das et al., 2019; Navon et al., 2020). Such organisms offer flexibility to
80 culture them in laboratories and often show quick responses from the changing
81 habitat (Kwok et al., 2015; Souissi and Soussi, 2021). Pelagic copepods have been
82 widely used in such assays by the exposure to dissolved toxicity, but not as often
83 to resuspended sediments. *Eurytemora affinis* is one such bioindicator species of
84 copepod that has endorsed its role in many studies that reported its varied
85 responses to a wide range of toxicants like, trace metals, organic pollutants,
86 hydrophobic compounds, etc. in acute and multigenerational exposure (Cailleaud
87 et al., 2007, 2009; Kadiene et al., 2019 a, b; Zidour et al., 2019; Das et al., 2020a).
88 However, a thorough assessment to report the response to sediment resuspension
89 is still in progress and the need of a sentinel species from the pelagic community
90 in response to resuspended sediment is however necessary especially near the
91 industrial and commercial sites, such as the Seine estuary. Previous studies
92 reported that *Eurytemora affinis*, on a larval assay has shown a sensitivity to
93 sediment bound hydrophobic compounds in a short-term toxicological experiment
94 (Lesueur et al., 2015). In the previous studies, experimental approaches using
95 extracts or elutriates of contaminants from the sediment has been used to report
96 the toxicity embedded in to the sediment column (Lesueur et al., 2013, 2015;
97 Buttino et al., 2018). Our previous study was the first documentation on the direct
98 resuspension of Seine estuarine sediment and its effect on the intergenerational
99 exposure to *E. affinis* (Das et al., 2020a). It highlights the adaptations and the
100 vulnerability induced from one generation to the next reporting the different

101 indicators of stress from sediment resuspension and dissolved trace metal (Das et
102 al., 2020a). Hence, in this study our primary objective was to detect the quick
103 responses of *E. affinis* through a 96 h exposure to anthropogenic resuspended
104 sediment.

105 For such indications, a comparison of the effect of a polluted area and sediment
106 from an unpolluted area (reference) with lower prevalence of contaminants, have
107 often proved necessary to comprehend the specific indicators of toxicity (Tackx et
108 al., 2003; Amara et al., 2007; Gillet et al., 2008; Mouneyrac et al., 2008;
109 Kerambrun et al., 2013). Therefore, another objective of this study was to
110 implement an inter-site comparison between Seine estuary and a relatively less
111 polluted area. Previous works on such comparisons between nearby estuaries in
112 Northern France has often marked the Canche estuary with proportionately lower
113 commercial activities, industries, human impact, and considerably a low
114 prevalence of pollutants by using it as a reference sediment (Henry et al., 2004,
115 2012; Amara et al., 2007; Kerambrun et al., 2013). Such studies also mentioned
116 the absence of a proper reference site in Northern France with significantly lower
117 toxicants as nearly all the estuaries are being polluted from the adjacent
118 manufacturing and/or industries, with the influence of urban pollution. Hence, in
119 this study we used sediment from the Canche estuary and referred it as a less
120 polluted site (LPS) comparing it with Seine estuarine sediment referred as a
121 polluted site (PS). In parallel, with the sediment resuspension, the effect of
122 dissolved exposure comprising of a cocktail with non-essential trace metals was
123 observed. The effect of a combined exposure of both essential and non-essential
124 trace metals have already been discussed in few studies that had posed a negative
125 effect on the population density and mortality of *E. affinis* (Zidour et al., 2019;

126 Kadiene et al., 2019b; Das et al., 2020a). Therefore, in this study we only used the
127 non-essential and ubiquitous trace metals in mixture comprising of Arsenic (As
128 V), Cadmium (Cd) and Lead (Pb) due to their high toxicity and presence in the
129 Seine estuary (Kerambrun et al., 2013) in dissolved form.

130 Hence, our study comprised of a control, dissolved trace metal exposure (TM),
131 sediment resuspension from a less polluted site (LPS, Canche estuary) and a
132 polluted site (PS, Seine estuary).

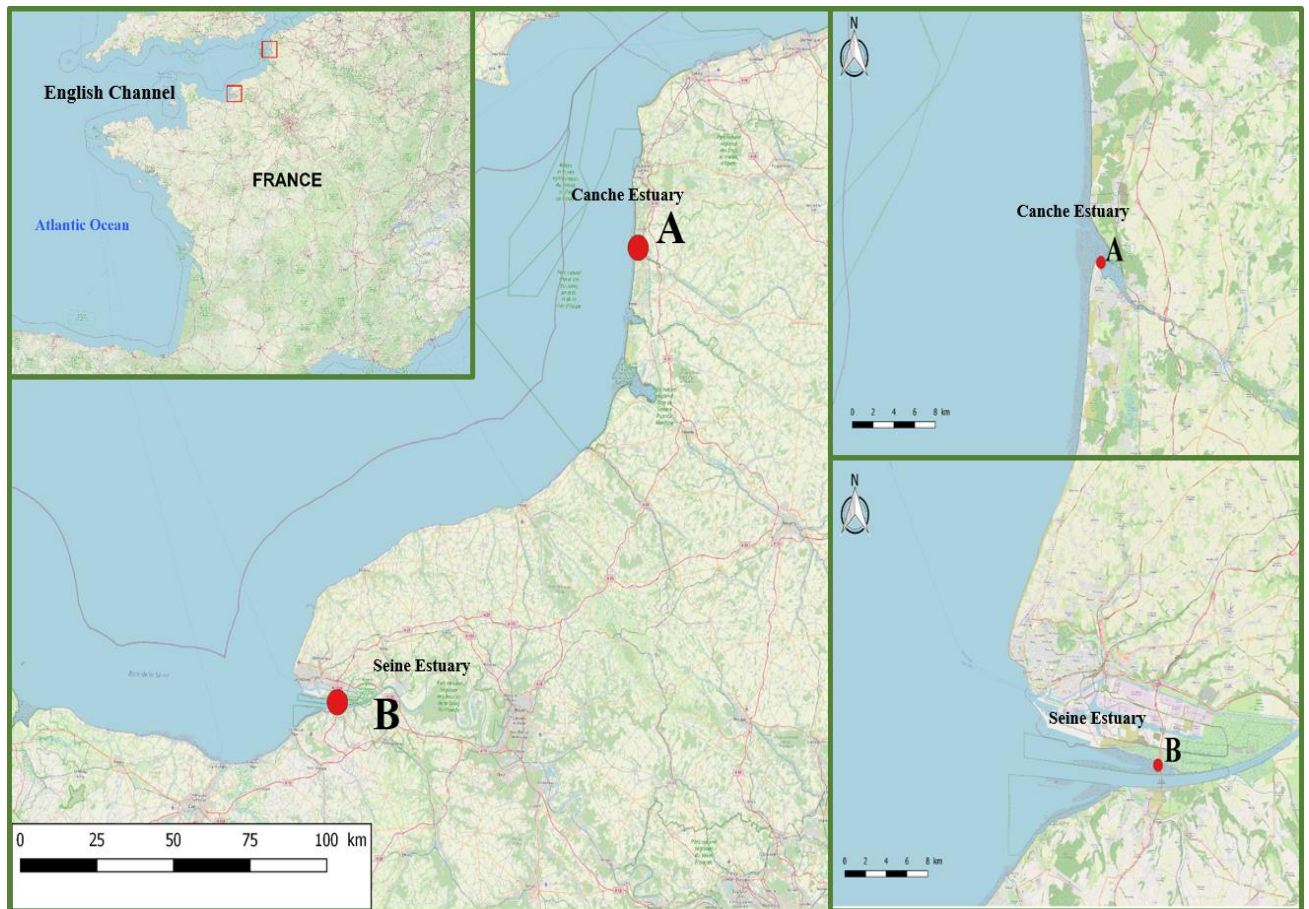
133

134 **2. Material and methods**

135 **2.1 Sediment sampling**

136 The two stations for collecting the sediment samples were chosen with an aim to
137 compare the effect of a less polluted (LPS, Fig 1. A) and polluted resuspension of
138 sediment (PS, Fig. 1B) on *E. affinis*. The Seine estuarine sediment sample was
139 collected towards the foreshore of the northern tidal-flat closer to the Normandy
140 Bridge (49°26'33.762"N; 0°16'8.475"E). On the other hand, the estuary of Canche
141 often classified as a Picard estuary located in the northwestern region of France, was
142 accounted as LPS and a reference site in this study (Henry et al., 2004, 2012;
143 Kerambrun et al., 2013). The sediment samples in Canche was collected near the
144 south shore, closer to the mouth of the estuary in the intertidal area (50°32'16.26"N;
145 1°35'34.02"E).

146 A basic grain size analysis was executed by using the Wentworth grade scale
147 after passing through the sieves of different size fractions (Das et al., 2017). The
148 Seine estuarine sediment was categorized as a very fine sand to silt (phi scale >4,
149 grain size < 0.125 mm) and the Canche estuarine sediment showed a medium sand to
150 fine sand texture (phi scale 2- 3, grain size between 0.062-0.031 mm).



152

153 **Fig. 1.** Map of the sampling area, where, A shows the Canche estuary and B shows the Seine
 154 estuary.

