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1 <Chemosphere>

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3 **Effects of different routes of exposure to metals on**
4 **bioaccumulation and population growth of the cyclopoid**
5 **copepod *Paracyclops nana****

6

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1 **Abstract**

2

3 We examined effects of the three metals cadmium (Cd), copper (Cu), and nickel (Ni) on two
4 subpopulations of the cyclopoid copepod *Paracyclopsina nana*. We sought to investigate the
5 effects of metal exposure on population growth and structure of *P. nana* and to understand the
6 parameters affecting the metal bioaccumulation in copepods. A first experiment tested the
7 hypothesis of competition between these metals in a mixture using a *P. nana* mass culture in
8 10 L beakers with the sublethal concentrations (1/3 of LC50) as determined for *E. affinis*. A
9 second experiment pursued the same with a *P. nana* population which was adapted to a higher
10 Cu concentration for several generations ($226.9 \pm 15.9 \mu\text{g}\cdot\text{g}^{-1}\text{ dw}$ Cu in copepods) and using
11 the proper sublethal concentrations for *P. nana*. After 96h of exposure, results from the first
12 experiment showed a decreasing population growth and instead of an increasing metal
13 accumulation in copepods. Cd also appeared to be more accumulated when it was alone,
14 confirming the hypothesis of metal competition in mixture. Results from the second
15 experiment revealed less marked effects. When metal concentrations increased in the
16 treatment it decreased in copepods, indicating depuration activity in the population already
17 adapted to metal exposure. This paper is the first one investigating the parameters affecting
18 the bioaccumulation capacity of *P. nana* in response to metals. It offers a better understanding
19 of copepod responses to metal contamination in a complex aquatic environment.

20

21 **KEYWORDS**

22 Bioaccumulation, copepod, ecotoxicology, trace metal, *Paracyclopsina nana*, population
23 growth.

24

25 **1. INTRODUCTION**

26

27 Most aquatic ecosystems are increasingly contaminated with a mixture of trace metals, such
28 as cadmium (Cd), copper (Cu), and nickel (Ni) which became common contaminants in
29 freshwater and oceanic environments (Rollin & Quiot, 2006). Their high concentrations in
30 natural waters are originating from several anthropogenic activities including agriculture and
31 aquaculture, industry, or urbanization (Thévenot et al., 2009; Förstner & Wittmann, 2012).
32 Some metals such as Cu and Ni appear to be functionally essential to the metabolism of cer-
33 tain organisms at low concentrations (Langston & Bebianno, 1998) but can become toxic
34 when they exceed a certain threshold concentration (Rainbow, Phillips & Depledge, 1990;
35 Ansari et al., 2004). Cadmium was shown to be toxic only and without known physiological
36 functions in any organism (Larsson et al., 1976; Yang et al., 1996; Godt et al., 2006). Cadmi-
37 um is recognized as one of the most toxic non-essential metals for aquatic organisms (Taylor,
38 1983), leading to alterations of the endocrine system, development, reproduction, and behav-
39 iour (Sullivan et al., 1983; Toudal & Riisgård, 1987). Besides showing these direct toxic ef-
40 fects, metals show combined effects and have a high potential of bioaccumulation within or-
41 ganisms and within ecosystems (Rainbow, 1990; Pavlaki et al., 2017). Metals commonly bio-
42 accumulate within organisms, and can reach harmful levels, causing physiological changes
43 and cascading problems in trophic webs (Dobbs et al., 1996; Kouba et al., 2010).

44 In aquatic ecosystems, copepods represent an important trophic link between lower trophic
45 levels such as phytoplankton producers and secondary consumers like fish or invertebrate
46 larvae (Breteler et al., 1990; Mauchline et al., 1998; Støttrup, 2000). Therefore, they can be
47 an important factor in the biomagnification of toxic contaminants in aquatic food webs (Fisher
48 et al., 2000; Stewart & Fisher, 2003). Due to their central position in marine food webs,
49 copepods are often used as bioindicators and are used as invertebrate models in aquatic eco-
50 toxicology studies (Raisuddin et al., 2007; Kwok et al., 2015; Dahms et al., 2016a). In addi-
51 tion, they are easy to cultivate because of their short life cycle and high fecundity (Raisuddin
52 et al., 2007; Kulkarni et al., 2013). Of them, the brackish water cyclopoid copepod *Paracy-*
53 *clopina nana* Smirnov, 1935 has demonstrated a high potential both for the aquaculture in-

54 dustry and for ecotoxicological research (Lee et al., 2006; Lee et al., 2013; Dahms et al.,
55 2016b). Native to the bays and estuaries of Eastern Asia (Japan, Taiwan, and South Korea), it
56 presents a small size at adult stage (600 μm of total length on average), a high tolerance to
57 temperature and salinity changes, and supports to be cultured at high densities (Lee et al.,
58 2012; Lee et al., 2017). Its whole genome has even been sequenced and annotated (Ki et al.,
59 2009; Han et al., 2015; Lee et al., 2015; Jeong et al., 2017) and is available at this web link:
60 <http://rotifer.skku.edu:8080/Pn>. The first exposure tests of *P. nana* to environmental contami-
61 nants and other stressors showed interesting results on its molecular and biochemical re-
62 sponses. For example, induction of vitellogenin is used as a biomarker of exposure to metals.
63 It could be shown that upon exposure to several metals (Cd, Cu, and As), the expression of
64 vitellogenin transcripts in *P. nana* was induced in a stronger and increasing manner through-
65 out its life cycle (Hwang et al., 2010). Also, in *P. nana* ovigerous females, increasing antiox-
66 idant enzyme and DNA repair activities coupled to an increasing mortality were shown in a
67 dose-dependent manner in response to gamma radiation (Won and Lee, 2014). However,
68 these studies were mostly carried out at a small scale with low individual numbers and often
69 using a single contaminant for each treatment. Given the current ecotoxicological challenges
70 with combined effects of several contaminants at the same time, it is demanding to use large
71 individual numbers in larger mesocosms and several contaminants which is closer to the real
72 world situation. An understanding of the effects of contaminant cocktails, such as metals, can
73 provide a better understanding about ecological risks in natural situations. To date, the com-
74 bined effects of metals on copepods are poorly known and some studies showed their additive
75 or synergetic effects (Hagopian-Schlekat et al., 2001; Wong & Pak, 2004). Similarly, the bio-
76 availability of metals is an important parameter to consider and only a few studies show that
77 this parameter is closely correlated to the chemical speciation of metals (Batley et al., 2004).
78 Recently, the bioaccumulation rates of Cd, Cu, and Ni were studied in the estuarine calanoid
79 copepod *E. affinis* (Zidour et al., 2019). The authors found that the accumulation rate of each
80 metal was higher in treatments alone than the combined effect of a three-metal mixture. A

81 lower bioaccumulation with the mixture could be explained by a competitive complexation
82 occurring between the three metals, resulting in a saturation phenomenon of metal-binding
83 sites. It would be interesting if this phenomenon applies also to another copepod species.
84 Moreover, quantitative studies about the bioaccumulation of metals in cyclopoid copepods
85 are still lacking. Furthermore, for tiny cyclopoids like *P. nana*, any experimental protocol for
86 bioaccumulation study should consider a high number of individuals to reach the threshold
87 value of a few mg of dry weight required for metal detection.

88 We firstly performed a bioaccumulation experiment with *P. nana* using 1/3 50% lethal
89 concentration (LC50) of each metal based on the values to *E. affinis* applied to *P. nana*, from
90 the point of view of pure comparison of the applied protocol and the attested effects. Then,
91 the LC50 concentrations of *P. nana* for Cd, Cu, and Ni were measured by using the same
92 protocol described in Tlili et al. (2016). This step allowed to perform a second
93 bioaccumulation experiment using 1/3 LC50 of *P. nana*. Furthermore, we slightly modified
94 the long-term mass culture conditions between both experiments by using a higher
95 concentration of Cu in the second culture, as Cu seems to be the most labile metal in copepod
96 bioaccumulation. It is important to compare both kinetics of bioaccumulation when initial
97 conditions, in terms of initial loading of Cu in copepods, are totally different. Thus, we first
98 replicated the experiment performed with *E. affinis* in the same conditions but by changing
99 the copepod species (Experiment 1). Then we performed another experiment focused on the
100 mixture using 1/3 LC50 of Cd, Cu, and Ni specific for *P. nana* but started the experiment
101 with individuals already saturated in terms of Cu bioaccumulation (Experiment 2).

102 In this study, we used the animal model copepod *P. nana* to quantify the bioaccumulation
103 of Cd, Cu, and Ni *via* waterborne uptake and the effect of these trace metals on population
104 growth and structure. We particularly challenged the competition hypothesis between these
105 metals when combined.

