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1 **Metal stable isotopes in transplanted oysters as a new tool for monitoring anthropogenic** 2 **metal bioaccumulation in marine environments: the case for copper**

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18 **Abstract**

19 Metal release into the environment from anthropogenic activities may endanger ecosystems and
20 human health. However, identifying and quantifying anthropogenic metal bioaccumulation in
21 organisms remain a challenging task. In this work, we assess Cu isotopes in Pacific oysters (*C. gigas*)
22 as a new tool for monitoring anthropogenic Cu bioaccumulation into marine environments. Arcachon
23 Bay was taken as a natural laboratory due to its increasing contamination by Cu, and its relevance as a
24 prominent shellfish production area. Here, we transplanted 18-month old oysters reared in an oceanic
25 neighbor area into two Arcachon Bay mariculture sites under different exposure levels to continental
26 Cu inputs. At the end of their 12-month long transplantation period, the oysters' Cu body burdens had
27 increased, and was shifted toward more positive $\delta^{65}\text{Cu}$ values. The gradient of Cu isotope
28 compositions observed for oysters sampling stations was consistent with relative geographic distance
29 and exposure intensities to unknown continental Cu sources. A binary isotope mixing model based on
30 experimental data allowed to estimate the Cu continental fraction bioaccumulated in the transplanted
31 oysters. The positive $\delta^{65}\text{Cu}$ values and high bioaccumulated levels of Cu in transplanted oysters
32 support that continental emissions are dominantly anthropogenic. However, identifying specific
33 pollutant coastal source remained unelucidated mostly due to their broader and overlapping isotope
34 signatures and potential post-depositional Cu isotope fractionation processes. Further investigations on
35 isotope fractionation of Cu-based compounds in an aqueous medium may improve Cu source
36 discrimination. Thus, using Cu as an example, this work combines for the first time a well-known
37 caged bivalve approach with metal stable isotope techniques for monitoring and quantifying the
38 bioaccumulation of anthropogenic metal into marine environments. Also, it states the main challenges
39 to pinpoint specific coastal anthropogenic sources utilizing this approach and provides the perspectives
40 for further studies to overcome them.

41 **Keywords:** metal bioaccumulation, non-traditional isotopes, bivalve mollusk, Arcachon Bay, MC-ICP-MS

42 **Introduction**

43 Metal pollution of aquatic environments inherently alters their chemical composition and poses
44 health risks to ecosystems and humans (Fu et al., 2016; Gaetke et al., 2014; Reilly, 2004).
45 Identifying the origins of metals that accumulate at any given site is a key step in developing
46 successful emission control strategies and targeting contaminated sites for remediation (Barletta et
47 al., 2019; de Souza Machado et al., 2016; Lu et al., 2018; Weiss et al., 2008). To this end, the
48 study of the composition of metal stable isotopes in low-cost biomonitoring organisms may be a
49 promising approach for identifying and quantifying anthropogenic inputs (Martín et al., 2018;
50 Shiel et al., 2012; Smith et al., 2021, 2020). However, a prerequisite step is to examine the ability
51 of this approach in discriminating between natural Cu (the geochemical background) and
52 anthropized Cu, the latter being Cu released to the environment after its transformation within the
53 anthroposphere.