155 2.2 Culturing of copepod *Eurytemora affinis*

156 The culture of *Eurytemora affinis* was initiated two months before the start of the
 157 experiment in a clean 300 L culture tank for a thorough acclimation. The
 158 copepods were collected from the mass cultures of our laboratory that are
 159 maintained for about two decades. The entire culturing process for this study was
 160 maintained under controlled conditions (salinity 15 with 18⁰ C ambient
 161 temperature and a light/dark cycle of 12h: 12h) and the red alga *Rhodomonas*
 162 *salina* was used as a feed to the copepods (Souissi et al., 2016 a, b; Michalec et
 163 al., 2017; Das et al., 2020a).

164

165 **2.3 Experimental approach**

166 The exposure of copepods to different conditions - sediment resuspension from
167 LPS, PS, TM (Cd + Pb + As) and control was done in 10 L beakers (8.5 L
168 seawater, for ease of handling) to ensure a higher quantity especially for analyzing
169 the trace metal concentrations from each replicate (Zidour et al., 2019 and Dayras
170 et al., 2020). The entire exposure was held in controlled temperature ($18 \pm 2^{\circ}$ C),
171 light/dark cycle and salinity, similar to the culture conditions with constant
172 resuspension and circulation inside the beakers for a nearly symmetrical exposure
173 of the contents of each treatment to *E. affinis*. The constant resuspension was
174 obtained by air bubbles through long glass pipettes generated from an air pump.
175 Two long glass pipettes from opposite sides were inserted inside the 10L beaker
176 that reached the bottom where the sediment settled from time to time and
177 thereafter got resuspended into the water column with the air bubbling. The
178 sediment exposure and concentration (1/2 10% LC50) from LPS and PS was done
179 by following Das et al. 2020a. In the trace metal mixture, only non-essential trace
180 metals (Cd + Pb + As) were dissolved according to their respective LC 50 %,
181 when exposed to *E. affinis*. The 10% LC50% of Cd, Pb and As was 10.88 μ g/L,
182 41.31 μ g/L and 44.9 μ g/L respectively as reported by Zidour et al., 2019 and Das
183 et al., 2020a, 2022 (reported from the same mass cultures of our laboratory).

184

185 **2.3.1 Population structure of *E. affinis***

186 Before starting the exposure at T0h, the population density in our mass culture
187 was analysed and the same density of *E. affinis* was introduced in each 8.5 L
188 beaker for a period of 96h. After T96h, the 8.5 L beaker contents in each treatment

189 was homogenised thoroughly and separated into a volume of 500 ml (fixed in
190 alcohol) for counting the density and examining the stages of the copepod; and the
191 rest of the copepods in 8L were filtered for trace metal analysis (described below,
192 section 2.4). The mortality% was calculated from the difference of the population
193 density (individuals/mL) in each treatment at T96h from the T0h population
194 density of the culture. The population density was the summation of all the stages
195 (early larval stages + adults) at T0h and T96h counted and calculated from each
196 condition with 3 replicates. The mortality% was calculated by following the
197 equation 1 for all the treatments.

198 Equation 1- Mortality% (96h) in all treatments =

$$199 \quad 100 \times \frac{\textit{Population density}_{T0h} - \textit{Population density}_{\textit{treatments (T96h)}}}{\textit{Population density}_{T0h}}$$

200

201 The sex ratio (% of males) was calculated by following equation 2, which is as follows-

202 Equation 2 - Sex ratio in all treatments =

203

$$204 \quad 100 \times \frac{\textit{C5male} + \textit{adult male}}{\textit{C5 male} + \textit{C5 female} + \textit{adult male} + \textit{adult female} + \textit{ovigerous female}}$$

205

206 **2.4 Trace metal analysis**

207 The trace metal analyses for the sediment and copepod samples were performed
208 by using an inductive coupled plasma quadrupole mass spectrometer (ICP-MS)
209 (ICP-MS 7900, Agilent Technologies, United States) following the same
210 procedure described in Das et al., 2020a. Copepods (approximately 6000
211 individuals, with a dry weight of 50-60 mg on an average in 8L volume) from our
212 culture that were well acclimated for months was collected on membrane filters

213 (0.45 μm) before the start of the exposure, at time 0 hours (T0h) in order to
214 analyze the trace metal concentration. Similarly, after sampling from both the
215 stations, sediment was collected on filters and dried in order to measure the trace
216 metal concentration at T0h before the exposure. After 96 hours (T96h) of
217 exposure, samples of sediment and copepod (in 8 L) were again collected from
218 each treatment and replicate to measure the final trace metal concentration bio-
219 accumulated in sediment and exposed *E. affinis* at T96h. The trace metal
220 concentration in the dissolved trace metal mixture treatment and the residual water
221 from all the treatments were analyzed by collecting the water samples in 15ml
222 falcon tubes fixed with 15 μL of ultrapure Nitric acid (HNO_3). All the
223 measurement of elemental standards as well as the procedures for quality
224 maintenance were executed by following Das et al., 2020a, 2021.

225

226 **2.5 Statistical analysis**

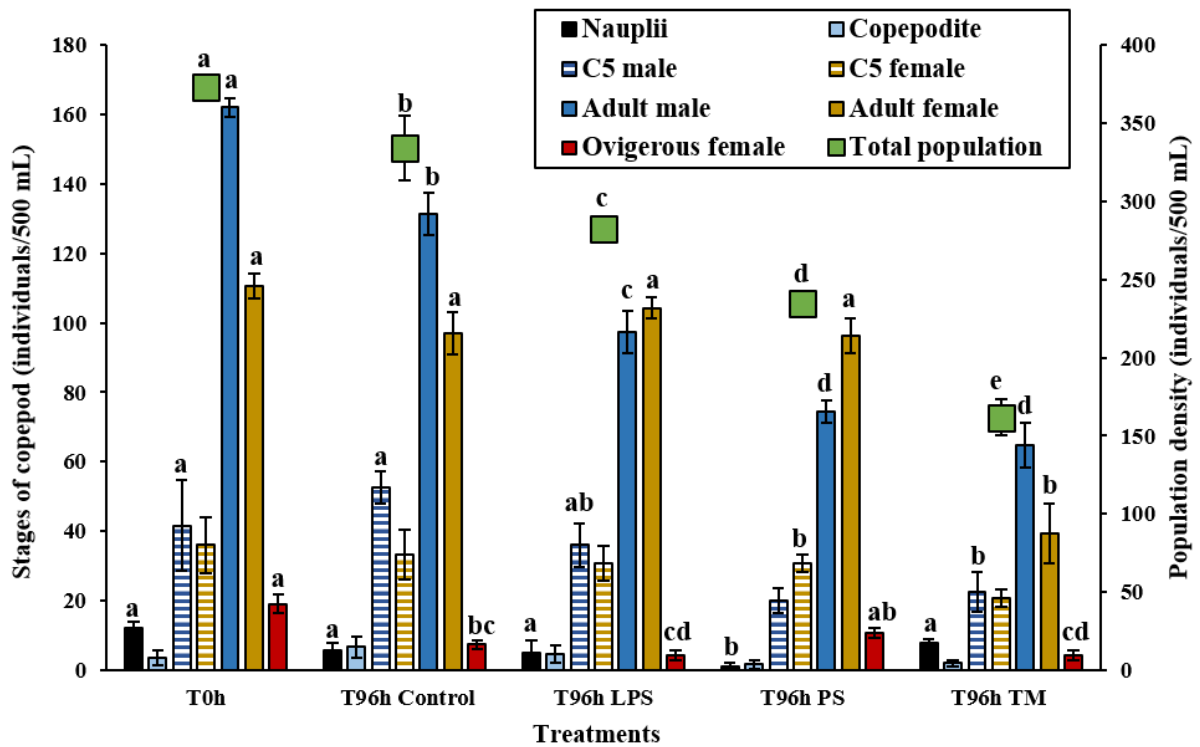
227 The statistical analyses for the comparison of all the parameters between the
228 treatments was executed by performing the analysis of variance (ANOVA) along
229 with Tukey multiple comparisons of means (family-wise confidence level of 95%
230 or less), on meeting the required criteria for the analysis. To perform ANOVA, a
231 test for normality using a Shapiro Wilk normality test was done and to test the
232 homogeneity of variance a Levene's test was done. All the analyses were executed
233 by using R studio (4.0.4). The conditions of testing ANOVA were satisfied and
234 no alternative non-parametric test was performed.

235

236 **3. Results**

237 **3.1 Population density and stages of *Eurytemora affinis***

238 The population density of *E. affinis* decreased gradually from T0h culture (373
239 individuals/500mL) > T96h control (334 individuals/500mL) > T96h LPS (281
240 individuals/500mL) > T96h PS (233 individuals/500mL) > T96h TM (158
241 individuals/500mL) significantly ($p < 0.05$) in each condition (Fig. 2). Among the
242 copepod stages, adult male [T0h (162 individuals/500mL) > T96h control (131
243 individuals/500mL) > T96h LPS (94 individuals/500mL)] in each treatment varied
244 significantly ($p < 0.05$), apart from PS (74 individuals/500mL) and TM (65
245 individuals/500mL). The C5 male showed significantly lower density in the PS
246 (20 individuals/500mL) and TM (23 individuals/500mL) treatment when
247 compared to T0h (42 individuals/500mL), T96h control (53 individuals/500mL),
248 and T96h LPS (36 individuals/500mL). The C5 female showed a significantly
249 lower no. of individuals in the TM exposure (21 individuals/500mL), but not for
250 PS (31 individuals/500mL) and / or LPS (30 individuals/500mL), as in both the
251 sediment exposed treatments, no. of adult female and C5 female increased
252 indicating a specific response due to the exposure of resuspended sediment
253 altering the sex-ratio (% of males) of the population. Another specific response
254 marking the sediment resuspension was the no. of nauplii that was lower in LPS
255 (~5 individuals/500mL) and PS (< 2 individuals/500mL) from all the other
256 treatments, however significantly lower in case of PS. Although PS treatment
257 showed lower no. of individuals in all the stages from T0h culture and T96h
258 control, only no. of ovigerous female was similar (11 individuals/500mL) and
259 significantly higher than TM (~4 individuals/500mL) and LPS (~5
260 individuals/500mL), presumably denoting a lower hatching success or a delay in
261 the embryonic development.