106

107 **2. MATERIALS AND METHODS**

108

109 **2.1. Copepod culture and experimental set-up**

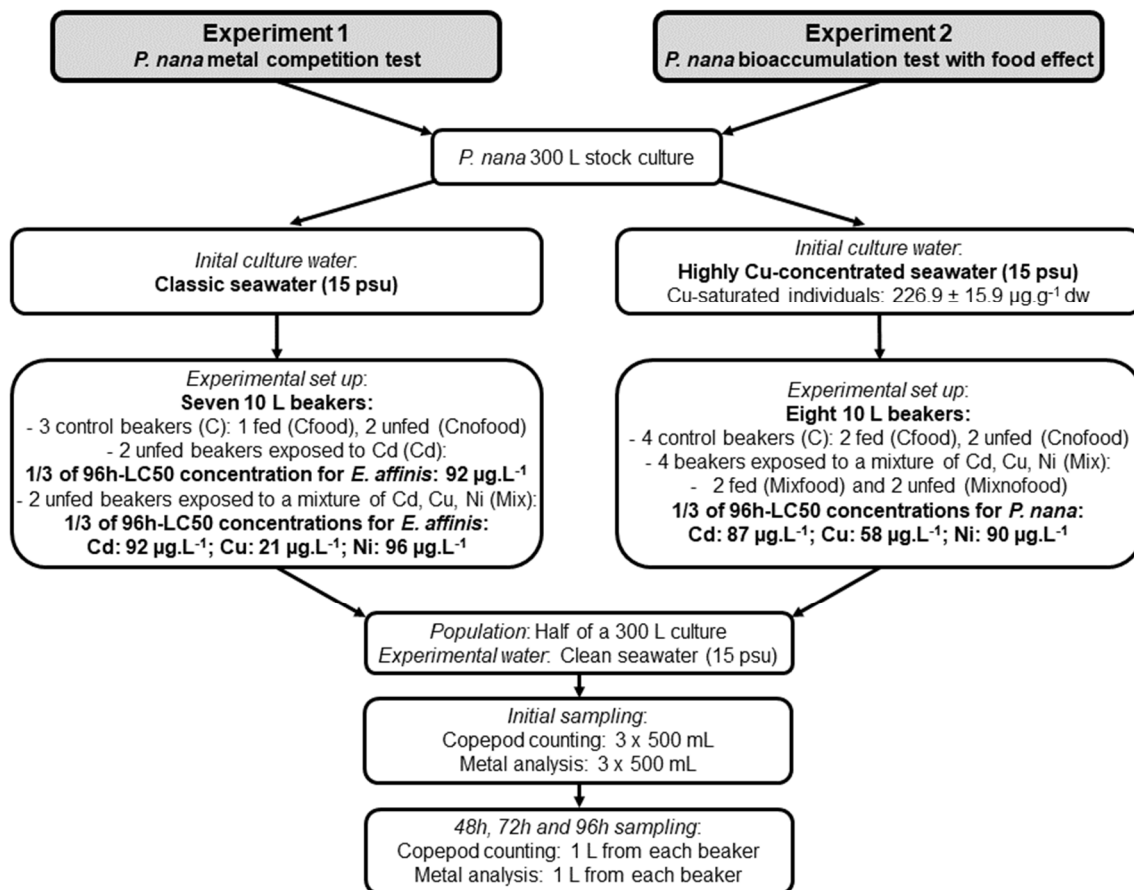
110

111 The copepod *P. nana* used in this experiment was initially obtained in 2015 from Prof. Jae-
112 Seong Lee's lab at Sungkyunkwan University, South Korea and has been added to the
113 copepod strain collection of Lille University in the Marine Station of Wimereux, France.
114 Copepods are grown in the laboratory in large-scale culture systems of 300 L cylindrical
115 acrylic tanks. The experimental conditions were the same as the regular culture conditions
116 and also the same as in Zidour et al. (2019): T = 18°C, salinity = 15 psu, 12h light: 12h dark
117 photoperiod, and fed with the microalga *Rhodomonas salina* as in Arias et al. (2017). This
118 widely distributed microalgae is the main species used to feed copepod cultures in the
119 laboratory because of its fast growth and its nutritional value (Dahl et al., 2009). The protocol
120 used for these two experiments is also the same as the one used in Arias et al. (2017) and as
121 described below, with only few minor differences between the two of them.

122 For each experiment, a whole column of 300 L of *P. nana* was filtered on a 33 µm mesh to
123 keep the whole population. Half of the amount of copepods was kept for the experiment and
124 concentrated in a 10 L beaker containing 15 psu seawater (filtered and autoclaved seawater +
125 distilled water). This salinity was kept constant using a multiparametric probe. The remaining
126 copepods was transferred to a new clean 300 L culture.

127 Several experimental 10 L beakers were set-up. We tested the effect of feeding copepods
128 with *P. nana* on the bioaccumulation capacity in Experiment 2. The respective experimental
129 set-ups and beaker labels used for each experiment were detailed in **Fig. 1**.

130



131

132 **FIGURE 1.** Flow chart of the experimental design and beaker labels used for each
 133 experiment.

134

135 Each beaker was filled with 8 L of seawater at a salinity of 15. For each experiment and
 136 after homogenization, 1 L of the volume of concentrated copepods was added in each beaker
 137 of the experiment, and 3 L were used for initial sampling. Three volumes of 500 mL (n = 3)
 138 were individually filtered on a 33 µm mesh and concentrated with 70% ethanol for initial
 139 counting of the population structure, and three volumes of 500 mL (n = 3) were filtered
 140 through cellulose nitrate filters of 0.45 µm porosity (Whatman International Ltd.,
 141 Maidstone, England). Filters containing copepods were dried in a desiccator for 72h and
 142 weighted for later metal analysis. Each time, 50 mL (n = 3) of the filtrate corresponding to
 143 culture water was collected and conditioned immediately in 50 µL pure nitric acid (HNO₃,
 144 Merck Suprapur 65%) for further analysis of residual metal concentrations. For each

145 experiment, the fed-treatments were initially fed with the microalga *R. salina* according to the
146 protocol provided by Souissi et al. (2010).

147

148 **2.2. Experimental monitoring**

149

150 Daily sampling was the same as the initial sampling and was performed at 48h, 72h and 96h.
151 The only difference was that a volume of 1 L was sampled from each beaker and filtered on a
152 cellulose nitrate filter for metal analysis. Obtained filters were also weighed and dried daily
153 in a desiccator throughout the whole experiment and the same volume of 50 µL of filtrate was
154 collected and fixed. A volume of 1 L was also sampled daily from each beaker and fixed with
155 70% ethanol for population structure analysis. Also, the fed beakers were given *R. salina*
156 daily following the same method as mentioned above.

157

158 **2.3. Copepod individual counting**

159

160 Copepods fixed with 70% ethanol were individually counted according to their sex and life
161 stage using a stereomicroscope (model Olympus SZX12, Tokyo, Japan) and a desk manual
162 counter with several units. Nauplii, copepodites (C1-C4), C5 males, C5 females, adult males,
163 adult females and ovigerous females were differentiated according to morphological details
164 (Hwang et al., 2010).

165

166 **2.4. Metal analysis**

167

168 Metal analysis was performed in the LASIR Laboratory, University of Lille (France) as
169 described by Ouddane et al. (1999) with some modifications specified below. Once filters
170 containing copepod samples were dry, they were mineralized with 3 mL ultrapure nitric acid
171 (HNO₃, Merck Suprapur, 65%) in a Block Digestion System (Environmental Express

172 HotBlock® SC100, Charleston, SC, USA) at 105°C for two hours. Obtained solutions were
173 then diluted with ultrapure water to a final volume of 10 mL. Respective elemental
174 concentrations of the three targeted metals in the copepod and water samples were measured
175 in triplicate against multi-element standards using an inductively coupled plasma atomic
176 emission spectrometer (Agilent Technologies ICP-AES 5110, dual view, Santa Clara, CA,
177 USA). Data obtained were expressed as the mean \pm standard deviation (SD).

178

179 **2.5. Statistical analysis**

180

181 In order to compare the concentration of each metal (Cd, Cu and Ni) in copepods and in wa-
182 ter, two similar databases were built for each experiment. In the first column, supposed to be
183 our dependent variable, we considered the metal concentration. Then we encoded in four dif-
184 ferent columns all sources of variability starting by replicate number, treatments (food, no
185 food), metal (Cd, Cu, Ni) and exposure duration (48, 72, 96h). Then for each metal we first
186 performed a multiway (n-way) analysis of variance (ANOVA) to test the effects of multiple
187 factors on the mean of metal concentration either in copepods or in water. Then in a second
188 step and because no significant differences between the treatments having two replicates were
189 detected, we performed a two-way analysis of variance by considering only two factors
190 treatment (food, no food) and exposure duration (48, 72, 96h). We also considered the inter-
191 action between these two factors in a two-way ANOVA model.

192 The same procedure was applied to the population structure of *P. nana* by using a similar
193 database with the number of individuals per L as dependent variable and 4 factors composed
194 of replicate, treatment, exposure duration, and life stage. The latter factor considered the fol-
195 lowing seven life stages: nauplii, copepodites (C1-C4), C5 males, C5 females, adult males,
196 adult females, and ovigerous females. An additional variable composed by the sum of pre-
197 adult (C5) and adult stages was also considered. The multi-way ANOVA and two-way ANO-

198 VA were applied in the same way as described for metals but by considering each develop-
199 mental stage separately as well as a combination of them (8 variables in total).

200 For all two-way ANOVA analyses, a post-hoc test was performed by using the interactive
201 graphical interface of MULTCOMPARE function of Matlab.

202 These analyses were performed using Matlab Software (Mathworks Inc., Version, 7.5).

203

204 3. RESULTS

205

206 3.1. Effects of metals on population growth of *Paracyclopsina nana*

207

208 *Total population growth*

209

210 The total number of *P. nana* individuals obtained for each condition of each experiment is
211 shown in **Table 1**.

212

213 **TABLE 1.** Detailed labels of the different conditions and their respective total population at
214 96h for each experiment.

215

Treatment description	Microcosm number	Label	Total population in Experiment 1	Total population in Experiment 2
Control with food	#1	Cfood1	8268	1960
Control with food	#2	Cfood2	-	1698
Control without food	#1	Cnofood1	7476	1532
Control without food	#2	Cnofood2	7616	1374

food			
Cadmium	#1	Cd1	5916
without food			
Cadmium	#2	Cd2	5956
without food			
Mixture			
(Cd+Cu+Ni)	#1	Mixfood1	2094
with food			
Mixture			
(Cd+Cu+Ni)	#2	Mixfood2	2152
with food			
Mixture			
(Cd+Cu+Ni)	#1	Mix1 / Mixnofood1	7216
without food			1340
Mixture			
(Cd+Cu+Ni)	#2	Mix2 / Mixnofood2	6440
without food			1252

216

217 For Experiment 1, the three control conditions showed more individuals than in the
218 conditions exposed to the contaminants, Cd and Mix. Nevertheless, the Cnofood1 and
219 Cnofood2 beakers corresponding to the unfed controls showed less individuals after 96h than
220 the fed control (Cfood) which led to the highest population density with 8268 individuals L⁻¹.
221 At exposed conditions, the Cd-exposed beakers (Cd1 and Cd2) final populations appeared
222 still below those of all other conditions, with 5916 individuals L⁻¹ and 5956 individuals L⁻¹,
223 respectively. Conversely, Mix1 and Mix2 beakers presented higher final populations than
224 those of beakers exposed only to Cd. At the end of Experiment 2 at 96h, all control beakers
225 (fed and unfed) showed a higher population density than the unfed exposed beakers

226 (Mixnfood1 and Mixnfood2), while populations in Cfood beakers were always higher than
227 in the Cnofood ones. However, the Mixfood1 and Mixfood2 beakers presented the highest
228 populations at 96h, even higher than all the control ones, with 2094 individuals L⁻¹ and 2152
229 individuals L⁻¹, respectively. Comparing the two experiments, overall results demonstrated
230 that the one based on *E. affinis* LC50 concentrations was presenting always more individuals
231 at each condition than the one using the proper *P. nana* LC50 concentrations.