54 In coastal and marine ecosystems, bivalve mollusks have been widely used in “Mussel
55 Watch programs” for monitoring among other pollutants, marine trace metal (Krishnakumar et al.,
56 2018; Lu et al., 2017; Zhou et al., 2008). Indeed, bivalves, such as oysters and mussels, combine
57 several features that are advantageous for biomonitoring purposes: they are suspended matter
58 filter-feeders, abundant, sessile, and relatively easy to collect and handle in the laboratory (Araújo
59 et al., 2021a). Datasets of elemental levels in these organisms help obtain qualitative information
60 about spatial and temporal trends on metal bioaccumulation, but physiological (e.g., body size,
61 homeostasis) and environmental (e.g., salinity, primary production, phytoplankton assemblages)
62 factors may be confounding, and prevent an accurate assessment of the influence of
63 anthropogenic metal inputs in the environment (Briant et al., 2017; Cossa and Tabard, 2020; Lu et
64 al., 2019, 2017; Pourmozaffar et al., 2019). In turn, metal stable isotopes and mixing models can
65 potentially help track metals from their respective natural and/or anthropogenic origins, thus
66 providing a more direct appreciation of anthropic influence (Araújo et al., 2021b). Indeed, isotope
67 signatures of metals within the anthroposphere are associated with manufactured materials or their
68 by-products. They result from their original sources (e.g., coal and ore deposits), modulated by
69 their transformation processes, such as electroplating and smelting (Borrok et al., 2010; Brocza et
70 al., 2019; Gonzalez and Weiss, 2015; Shiel et al., 2010; Sun, 2019; Tonhá et al., 2020; Zeng and
71 Han, 2020; Zhong et al., 2021). Isotope signatures of metals circulating in the anthroposphere tend
72 to differ from isotope compositions of metals occurring naturally in waters and sediments, which
73 are modulated by weathering and biological activity (Araújo et al., 2019a; Babcsányi et al., 2014;
74 Guinoiseau et al., 2017; Mulholland et al., 2015; Vance et al., 2016). In previous studies, Zn and
75 Cd isotope records in wild bivalve’s soft tissues allowed to gauge and/or quantify anthropogenic
76 inputs in marine environments (Araújo et al., 2021b; Shiel et al., 2013, 2012). These previous

77 works used bivalve samples provided by samples banks feed by Mussel Watch programs from
78 France and United States, operating continuously since the 70s. Unfortunately, these national
79 biomonitoring programs do not cover all marine sites, and they are inexistent in most countries.
80 Transplanting bivalves from one ground to another is an alternative to circumvent the
81 unavailability of bank samples. It has been commonly practiced to observed metal
82 bioaccumulation trends in these organisms over determined periods (Geffard et al., 2002; Regoli
83 and Orlando, 1994; Riget et al., 1997; Roméo et al., 2003; Séguin et al., 2016; Senez-Mello et al.,
84 2020, 2020; Wallner-Kersanach et al., 2000). We are unaware of studies using “non-traditional”
85 isotopes in transplanted organisms.

86 A first examination on the variations of Cu isotope abundances (^{65}Cu and ^{63}Cu) in soft
87 tissues of wild bivalves (mussels and oysters) revealed the potential to obtain information related
88 to Cu sources, bioaccumulation mechanisms, and physiological status (Araújo et al., 2021a). The
89 latter study was conducted in a low-contaminated Atlantic site (Vilaine Bay) and integrated a 10-
90 year biomonitoring period. It attributed the observed temporal isotope fractionation patterns of
91 mussels to homeostatic regulation processes, involving changes in uptake and excretion rates with
92 increasing Cu bioavailability. For oysters, Cu isotope compositions evolved linearly with Cu body
93 burden, indicating a conservative isotope fractionation with Cu bioaccumulation over time.
94 However, the use of this particular isotope bioaccumulation pattern observed in oysters to identify
95 and quantify the biological incorporation of anthropogenic Cu remained untested. To continue this
96 work, we conducted an *in-situ* experiment with transplanted and caged oysters in Atlantic oyster-
97 rearing site recognized by its Cu-contamination history.

98 With up to 12000 tons/year, France is at present Europe’s top producer and consumer of
99 Pacific oysters (*Crassostrea gigas*, Buestel et al., 2009), and environmental concerns with high Cu
100 anthropogenic bioaccumulation in France’s farmed oysters dates back to the 1930s (Hinard, 1932).
101 Arcachon Bay (AB) is the oldest French oyster-producing basin, where a continuous increase in
102 Cu concentrations since 1982 is observed (Claisse and Alzieu, 1993; Fig. 1). Although Cu is an
103 essential micronutrient for oysters, excessive environmental concentrations of this metal can
104 damage endocrine systems and affect larval life stages, thus impacting ecological services
105 provided by these organisms, and up to compromising mariculture production and food safety
106 (Gamain et al., 2017; Mai et al., 2012; Sussarellu et al., 2018; Wang et al., 2011; Wijsman et al.,
107 2019). The origin of the increased Cu observed in AB oysters was putatively attributed to the
108 growing use by nautical activities of Cu-based antifouling paints after the ban of tributyltin (TBT),
109 a biocide that had long entered in the composition of such paints (Claisse and Alzieu, 1993).
110 Nevertheless, previous studies using Cu concentration data in sediment, water, and bivalves did
111 not allow to pinpoint the origin of this increased Cu, and/or ascertain its bioaccumulation.