262

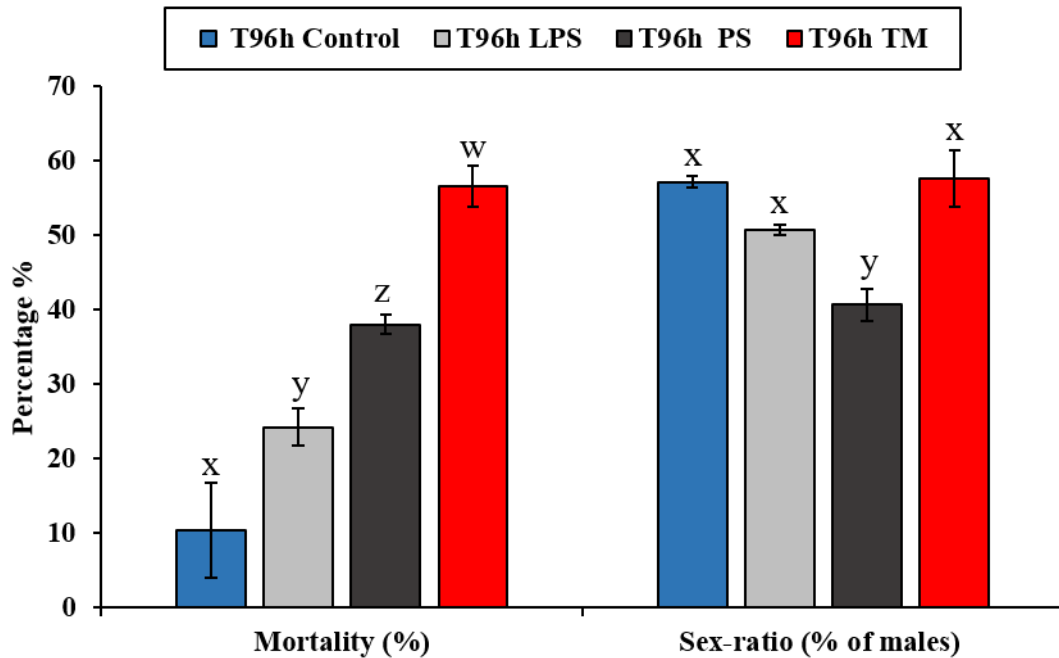
263

264 **Fig. 2.** Comparison of the stages of copepod and the total population of the copepod from
 265 culture at T0h and all the treatments [control, less polluted sediment (LPS), polluted sediment
 266 (PS) and dissolved trace metal (TM)] after 96h (T96h) of exposure. The total population is
 267 plotted on the secondary axis. The error bar shows the standard deviation between the three
 268 replicates in each treatment. The letters a, b, c, denotes the significant differences in each
 269 treatment within one stage of *E. affinis*.

270 **3.2 Mortality and sex-ratio (% of males)**

271 The mortality % in control (10.4%) was significantly lower than the other
 272 treatments (Fig.3). LPS showed the lowest mortality (24 %) within the
 273 exposed treatments (both dissolved and resuspended sediment), followed by
 274 PS (38.5%) with dissolved TM showing significantly the highest mortality
 275 (57.5 %). In the right panel of Fig.3, The PS treatment showed significantly
 276 lower sex-ratio (39.2% of males) than the other treatments. The LPS treatment

277 showed lower sex-ratio (52.73 % of males) than control (57.2%) and dissolved
 278 TM (57.6%). The sex- ratio (% of males) of *E. affinis* at T0h from the mass
 279 cultures was in the same range as the control (57.67%).

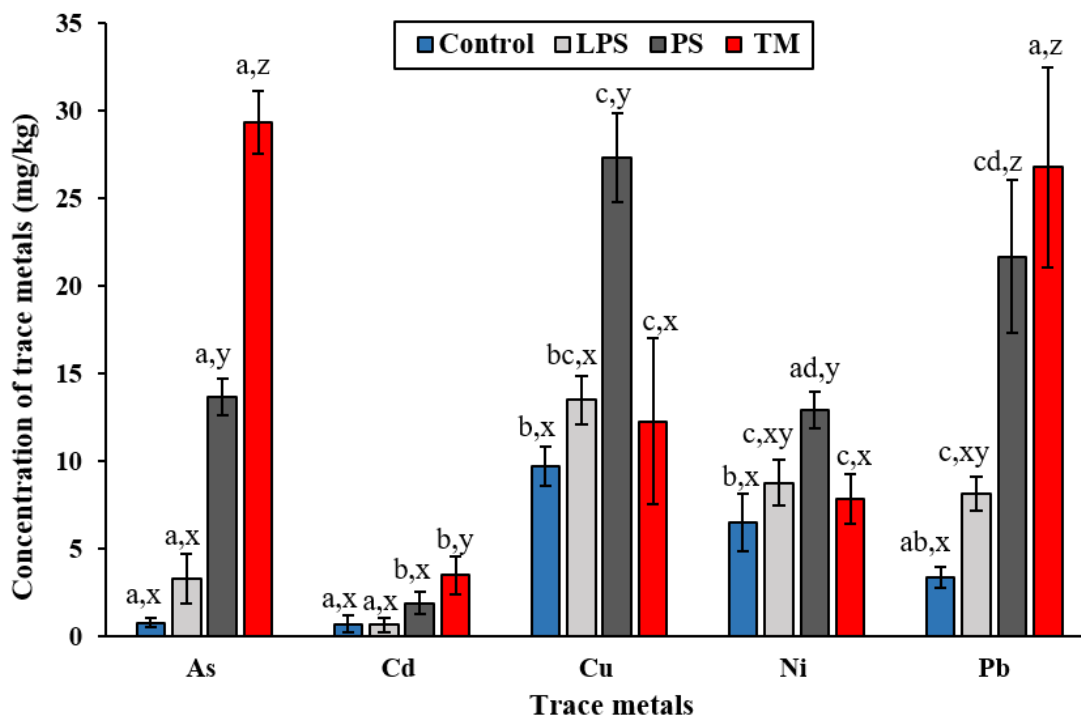


280
 281 **Fig. 3.** (Left side) showing the mortality % in all the treatments with significant differences in
 282 each treatment marked by different letters (x, y, z, w). (Right side) showing the sex-ratio (%
 283 of males) in all the treatments with significant differences in each treatment marked by
 284 different letters (x, y, z, w) at $p < 0.05$. The error bar shows the standard deviation between
 285 the three replicates in each treatment.

286 3.3 Trace metal concentration

287 The total concentration of As (29.5 mg/kg), Cd (3.4 mg/kg) and Pb (26.8 mg/kg)
 288 bioaccumulated by *E. affinis* was significantly higher in dissolved TM than in
 289 control [As (0.75 mg/kg), Cd (0.68 mg/kg), Pb (3.34 mg/kg)], LPS [As (3.26
 290 mg/kg), Cd (0.63 mg/kg), Pb (8.15 mg/kg)], and PS [As (13.7 mg/kg), Cd (1.88
 291 mg/kg), Pb (21.81 mg/kg)], however the concentration of Pb had no significant
 292 difference between PS (21.81 mg/kg) and TM (26.8 mg/kg) after 96h of exposure

293 (Fig.4). In between the two sediment exposed treatments, total As (3.26 mg/kg),
 294 Cu (13.29 mg/kg) and Pb (8.15 mg/kg) from LPS showed significantly lower
 295 values than PS [As (13.7 mg/kg), Cu (27.27 mg/kg), Pb (21.81 mg/kg)]. The
 296 concentration of Cu (12.82 mg/kg) and Ni (7.81 mg/kg) were detected in the
 297 dissolved TM (without any addition) as they play essential roles in the metabolism
 298 of organisms that was significantly lower ($p < 0.05$) than the concentrations of Cu
 299 (27.27 mg/kg) and Ni (12.8 mg/kg) found in the PS. The highest concentration of
 300 bioaccumulation was observed for As (29.5 mg/kg) from the dissolved phase
 301 (TM), followed by Pb (26.8 mg/kg), Cu (12.82 mg/kg), Ni (7.81 mg/kg) and Cd
 302 (3.4 mg/kg). On the other hand, from resuspended source of exposure (PS and
 303 LPS), highest bioaccumulation was observed for Cu followed by Ni, Pb, As and
 304 Cd.

