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1 **Graphene oxide worsens copper-mediated embryo-larval toxicity in the Pacific oyster while**  
2 **reduced graphene oxide mitigates the effects**

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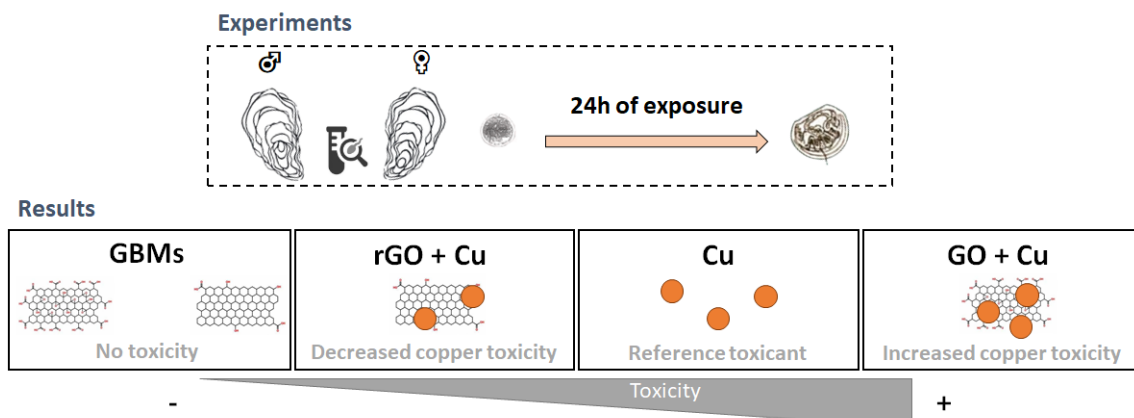
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12 **Abstract**

13 Due to their properties, graphene-based nanomaterials (GBMs) are triggering a great interest leading  
14 to an increase of their global production and use in new applications. As a consequence, their release  
15 into the environment is expected to increase in the next years. When considering the current  
16 knowledge in the evaluation of GBMs ecotoxic potential, studies aiming to evaluate the hazard  
17 associated to these nanomaterials towards marine species and particularly considering potential  
18 interactions with other environmental pollutants such as metals are scarce. Here we evaluated the  
19 embryotoxic potential of GBMs, which include graphene oxide (GO) and its reduced form (rGO), both  
20 individually and in combination with copper (Cu) as a referent toxicant, towards early life stages of the  
21 Pacific oyster through the use of a standardized method (NF ISO 17244). We found that following  
22 exposure to Cu, dose-dependent decrease in the proportion of normal larvae was recorded with an  
23 Effective Concentration leading to the occurrence of 50 % of abnormal larvae (EC<sub>50</sub>) of 13.85 ± 1.21  
24 µg/L. Interestingly, the presence of GO at a non-toxic dose of 0.1 mg/L decreased the Cu EC<sub>50</sub> to 12.04  
25 ± 0.85 µg/L while it increased to 15.91 ± 1.57 µg/L in presence of rGO. Based on the measurement of  
26 copper adsorption, the obtained results suggest that GO enhances Cu bioavailability, potentially  
27 modifying its toxic pathways, while rGO mitigates Cu toxicity by decreasing its bioavailability. This  
28 research underscores the need to characterize the risk associated to GBMs interactions with other  
29 aquatic contaminants and supports the adoption of a safer-by-design strategy using rGO in marine  
30 environments. This would contribute to minimize the potential adverse effects on aquatic species and  
31 to reduce the risk for economic activities associated to coastal environments.

32 **Keywords:** Ecotoxicology, nanomaterials, combined toxicity, safer-by-design, marine environment