112 As a new approach for our study, bivalves originating from a coastal neighboring site were
113 transplanted into AB and monitored for one year. Parameters included their elemental Cu levels,

114 Cu isotope compositions, and other biometric data, including shell length and weight of soft tissue
115 parts. Transplanted, caged bivalves share their previous and identical environmental chemical
116 exposure and life histories, and hence, they are advantageous to reduce possible isotope
117 variabilities related to variations in environmental and biological factors (Benedicto et al., 2011;
118 Caro et al., 2015; Ostrander, 1996; Senez-Mello et al., 2020). Since oysters were reared together
119 and at the same site since their larval stage, we hypothesize that the differences in isotope
120 fingerprints observed in transplanted oysters after a one-year exposure period reflect the different
121 local Cu isotope signatures at the new site. In our study of the AB, observed isotope shifts in
122 bivalves' soft tissues are attributable mainly to the bioaccumulation of Cu coming from coastal
123 anthropogenic sources, rather than isotope changes affecting marine Cu. Thus, using Cu as an
124 example, this work combines for the first time a well-known caged bivalve approach with metal
125 stable isotope techniques for monitoring and quantifying the bioaccumulation of anthropogenic
126 metals by these organisms into marine environments. Also, it states the main challenges to
127 pinpoint specific coastal anthropogenic sources utilizing this approach and provides the
128 perspectives for further studies to overcome them.

129

130 **Methods**

131 *Study area*

132 The Arcachon Bay (AB, 44°40'N, 01°10'W, Fig. 1) is a French macrotidal coastal lagoon (1 - 5 m
133 tidal range, 156 km²) connected to the Atlantic Ocean by a 5 km long channel (Deborde et al.,
134 2008). Surface seawater temperature ranges between 1 and 30 °C, and salinity is between 22 and
135 32 psu, with a significant difference between western and eastern basins that are under the
136 influence of oceanic and continental waters, respectively (Deborde et al., 2008). Shellfish farming
137 activities are characteristic of the local culture and economy, which started at the end of the 19th
138 century. The perimeter of AB is entirely lined by suburban centers and associated marinas (Fig. 1).
139 The port of Arcachon is one main leisure port of of the French Atlantic coast, accounting about
140 12,000 registered boats, which 95% of pleasure crafts, and the rest are used for fishing, oyster
141 farming and maritime transport (Le Berre et al., 2010). A census performed in the early 2000's
142 showed that most of these embarkations now use annually about 4,3m³ of copper-based
143 antifouling paints since TBT paints were banned (Auby and Maurer, 2004).. Thus, the sheer
144 intensity of leisure boating activities interacts strongly with AB's natural environment and its
145 professional users (Le Berre et al., 2010). The inner basin is affected by a major source of fresh
146 water by the Leyre River, and other small rivers that flow into the lagoon (Rimmelin et al., 1998).
147 The farm activities in the Leyre river watershed (Fig. 1) are a source of pesticides in the AB
148 (Fauvelle et al., 2018).

149

150 *Oyster transplantation experiment and wild oyster collection*

151 One thousand 18-month-old *C. gigas* oysters were used for the transplantation experiment. They
152 originated from the “Arguin Banc” site in the open Bay of Biscay, and presumably fully under
153 oceanic influence (Fig. 1), and were transplanted in AB where they were held in polyethylene
154 mesh bags used as cages. This pool of oysters was sub-divided into two 500-organism batches
155 that were transplanted on 30th March 2017 (beginning of the study, T₀) at the sites of “Comprian”
156 (inner AB) and “Grand Banc” (outer AB, Fig. 1). At each time point, ca. 80 individual oysters
157 were collected from each site after 3-, 6-, and 12-month exposure periods.

158 After collection, bivalves were depurated in laboratory tanks during 24 h, using filtered
159 local seawater. Then, 30 specimens were taken for biometric measurements, including shell length
160 and weight, total body weight, and the weight of their lyophilized soft tissues (namely dry tissue),
161 to quantify their growth using a body condition index: (weights of dry tissue / (total body tissue –
162 shell) * 1000; (Lawrence and Scott, 1982). Depending on the weight of the organisms, soft parts
163 of between 20 and 50 individuals were pooled, homogenized, and dried for the metal analyses
164 presented here. The average bioaccumulated Cu body burden (µg Cu per individual bivalve) in the
165 soft tissues of each pool was estimated by multiplying Cu concentrations of the pool by the
166 corresponding mean dry weight of the soft tissues. This is meant to accommodate differences in
167 growth rates and neutralize biodilution.