232

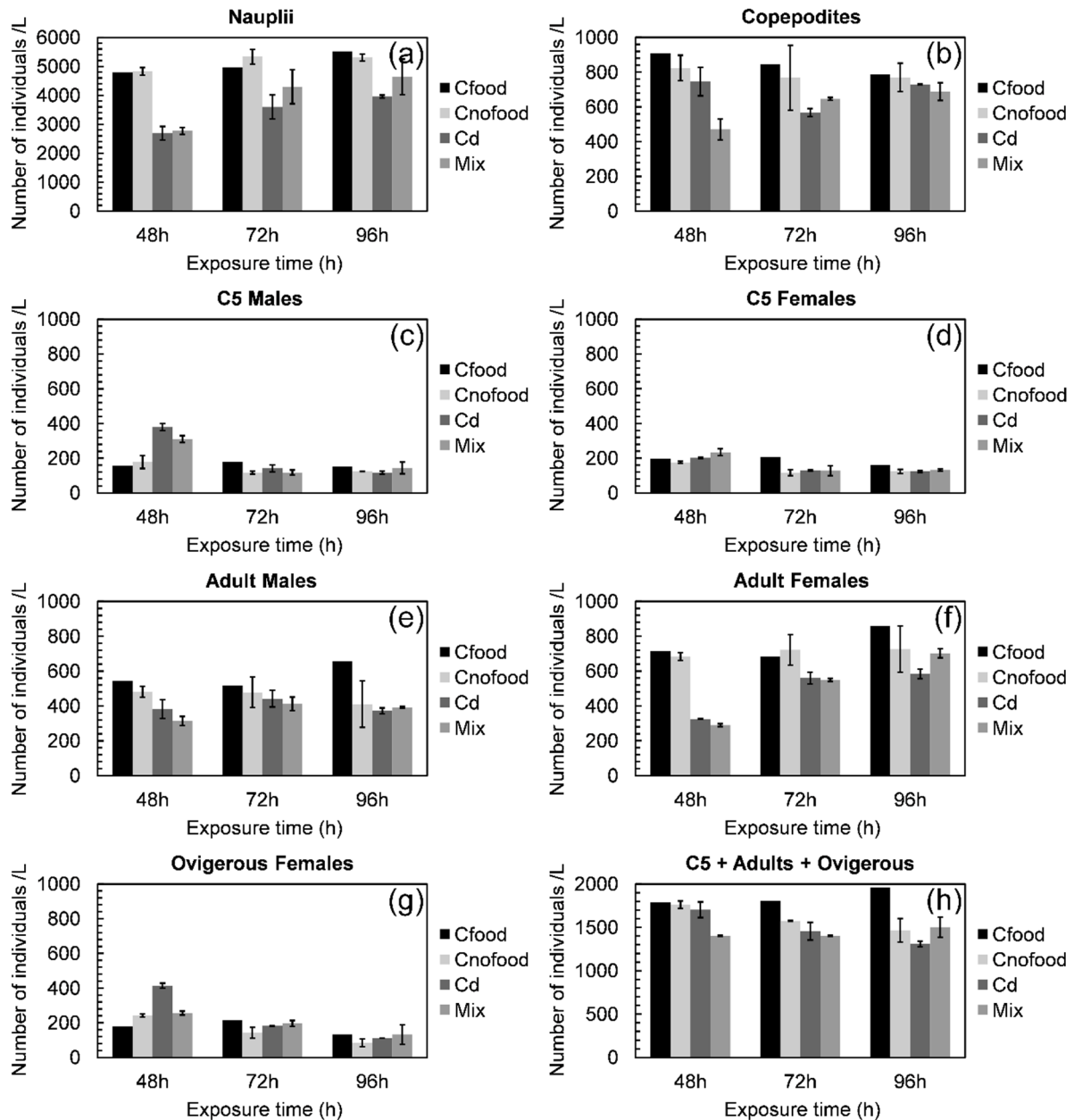
233 *Stage-specific composition*

234

235 For the first experiment, all 3-way ANOVA (replicates, treatments and exposition duration as
236 factors) performed on the 8 life stages (or aggregated stages) showed significant levels
237 (p<0.05) for only two factors (treatment and duration). Consequently, we performed 2-way
238 ANOVA using only those factors and by adding their interaction.

239 **Fig. 2** presents the stage-specific composition of Experiment 1 over time.

240



241

242 **FIGURE 2.** Stage-specific composition of the *Paracyclopsina nana* populations for each
 243 condition during Experiment 1: Nauplii (a), Copepodites (b), C5 Males (c), C5 Females (d),
 244 Adult males (e), Adult females (f), Ovigerous females (g), C5+Adults+Ovigerous (h).

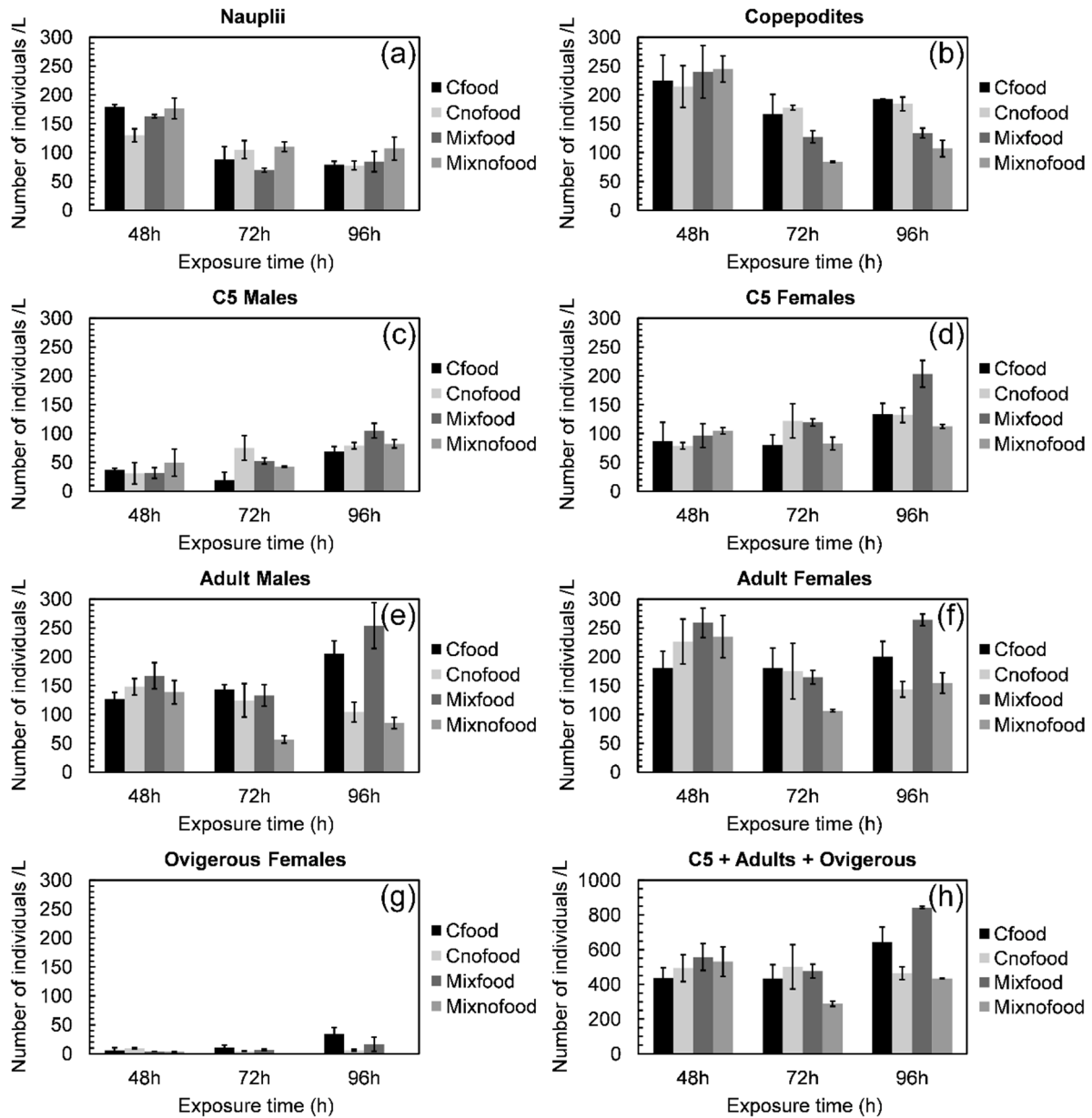
245

246 The density of nauplii (**Fig. 2a**) was relatively high in every condition of the experiment.
 247 Both factors were significant but not their interaction ($p = 0.188$). In fact, nauplii showed an
 248 increasing density over time, and particularly in contaminant-exposed beakers, they reached
 249 3956 ± 51 individuals L^{-1} for the Cd beakers and 4638 ± 614 individuals L^{-1} for the combined
 250 treatments. Copepodite (**Fig. 2b**) populations gradually aligned over time between the

251 different conditions, slightly decreasing in the control but increasing in the treatments,
252 reaching 730 ± 3 individuals L^{-1} for the Cd and 688 ± 51 individuals L^{-1} for the Mix
253 treatments. However, only the treatment factor was statistically significant ($p < 0.05$). In the
254 pre-adult stages (C5), the observed patterns in males (**Fig. 2c**) and females (**Fig. 2d**) were
255 similar to the highest densities observed in the treatments of Cd and Mix at the beginning of
256 the experiment, and then their numbers decreased over time, reaching the lowest density after
257 96h of exposure. Both tested factors and their interaction were statistically significant
258 ($p < 0.05$). The density of adult males (**Fig. 2e**) increased during the experiment in the fed
259 control (Cfood), but their number varied slightly in other conditions over time. Only a slight
260 decrease in their number was observed both in the unfed control (Cnofood) and in Cd and
261 Mix conditions (372 ± 17 individuals L^{-1} and 392 ± 6 individuals L^{-1} , respectively). Only the
262 treatment factor was statistically significant. Contrary to adult females (ovigerous and non-
263 ovigerous), the interaction of both factors were statistically significant. Adult females (**Fig.**
264 **2f**) showed an increasing number during the experiment in the fed control (Cfood) and a
265 rather stable population in the unfed control (Cnofood). Their density was increasing over
266 time in both Cd and Mix-exposed conditions (584 ± 28 individuals L^{-1} and 702 ± 25
267 individuals L^{-1} , respectively). The number of ovigerous females (**Fig. 2g**) globally decreased
268 during the experiment in the control beakers (Cfood and Cnofood) but it decreased even more
269 in the Cd and Mix-exposed beakers (112 individuals L^{-1} and 132 ± 57 individuals L^{-1} ,
270 respectively, after 96h).

271 The stage-specific composition of Experiment 2 over time is shown in **Fig. 3**.

272 When 3-way ANOVA was applied to all stages only two factors (treatment and duration)
273 showed significant levels. Consequently, we performed 2-way ANOVA using only those
274 factors and their interaction.