## 34 1. Introduction

35 The development of technologies involving the use of graphene-based nanomaterials (GBMs) is  
36 supported by their unique properties, opening up possibilities for new applications in a wide range of  
37 areas (Jiříčková et al. 2022; Zhang et al. 2022). Among applications triggering interest, GBMs such as  
38 graphene oxide (GO) possess a high potential for pollution removal from water systems due to their  
39 sorption capacities (Adel et al. 2022; Akash et al. 2022; Kamran et al. 2022). However, while GO and its  
40 reduced form (rGO) constitute the major graphene-related products in the graphene market, they are  
41 likely to be mass-produced within the next years (Lin et al. 2019), conducting undoubtedly to their  
42 release in the environment at multiple phases of their life cycle (Mottier et al. 2017). Among GBMs,  
43 GO and rGO are the main products in the graphene market, constituting the GBMs products that are  
44 most likely to be released in the environment (Lin et al. 2019). When reaching the aquatic environment  
45 which act as a reservoir for nanomaterials (Scown et al. 2010), GBMs are likely to interact with other  
46 pollutants and potentially modify their fate, behaviour as well as their bioavailability for aquatic  
47 organisms, influencing their toxicity (Chowdhury et al. 2014; Martínez-Álvarez et al. 2021; Wang and  
48 Chen 2015). While based on modelling studies, GBMs concentrations in the aquatic environment are  
49 predicted to range from 0.001 to 1000 µg/L (De Marchi et al. 2018; Sun et al. 2016), to our knowledge,  
50 no estimation was provided yet for estuarine or marine ecosystems. Moreover, GBMs entry in  
51 estuarine areas is of particular concern as these ecosystems host an important biodiversity, provide  
52 essential ecosystem services and support human activities of high economic value such as navigation,  
53 fisheries and aquaculture (Barbier et al. 2011; Basset et al. 2013; Forrest et al. 2009).

54 Despite that data availability on the ecotoxic potential evaluation of GBMs is increasing (Ding et al.  
55 2022; Fadeel et al. 2018), GBMs hazard evaluation on marine species has been much less investigated.  
56 In addition, while considering the evaluation of interactive effects between GBMs and environmental  
57 contaminants, the main body of literature available present studies performed in freshwater  
58 organisms (De Marchi et al. 2018; Hu et al. 2016; Medeiros et al. 2020). Thus, knowledge gaps are  
59 remaining concerning these effects in marine ecosystems. Otherwise, the available data mainly  
60 focused on the effects of GBMs alone towards adult marine organisms (Khan et al. 2019a, b; Li et al.  
61 2021), while in marine invertebrates, early life stages were shown to be more sensitive to pollutants  
62 (Lasota et al. 2018). For this reason, there is a need to carefully evaluate their potential impact on  
63 marine species and interactions with other pollutants to properly regulate the use and disposal of  
64 GBMs, in order to prevent unexpected issues related to these nanomaterials (Hull 2019). We suggest  
65 that GBMs are able to modulate the ecotoxic potential of environmental contaminants due to their  
66 sorption capacities. For this purpose, the effects of GO alone or associated to copper as referent  
67 contaminant were evaluated through the assessment of their embryotoxic potential towards early life  
68 stages of the pacific oyster *Crassostrea gigas* also known as *Magallana gigas* (Bayne et al. 2017), using  
69 a standardized procedure (NF ISO 17244). In addition, as graphene-metal interactions were shown to  
70 depend upon the oxygen-containing surface groups (Nirmala et al. 2022), the consequences of a  
71 thermal reduction of GO into rGO on the toxic potential of the nanomaterials were evaluated as the  
72 loss of oxygen-containing functions might lead to decrease sorption capacities of the nanomaterial.

## 73 2. Material and methods

### 74 2.1. Tested graphene-based nanomaterials

75 Graphene oxide (GO) used in this study was provided by Antolin Group and prepared by oxidizing  
76 Grupo Antolin Carbon Nanofibers (GANF®; Grupo Antolín, Burgos, Spain) using the Hummer's method  
77 (Hummers and Offeman 1958; Lobato et al. 2016). The GO provided was thermally reduced at 200°C  
78 under H<sub>2</sub> atmosphere to produce rGO. The methodology applied for the production of rGO and the

79 procedure for the characterization of the GBMs are detailed in a previous work (Evariste et al. 2019).  
80 The results obtained from the characterization of the nanomaterials tested in the current study are  
81 summarized in Table 1. For any of the experiment carried out, GBMs were dispersed by sonication  
82 through the use of an ultrasonic bath for 10 min before contamination of the exposure medium.

83 GBMs behaviour in seawater was evaluated through the monitoring of dispersion stability using  
84 Turbiscan™ LAB Stability Analyzer (Formulation SA, Toulouse, France). Dispersion stability was  
85 monitored for 24 hours in seawater, corresponding to the whole embryonic development duration  
86 and the exposure media used during the bioassay. To allow detection of particles by the device, GBMs  
87 suspensions were prepared at 10 mg/L in seawater in presence or not of Cu at 2.5 mg/L. This condition  
88 was selected as it corresponds to the same GBMs/Cu mass concentration ratio performed in the  
89 embryotoxicity assessment based on the highest copper concentration to be tested according to the  
90 test guidelines (ISO 2015). This test was performed to determine the potential impact of the metal on  
91 the dispersion stability in the exposure media.