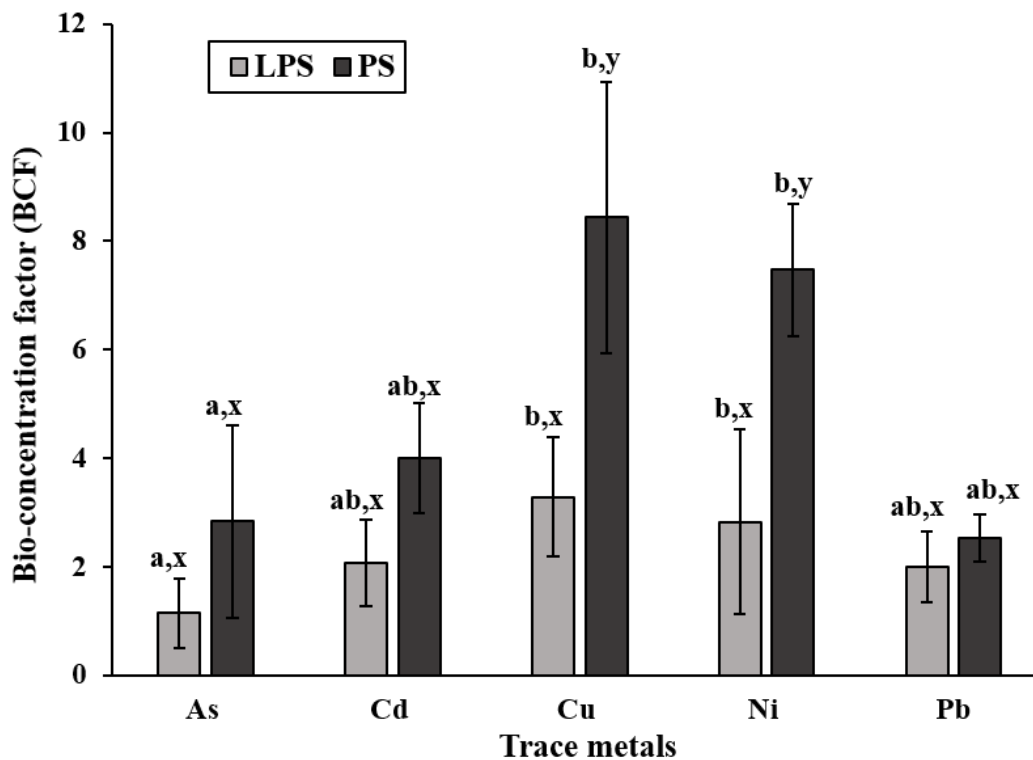


305
 306 **Fig. 4.** Trace metal concentrations bio-accumulated in *E. affinis* after 96 hours of exposure
 307 (T96h) from control, less polluted sediment (LPS), polluted sediment (PS), and dissolved

308 trace metal (TM). The error bar shows the standard deviation between the three replicates in
 309 each treatment. The letters a, b, c denote the significant difference between the trace metals in
 310 one single treatment and the letters x, y, z denote the significant differences between the
 311 treatments in a single trace metal.

312 3.4 Bioconcentration factor of trace metals

313 The BCF analyzed in *E. affinis* exposed from the sediment resuspension of PS was
 314 higher than LPS for all the trace metals, however being statistically significant in
 315 case of Ni [PS (7.21), LPS (2.83)] and Cu [PS (8.59), LPS (3.21)] (Fig. 5). The
 316 BCF of dissolved trace metal mixture is enclosed in the supplementary info (Fig.1
 317 S).



318
 319 **Fig. 5.**The bio-concentration factor (BCF) in *E. affinis* exposed to the sediment resuspension
 320 (LPS and PS) treatments. The letters a, b denote the significant differences between the trace
 321 metal in one treatment; letter x, y denote significant differences between each treatment for a

322 single trace metal, $p < 0.05$. The error bar shows the standard deviation between the three
323 replicates in each treatment.

324 **4. Discussion**

325 **4.1 Life cycle traits of *Eurytemora affinis***

326 Comparison of toxicity from different matrices remains crucial to comprehend the
327 mechanisms adapted by bioindicator organisms that are prevalent in nature.
328 *Eurytemora affinis* has conveyed many such adaptive mechanisms to toxicity
329 through its life cycle traits although mostly in studies with respect to dissolved
330 toxicity in an artificially contaminant spiked exposure (Legrand et al., 2016;
331 Kadiene et al., 2017; Kadiene et al., 2019 b; Zidour et al., 2019; Choi et al., 2021).
332 Zidour et al. (2019) reported that a combination of trace metal exposed from the
333 dissolved phase decreases the population density of *E. affinis* in a 96 hour
334 exposure. In some studies, an acute and chronic toxicological exposure of trace
335 metals from the dissolved phase and dietary pathway to copepod species like
336 *Pseudodiaptomas annandalei* and *Paracyclopsina nana* affected the population
337 growth, developmental stages with sex specific responses (males more affected
338 than females related to differentially expressed genes) highlighting the differences
339 of toxicity originating from varied pathways (Kadiene et al., 2019, 2020, Dayras
340 et al., 2020). These observations was similar to our finding, where in a 96 h of
341 exposure to dissolved trace metal mixture, the population density of *E. affinis*
342 significantly reduced with a significant increase in mortality, further affecting all
343 the stages of the copepod. Studies comparing exposure from diverse matrices like
344 sediment resuspension, sediment elutriates, dissolved phases are considerably less,
345 with only few literature reporting the effect of pollutants originating from dietary
346 pathways, whole resuspended sediment and also from elutriates of natural

347 sediments (Lesueur et al., 2013; 2015, Charry et al., 2018; Buttino et al., 2018;
348 Kadiene et al., 2019a, Das et al., 2020a).

349 In this study we found that the dissolved trace metals posed more adverse effect
350 on the population density (T96h) of *E. affinis* than the indirect exposure of
351 resuspended sediments. This observation was similar to our previous study where
352 an intergenerational exposure to trace metals from sediment resuspension (Seine
353 estuary) showed higher population density after the second generation of exposure
354 and a higher recovery in the non-exposed generation when compared to trace
355 metal exposure in dissolved form (Das et al., 2020a). Such similar observations
356 reflect on the plasticity and the quick responses that is generated by *E. affinis*
357 when exposed to resuspended anthropogenic sediment.

358 Furthermore, in some previous studies a lower number of nauplii was observed
359 when exposed to sediment bound contaminants and Perez-Landa and Simpson
360 (2011) reported higher sensitivity of nauplii to sediment exposure than dissolved
361 toxic compounds (Fleeger and Lotufo, 1999; Kovatch et al., 1999; Chandler and
362 Green, 2001; Spadaro et al., 2008; Perez-Landa and Simpson, 2011; Das et al.,
363 2020a). This study also reports a similar trend of lower no. of nauplii in the
364 sediment exposed treatments (PS, significantly lower and LPS not significantly
365 lower) in the *E. affinis* population just after 96 hours of exposure. In parallel to
366 lower no. of nauplii we observed a significantly higher no. of ovigerous females
367 remaining in the PS treatment (Seine estuary) after 96 hour of exposure, which in
368 the previous studies have been indicated as an effect of endocrine disruptors that
369 is prevalent in the Seine estuarine sediment and is often reported to cause
370 imbalances in hormones and reproduction, affecting the hatching success rate and
371 impeding the embryonic development (Spadaro et al., 2008; Cailleaud et al., 2010;

372 Perez-Landa and Simpson, 2011; Das et al., 2020a). Along with the lower no. of
373 nauplii we report that sex-ratio (% of males) was another biomarker specifically
374 observed in the resuspended sediment exposure from Seine estuary (PS) that
375 showed significantly lower sex-ratio (% of males) than that of control, and
376 dissolved trace metals. This observation was consistent in our previous study on
377 intergenerational exposure and also the present study on a 96 hour exposure,
378 which further substantiates the presence of sex steroids in the seine estuarine
379 sediment as reported in previous works (Cailleaud et al., 2007, 2009, and 2010).
380 Another hypothesis for a lower sex-ratio (% of males) in the resuspended
381 sediment exposed treatments is the sex specific mortality in *E. affinis* along with
382 the varied pathways and also gender specific bioaccumulation of trace metals as
383 reported in previous studies from our group, working with the same mass culture
384 in our laboratory (Kadiene et al., 2017, 2019a). Hence, sediment resuspension and
385 dissolved toxicity when compared based on the life cycle traits possess varied
386 responses (supplementary info Table. 3S), of which, few are specific to particular
387 pathway of contamination and *E. affinis* features its suitability to be used as an
388 indicator in such toxicological assays (Cailleaud et al., 2009, 2010; Lee et al.,
389 2018; Kadiene et al., 2019 b; Zidour et al., 2019, Das et al., 2020a).

390 We report that sediment resuspension from the reference site of Canche estuary
391 (LPS) caused lower mortality, with higher population density of *E. affinis* after 96
392 h of exposure when compared to PS. Although studies on pelagic copepod
393 comparing the effect of reference and polluted sediment resuspension is scarce, a
394 similar observation was noted in a population of juvenile European flounder,
395 where Canche estuary comprised of healthier individuals than Seine, Scheldt and
396 Loire estuaries analyzed based on Fulton's K condition index (Suthers, 2000;

397 Kerambrun et al., 2013). In addition, a study comparing the influence of coastal
398 pollution in the Seine and Authie estuaries (Authie estuary is situated in the same
399 coast as Canche, approximately 20 km south) when exposed to a benthic indicator,
400 *Hediste diversicolor*, reported that due to the higher pollution in the Seine with
401 reference to chemical contaminants, the population of *H. diversicolor* was
402 significantly lower than that of the Authie estuary (Gillet et al., 2008). In this
403 study, we also highlight the difference in sediment grain sizes in Canche estuary
404 (medium to fine sand) and the Seine (very fine sand to silt) with reference to the
405 sediment toxicity. Previous studies reported that finer sediments often tend to
406 adsorb higher amount of chemical contaminants like trace metals, organic
407 hydrophobic compounds, etc., than coarse sediment particles (Mao et al., 2019;
408 Das et al., 2020b; Vane et al., 2020). Our study also reports the same observation
409 where the Seine estuarine sediment comprised of finer grain sizes with higher
410 amount of trace metals than the Canche estuary characterizing a sandy grain size
411 with lower aggregates of contaminants. The above observations further highlight
412 the need for the comparison of numerous matrices of toxicity and the
413 consideration of different levels of contamination existing in several estuaries for
414 developing new tools to evaluate the health of the ecosystem.