275

276 **FIGURE 3.** Stage-specific compositions of the *Paracyclops nana* populations for each

277 condition during Experiment 2: Nauplii (a), Copepodites (b), C5 Males (c), C5 Females (d),

278 Adult males (e), Adult females (f), Ovigerous females (g), C5+Adults+Ovigerous (h).

279

280 The density of nauplii (**Fig. 3a**) was decreasing until the end of the experiment for all

281 conditions. Only a slight increase was noted in the Mixfood beakers at 96h (Mixfood: 84 ± 18

282 individuals L^{-1} ; Mixnofood: 107 ± 20 individuals L^{-1}). Both factors were significant ($p < 0.05$)

283 but not their interaction. Copepodites observed a fall in all the beakers at 72h, particularly for

284 Mix conditions but their number slightly increased at each condition at 96h with the Mix ones

285 always lower than the control ones (Mixfood: 134 ± 8 individuals L^{-1} ; Mixnofood: 107 ± 14

286 individuals L⁻¹) (**Fig. 3b**). However, statistically only the treatment factor was significant
287 (p<0.05). The density of C5 males (**Fig. 3c**) was increasing in all the beakers during the
288 experiment with Mixfood and Mixnofood being the highest after 96h with 105 ± 13
289 individuals L⁻¹ and 82 ± 7 individuals L⁻¹, respectively. Both factors and their interaction
290 were significant. C5 females observed a global increase also in each condition, with a very
291 high final number in Mixfood beakers (203 ± 23 individuals L⁻¹) (**Fig. 3d**), while Mixnofood
292 beakers were lower than the control ones (112 ± 4 individuals L⁻¹). The two-way ANOVA
293 provided similar results for C5 females. The density of adult males increased after 96h in
294 both the fed conditions Cfood and Mixfood (205 ± 22 individuals L⁻¹ and 254 ± 40
295 individuals L⁻¹, respectively) (**Fig. 3e**), whereas it decreased in the unfed treatments Cnofood
296 and Mixnofood (104 ± 17 individuals L⁻¹ and 85 ± 10 individuals L⁻¹, respectively). Only the
297 treatment factor was significant for males whereas both factors and their interaction were
298 significant for females. The numbers of adult females increased in the fed treatments after
299 96h (Cfood: 200 ± 26 individuals L⁻¹; Mixfood: 264 ± 10 individuals L⁻¹) but decreased in
300 Cnofood (143 ± 13 individuals L⁻¹) and Mixnofood (154 ± 18 individuals L⁻¹) (**Fig. 3f**).
301 Initial densities of ovigerous females were very low but increased substantially after 96h in
302 the fed conditions Cfood and Mixfood (34 ± 11 individuals L⁻¹ and 16 ± 12 individuals L⁻¹,
303 respectively) (**Fig. 3g**). Both factors and their interaction were significant (p<0.05).

304

305 **3.2. Bioaccumulation of metals in *Paracyclopina nana***

306

307 *Initial concentrations in copepods and water*

308

309 The concentrations of the three metals were measured both in copepods and water from the
310 initial copepod stock cultures used for each experiment (**Table 2**).

311 **TABLE 2.** Concentrations of cadmium (Cd), copper (Cu) and nickel (Ni) both in copepods
312 and water from initial stock cultures for each experiment.

Experiment 1	Initial concentration in copepods ($\mu\text{g.g}^{-1}$ dw)	Initial concentration in water ($\mu\text{g.L}^{-1}$)	Concentration used (1/3 LC50 of <i>E. affinis</i>) ($\mu\text{g.L}^{-1}$)
Cd	0.02 ± 0.04	0.26 ± 0.5	92
Cu	20.7 ± 1.6	44.3 ± 5.9	21
Ni	3.7 ± 0.2	36.5 ± 0.9	96
Experiment 2	Initial concentration in copepods ($\mu\text{g.g}^{-1}$ dw)	Initial concentration in water ($\mu\text{g.L}^{-1}$)	Concentration used (1/3 LC50 of <i>P. nana</i>) ($\mu\text{g.L}^{-1}$)
Cd	0.08 ± 0.02	0.45 ± 0.05	87
Cu	226.9 ± 15.9	2.4 ± 0.1	58
Ni	4.4 ± 0.6	1.1 ± 0.4	90

314

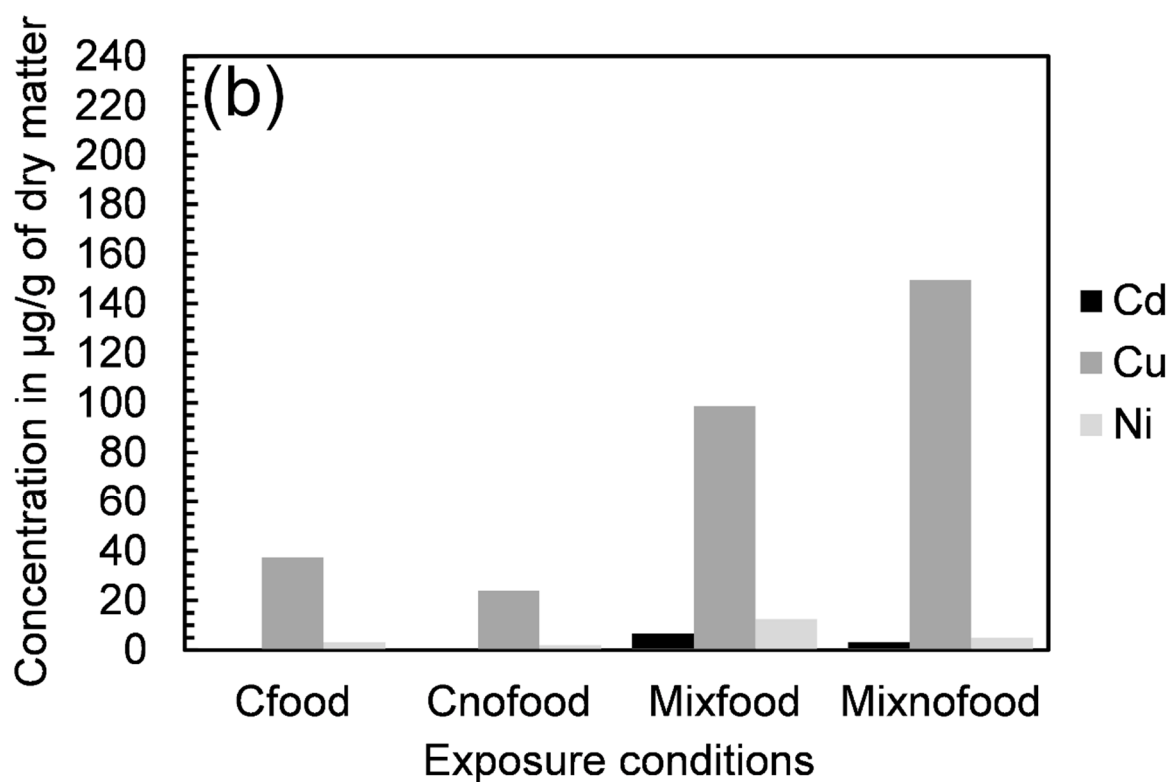
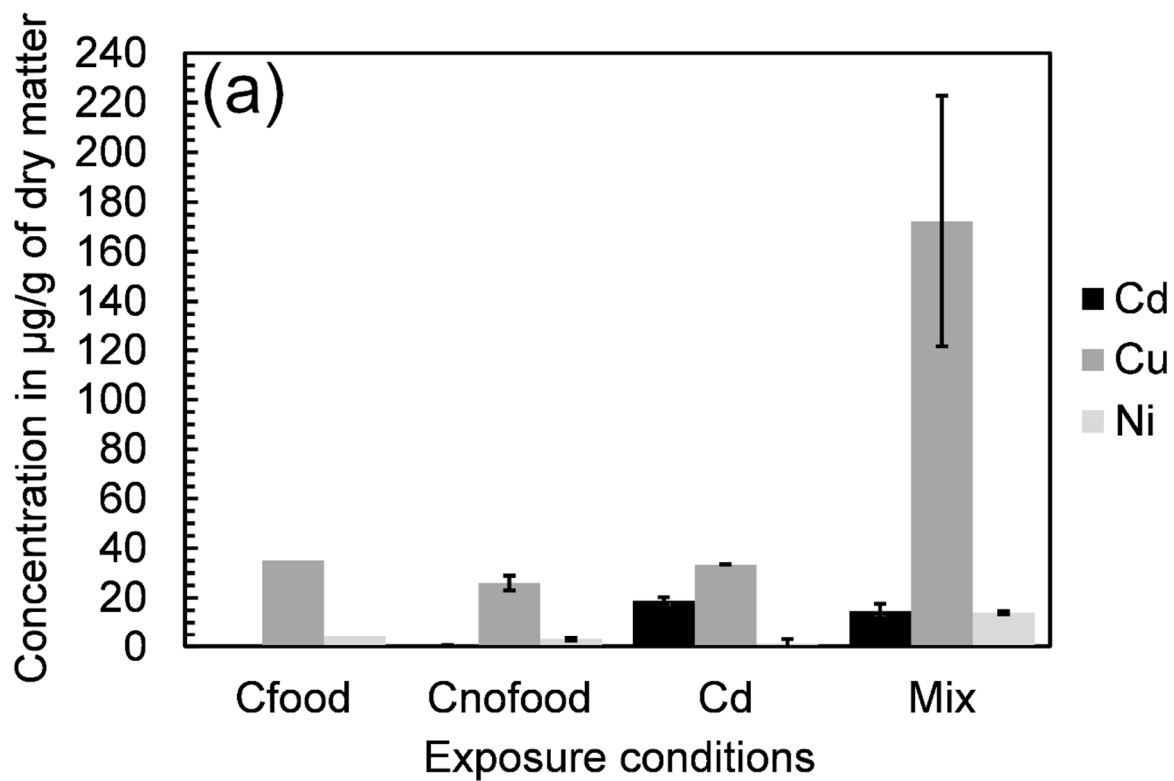
315 Concentrations in copepods for the pre-exposed population always showed higher values for
316 each metal than the copepods used for Experiment 1, particularly for Cu which was reaching
317 $226.9 \pm 15.9 \mu\text{g.g}^{-1}$ dw, but was $20.7 \pm 1.6 \mu\text{g.g}^{-1}$ dw for Experiment 1. Conversely,
318 concentrations in the water of each initial culture were generally higher for Experiment 1 than
319 for Experiment 2, except for Cd, which reached $0.45 \pm 0.05 \mu\text{g.L}^{-1}$ in the culture intended for
320 Experiment 2 but was $0.26 \pm 0.5 \mu\text{g.L}^{-1}$ for Experiment 1. Initial Cu and Ni concentrations in
321 water for Experiment 1 were really high, with $44.3 \pm 5.9 \mu\text{g.L}^{-1}$ and $36.5 \pm 0.9 \mu\text{g.L}^{-1}$,
322 respectively, whereas it was at $2.4 \pm 0.1 \mu\text{g.L}^{-1}$ and $1.1 \pm 0.4 \mu\text{g.L}^{-1}$, respectively, in the water
323 of the pre-exposed culture.