92 Table 1: Summary of the physico-chemical characteristics of the graphene oxide (GO) and reduced  
93 Graphene oxide (rGO) tested in the study. BET: Brunauer-Emett-Teller

	GO	rGO
Carbon content (at. %)	68.62	83.01
Oxygen content (at. %)	30.37	16.81
Number of layers	1-5	1-5
Lateral size (µm)	0.2-8	0.2-8
Specific surface area (BET; m <sup>2</sup> .g <sup>-1</sup> )	153.8	155.7

## 94 2.2. GBMs adsorption capacity

95 Copper adsorption capacity of the tested GBMs was evaluated. For this purpose, GO and rGO  
96 dispersions were prepared in deionized water at 10 mg/L and with Cu at 2.5 mg/L (added as copper  
97 sulfate) without agitation. Following 24 hours of incubation at room temperature, 10 mL of the solution  
98 were sampled prior filtration at 0.22 µm to remove GBMs from the solution.

99 Copper levels were analysed in samples using Inductively Coupled Plasma Emission Spectroscopy (ICP-  
100 OES, quantification limit: 2–10000 µg kg<sup>-1</sup>, precision measure: 1–5%; AMETEK Spectro ARCOS FHX22,  
101 Kleve, Germany). Sample solutions were diluted with a HNO<sub>3</sub> 2% solution to reduce the spectral  
102 interference and viscosity effects. With this method, the amount of copper in a quality control sample  
103 was recovered at 96% (nominal concentration: 50 µg L<sup>-1</sup>, actual concentration: 48 µg L<sup>-1</sup>). The solution  
104 was acidified at 2% of HNO<sub>3</sub> with ultra-pure 69% HNO<sub>3</sub> to avoid precipitation or adsorption in  
105 containers. Cu adsorption was determined based on the difference between the Cu concentration  
106 recovered in solutions devoid of GBMs compared to the concentration measured in the supernatant  
107 of GBMs-containing solutions.

## 108 2.3. Embryotoxicity assessment

109 The embryo-larval toxic potential of GBMs and its influence on copper toxicity was evaluated using the  
110 standardised NF ISO 17244 procedure (ISO 2015), previously AFNOR XP-T90-382. For this purpose,  
111 males (68.3 ± 13 g) and females (82.8 ± 10.8 g) conditioned genitor oysters were purchased from  
112 Guernesey Sea Farm Ltd. hatchery (Guernsey, UK). Gamete emissions were induced by thermal  
113 stimulation through successive baths at 16 °C and 28 °C for 30 minutes. Male gametes were mixed with  
114 oocytes at a ratio of 6:1. 20-minutes post-fertilization embryos are then distributed at a density of  
115 50,000 oocytes/L in wells of 6-wells microplates (300/wells) containing 6 mL of exposure media

116 composed of natural sterile seawater (Agua de mar, Vizmaragua grupo, Spain), pH = 7.97, with salinity  
 117 adjusted to 35 ‰ to fit with the salinity of the site of origin of the genitors. Embryo were exposed to a  
 118 positive control composed of Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O) at concentrations ranging from 2.5 to 25 µg/L as  
 119 recommended by the test guidelines. While copper was shown to mainly be associated to carbonate  
 120 ions in seawater (Millero 2005), the term Cu will be used for the different forms of dissolved copper.