415

416 **4.2 Bioaccumulation of trace metals**

417 The bioaccumulation of trace metals in *E. affinis* has been studied from the
418 sediment resuspended toxicity (LPS and PS), from the dissolved toxicity, and
419 control treatment at 0h and after 96h of exposure. Our results show that Seine
420 estuarine sediment [As (10.49 mg/kg), Ba (16.1 mg/kg), Mn (45.2 mg/kg), Cu
421 (13.83 mg/kg), Zn (56.1 mg/kg)] including the seawater analysed after

422 resuspension, comprised of higher trace metals than Canche sediment [As (2.48
423 mg/kg), Ba (9.9 mg/kg), Mn (15.8 mg/kg), Cu (5.62 mg/kg), Zn (25.7 mg/kg)]
424 (Table 1S, and Table 2S, supplementary info) as reported in previous studies
425 comparing the two sites (Gilliers et al., 2006; Amara et al., 2007; Kerambrun et
426 al., 2013; Francescangeli et al., 2020). Meybeck et al. (2007) widely discussed the
427 significance of metal contamination in the Seine estuary from 1930 that posed a
428 notable concern for the prevalence of high concentration of toxic compounds for
429 decades. In 2013, another study on the trace metal concentration of Seine estuary
430 reported that none of the metals detected had surpassed the ERL (Effect Range
431 Low) guideline other than mercury (Hg) (Kerambrun et al., 2013). Our study
432 reports a similar range of trace metal concentration reported in previous works, for
433 both the Canche and Seine estuarine sediment (Negrel, 1997; Gilliers et al., 2006;
434 Amara et al., 2007; Kerambrun et al., 2013). A recent article on comparison of
435 coastal pollution in different harbours and estuaries in Northern France, reports a
436 high total organic carbon (1- 3.5) % and no significant trace metal contamination
437 (analysed using enrichment factor (EF <3 for Co, Cu, Fe, Mn, Vn and Zn)
438 prevailing in the sediment at the Canche estuary (Birth, 2003; Francescangeli et
439 al., 2020). Similar to the above finding we report that exposure to Canche
440 estuarine sediment caused a significantly lower mortality of *E. affinis* than those
441 exposed to Seine estuarine sediment, even when the exposed sediment
442 concentration were same for both the estuaries. In comparison between the three
443 exposed treatments (LPS, PS and TM), dissolved TM treatment showed
444 significantly higher As (29.5 mg/kg) and Cd (3.4 mg/kg) concentration
445 bioaccumulated by *E. affinis*, when compared to other treatments, reflecting on the
446 higher adversity from dissolved toxicity in comparison to indirect exposure of

447 trace metals resuspended through sediment exposure. This phenomena was similar
448 with our previous findings along with some other studies that focused on various
449 matrices of contamination, reporting higher ability of bioaccumulation of
450 contaminants from dissolved phase when compared to dietary pathways or
451 sediment resuspension (Kadiene et al., 2019a, b; Das et al., 2020a; Dayras et al.,
452 2020). The inter-site comparison of sediment exposure (LPS and PS), showed
453 significantly higher accumulation of As (13.7 mg/kg), Cu (27.2 mg/kg) and Pb
454 (21.8 mg/kg) from PS sediment when compared to the LPS sediment [As (3.26
455 mg/kg), Cu (13.29 mg/kg), Pb (8.15 mg/kg)]. Pb (21.8 mg/kg) and Ni (12.8 mg/kg)
456 accumulation from the Seine estuary almost reached the range of 10% LC50
457 [sublethal concentration; Pb (10%LC50% = 41.3 mg/kg); Ni (10%LC50% = 12.55
458 mg/kg)] of *E. affinis* marking the negative impact of higher prevalence of trace
459 metals in the Seine sediment column, and the chances of their uptake by nearby
460 organisms through resuspension causing significantly higher mortality than low
461 polluted quarters (like, LPS). In addition, the Cu accumulation by *E. affinis* from
462 the PS sediment exposure was even more toxic than Pb and Ni, as it reached the
463 similar range of the lethal concentration (LC 50% Cu- 33.5 µg/L) of *E. affinis* in
464 just 96 hours of exposure (Zidour et al., 2019). Similar to our results, in a
465 comparison with the nearby tidal flats, Scheldt estuary was reported to contain
466 varied trace metals (Ag, As, Cd, Cr Co, Zn, and many others) in concentrations
467 similar to the range present in the Seine and the Loire estuaries, further reporting a
468 high risk of Arsenic in the edible seafood like shrimps (Kerambrun et al., 2013;
469 Van Ael et al., 2017). Likewise, our study highlights the capacity of *E. affinis* to
470 significantly bioaccumulate trace metals like Cu, Pb, As, and Ni in concentrations
471 that are sub lethal to lethal from the resuspension of Seine estuarine sediment

472 indicating the chances of biomagnification of trace metals in higher trophic
473 organisms. Van Ael et al. (2017) further discussed about the importance of
474 studying the bioavailability of trace metals from sediment column and
475 bioaccumulation by inhabiting organisms along with the importance of
476 considering several matrices of contamination that helps us to better assess the
477 aspects concerning pollution of a particular area (Culioli et al., 2009; Van
478 Campenhout et al., 2009). In this study we considered the bioaccumulation along
479 with the bioconcentration factor of trace metals by *E. affinis* that was reported to
480 be significantly higher from the dissolved combined TM treatment (> 2000) than
481 the resuspended sediment exposure, similar to our previous study (Das et al.,
482 2020a). The sediment exposure to *E. affinis* showed some specific markers in this
483 study in just a 96h exposure, although most significant indicators were found in
484 case of Seine sediment exposure than that of Canche sediment. This observation
485 was due to the contaminants that are prevalent in the Seine sediment, and previous
486 works have reported the presence of a range of organic pollutants, endocrine
487 disruptors, sex steroids apart from the trace metals that implied certain significant
488 responses in the Seine sediment resuspension treatment like the lower no. of
489 nauplii, shift in the sex-ratio with less % of male with respect to females,
490 population density along with higher mortality of *E. affinis* in this study
491 (Cailleaud et al., 2007, 2009, 2010; Kinani et al., 2010; Gerbron et al., 2014; Das
492 et al., 2020a; Arcanjo et al., 2021). On the other hand, the exposure of the Canche
493 sediment did not significantly affect the sex-ratio (% of males) and the no. of
494 nauplii in *E. affinis*, presumably due to the lower concentration of contaminants in
495 this estuary as reported in previous studies (Evrard et al., 2010; Kerambrun et al.,
496 2013; Van Ael et al., 2017; Francescangeli et al., 2020). The responses from the

497 different matrices of toxic exposure has been documented in a table (Table. 3S) in
498 the supplementary info, listing all the treatments and parameters analyzed in this
499 study.

500

501 **5. Conclusion**

502 This study shows the responses of *E. affinis* in a 96h exposure from different
503 matrices of contamination. In a 96-hour assay, the population of *E. affinis* showed
504 38% higher mortality from the Seine sediment (PS) when compared to the low
505 polluted site (LPS, Canche estuary). Apart from significantly higher mortality%,
506 the population of *E. affinis* exposed to PS had lower no. of nauplii and higher no.
507 of ovigerous female, reflecting a low hatching success and delayed embryonic
508 development, due to the presence of a wide variety of contaminants in the Seine.
509 The exposure to Seine sediment also significantly altered the sex-ratio (% of
510 males) when compared to other treatments like the low polluted sediment
511 resuspension and the dissolved toxicity.

512 The dissolved non-essential trace metal treatment (As + Cd + Pb) induced the
513 highest mortality and the bioconcentration factor. Although the trace metals
514 mixture treatment showed a higher bioaccumulation in *E. affinis*, trace metals
515 accumulated by *E. affinis* from the Seine sediment almost reached similar values
516 for the concentration of Pb. The most remarkable aspect is the ability of *E. affinis*
517 to bioaccumulate trace metals (Cu, Ni, As) in just 96 hours reaching
518 concentrations that are lethal to sub lethal from resuspension of sediment and bio-
519 indicating certain responses that corroborate its role as a Sentinel to sediment
520 resuspension. The specific indicators from the sediment resuspension can further
521 be utilised in other studies for a deeper understanding of the stress in

522 anthropogenic domains dominated with high resuspension of sediments, naturally
523 or due to human activities like dredging and coastal fabrications for human
524 motive. This study provides important tools to compare toxicity of sediment
525 resuspension from different estuaries all around the globe with varying levels of
526 contamination.