324

325 *Concentration in copepods*

326

327 Final concentrations of the three tested metals in the copepod samples are presented in **Fig. 4**.

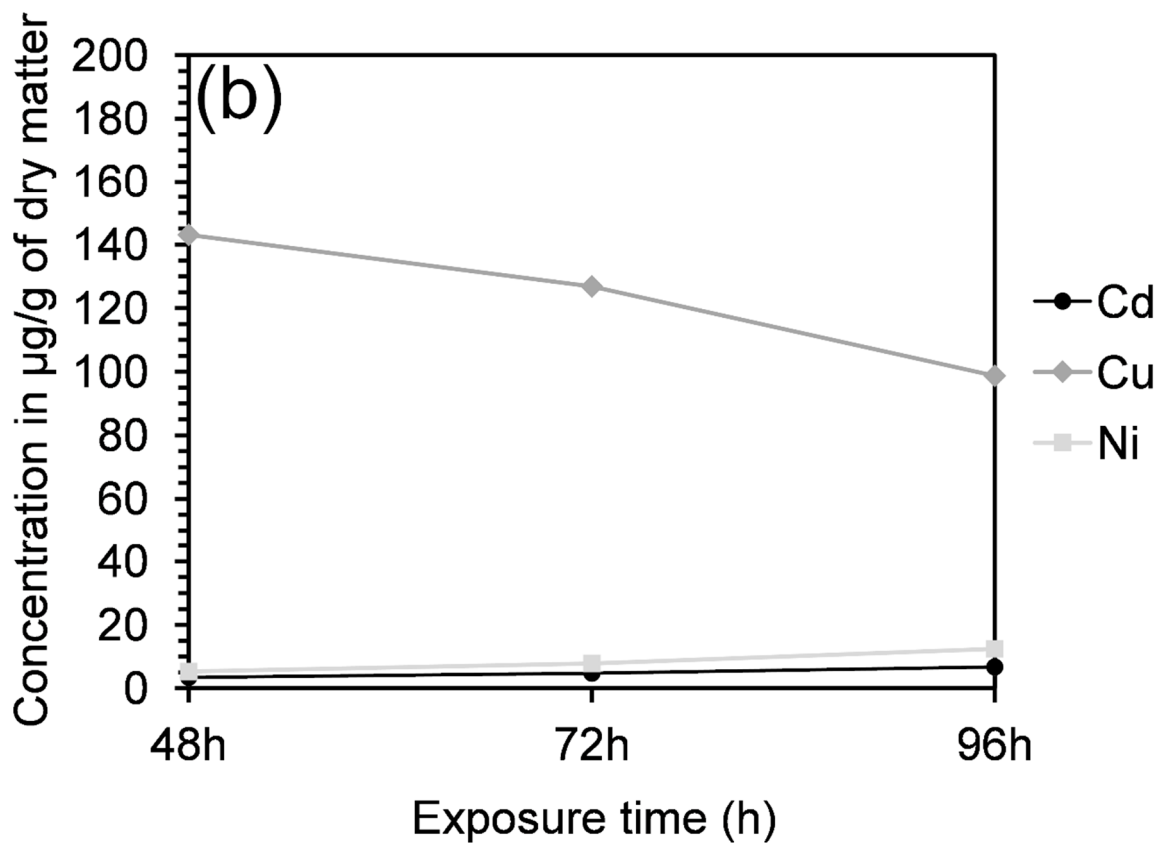
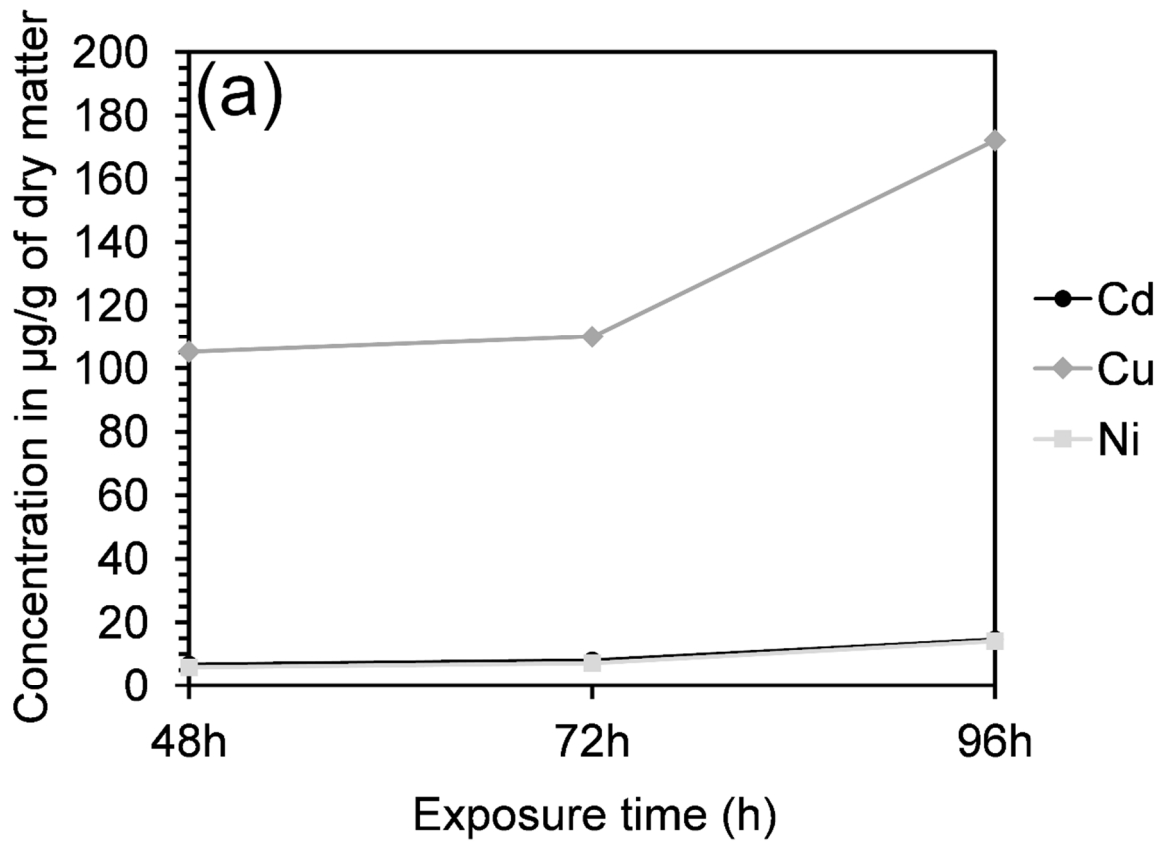


328
 329 **FIGURE 4.** Final concentrations of cadmium (Cd), copper (Cu) and nickel (Ni) in copepods
 330 of each treatment of Experiment 1 (a) and Experiment 2 (b) after 96h of exposure.

331
 332 Looking at the results of Experiment 1 (**Fig. 4a**), cadmium was present at very low
 333 concentrations in controls (Cfood: $0.16 \mu\text{g}\cdot\text{g}^{-1}$ dw; Cnofood: $0.21 \mu\text{g}\cdot\text{g}^{-1}$ dw) and at high

334 concentrations in the Cd and Mix conditions ($18.8 \pm 1.5 \mu\text{g.g}^{-1} \text{ dw}$ and $14.8 \pm 2.8 \mu\text{g.g}^{-1} \text{ dw}$,
335 respectively), but still higher for Cd alone. The two-way ANOVA performed on Cd loadings
336 showed that only the treatment factor was significant ($p < 0.05$). Ni was only noticeably
337 present at separate exposure; Mix ($14 \pm 0.6 \mu\text{g.g}^{-1} \text{ dw}$). Statistical analyses showed that only
338 treatment and the interaction 'treatment x duration' were significant ($p < 0.05$). Cu appeared as
339 the most detected metal in copepods, showing high concentrations in the individuals from the
340 Mix beakers, the only ones exposed to it ($172.2 \pm 50.6 \mu\text{g.g}^{-1} \text{ dw}$). However, Cu was also
341 present in high and quite similar concentrations in both controls and Cd condition ($33.5 \pm$
342 $0.03 \mu\text{g.g}^{-1} \text{ dw}$ for the Cd treatment). Both factors were significant but not their interaction.
343 Additionally, both Cu and Ni appeared to be higher in the fed control copepods than in the
344 unfed ones. In Experiment 2, Cd was present at very low concentrations in the control
345 (Cfood: $0.04 \mu\text{g.g}^{-1} \text{ dw}$; Cnofood: $0.06 \mu\text{g.g}^{-1} \text{ dw}$) but more concentrated in copepods from
346 the Mix conditions (**Fig. 4b**). Thus, its accumulation was higher when copepods were fed
347 (Mixfood: $6.7 \mu\text{g.g}^{-1} \text{ dw}$; Mixnofood: $3.3 \mu\text{g.g}^{-1} \text{ dw}$). The same tendency appeared for Ni,
348 which was present at elevated concentrations under Mix conditions as well, and was very
349 high in the Mixfood one (Mixfood: $12.5 \mu\text{g.g}^{-1} \text{ dw}$; Mixnofood: $4.9 \mu\text{g.g}^{-1} \text{ dw}$). Cu was also
350 the most detected metal in copepods, showing important concentrations in the exposed Mix
351 conditions, with $98.7 \mu\text{g.g}^{-1} \text{ dw}$ for Mixfood and $149.5 \mu\text{g.g}^{-1} \text{ dw}$ for Mixnofood. Like Cd
352 and Ni, Cu was higher in the fed controls than in the unfed ones, but was more concentrated
353 in the copepods from Mixnofood than in the ones from Mixfood. Only for all metals the
354 treatment factor was statistically significant ($p < 0.05$).

355 The concentrations of the three tested metals during the whole experiments in the
356 copepods from the Mixfood conditions are shown in **Fig. 5**.



357

358 **FIGURE 5.** Concentrations of cadmium (Cd), copper (Cu) and nickel (Ni) in copepods of the
 359 Mixfood condition during Experiment 1 (a) and Experiment 2 (b).