168 In AB, there are three oyster sampling stations from the French marine chemical
169 contamination biomonitoring network ROCCH. These stations, which include the Comprian site,
170 have been using indigenous oysters to monitor marine contaminants, including TBT and Cu, since
171 1980 (<https://wwz.ifremer.fr/surval/>). For an example, Fig. 1 shows a time series of Cu and TBT
172 levels in oysters from Comprian. For our study, an additional sample made of lyophilized soft
173 tissues of ca. ten indigenous oysters from Comprian and collected in the winter of 2019 and
174 prepared identically to the transplanted oysters within the biomonitoring framework of the
175 ROCCH.

176

177 *Sample preparation and analyses*

178 Bivalve sample digestion and chemical analysis have already been detailed in previous
179 publications (Araújo et al., 2019,a,b; 2021a,b). Briefly, aliquots of freeze-dried bivalve tissues
180 (~200 mg) were digested in closed vessels by a concentrated nitric acid solution and using
181 microwave energy. Copper elemental analyses were performed by quadrupole inductively coupled
182 plasma mass spectrometry (ICP-MS). For isotope analyses, aliquots of digested samples
183 containing 500 ng of Cu were purified using an AG-MP1 resin, and Cu isotope abundances
184 determined by multicollector ICP-MS (Neptune, Thermo Scientific) at the Pôle Spectrométrie
185 Océan (PSO) laboratory (Ifremer, France). Reference materials (RMs) of other animal tissues
186 (oyster SRM 1566b-NIST®; protein fish DORM-4, NRC-CNRC®) and procedural blanks were

187 included in each sample batch for analytical control. All sample preparation procedures were
188 carried out with ultrapure water and high-purity acid blends.

189 Isotope analyses samples were dissolved in diluted acid nitric (2% v/v) and analyzed at
190 concentrations around 250 ng g⁻¹. A Stable Introduction System (SIS: cyclonic spray chamber and
191 PFA nebulizer at 50 μL min⁻¹, ESI) was used to introduce samples into spectrometer. The raw Cu
192 isotope ratios were corrected for mass bias using the standard bracketing technique and the final
193 Cu isotope compositions expressed using the conventional δ-notation relative to the isotope
194 certified reference material NIST SRM-976 (Eq. 1):

$$195 \quad \delta^{65}\text{Cu}_{\text{SRM-976}}(\text{‰}) = \left(\frac{R\left(\frac{^{65}\text{Cu}}{^{63}\text{Cu}}\right)_{\text{sample}}}{R\left(\frac{^{65}\text{Cu}}{^{63}\text{Cu}}\right)_{\text{SRM-976}}} - 1 \right) \times 1000 \quad (\text{Eq. 1})$$

196
197 For unknown samples and RMs, δ⁶⁵Cu_{SRM-976} values represent the average and the two-standard
198 deviation (2s) of two or three individual measurements performed during a single analytical
199 session. The precision average obtained for individual samples and RMs was better than ±0.05‰.
200 The obtained δ⁶⁵Cu_{SRM-976} value for the RM fish protein DORM-4 (+0.55 ± 0.02‰, Table 1) fell
201 in the same range of values published for this material (+0.52 ± 0.08‰, Sullivan et al., 2020;
202 +0.48 ± 0.06‰, Sauzéat et al., 2021). The δ⁶⁵Cu_{SRM-976} value obtained for three full replicates of
203 the oyster tissue SRM 1566b of +0.25 ± 0.03‰ is in line with the long-term reproducibility for
204 this RM (+0.22 ± 0.03‰, 2s, n = 8, Araújo et al., 2021) at PSO laboratory.

205

206 **Results and discussion**

207

208 *Evolution of bioaccumulated Cu and its isotope composition in transplanted oysters*

209 The dataset is presented in Table 1, and all biometric data are included in the Supplementary
210 Material (Table 1S). The biometric data indicate a faster and higher body growth of oysters in
211 Grand Banc (outer AB) compared to those in Comprian (inner AB, Table 1S). Hence, to cancel
212 out effects of biodilution in Cu concentrations and to be able to compare Cu bioaccumulation
213 despite growth rate differences between these sites, we computed the oysters' Cu body burden (μg
214 Cu/per individual, Table 1) rather than Cu concentrations.