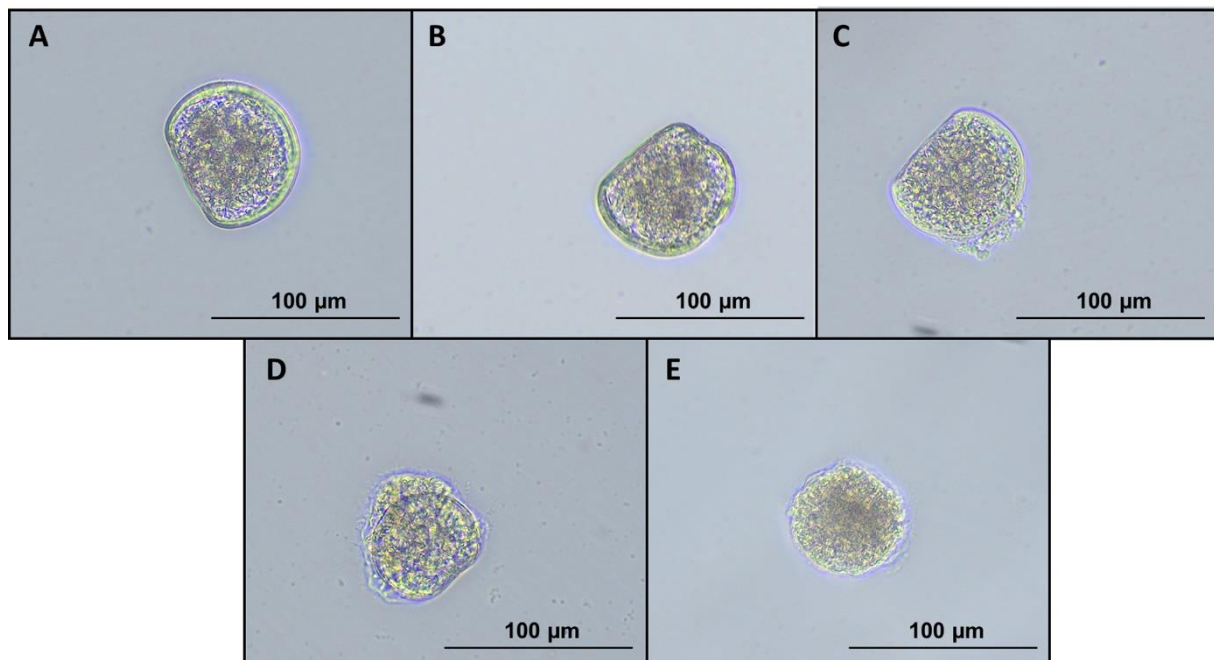
121 The effects of GO and rGO were tested using the GBMs alone at concentrations of 0.1, 1 or 10 mg/L or  
 122 at 0.1 mg/L associated with the tested Cu doses. The table 2 summarize the experimental conditions  
 123 tested. GBMs-Cu stock solutions were prepared 24 hours prior to the experiment and each  
 124 experimental condition was assessed in triplicate for each couple. After 24 h at 23.5 ± 0.5 °C, embryos  
 125 reached the D-shaped larvae stage in the negative control and were fixed using 100 µL of an 8%  
 126 formalin solution. Compared to normal larvae, anomalies were categorized as previously described as  
 127 shell abnormality, mantle abnormality, shell plus mantle abnormalities and development arrest (Figure  
 128 1) (Mottier et al. 2013).

129 Table 2: Summary of the experimental conditions tested, indicating the nominal concentrations of Cu  
 130 and GBMs tested in each experimental group.

Ctrl		GO		rGO		Cu		Cu+GO		Cu+rGO	
[Cu]	[GBM]	[Cu]	[GBM]	[Cu]	[GBM]	[Cu]	[GBM]	[Cu]	[GBM]	[Cu]	[GBM]
-	-	-	0.1 mg/L	-	0.1 mg/L	2,5 µg/L	-	2,5 µg/L	0.1 mg/L	2,5 µg/L	0.1 mg/L
		-	1 mg/L	-	1 mg/L	6.25 µg/L	-	6.25 µg/L	0.1 mg/L	6.25 µg/L	0.1 mg/L
		-	10 mg/L	-	10 mg/L	12.5 µg/L	-	12.5 µg/L	0.1 mg/L	12.5 µg/L	0.1 mg/L
						18.75 µg/L	-	18.75 µg/L	0.1 mg/L	18.75 µg/L	0.1 mg/L
						25 µg/L	-	25 µg/L	0.1 mg/L	25 µg/L	0.1 mg/L

131

132



133

134 Figure 1: Photomicrographs of D-shaped larvae of *Crassostrea gigas* following 24 hours of  
 135 development showing the anomalies considered for the analysis. A: normal larvae; B: shell anomaly;  
 136 C: mantle anomaly; D: shell + mantle anomaly; E: embryo

137

138

## 139 **2.4. Statistical analysis**

140 Statistical analysis was performed using Graphpad Prism 9 software. The results of embryo-larval  
141 development of exposed larvae are expressed as net percentages of normal larvae from the control  
142 condition. Non-linear four parameters regression was applied on the obtained data to calculate the  
143 EC<sub>50</sub> values, corresponding to the Effective Concentration leading to the occurrence of 50 % of larvae  
144 with development anomaly. Data were statistically tested by one-way ANOVAs after ensuring that the  
145 assumption of normal distribution and variance homogeneity are met.