527

528 **Acknowledgement**

529 We acknowledge the project SARTRE, GIP Seine Aval, for financial support. This study is a
530 contribution to the International Associated Laboratory between Université de Lille and
531 National Taiwan Ocean University (IAL MULTIFAQUA). The study is a contribution to the
532 COPEFISH project and long-term partnership between the Université de Lille and the
533 Aquarium Nausicaa in Boulogne sur Mer. We especially thank Dr. Emilie Moisez and Mister
534 Camille Hennion for sampling the sediment from the Canche estuary. We also thank Miss
535 Capucine Bialais for the technical assistance and maintenance of algae and copepod cultures
536 in the laboratory. Our gratitude to all the past and present members of S. Souissi's group for
537 nurturing microalgae and copepod cultures for two decades. We are grateful to the
538 Communauté d'Agglomération du Boulonnais (CAB) for the implementation of a copepod-
539 rearing pilot project (agreement 'HALIOCAP' Université de Lille-CAB). This study
540 acknowledges the French GDR "Aquatic Ecotoxicology" for stimulating scientific exchanges
541 and collaborations on various toxicological assays and the Chevreul Institute for the trace
542 metals analysis.

543

544 **Reference**

545 Allen, G.P., Salomon, J.C., Bassoulet, P., Du Penhoat, Y., De Grandpre, G., 1980. Effects of
546 tides on mixing and suspended sediment transport in macrotidal estuaries. *Sediment. Geol.*
547 26, 69–90.
548 [https://doi.org/10.1016/0037-0738\(80\)90006-8](https://doi.org/10.1016/0037-0738(80)90006-8)

549
550 Amara, R., Meziane, T., Gilliers, C., Hermel, G., Laffargue, P., 2007. Growth and condition
551 indices in juvenile sole *Solea solea* measured to assess the quality of essential fish habitat.
552 Mar. Ecol. Prog. Ser. 351, 201–208.
553 <https://doi.org/10.3354/meps07154>
554
555 Arcanjo, C., Trémolet, G., Giusti-Petrucciani, N., Duflot, A., Forget-Leray, J., Boulangé-
556 Lecomte, C., 2021. Susceptibility of the Non-Targeted Crustacean *Eurytemora affinis* to the
557 Endocrine Disruptor Tebufenozide: A Transcriptomic Approach. Genes, 12(10), 1484.
558 <https://doi.org/10.3390/genes12101484>
559
560 Avoine, J., Allen, G.P., Nichols, M., Salomon, J.C., Larssonneur, C., 1981. Suspended
561 sediment transport in the Seine estuary, France: effect of man-made modifications on estuary-
562 shelf sedimentology. Mar. Geol. 40, 119–137.
563 [https://doi.org/10.1016/0025-3227\(81\)90046-3](https://doi.org/10.1016/0025-3227(81)90046-3)
564
565 Birth, G., 2003. A scheme for assessing human impacts on coastal aquatic environments
566 using sediments. In: Woodcoffe, C.D., Furness, R.A. (Eds.), Coastal GIS 2003. Wollongong
567 University Papers in Centre for Maritime Policy, 14, Wollongong.
568
569 Borcier, E., Artigaud, S., Gaillard, J.C., Armengaud J., Charrier, G., Couteau, J., Receveur, J.,
570 Ouddane, B., Diop, M., Amara, R., Laroche, J., Pichereau, V., 2019. Coupling caging and
571 proteomics on the European flounder (*Platichthys flesus*) to assess the estuarine water quality
572 at micro scale. Science of the Total Environment, 695, 133760.
573 <https://doi.org/10.1016/j.scitotenv.2019.133760>
574
575 Borcier, E., Charrier, G., Couteau, J., Maillet, G., Le Grand, F., Bideau, A., Waeles, M., Le
576 Floch, S., Amara, R., Pichereau, V., Laroche, J., 2020. An Integrated Biomarker Approach
577 Using Flounder to Improve Chemical Risk Assessments in the Heavily Polluted Seine
578 Estuary. Journal of Xenobiotics, 10 (2), 14-35.
579 <https://doi.org/10.3390/jox10020004>
580
581 Buttino, I., Vitiello, V., Macchia, S., Scuderi, A., Pellegrini, D., 2018. Larval development
582 ratio test with the calanoid copepod *Acartia tonsa* as a new bioassay to assess marine
583 sediment quality. Ecotoxicology and environmental safety, 149, 1- 9.
584 <https://doi.org/10.1016/j.ecoenv.2017.10.062>
585
586 Cailleaud, K., Forget-Leray, J., Souissi, S., Hilde, D., Lemenach, K., Budzinski, H., 2007.
587 Seasonal variations of hydrophobic organic contaminant concentrations in the water column
588 of the Seine Estuary and their transfer on a planktonic species *Eurytemora affinis* (Calanoïda,
589 copepoda). Part1: PCBs and PAHs. Chemosphere 70 (2), 270-280.
590 <https://doi.org/10.1016/j.chemosphere.2007.05.095>
591
592 Cailleaud, K., Forget-Leray, J., Peluhet, L., Lemenach, K., Souissi, S., Budzinski, H., 2009.
593 Tidal influence on the distribution of hydrophobic organic contaminants in the Seine Estuary
594 and biomarker responses on the copepod *Eurytemora affinis*. Environ. Pollut. 157, 64-71.
595 <https://doi.org/10.1016/j.envpol.2008.07.026>
596
597 Cailleaud, K., Budzinski, H., Lardy, S., Augagneur, S., Barka, S., Souissi, S., Forget- Leray,
598 J., 2010. Uptake and elimination, and effect of estrogen-like contaminants in estuarine

599 copepods: an experimental study. Environ. Sci. Pollut. Res. 18, 226-236.
600 <https://doi.org/10.1007/s11356-010-0355-6>.
601
602 Chandler, G.T., Green, A.S., 2001. Developmental stage-specific life-cycle bioassay for
603 assessment of sediment-associated toxicant effects on benthic copepod production. Environ.
604 Toxicol. Chem. 20, 171-178.
605 <https://doi.org/10.1002/etc.5620200119>.
606
607 Charry, M. P., Keesing, V., Costello, M., Tremblay, L. A., 2018. Assessment of the
608 ecotoxicity of urban estuarine sediment using benthic and pelagic copepod bioassays. PeerJ, 6
609 (e4936).
610 <https://doi.org/10.7717/peerj.4936>
611
612 Chiarelli, R., Martino, C., & Roccheri, M. C., 2019. Cadmium stress effects indicating marine
613 pollution in different species of sea urchin employed as environmental bioindicators. Cell
614 Stress and Chaperones, 24(4), 675-687.
615 <https://doi.org/10.1007/s12192-019-01010-1>
616
617 Choi, B. S., Kim, D. H., Kim, M. S., Park, J. C., Lee, Y. H., Kim, H. J., et al., 2021. The
618 genome of the European estuarine calanoid copepod *Eurytemora affinis*: Potential use in
619 molecular ecotoxicology. Marine Pollution Bulletin, 166, 112190.
620 <https://doi.org/10.1016/j.marpolbul.2021.112190>
621
622 Culioli, J.-L., Fouquoire, A., Celendini, S., Mori, C., Orsini, A., 2009. Trophic transfer of
623 arsenic and antimony in a freshwater ecosystem. Aquat. Toxicol. 94, 286-293.
624 <https://doi.org/10.1016/j.aquatox.2009.07.016>
625
626 Das, S., Tseng, L.-C., Wang, L., Hwang, J.-S., 2017. Burrow characteristics of the mud
627 shrimp *Austinoegbia edulis*, an ecological engineer causing sediment modification of a tidal
628 flat. PLoS ONE 12(12) : e0187647.
629 <https://doi.org/10.1371/journal.pone.0187647>
630
631 Das, S., Tseng, L.C., Chou, C., Wang, L., Souissi, S., Hwang, J.S., 2019. Effects of cadmium
632 exposure on antioxidant enzymes and histological changes in the mud shrimp *Austinoegbia*
633 *edulis* (Crustacea: Decapoda). Environ. Sci. Pollut. Res. Int. 26 (8), 7752-7762.
634 <https://doi.org/10.1007/s11356-018-04113-x>.
635
636 Das, S., Ouddane, B., Hwang, J.S., Souissi, S., 2020a. Intergenerational effects of
637 resuspended sediment and trace metal mixtures on life cycle traits of a pelagic copepod.
638 Environ. Pollut. 267, 115460.
639 <https://doi.org/10.1016/j.envpol.2020.115460>
640
641 Das, S., Aria A.H., Cheng, J.O., Souissi, S., Hwang, J.S., Ko, F.C., 2020b. Occurrence and
642 distribution of anthropogenic persistent organic pollutants in coastal sediments and mud
643 shrimps from the wetland of central Taiwan, PloS One, 15, 1–17.
644 <https://doi.org/10.1371/journal.pone.0227367>
645
646 Das, S., Gevaert, F., Ouddane, B., Duong, G., & Souissi, S., 2021. Single toxicity of arsenic
647 and combined trace metal exposure to a microalga of ecological and commercial interest:
648 *Diacronema lutheri*. Chemosphere, 132949.