215 Oysters transplanted in Comprian and Grand Banc sites shared temporal patterns of Cu
216 concentration, body burden, and isotope compositions, i.e., a significant increase in the
217 concentration of bioaccumulated Cu accompanied by a shift toward more positive δ⁶⁵Cu values
218 (Fig. 2, Table 1). In Comprian, over the one-year exposure period, mean oyster Cu body burdens
219 increased continuously from 44 to 189 μg (four-fold increase), while δ⁶⁵Cu values shifted from
220 +0.35 to +0.60‰. The latter is remarkably close to indigenous oysters from Comprian harvested
221 in winter 2019 (+0.59‰, Fig. 2). In Grand Banc, the Cu body burden levels are lower than in

222 Comprian at the 3-month and 6-month time steps, but reach a very similar 188 $\mu\text{g}/\text{per individual}$
223 (Fig. 2a) at the end of the exposure period. In turn, the Cu isotope composition does not evolve
224 monotonously, with an initial shift to lower $\delta^{65}\text{Cu}$ values at the first-time step followed by an
225 increase and stabilization near 0.4‰ (Fig. 2b). Despite comparable Cu body burdens at the 2 AB
226 sites reached at the end of the transplantation experiment, the oysters' final isotope compositions
227 differ by 0.17‰ (Fig. 3a). It is also notable that the largest isotope shift occurs at Comprian, about
228 0.25‰.

229 The temporal changes from initial $\delta^{65}\text{Cu}$ values in oyster soft parts, and the increase of Cu
230 body burden and concentration suggest that bioaccumulated Cu in AB has a distinct origin from
231 that offshore, at the Arguin Banc site (Fig. 3a). In line with our initial hypothesis, the simultaneous
232 and linear increases of elemental body burden and isotope compositions shows that there exists a
233 pool of bioavailable Cu inside the AB which is distinct from the offshore marine environment.
234 The faster Cu bioaccumulation and larger magnitudes of isotope variation in oysters from
235 Comprian (inner AB) suggest that this area is under greater exposure to continental Cu emissions.
236 (Fig. 3a). This is possibly due to its geographic proximity to agriculture and urban sources from
237 the Leyre watershed and/or the influence from nautical releases (antifouling paints) from the inner
238 bay. In contrast, the more restricted isotope variations and lower Cu bioaccumulation rates in
239 Grand Banc (outer AB) are consistent with the more oceanic character of this site, which also
240 captures an attenuated and temporally-delayed continental Cu signal. The observed shift to lower
241 $\delta^{65}\text{Cu}$ values in the first three months of the transplantation experiment (GB-T1 sample in outer
242 AB) is attributed to off-shore seawater during the summer (low river flow). Indeed, estuarine
243 waters from the neighboring Gironde estuary (Atlantic French coast) with low anthropogenic Cu
244 displayed enrichments in light isotope about $+0.12 \pm 0.08\text{‰}$ (1s, n= 8, Petit et al., 2013), which
245 supports this proposition. Indeed, as will see in the further discussion, the estimated end-member
246 of marine bioaccumulated Cu pool for oysters matches well with a low $\delta^{65}\text{Cu}$ value for oceanic
247 waters.

248
249 *Inferring a Cu binary source model to apportion the continental and natural Cu fractions*
250 *bioaccumulated in oysters*

251 The gradient of Cu isotope compositions observed for oyster soft parts at Comprian and Grand
252 Banc sampling stations was consistent with exposure intensities to a continental Cu source.
253 Plotting $\delta^{65}\text{Cu}$ values against its reciprocal concentration ($1/[\text{Cu}]$) is a useful approach to identify
254 source mixing processes (El Azzi et al., 2013; Křibek et al., 2018; Mihaljevič et al., 2019). The
255 good fit of all oyster samples (indigenous and transplanted) on a straight line ($R^2 = 0.93$, $p < 0.05$)
256 indicate that bioaccumulated Cu and associated $\delta^{65}\text{Cu}$ values can be described in terms of a simple
257 binary mixing source model involving two end-members, defined here as representing the
258 continental and marine bioavailable Cu pools in oysters, respectively (Fig. 3b). The “mixture line”

259 obtained by regression analysis allow to estimate the values of these two end-members, which in
260 turn can then be used to quantify the continental Cu fraction bioaccumulated in oysters. As noted,
261 these end-members represent bioaccumulated pools of Cu, rather than actual Cu sources to this
262 environment. Since they are based on the oyster dataset, they already include any potential
263 biological isotope fractionation induced by oyster Cu bioaccumulation. Therefore, they can be
264 used to quantify the continental Cu fraction bioaccumulated in oysters' soft tissues irrespectively
265 of any biological fractionation. The implications in their use for source identification are discussed
266 in next section.