360

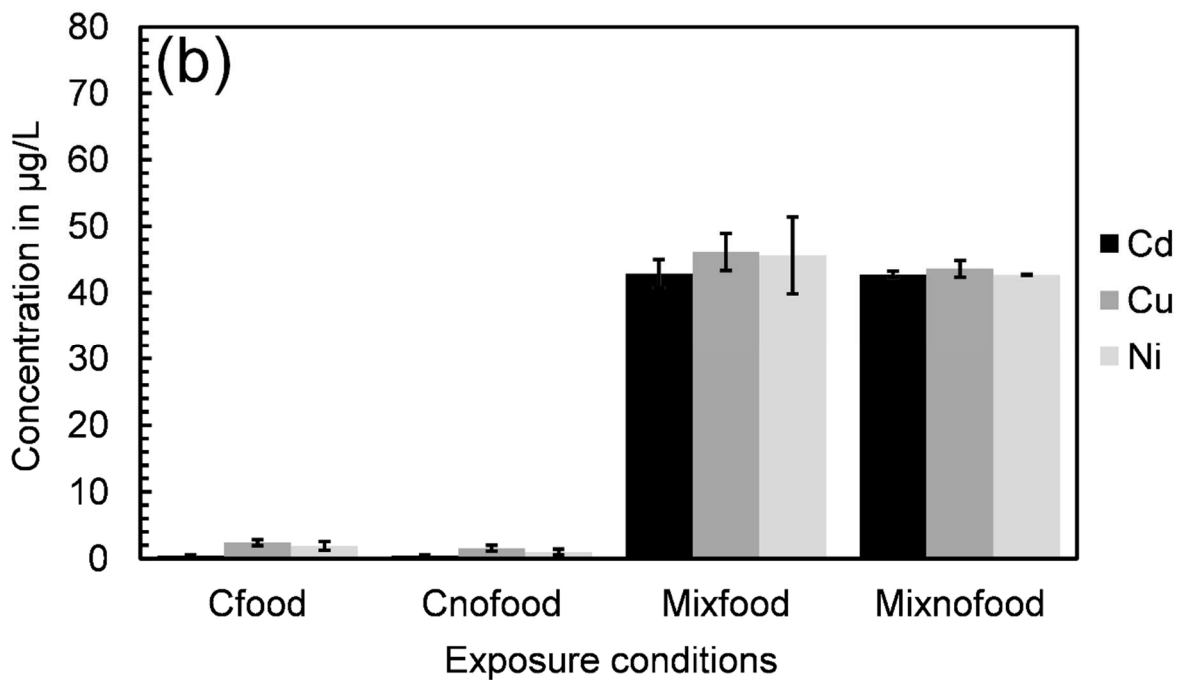
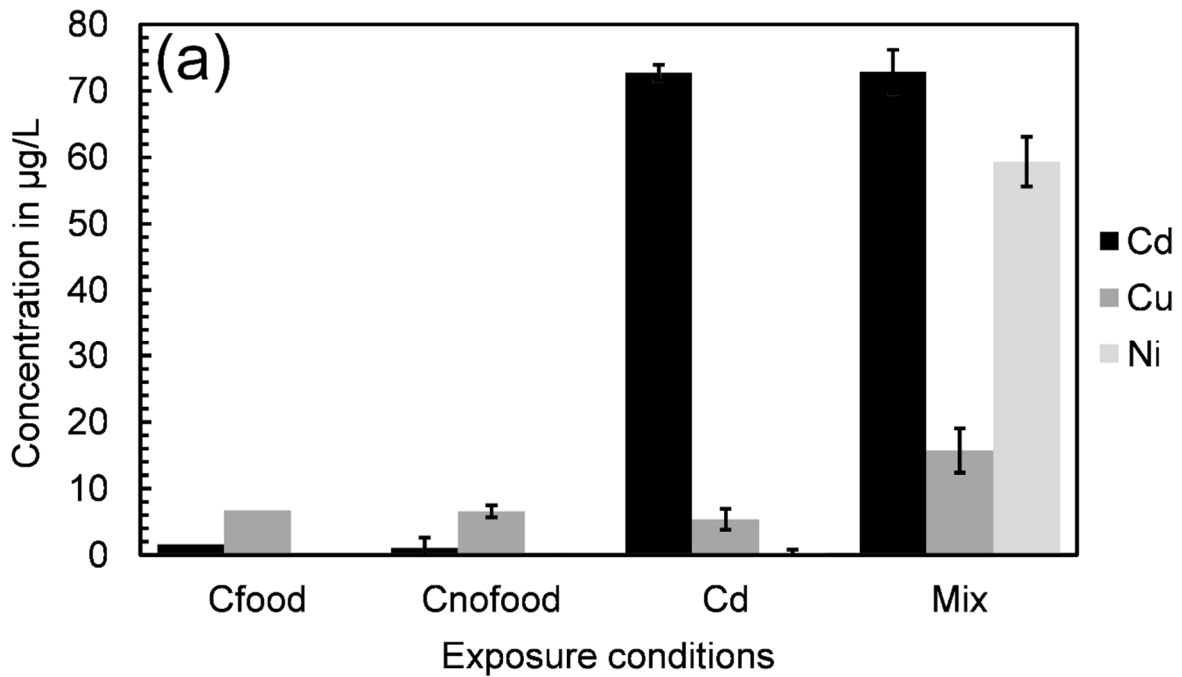
361 The relative bioaccumulation values for Experiment 1 (**Fig. 5a**) showed that Cu
362 concentrations in copepods always increased over time. It was by far the most accumulated
363 metal in copepods, whereas it was the one introduced at the lowest concentration. Cd and Ni,
364 for their part, showed similarly increasing bioaccumulation rates, in a much lower range than
365 Cu. Looking at the results for Experiment 2 (**Fig. 5b**), Cu appeared once again as the most
366 concentrated metal in copepods, but its concentration in the sample was decreasing over time.
367 The accumulation of Cd and Ni was quite identical as for the other experiment and still low
368 compared to Cu. Both of their concentrations increased in copepods during the experiment.

369

370 *Concentration of metals in water*

371

372 The final respective concentrations of the three tested metals in the water samples are shown
373 in **Fig. 6**.



374

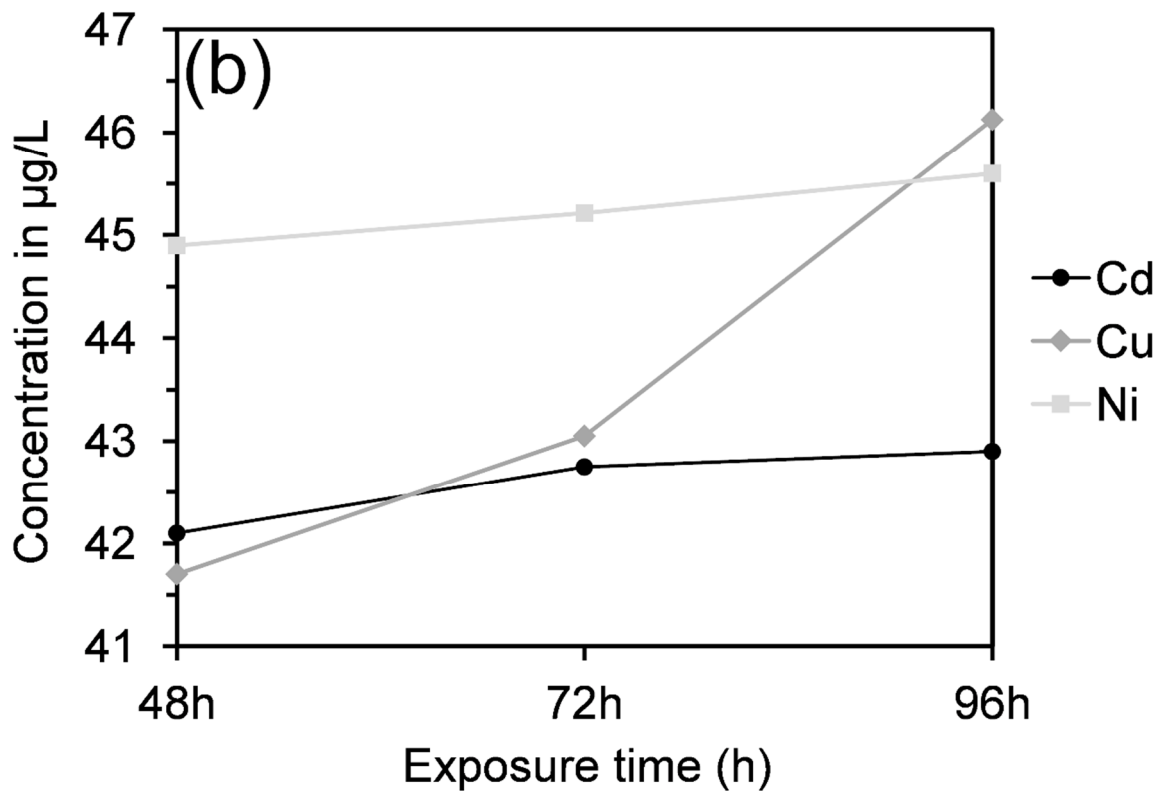
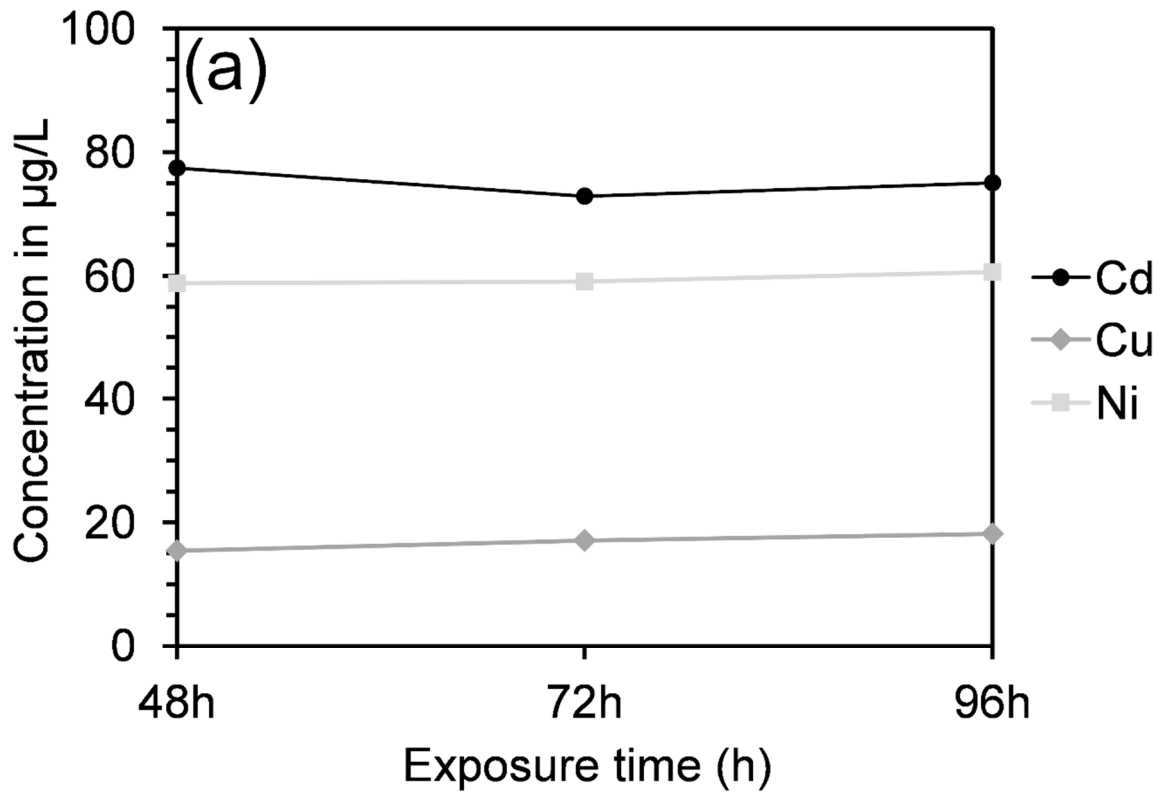
375 **FIGURE 6.** Final concentrations of cadmium (Cd), copper (Cu) and nickel (Ni) in the water
 376 of each condition of Experiment 1 (a) and Experiment 2 (b) after 96h of exposure.