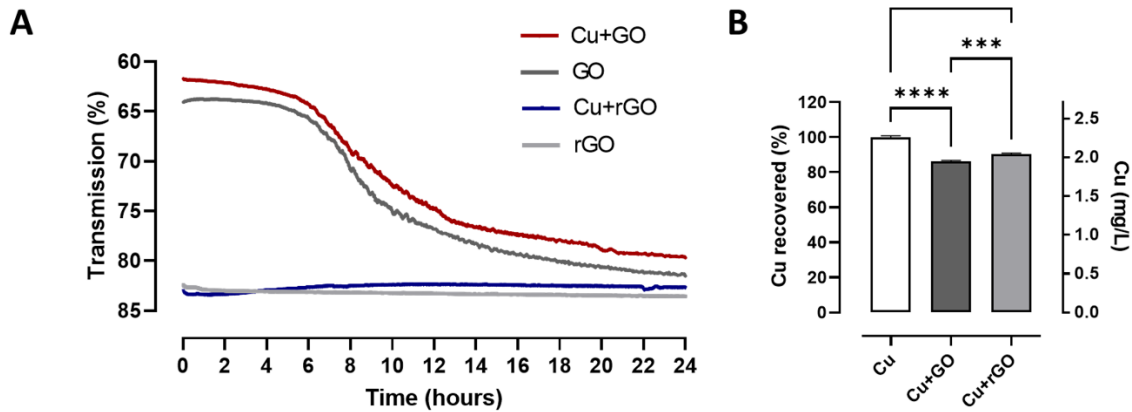
## 146 **3. Results and discussion**

### 147 **3.1. GBMs behaviour in the exposure media and copper adsorption capacities**

148 The dispersion stability of GBMs monitored over 24 hours in seawater is presented in Fig. 2A. While  
149 rGO nanoparticles in the seawater are almost not detected at a concentration of 10 mg/L based on the  
150 high and constant transmission value ( $83.2 \pm 0.2\%$ ), GO dispersion showed sedimentation starting after  
151 6 hours of incubation as indicated by the transmission percentage increase over time (Figure 2A). The  
152 presence of copper ions did not significantly influence the GBMs stability in seawater.

153 Bioavailability of GBMs in the environment is driven by their behaviour in aqueous media. Multiple  
154 parameters including nanomaterial concentration, size, physicochemical properties, natural organic  
155 matter or salt composition were previously shown to influence GBMs colloidal properties (Castro et al.  
156 2018; Chowdhury et al. 2013, 2014, 2015; Ren et al. 2018; Su et al. 2017). Aggregation and  
157 sedimentation processes were previously shown to occur in seawater (Ding et al. 2018) which is  
158 consistent with the present data. Interestingly, the GO sedimentation pattern over time observed in  
159 this study is close to the data previously obtained using mineral water containing a much lower amount  
160 of salts while GO dispersion is stable in deionised water (Evariste et al. 2020). This, suggest that the  
161 critical amount of salt/ions leading to GO sedimentation is low and interactions with ions from  
162 seawater might be responsible of the GBMs sedimentation. However, as GBMs concentration was  
163 shown to influence its colloidal properties (Su et al. 2017), we cannot exclude that the stability of the  
164 tested materials would be higher at lower concentrations, including at 0.1 mg/L used in the  
165 embryotoxicity assessment.

166 Based on ICP-OES analysis, stock solution of copper used for serial dilutions, prepared at 10 mg/L of  
167 Cu, were dosed at  $9.5 \pm 0.06$  mg/L which is consistent with the nominal concentration. Monitoring of  
168 the water Cu concentration over time indicated a stable concentration of copper in absence of GBMs  
169 as indicated by the recovery of  $95.4 \pm 0.7\%$  of the initial copper concentration after 24 hours of  
170 incubation. However, after 24 hours in presence of GBMs, Cu concentration decreased by  $13.69 \pm 0.5\%$   
171 or  $9.52 \pm 0.5\%$  in presence of GO or rGO respectively (Figure 2B). As a consequence, the adsorption  
172 capacity of Cu on the tested materials was 31 mg/g of GO and 21.6 mg/g of rGO. Previous study  
173 indicated Cu adsorption process was controlled by chemical adsorption involving complexation  
174 between copper ions with oxygen-containing groups of the GBMs surface, which is consistent with the  
175 current results (De Beni et al. 2022; Liu et al. 2018). However, these results are a bit lower compared  
176 to the available data ranging from 55.47 to 294 mg/g for pristine nanomaterials and higher for  
177 modified composites (Adel et al. 2022; Sitko et al. 2013; White et al. 2018). Such discrepancies between  
178 studies may be due to the variability of the material characteristics in term of size, the types of oxygen-  
179 containing functions or the atomic percentage of oxygen in the materials, the specific surface area  
180 which are influenced by the synthesis and reduction process applied, influencing reciprocally their  
181 adsorption capacities (Bai et al. 2016; Botas et al. 2013; Leão et al. 2023; Liu and Speranza 2021).