267 By substituting a global natural concentration baseline of Cu in oysters ($\sim 34 \text{ mg kg}^{-1}$, Lu et
268 al., 2019) in the linear regression equation, we obtain $+0.02\text{‰}$ for an isotopic end-member
269 representing the natural marine Cu bioaccumulated pool at our study site. Interestingly, this value
270 is close to some relatively unpolluted estuarine water samples from the neighboring Gironde
271 estuary (Atlantic French coast, $+0.12 \pm 0.08\text{‰}$, 1s, $n = 8$, Petit et al., 2013). For the other end-
272 member, we use the highest Cu concentration reported in oysters from the French Mussel Watch
273 program (of approximately $2,500 \text{ mg.kg}^{-1}$) to obtain a value about $+0.65\text{‰}$ for the continental
274 bioaccumulated Cu end-member (Fig. 3b). This extrapolation towards low and elevated Cu
275 concentrations is consistent with the exceptional capacity of oysters to bioaccumulate high loads
276 of Cu, as high as 4 % d.w. of whole-body tissue (Wang et al., 2011). The standard error of the
277 regression analysis (S), which represents the average distance that the observed values fall from
278 the regression curve, is about $\pm 0.05 \text{‰}$ and is considered the uncertainty associated with the
279 estimation of the two end-member values.

280 The calculated $\delta^{65}\text{Cu}$ end-member values of $+0.65 \text{‰}$ and $+0.02 \text{‰}$ enable the use of a
281 simple binary mixing model to quantify respectively the relative fractions of continental and
282 marine Cu fractions bioaccumulated in their soft tissues during the time-course of the
283 transplantation:

284

$$285 \quad \text{Cu}_{\text{continental}}(\%) = \left(\frac{\delta^{65}\text{Cu}_{\text{sample}} - \delta^{65}\text{Cu}_{\text{marine}}}{\delta^{65}\text{Cu}_{\text{continental}} - \delta^{65}\text{Cu}_{\text{marine}}} \right) * 100 \text{ (Eq. 2)}$$

286

287 where $\delta^{65}\text{Cu}_{\text{sample}}$, $\delta^{65}\text{Cu}_{\text{natural}}$, and $\delta^{65}\text{Cu}_{\text{anthropogenic}}$ stand for the $\delta^{65}\text{Cu}$ values obtained for the
288 sample of interest, and the estimated values for natural and anthropogenic Cu end-members,
289 respectively. The computed values are included in Table 1. Uncertainty values were computed by
290 error propagation in Equation 2 using analytical uncertainties of oyster samples (Table 1) and
291 estimate uncertainties of end-members ($\pm 0.05\text{‰}$) were below 1% for transplanted oysters.

292 The Cu of continental origin bioaccumulated in oysters before transplantation amounts to
293 52 %, revealing the transport of continental emissions to the marine environments beyond AB. In
294 the transplanted oysters from Comprian, this percentage climbs to 92%, matching closely this of

295 indigenous oysters (90%). Even if oysters from Grand Banc display similar Cu bioaccumulation
296 loads (Table 1), the continental Cu fraction is significant, with a contribution of 65 % to the Cu
297 body burden. As a consistency check, we use the concentration presented in Fig. 1. It shows that
298 the Cu concentrations in contemporary oysters have nearly quadrupled over the last 40 years,
299 indicating the “new” Cu incoming AB unlikely to derive from natural sources. It is reassuring that
300 the $\frac{3}{4}$ of continental Cu in present-day oysters estimated from the time series is close to the
301 isotopically-calculated fraction. Most anthropic sources reported in the literature, including
302 antifouling and urban sources, display more positive Cu isotope composition averages than the
303 Upper Continental Crust, which isotope range is about 0‰ (Fig. 4). Therefore, it is plausible that
304 the bioaccumulation of Cu emitted from coastal anthropogenic sources shifts oyster’s isotope
305 compositions to positive values, such as observed in our study. Thus, we consider continental Cu
306 emissions in AB dominantly anthropogenic. The following section discusses the potential use of
307 oyster’s isotope signatures to pinpoint anthropogenic Cu sources.