377

378 Results for Experiment 1 (**Fig. 6a**) showed that Cd concentrations in the water of the beakers
 379 at the end of the experiment was low in all the controls but were very high in all the exposed
 380 ones, with $72.7 \pm 1.2 \mu\text{g.L}^{-1}$ for Cd and $72.9 \pm 3.3 \mu\text{g.L}^{-1}$ for Mix. The same trend was found
 381 with Ni, which was very low in all controls and the Cd-exposed beakers (Cd) but very high in
 382 the water of the exposed Mix beakers ($59.4 \pm 3.7 \mu\text{g.L}^{-1}$). Cu was the highest in the Mix

383 beakers ($15.7 \pm 3.3 \mu\text{g.L}^{-1}$) but generally low compared to the Cd and Ni concentrations.
384 These trends were obviously due to the initial addition of high concentrations of metals in the
385 exposed beakers. The two-way ANOVA performed on each metal showed similar results for
386 Cd and Ni, with treatment, duration and their interaction significant ($p < 0.05$). But for Cu only
387 treatment factor was significant. **Fig. 6b** shows the results for Experiment 2. Very low
388 concentrations of the three tested metals were observed in the water of all the control beakers,
389 fed and unfed, but high concentrations appeared in both Mixfood and Mixnofood beakers at
390 the end of the experiment, with similar values for the three metals, all of them between 42
391 and $46 \mu\text{g.L}^{-1}$. Only the treatment factor was statistically significant in all metals.

392 The respective concentrations in the water of the Mix and Mixfood beakers during the
393 experiments were compared in **Fig. 7**.



394

395 **FIGURE 7.** Concentrations of cadmium (Cd), copper (Cu) and nickel (Ni) in the water of the

396 Mix condition during Experiment 1 (a) and the Mixfood condition during Experiment 2 (b).

397

398 Results for Experiment 1 (**Fig. 7a**) indicate that all concentrations in the Mix beakers were
399 quite steady throughout the whole experiment, with Cd and Ni being the highest compared to
400 Cu. **Fig. 7b** shows the corresponding results for Experiment 2. The concentration of all of the
401 three tested metals in the water of the Mixfood beakers was increasing throughout the whole
402 experiment, slightly for Cd and Ni but considerably for Cu, which presented the lowest
403 concentration among the three at the beginning but became the highest after 96h of exposure.

404

405 **4. DISCUSSION**

406

407 **4.1. Effects of metals on population growth of *Paracyclopsina nana***

408

409 ***Total population growth***

410

411 Overall results for Experiment 1 and for Experiment 2 showed that the total number of
412 individuals was always higher in the control than in the treatments with metals. It is
413 recognized that trace metals have toxic effects on marine organisms and can even affect their
414 survival (Bryan, 1971; Calabrese et al., 1977; Florence et al., 1994; Zyadah & Abdel-Baky,
415 2000; Berasategui et al., 2018). Here, the decreasing populations observed among the
416 different conditions of the experiments were due to mortality that occurred during the *P. nana*
417 life cycle.

418 Results of Experiment 1 showed that the lowest final population size among all conditions
419 was obtained when copepods were exposed to Cd alone, whereas the mixture led to an
420 increasing and higher final population density. Zidour et al. (2019) also found that the metal
421 mixture composed of sublethal concentrations of Cd, Cu, and Ni did not present additive or
422 synergistic effect in terms of mortality to the calanoid copepod *E. affinis*, whereas Cu alone
423 induced a very high mortality rate. Brand et al. (2004) also showed that the total population

424 size of the copepod *Tisbe holothuriae* was always reduced when exposed to Cd only, even at
425 low concentrations.

426 All conditions from Experiment 2 provided increasing population densities after 96h of
427 exposure, except the populations from the unfed controls (Cnofood). The highest final
428 populations were even obtained from the Mixfood beakers, being higher than all the control
429 populations. The exposure to the mixture, therefore, did not affect the growth of the copepod
430 population used for Experiment 2. This initial copepod cultures were grown for several
431 months in 15 psu seawater made of the classic seawater used in the laboratory and tap water
432 containing above average concentrations of Cu. Therefore, copepods growing in this culture
433 were chronically exposed to the metal and already accumulated it in an abnormally high
434 concentration, and were even already saturated in Cu before the beginning of the experiment.
435 This led to the fact that copepods were not able to bioaccumulate more Cd, Cu, and Ni used
436 at sublethal concentrations in the Mix beakers, and they were also not able to fully release
437 their high accumulated metal concentrations due to a lack of completely uncontaminated
438 water. These results may shed light on a phenomenon of tolerance to metals, and particularly
439 Cu, that appeared in the *P. nana* population before the experiment. Moraitou-Apostolopoulou
440 et al. (1983) observed a tendency for higher tolerance to Cu over generations of the marine
441 copepod *Tisbe holothuriae* due to acclimation. The delay in maturation time induced by Cu in
442 the F2 generation appeared less pronounced in F3 and similar to that of untreated individuals.
443 The initial 48h-LC50 also presented an increase in the F3 and F5 generations. The *P. nana*
444 population used for our experiment may have developed a metal tolerance over generations
445 during its several months of acclimation. In addition, the Mixfood beakers eventually turned
446 out to be those offering a most similar environment to the one where copepods were adapted
447 to, containing metals and food. Therefore, were not affected by these conditions but
448 developed at these particular treatments. Another hypothesis proposed by Kadiene et al.
449 (2019) was followed here as well. The authors showed that copepods are able to take in water
450 orally thanks to a dyed medium and that the dye concentrated into the gut epithelium of the

451 copepods. This phenomenon supported the idea that oral water intake may be involved in the
452 higher metal body accumulation observed in copepods exposed to dissolved metals compared
453 to those exposed to dietary metals. Feeding would then reduce the stress associated with
454 exposure to dissolved metals in copepods by filling the guts, and preventing it from
455 additional intake of metal-loaded water.

456 In both experiments, the unfed control Cnofood presented a decreasing population
457 whereas the population for the fed control Cfood was always increasing to become one of the
458 highest. In the Cfood beakers, these already contaminated copepods were fed and able to
459 eliminate a part of their accumulated metals to the ambient water. It became obvious that
460 starved copepods had decreasing population densities due to mortality (Cowey & Corner,
461 1963; Threlkeld, 1976). The mortality rate of *Calanus helgolandicus* was 5.0-6.5% per day
462 for starved copepods and below 1% for fed ones in the starvation experiment performed by
463 Cowey & Corner (1963). But this effect was reinforced when subjects are exposed to
464 contaminants or additional stress such as non-optimal salinity (Devreker et al., 2004). Results
465 of Experiment 2 showed that copepod populations of the Mixnofood beakers were lowest.
466 The exposure to metals coupled to a lack of food amplified copepod mortality and population
467 decrease. Pedroso et al. (2007) exposed the euryhaline copepod *Acartia tonsa* to silver and
468 proved that the mortality observed in starved copepods was explained by a decrease in the
469 body concentration of Mg^{2+} due to the inhibition of the associated enzymes by increasing
470 silver concentrations.

471 Comparing results from Experiment 1 and Experiment 2 demonstrated higher population
472 densities in *P. nana* of Experiment 1 using the LC50 concentrations of *E. affinis*. This proves
473 that LC50 concentrations for the same metals were higher than for *P. nana* than those for *E.*
474 *affinis*, and so that *P. nana* appears as a less sensitive copepod species to metals compared to
475 *E. affinis* (Dahms et al., 2016).

476

477 ***Stage-specific composition***

478

479 Our results from Experiment 1 showed an abundance of nauplii in all treatments with an
480 increasing density over time, particularly in the conditions exposed to metals, Cd and Mix.
481 Zidour et al. (2019) also demonstrated an increasing number of nauplii of *E. affinis* in the Cd
482 treatment. In their study, the copepodite density also increased at the end of the experiment
483 particularly with Cd. Increased copepodites likely correspond to the high number of resisting
484 nauplii who turned into copepodites. The increasing densities of larval and juvenile stages
485 despite the exposure to metals may also be explained by the concentrations used for this
486 experiment corresponded to the respective LC50 values of *E. affinis* and were too low for *P.*
487 *nana*, which appeared as less sensitive to metals. Pre-adult individuals (C5 males and
488 females) appeared to be much affected by metal exposure as their densities decreased over
489 time in the metal treatments. The same pattern was found by Zidour et al. (2019), the number
490 of pre-adults of *E. affinis* decreased after exposure to Cd, Cu, and Ni, studied alone and in
491 mixture. Copepod C5 individuals, therefore, appeared as a metal-sensitive life stage. This
492 sensitivity could be related to the physiological requirements and the energetic cost of
493 maturation from C5 to the final reproductive C6 stage. A slight decrease appeared in the
494 densities of adult males at the end of the experiment in Cd and Mix conditions. Conversely,
495 the number of adult females globally increased at all conditions, particularly the exposed
496 ones. In copepods, male sensitivity was often described as higher than those of females. The
497 male survival of the harpacticoid copepod *Amphiascus tenuiremis* exposed to Cu, Pb and Ni
498 was significantly lower than that of female (Hagopian-Schlekat et al., 2001). Finally, our
499 results showed that the densities of *P. nana* ovigerous females globally dropped at all the
500 conditions of the experiment, and even more at metal exposure. The number of *E. affinis*
501 ovigerous females decreased considerably when exposed both to Cd and Ni compared to the
502 control, whereas no effect was observed in the combined treatment (Zidour et al., 2019). A
503 similar phenomenon happened in our study as the population of ovigerous females in the Mix
504 beakers decreased after 96h but appeared always higher than that of the Cd beakers and even

505 reached one of the highest abundance, comparable to the controls. Ovigerous female densities
506 were generally low indicating that this life stage was most sensitive to metals as shown
507 earlier (Dur et al., 2009).