182

183 Figure 2: GBMs behaviour in seawater and copper adsorption capacity. A: monitoring of the stability  
 184 of GO and rGO dispersion in seawater over 24 hours, expressed by the % of transmission detected  
 185 after the light goes through the sample. B: adsorption of Cu over 24 hours onto GBMs at 10 mg/L. The  
 186 values are given as the mean  $\pm$  SD ( $n = 3$ ). Statistical differences are evaluated using ANOVA followed  
 187 by post-hoc Tukey test as  $p < 0.05$ . \*\*\*:  $p < 0.001$ ; \*\*\*\*:  $p < 0.0001$ .

### 188 3.2. Embryotoxic potential of GBMs alone or associated with copper

189 Following exposure to GO or rGO at concentrations up to 10 mg/L, no significant induction of larval  
 190 development anomaly was observed (ANOVA,  $p = 0.642$ ) (Figure 3A). In previous studies, sperm  
 191 exposure from 0.0001 to 1 mg/L of GO induced fecundity defects and led to an increased occurrence  
 192 of early development abnormalities in the sea urchin (Mesarič et al. 2015). On the contrary, others  
 193 carbon-based nanomaterials such as carbon nanotubes (CNT) or nanofibers (CNF) were show to  
 194 possess a weak potential of egg fertilization inhibition in sea urchin embryos while it induced mortality  
 195 after 24 hours or exposure, leading to a  $LC_{50}$  of  $\sim 90$  and  $\sim 30$  mg/L respectively, (Pikula et al. 2020).  
 196 These data suggest that the reprotoxic potential of GO towards sea urchin embryo, is higher than other  
 197 carbon-based materials. In the present study, based on the test guidelines, the experimental design  
 198 used allow to determine the effects of GBMs specifically on early-life development as fecundations are  
 199 performed in uncontaminated water prior exposure to the contaminants. Thus, based on the literature  
 200 and the current results, it is suggested that exposure to GO during the fecundation constitute a critical  
 201 period for the induction of development anomalies as no developmental defects are observed in *C.*  
 202 *gigas* when embryos are exposed post-fecundation to the GBMs alone.