649 <https://doi.org/10.1016/j.chemosphere.2021.132949>
650

651 Dauvin, J.C., 2008. Effects of heavy metal contamination on the macrobenthic fauna in
652 estuaries: the case of the Seine estuary. *Mar. Pollut. Bull.* 57 (1e5), 160-169.
653 <https://doi.org/10.1016/j.marpolbul.2007.10.012>
654

655 Dayras, P., Bialais, C., Ouddane, B., Lee, J.-S., Souissi, S., 2020. Effects of different routes
656 of exposure to metals on bioaccumulation and population growth of the cyclopoid copepod
657 *Paracyclops nana*. *Chemosphere*, 248, 125926.
658 <https://doi.org/10.1016/j.chemosphere.2020.125926>
659

660 Deloffre, J., Lafite, R., Lesueur, P., Lesourd, S., Verney, R., & Guézennec, L., 2005.
661 Sedimentary processes on an intertidal mudflat in the upper macrotidal Seine estuary, France.
662 *Estuarine, Coastal and Shelf Science*, 64(4), 710–720.
663 <https://doi.org/10.1016/j.ecss.2005.04.004>
664

665 Deloffre, J., Lafite, R., Lesueur, P., Verney, R., Lesourd, S., Cuvilliez, A., Taylor, J., 2006.
666 Controlling factors of rhythmic sedimentation processes on an intertidal estuarine mudflat—
667 role of the turbidity maximum in the macrotidal Seine estuary, France. *Marine*
668 *Geology*, 235(1-4), 151-164.
669 <https://doi.org/10.1016/j.margeo.2006.10.011>
670

671 Deloffre, J., Verney, R., Lafite, R., Lesueur, P., Lesourd, S., & Cundy, A. B., 2007.
672 Sedimentation on intertidal mudflats in the lower part of macrotidal estuaries: Sedimentation
673 rhythms and their preservation. *Marine Geology*, 241(1–4), 19–32.
674 <https://doi.org/10.1016/j.margeo.2007.02.011>
675

676 Evrard, E., Devaux, A., Bony, S., Burgeot, T., Riso, R., Budzinski, H., Le Du, M., Quiniou,
677 L., Laroche, J., 2010. Responses of the European flounder *Platichthys flesus*, to the chemical
678 stress in estuaries: load of contaminants, gene expression, cellular impact and growth rate.
679 *Biomarkers* 15 (2), 111–127.
680 <https://doi.org/10.3109/13547500903315598>
681

682 Farias, D. R., Hurd, C. L., Eriksen, R. S., & Macleod, C. K. (2018). Macrophytes as
683 bioindicators of heavy metal pollution in estuarine and coastal environments. *Marine*
684 *Pollution Bulletin*, 128, 175-184.
685 <https://doi.org/10.1016/j.marpolbul.2018.01.023>
686

687 Fleeger, J.W., Lotufo, G.R., 1990. Development and Application of a Sublethal Toxicity
688 Test to PAH Using Marine Harpacticoid Copepods. Final Report (United States:
689 Web).
690

691 Francescangeli, F., Quijada, M., Du Châtelet, E. A., Frontalini, F., Trentesaux, A., Billon, G.,
692 & Bouchet, V. M. P., 2020. Multidisciplinary study to monitor consequences of pollution on
693 intertidal benthic ecosystems (Hauts de France, English Channel, France): Comparison with
694 natural areas. *Marine Environmental Research*, 160, 105034.
695 <https://doi.org/10.1016/j.marenvres.2020.105034>
696

697 Gerbron, M., Geraudie, P., Fernandes, D., Rotchell, J. M., Porte, C., & Minier, C., 2014.
698 Evidence of altered fertility in female roach (*Rutilus rutilus*) from the River Seine
699 (France). *Environmental pollution*, 191, 58-62.
700 <https://doi.org/10.1016/j.envpol.2014.04.015>
701

702 Gillet, P., Mouloud, M., Durou, C., & Deutsch, B., 2008. Response of *Nereis diversicolor*
703 population (Polychaeta, Nereididae) to the pollution impact–Authie and Seine estuaries
704 (France). *Estuarine, Coastal and Shelf Science*, 76(2), 201-210.
705 <https://doi.org/10.1016/j.ecss.2007.07.004>
706

707 Gilliers, C., Le Pape, O., Desaunay, Y., Morin, J., Guérault, D., & Amara, R., 2006. Are
708 growth and density quantitative indicators of essential fish habitat quality? An application to
709 the common sole *Solea solea* nursery grounds. *Estuarine, Coastal and Shelf Science*, 69(1-2),
710 96-106.
711 <https://doi.org/10.1016/j.ecss.2006.02.006>
712

713 Grasso, F., Verney, R., Le Hir, P., Thouvenin, B., Schulz, E., Kervella, Y., Khojasteh Pour
714 Fard, I., Lemoine, J. P., Dumas, F., Garnier, V., 2018. Suspended sediment dynamics in the
715 macrotidal Seine Estuary (France): 1. Numerical modelling of turbidity maximum
716 dynamics. *Journal of Geophysical Research: Oceans*, 123(1), 558-577.
717

718 Henry, F., Amara, R., Courcot, L., Lacouture, D., Bertho, M. L., 2004. Heavy metals in four
719 fish species from the French coast of the Eastern English Channel and Southern Bight of the
720 North Sea. *Environment International*, 30 (5), 675-683.
721 <https://doi.org/10.1016/j.envint.2003.12.007>

722 Henry, F., Filipuci, I., Billon, G., Courcot, L., Kerambrun, E., & Amara, R., 2012. Metal
723 concentrations, growth and condition indices in European juvenile flounder (*Platichthys*
724 *flesus*) relative to sediment contamination levels in four Eastern English Channel
725 estuaries. *Journal of environmental monitoring*, 14(12), 3211-3219.
726 <https://doi.org/10.1039/C2EM30765K>
727

728 Kadiene, E.U., Bialais, C., Ouddane, B., Hwang, J.S., Souissi, S., 2017. Differences in lethal
729 response between male and female calanoid copepods and life cycle traits to cadmium
730 toxicity. *Ecotoxicology* 26 (9), 1227-1239.
731 <https://doi.org/10.1007/s10646-017-1848-6>
732

733 Kadiene, E.U., Meng, P.-J., Hwang, J.-S., Souissi, S., 2019a. Acute and chronic toxicity of
734 Cadmium on the copepod *Pseudodiaptomus annandalei*: a life history traits approach.
735 *Chemosphere* 233, 396-404.
736 <https://doi.org/10.1016/j.chemosphere.2019.05.220>
737

738 Kadiene, E.U., Ouddane, B., Hwang, J.S., Souissi, S., 2019b. Bioaccumulation of metals in
739 calanoid copepods by oral intake. *Sci. Rep.* 9, 9492.
740 <https://doi.org/10.1038/s41598-019-45987-2>
741

742 Kadiene, E.U., Ouddane, B., Gong, H.Y., Kim, M.S., Lee, J.S., Pan, Y.J., Hwang, J.S.,
743 Souissi, S., 2020. Differential gene expression profile of male and female copepods in
744 response to cadmium exposure. *Ecotoxicol. Environ. Saf.* 204, 111048.
745 <https://doi.org/10.1016/j.ecoenv.2020.111048>.

746
747 Kerambrun, E., Henry, F., Cornille, V., Courcot, L., Amara, R., 2013. A combined
748 measurement of metal bioaccumulation and condition indices in juvenile European flounder,
749 *Platichthys flesus*, from European estuaries. *Chemosphere*, 91(4), 498-505.
750 <https://doi.org/10.1016/j.chemosphere.2012.12.010>
751
752 Kinani, S., Bouchonnet, S., Creusot, N., Bourcier, S., Balaguer, P., Porcher, J. M., & Aït
753 Aïssa, S., 2010. Bioanalytical characterisation of multiple endocrine-and dioxin-like activities
754 in sediments from reference and impacted small rivers. *Environmental Pollution*, 158(1), 74
755 83.
756 <https://doi.org/10.1016/j.envpol.2009.07.041>
757
758 Kovatch, C.E., Chandler, G.T., Coull, B.C., 1999. Utility of a full life-cycle copepod bioassay
759 approach for assessment of sediment-associated contaminant mixtures. *Mar. Pollut. Bull.* 38,
760 692-701.
761 [https://doi.org/10.1016/S0025-326X\(99\)00029-6](https://doi.org/10.1016/S0025-326X(99)00029-6).
762
763 Kwok, K.W., Souissi, S., Dur, G., Won, E.J., Lee, J.S., 2015. Copepods as references species
764 in estuarine and marine waters. In: AmiardTriquet C, Amiard JC, Mouneyrac C (eds) *Aquatic*
765 *ecotoxicology: advancing tools for dealing with emerging risks*. London: Academic Press, p
766 281 –308.
767 <https://doi.org/10.1016/B978-0-12-800949-9.00012-7>
768
769 Lafite, R., & Romaña, L. A. (2001). A man-altered macrotidal estuary: the Seine estuary
770 (France): introduction to the special issue. *Estuaries and Coasts*, 24(6), 939.
771
772 Le Hir, P., Ficht, A., Da Silva Jacinto, R., Lesueur, P., Dupont, J.P., Lafite, R., Brenon, I.,
773 Thouvenin, B., Cugier, P., 2001. Fine sedimentation transport and accumulations at the
774 mouth of the Seine Estuary (France). *Estuaries* 24 (6B), 950–963.
775 <https://doi.org/10.2307/1353009>
776
777 Lee, B. Y., Lee, M. C., Jeong, C. B., Kim, H. J., Hagiwara, A., Souissi, S., et al., 2018. RNA-
778 Seq-based transcriptome profiling and expression of 16 cytochrome P450 genes in the benzo
779 [α] pyrene-exposed estuarine copepod *Eurytemora affinis*. *Comparative Biochemistry and*
780 *Physiology Part D: Genomics and Proteomics*, 28, 142-150.
781 <https://doi.org/10.1016/j.cbd.2018.08.002>
782
783 Legrand, E., Forget-Leray, J., Duflot, A., Olivier, S., Thomé, J. P., Danger, J. M., &
784 Boulangé-Lecomte, C., 2016. Transcriptome analysis of the copepod *Eurytemora affinis* upon
785 exposure to endocrine disruptor pesticides: focus on reproduction and development. *Aquatic*
786 *toxicology*, 176, 64-75.
787 <https://doi.org/10.1016/j.aquatox.2016.04.010>
788
789 Lesueur, T., Boulangé-Lecomte, C., Xuereb, B., Budzinski, H., Cachot, J., Vicquelin, L., et
790 al., 2013. Development of a larval bioassay using the calanoid copepod, *Eurytemora affinis* to
791 assess the toxicity of sediment-bound pollutants. *Ecotoxicology and environmental*
792 *safety*, 94, 60-66.
793 <https://doi.org/10.1016/j.ecoenv.2013.04.025>