508 For Experiment 2, the densities of nauplii and copepodites started very low compared to
509 Experiment 1 and had lower densities at all conditions. There was only a slight increase of
510 population densities at 96h in some exposed beakers. It has been widely reported that nauplii
511 are a particularly sensitive life stage when exposed to high metal concentrations (Kadiene et
512 al., 2019). The most sensitive life stage of the copepod *Tisbe holothuriae* to both Cu and Cd
513 was the one-day-old nauplius (Verriopoulos and Moraitou-Apostopoulou, 1982). Similarly,
514 nauplii of the marine copepod *Tigriopus brevicornis* appeared to be two to four times more
515 sensitive than adults and copepodites for exposure to all tested contaminants, including Cd,
516 arsenic (As), and four different pesticides (Forget et al., 1998). The Cd LC50 of the naupliar
517 and copepodites stages of the asiatic copepod *Pseudodiaptomus annandalei* were very low,
518 with 40.3 $\mu\text{g}\cdot\text{L}^{-1}$ Cd and 120.4 $\mu\text{g}\cdot\text{L}^{-1}$ Cd, respectively (Kadiene et al., 2019). Accordingly, in
519 this study, the newly hatched nauplii showed a drastic decrease. The reduction of larval
520 survival affected the recruitment potential of nauplii and resulted in a decreasing copepodites
521 population. Conversely, the densities of C5 males and C5 females increased at all conditions
522 during the experiment, particularly in the fed ones, with very high final densities for the
523 Mixfood beakers. Moreover, a similar trend was observed for the adult stage: the numbers of
524 adult males, females, and ovigerous females all increased overall at fed conditions, and
525 particularly in the Mixfood treatment. This phenomenon can be explained by the fact that C5
526 individuals introduced in the experiment were already used to metal-contaminated water
527 because of their prior acclimation. The pre-adult individuals from this population appeared to
528 be tolerant to metals and thus developed within the experiment, especially in the exposed and
529 fed beakers that correspond to their acclimation living conditions, and lead to thriving adults.
530 The introduction of the food parameter in the experiment also confirmed this acclimation
531 phenomenon in copepods since these individuals developed better under fed conditions than

532 unfed and were, therefore, more affected by the lack of food than by exposure to metals. This
533 can be explained by the necessity of energy supplies assisting in the energetic costs of
534 detoxification. The occurrence of metal tolerance has already been reported in copepods.
535 Kwok et al. (2009) studied the marine copepod *Tigriopus japonicus* and showed that it can
536 develop a Cu resistance through multigeneration acclimation to high Cu levels and that this
537 resistance was increased from the very first generation of acclimation. Similarly, chronically
538 exposed lines of the copepod *Tigriopus californicus* to sub-lethal Cu concentrations for 12
539 generations showed an increasing Cu tolerance over time from generation 3 onwards (Sun et
540 al., 2014). Multigenerational exposure to Cu, therefore, generated a response consistent with
541 the procedure of long-term acclimation in our study.

542 For both experiments, our results showed much lower adult male numbers than females.
543 Particularly in Experiment 2, which led to about half as many males as females. This
544 parameter is affected by several factors such as species-specificity, but it was shown before
545 that copepod males are more sensitive than females to contaminants. The study of the
546 different lethal responses between male and female copepods to Cd toxicity revealed that
547 males of *P. annandalei* were almost twice as sensitive as females when comparing their
548 respective LC50 (120.6 $\mu\text{g.L}^{-1}$ Cd for males and 239.5 $\mu\text{g.L}^{-1}$ Cd for females) (Kadiene et al.,
549 2017). The same conclusion was established for *E. affinis* before in terms of Cu and Ni
550 toxicity as LC50 for males were 25.0 mg.L^{-1} Cd and 90.0 mg.L^{-1} Ni, whereas female LC50
551 were 38.0 mg.L^{-1} Cd and 161.0 mg.L^{-1} Ni (Zidour et al., 2019).

552 When comparing results from both experiments, we clearly saw that Experiment 2 gave
553 far fewer individuals of each life stage in each condition than Experiment 1. The second
554 experiment resulted in more than half as many adult males and females, and even more than
555 ten time less ovigerous females than the first one. This is explained by the lack of survival of
556 newly hatched nauplii, resulting in low recruitment and low population growth.

557

558 **4.2. Bioaccumulation of metals in *Paracyclopsina nana***

559

560 Copepods from Experiment 1 accumulated high concentrations of metals at exposed
561 conditions, and particularly in combined treatments as mixtures. This is explained by the fact
562 that the population was originally grown in uncontaminated water without any acclimation to
563 high metal concentrations. Results showed low initial concentrations of each metal in these
564 copepods. We observed an upward trend with exposure time in the accumulation of the three
565 tested metals. At the same time, a slight decrease of the three metal concentrations in water
566 was noticed. All three metals were highly concentrated in copepods from the combined
567 treatments, but Cu concentrations were always increasing over time and became particularly
568 high after 96h. Besides, Cu concentrations in the water of the combined treatments were
569 lowest compared to Cd and Ni which were highly concentrated, thus showing the opposite
570 trend. However, Cu was the metal introduced at the lowest concentration at the beginning of
571 the experiment. Cu therefore appeared as a more easily accumulable metal than Cd and Ni.
572 This is explained by Cu being an essential trace metal and marine invertebrates present the
573 ability to accumulate dissolved Cu from water directly by absorption through body surfaces
574 (Pinho et al., 2007). The calanoid copepods *E. affinis* and *P. annandalei* both demonstrated a
575 higher uptake of Cu than Cd and Ni from the dissolved phase, whereas it was the lowest
576 concentrated in the dissolved metal mixture at the beginning of the experiment (Kadiene et
577 al., 2019). This may also introduce the idea of a competition for the absorption between the
578 metals present in a mixture, as found for the same three metals with *E. affinis* (Zidour et al.,
579 2019). The metal presenting the highest affinity with the metal-binding sites in the body of
580 copepods may thus saturate these sites, preventing other metals to fix themselves, and
581 resulting in being the most accumulated metal among the combined treatments. This idea is
582 confirmed when comparing the respective Cd bioaccumulation levels in both the treatment
583 with Cd alone and with the mixture. Higher Cd concentrations were found in copepods from
584 the Cd alone beakers than in copepods from the mixture. Copepods were not able to

585 accumulate as much Cd in the mixture as the diversity of metals introduced a competition for
586 binding sites and their rapid saturation preventing a higher Cd absorption.

587 In Experiment 2, copepods also accumulated metals more at the exposed conditions than
588 the controls, but to a lesser extent. Cu was the most detected metal in copepod samples but
589 lower concentrations of each metal were found in copepods from Mixfood than in
590 Experiment 1. Cd was even half as accumulated as in the first experiment. Copepods from the
591 second experiment were growing for months in water containing sub-lethal concentrations of
592 metals and, therefore, had acclimated to metal exposure. Initial concentrations showed that
593 individuals from this population had already concentrated metal levels, particularly in Cu,
594 and so their bioaccumulation capacity against the exposure conditions of the experiment was
595 not as high as the population used in Experiment 1. That explains the three tested metals had
596 high concentrations (more than $40 \mu\text{g}\cdot\text{L}^{-1}$) in the water of all the Mix beakers of the second
597 experiment. Interestingly, Cu concentrations were always decreasing over time in copepods
598 from Mixfood. It was also the lower concentrated metal in water of these beakers at the
599 beginning of the experiment. However, it constantly increased over time to concentrations
600 more than twice as high as that of Experiment 1. At the beginning of this experiment,
601 copepods were actually more concentrated in metals than the water they were transferred to
602 where they depurated and released their accumulated metals into the experimental water,
603 particularly for Cu. Such rapid uptake and depuration rates of contaminants suggest that
604 copepods are able to quickly respond to changing concentrations in their environment,
605 confirming their strong potential as bioindicators in aquatic environments.

606 When comparing overall results for the feeding parameter, metal concentrations in
607 copepods were mainly higher for the fed conditions than the unfed ones, and for both
608 experiments. Each of the three metals was more concentrated in copepods from the fed
609 controls than in the unfed ones for the two experiments. Looking at exposed conditions, Cd
610 and Ni were also higher in copepods from Mixfood than Mixnofood of Experiment 2.
611 Copepods therefore, accumulate more metals when they are fed, for the same exposure

612 concentrations. Some studies already proved the existence of an increased effect of food
613 presence on the bioaccumulation of contaminants and that food-borne contamination is a
614 major factor to consider for the body concentrations of several elements (Kadiene et al.,
615 2019). Assimilation efficiency of trace elements is expected to increase with longer gut
616 passage time, therefore corresponding to feeding and digestive intervals.

617

618 **5. CONCLUSIONS**

619

620 The contrasting results from the two different populations revealed the plastic survival and
621 bioaccumulation capacity of *P. nana* for metals. The first experiment based on a regular
622 population showed that metal exposure induces reduction in population growth and increased
623 bioaccumulation in individuals. Cd was even more accumulated when it was treated
624 separately than when in combined mixture. This unveils a phenomenon of competition
625 between metals in mixture for the absorption in copepods. The second experiment started
626 with a population already pre-exposed to metals over several generations and revealed a
627 population growth that was less affected by metals and lower metal concentrations in exposed
628 individuals. The overall metal concentrations decreased in the copepods and increased in
629 experimental waters, demonstrating a depuration phenomenon in this population that was
630 already loaded with metals and acclimated to metal exposure. The overall results also showed
631 that food attenuated the effects of metal exposure on population growth but increased the
632 bioaccumulation capacity of metals. This study sheds light on the parameters that affect the
633 bioaccumulation of metals of copepods in contaminated aquatic ecosystems.

634

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636

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652

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