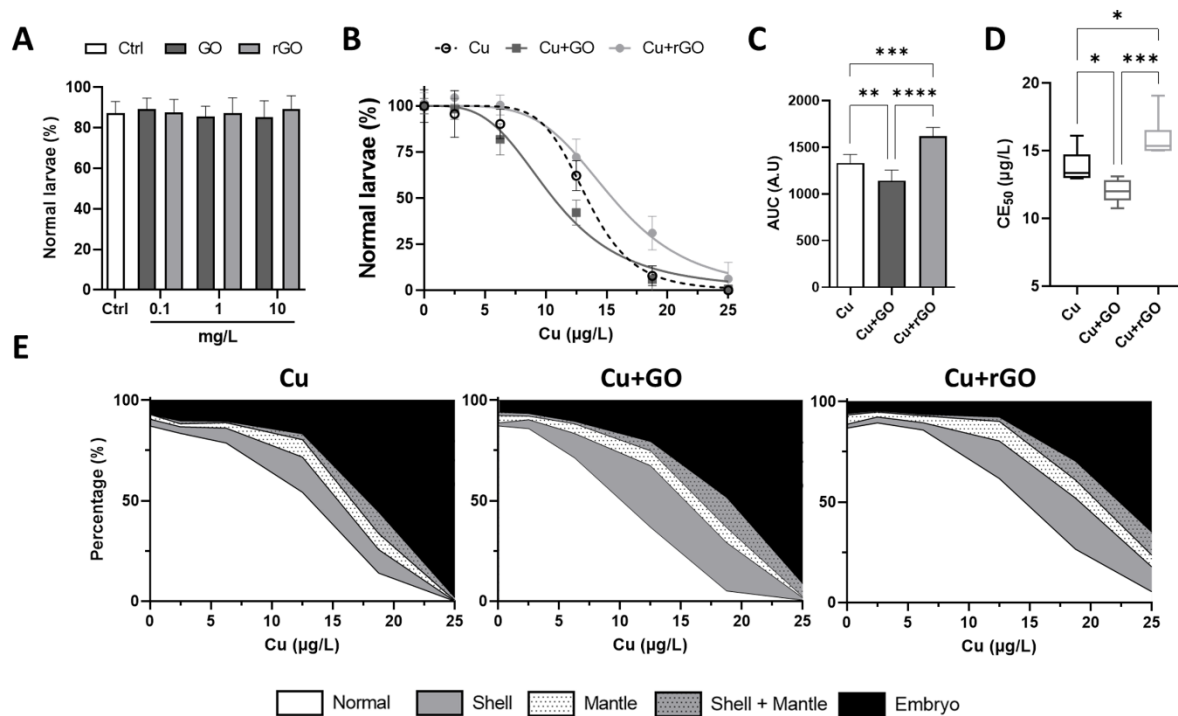
203 As expected based on the NF ISO 17244 procedure, a dose-dependent decrease in the proportion of  
 204 normal larvae was recorded following exposure to Cu with an  $EC_{50}$  of  $13.85 \pm 1.21$   $\mu\text{g/L}$  (Figure 3, B-D).  
 205 *C. gigas* larvae were shown to be sensitive to a wide range of environmental pollutants including trace  
 206 metal elements (Xie et al. 2017), pesticides (Mottier et al. 2014), PAHs (Nogueira et al. 2017),  
 207 pharmaceuticals (Di Poi et al. 2014), nanoparticles (Ringwood et al. 2010) as well as microplastics  
 208 (Bringer et al. 2020). Copper is used as a positive control to evaluate the sensitivity of the test  
 209 organisms as bivalve embryos are known to be sensitive to this model contaminant (Fitzpatrick et al.  
 210 2008; Gamain et al. 2016), exerting embryotoxic and genotoxic effects. In addition, *C. gigas* embryos  
 211 from hatchery-raised animals or from the field were previously shown to display the same sensitivity  
 212 to pollutants (Gamain et al. 2017). Thus, as the obtained results following copper exposure are in  
 213 accordance with the guideline validity requirements indicating that the Cu  $EC_{50}$  must fall between 6  
 214 and 16  $\mu\text{g/L}$  (ISO 2015), the data from other experimental conditions cannot be associated to a  
 215 particular sensitivity or resistance of test organisms to contaminants.

216 Interestingly, when Cu is associated to GO at the non-toxic dose of 0.1 mg/L, the calculated EC<sub>50</sub>  
217 significantly decrease to 12.04 ± 0.85 µg/L while on contrary it significantly increases to 15.91 ± 1.57  
218 µg/L in presence of rGO at the same dose (ANOVA, p < 0.001) (Figure 3 B-D). The lower EC<sub>50</sub> of Cu  
219 measured in presence of GO at 0.1 mg/L while the nanomaterial alone had no effect on embryo larval  
220 development suggest that GO increase Cu bioavailability while not for rGO. This hypothesis is  
221 supported by the data obtained with the assessment of GBMs copper adsorption capacity. Indeed, we  
222 demonstrated on the one hand that the Cu concentration in the water decreased by 13.69 ± 0.5% or  
223 9.52 ± 0.5% in the presence of GO or rGO respectively. On the other hand, the toxicity of Cu increased  
224 by 13.04 ± 6.15% in the presence of GO and decreased by 10.36 ± 2.43% in presence of rGO. This  
225 suggests that following adsorption, the bioavailability *i.e.* toxicity of Cu ions adsorbed on GO increases  
226 while it decreases in the case of rGO which possesses lower dispersion capacities in aqueous media.

227 Focusing on the type of anomalies recorded, the association of Cu with GO led to an earlier occurrence  
228 of shell anomalies while in presence of rGO, the proportion of the more severe anomalies are  
229 decreased following exposure to high copper concentrations (Figure 3E). The increase of shell  
230 anomalies specifically occurring following exposure to low Cu concentrations and the decreased  
231 proportion of development arrest following exposure to the highest copper dose suggest that the  
232 copper-associated toxic pathways are modified in presence of GO.