794 Lesueur, T., Boulangé-Lecomte, C., Restoux, G., Deloffre, J., Xuereb, B., Le Menach, K., et
795 al., 2015. Toxicity of sediment-bound pollutants in the Seine estuary, France, using a
796 *Eurytemora affinis* larval bioassay. *Ecotoxicology and environmental safety*, 113, 169-175.
797 <https://doi.org/10.1016/j.ecoenv.2014.11.033>
798

799 Mao, L., Ye, H., Li, F. *et al.* 2019. Enrichment assessment of Sb and trace metals in
800 sediments with significant variability of background concentration in detailed scale. *Environ*
801 *Sci Pollut Res* 26, 2794–2805.
802 <https://doi.org/10.1007/s11356-018-3836-7>
803

804 Meybeck, M., Lestel, L., Bonte, P., Moilleron, R., Colin, J.-L., Rousselot, D., de Ponteves,
805 C., Grosbois, C., Thevenot, D.R., 2007. Historical perspective of heavy metals contamination
806 (Cd, Cr, Cu, Hg, Pb, Zn) in the Seine River basin (France) following a DPSIR approach
807 (1050–2005). *Sci. Total Environ.* 375, 204–231.
808 <https://doi.org/10.1016/j.scitotenv.2006.12.017>
809

810 Michalec, F. G., Fouxon, I., Souissi, S., & Holzner, M., 2017. Zooplankton can actively
811 adjust their motility to turbulent flow. *Proceedings of the National Academy of*
812 *Sciences*, 114(52), E11199-E11207.
813 <https://doi.org/10.1073/pnas.1708888114>
814

815 Minier, C., Abarnou, A., Jaouen-Madoulet, A., Le Guellec, A.M., Tutundjan, R., Bocquené,
816 G., Leboulenger, F., 2006. A pollution monitoring pilot study involving contaminant and
817 biomarker measurements in the Seine estuary, France, using Zebra Mussels (*Dreissena*
818 *polymorpha*). *Environ. Toxicol. Chem.* 25, 112-119.
819 <https://doi.org/10.1897/05-161R.1>
820

836 Mitchell, S. B., and Uncles, R. J., 2013. Estuarine sediments in macrotidal estuaries: Future
837 research requirements and management challenges. *Ocean & Coastal Management*, 79, 97-
838 100
839 <https://doi.org/10.1016/j.ocecoaman.2012.05.007>
840

841 Mouneyrac, C., Linot, S., Amiard, J. C., Amiard-Triquet, C., Métais, I., Durou, C., et al.,
842 2008. Biological indices, energy reserves, steroid hormones and sexual maturity in the
843 infaunal bivalve *Scrobicularia plana* from three sites differing by their level of
844 contamination. *General and Comparative Endocrinology*, 157(2), 133-141.
845 <https://doi.org/10.1016/j.ygcen.2008.04.010>
846

847 Navon, G., Kaplan, A., Avisar, D., & Shenkar, N., 2020. Assessing pharmaceutical
848 contamination along the Mediterranean and Red Sea coasts of Israel: Ascidiaceae (Chordata,
849 Ascidiaceae) as bioindicators. *Marine pollution bulletin*, 160, 111510.
850 <https://doi.org/10.1016/j.marpolbul.2020.111510>
851

852 Negrel, P., 1997. Multi-element chemistry of Loire estuary sediments: anthropogenic vs.
853 natural sources. *Estuarine, Coastal and Shelf Science*, 44(4), 395-410.
854 <https://doi.org/10.1006/ecss.1996.0139>
855

856 Ouddane, B., Hamzeh, M., Daye, M., 2018. Implications of Sulphide on Trace Metal
857 Pollution Mobility in Sediment. In: Kallel A., Ksibi M., Ben Dhia H., Khélifi N. (eds) *Recent*
858 *Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions.*

859 EMCEI 2017. Advances in Science, Technology & Innovation (IEREK Interdisciplinary
860 Series for Sustainable Development). Springer, Cham.
861 https://doi.org/10.1007/978-3-319-70548-4_7
862

863 Parmar, T. K., Rawtani, D., & Agrawal, Y. K., 2016. Bioindicators: the natural indicator of
864 environmental pollution. *Frontiers in life science*, 9(2), 110-118.
865 <https://doi.org/10.1080/21553769.2016.1162753>
866

867 Perez-Landa, V., Simpson, S.L., 2011. A short life-cycle test with the epibenthic copepod
868 *Nitocra spinipes* for sediment toxicity assessment. *Environ. Toxicol. Chem.* 30, 1430-1439.
869 <https://doi.org/10.1002/etc.513>.
870

871 Sew, G., Calbet, A., Drillet, G., Todd, P. A., 2018. Effects of concentration and size of
872 suspended particles on the ingestion, reproduction and mortality rates of the copepod, *Acartia*
873 *tonsa*. *Marine environmental research*, 140, 251-264.
874 <https://doi.org/10.1016/j.marenvres.2018.06.016>
875

876 Souissi, A., Souissi, S., Hwang, J.S., 2016 a. Evaluation of the copepod *Eurytemora affinis*
877 life history response to temperature and salinity increases. *Zool. Stud.* 55
878 (4), 2016-2055.
879 <https://doi.org/10.6620/ZS.2016.55-04>.
880

881 Souissi, A., Souissi, S., Hansen, B.W., 2016 b. Physiological improvement in the copepod
882 *Eurytemora affinis* through thermal and multi-generational selection. *Aquacult. Res.* 47 (7),
883 2227-2242.
884 <https://doi.org/10.1111/are.12675>
885

886 Souissi, S., Souissi, A., 2021. Promotion of the Development of Sentinel Species in the Water
887 Column: Example Using Body Size and Fecundity of the Egg-Bearing Calanoid
888 Copepod *Eurytemora affinis*. *Water*, 13, 1442.
889 <https://doi.org/10.3390/w13111442>
890

891 Spadaro, D., Micevska, T., Simpson, S.L., 2008. Effect of nutrition on toxicity of
892 contaminants to the epibenthic amphipod, *Melita plumulosa*. *Arch. Environ. Contam.*
893 *Toxicol.* 55, 593-602. <https://doi.org/10.1007/s00244-008-9153-2>
894

895 Suthers, I., 2000. Significance of larval condition: comment on laboratory
896 experiments. *Canadian Journal of Fisheries and Aquatic Sciences*, 57(7), 1534-1536.
897 <https://doi.org/10.1139/f00-098>
898

899 Tackx, M. L., Herman, P. J., Gasparini, S., Irigoien, X., Billiones, R., Daro, M. H., 2003.
900 Selective feeding of *Eurytemora affinis* (Copepoda, Calanoida) in temperate estuaries: model
901 and field observations. *Estuarine, Coastal and Shelf Science*, 56(2), 305-311.
902 [https://doi.org/10.1016/S0272-7714\(02\)00182-8](https://doi.org/10.1016/S0272-7714(02)00182-8)
903

904 Van Campenhout, K., Bervoets, L., Steen Redeker, E., Blust, R., 2009. A kinetic model for
905 the relative contribution of waterborne and dietary cadmium and zinc in the common carp
906 (*Cyprinus carpio*). *Environ. Toxicol. Chem.* 28, 209-219.
907 <https://doi.org/10.1897/08-136.1>
908

909 Van Ael, E., Blust, R., & Bervoets, L., 2017. Metals in the Scheldt estuary: from
910 environmental concentrations to bioaccumulation. *Environmental pollution*, 228, 82-91.
911 <https://doi.org/10.1016/j.envpol.2017.05.028>
912

913 Wang, Y. P., Voulgaris, G., Li, Y., Yang, Y., Gao, J., Chen, J., Gao, S., 2013. Sediment
914 resuspension, flocculation, and settling in a macrotidal estuary. *Journal of Geophysical*
915 *Research: Oceans*, 118(10), 5591-5608.
916 <https://doi.org/10.1002/jgrc.20340>
917

918 Zidour, M., Boubechiche, Z., Pan, Y.J., Bialais, C., Cudennec, B., Grard, T., Drider, D.,
919 Flahaut, C., Ouddane, B., Souissi, S., 2019. Population response of the estuarine copepod
920 *Eurytemora affinis* to its bioaccumulation of trace metals. *Chemosphere* 220, 505-513.
921 <https://doi.org/10.1016/j.chemosphere.2018.12.148>
922
923