233 It was previously indicated that copper exposure induces genotoxic effects and alteration of the  
234 homeotic gene transcriptome leading to development anomalies (Sussarellu et al. 2018). Based on  
235 studies focusing on the effects of ocean acidification on oyster larvae development, it was indicated  
236 that shell development alterations occurred in *C. gigas* when carbonate ions availability decreased  
237 (Gazeau et al. 2011). Even if it was previously suggested that carbonate ions (CO<sub>3</sub><sup>2-</sup>) strongly interact  
238 with GO surface (Reynosa-Martínez et al. 2020), the increased occurrence of shell anomalies observed  
239 in presence of copper is unlikely to be associated to a decreased CO<sub>3</sub><sup>2-</sup> availability due to GBMs  
240 adsorption as those effects would also been noticed when embryos are exposed to GBMs alone.  
241 Further studies would be needed to determine which toxic pathways are associated with the co-  
242 exposure to GO and copper. Also, while technologies allowing an accurate detection and quantification  
243 of GBMs in complex environmental matrices at low concentrations are not available yet (Goodwin et  
244 al. 2018), their future development would help to decipher the understanding of interactions between  
245 GBMs, environmental contaminants and biota.





246

247 Figure 3: Effects of increasing concentrations of GBMs alone or at 0.1 mg/L associated with copper on  
 248 the embryo-larval development of the oyster *Crassostrea gigas*. A: histogram presenting the  
 249 percentage of normal larvae following 24 hours of exposure to GBMs; B: dose-response curves of  
 250 exposure to copper alone or associated to GO or rGO; C: resulting area under the curve calculated;  
 251 effective concentration inducing induces 50% of abnormal larvae; D: Occurrence of the abnormality  
 252 types determined after exposure to the contaminants. ANOVA followed by Tukey test. \*: p < 0.05; \*\*:  
 253 p < 0.01 and \*\*\*: p < 0.001.

254

255 In the case of GO, previous studies described a similar trend in toxicological responses in the shrimp  
 256 *Palaemon pandaliformis* (Batista de Melo et al. 2019), indicating that GO at 1 mg/L potentiates the  
 257 ecotoxicity of Cd and Zn, leading to an increased acute lethal toxicity. Similar effects to those measured  
 258 in presence of rGO were observed in presence of chelators which led to an increased larvae survival  
 259 (Knezovich et al. 1981). This suggest that on contrary to GO which is suggested to increase metals  
 260 bioavailability following adsorption, rGO behaviour in seawater leads to a decreased copper  
 261 bioavailability and protects marine organisms from copper toxicity. Of all the contaminants  
 262 transported to marine areas via the river-estuary continuum, copper occupies a predominant position  
 263 as a strong marker of human contamination linked to industrial activities (metallurgy, electronics,  
 264 surface industry), agriculture (wine growing, arboriculture), aquaculture (shrimp production) and  
 265 urban activities (wastewater treatment plant discharges) (Lyle-Fritch et al. 2006; Panagos et al. 2018;  
 266 Richardson 2014). Because of the worldwide growing development of GBMs, mixed GO/rGO and  
 267 metals contaminations in the aquatic environment have to be considered in the future.

268 Based on the results presented in the present study, we suggest that the thermal treatment of GO to  
 269 produce rGO constitutes a strategy promoting a safer by design approach in marine environment (Corsi  
 270 et al. 2021), through mitigation of toxicity associated to the decreased bioavailability of adsorbed  
 271 environmental pollutants. In this way, the reduction of GO to rGO could contribute to a better control  
 272 of toxic risks in coastal waters while helping to maintain economic activities such as shellfish farming.

273 **Author statement**

274 Antoine Mottier: Formal analysis, Investigation, Methodology; Morgan Légnani: Formal analysis;  
275 Frédéric Candaudap: Formal analysis; Emmanuel Flahaut: Funding acquisition, Resources; Florence  
276 Mouchet: Funding acquisition, Project administration; Laury Gauthier: Funding acquisition, Writing –  
277 review & editing; Lauris Evariste: Writing – original draft, Conceptualization, Methodology

278 **Declaration of competing interest**

279 The authors declare that they have no known competing financial interests or personal relationships  
280 that could have appeared to influence the work reported in this paper.

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