308

309 *A critical assessment of using recorded Cu isotope signals in oysters for source identification*

310 Using oyster isotope signatures for source identification requires verifying if biological uptake and
311 biogeochemical processes in sediment-water interface can overprint original isotope signatures of
312 sources. Here, we assess the potential effect of these factors on Cu isotope signals recorded in
313 oysters.

314 Oysters can accumulate Cu (and Zn) at high concentrations without serious toxic effects,
315 due to great capacity for detoxifying excess Cu and Zn by making these metals under inert and
316 non-toxic forms. Presumably, this mechanism compensates for their lack of significant cellular Zn
317 and Cu excretion (Kunene et al., 2021; Rainbow, 2018; Wang et al., 2018, 2011). Indeed, the
318 “half-lives” calculated for Cu and Zn excretion from the same *C. gigas* species from the
319 neighboring Gironde estuary Atlantic Coast, are about 1,500 and 3,000 days, respectively
320 (Geffard et al., 2002). These durations are very long compared to the life-spans of our oysters, and
321 are consistent with the limited elimination of these elements into inert, intracellular, metal-rich
322 granules (Geffard et al., 2002; Wang et al., 2018). Thus, these very low excretion rates of Cu and
323 Zn in oysters likely to not affect isotope budget of whole soft tissues, which makes these
324 organisms “integrative isotope recorders” of the source contributions in the bioaccumulated Cu
325 and Zn from their surrounding environment. While this has already been shown for Zn isotopes in
326 oysters in aquarium-based experiments (Ma et al., 2019), it is still to be rigorously confirmed for
327 Cu. However, based on the similarity of bioaccumulation mechanisms for these both
328 micronutrient elements (Kunene et al., 2021; Tan et al., 2015, Weng et al. 2018), and the data
329 presented above, we can speculate that it is also true for Cu.

330 Thus, we attribute the greatest difficulty to pinpoint anthropogenic Cu not to biological
331 fractionation processes, but rather to the gaps in ours constrain about Cu isotope fractionation in

332 anthroposphere (Tonhá et al., 2020; Viers et al., 2018; Yin et al., 2018). Anthropogenic isotope
333 signatures latter derive mainly from mineral deposits of this element, which present the most
334 extensive range among the natural compartments (-16.5 to $+10$ ‰, Klein and Rose, 2020; Mathu
335 and Wang, 2019; Moynier et al., 2017; Wang et al., 2017). Consequently, anthropogenic sources
336 also display a wide isotope variability that can overlap each other, hampering the discrimination of
337 individual Cu anthropogenic sources (Fig. 4). This drawback becomes more critical in complex
338 coastal environments where several anthropogenic metal sources normally coexist.

339 It is worthy also to argue that the direct comparison of Cu isotope compositions between
340 distinct, but connected marine biogeochemical reservoirs (e.g., sediment, water, biota) and
341 anthropogenic materials is also fraught with uncertainties due to potential isotope fractionation
342 that may occur during release and transfer between these environmental compartments before
343 bioaccumulation. As an example, in Cu-polluted soils by Cu-based fungicide, soil particle
344 leaching and surface runoff exhibit a shift up to 0.40 ‰ in comparison to the particulate phase
345 source (Babcsányi et al., 2016; Blotevogel et al., 2018; El Azzi et al., 2013). In marinas and
346 harbors, sediments that have been Cu-contaminated by antifouling paints show isotope signatures
347 slightly lighter than the source anti-fouling paints themselves, but significantly different from the
348 natural background (Briant, 2014). This suggests a preferential release of heavy isotopes from
349 these compounds when solubilized in seawater, or the occurrence of an unaccounted fractionation
350 process between the paint chip and its host sediment. Similar modifications on source isotope
351 signatures induced by changes in the metal speciation have also been observed for Zn, Hg, and Cd
352 isotope systems in sites with industrial and metallurgical contamination legacies or in laboratory
353 involving photochemical reactions of Ag-nanoparticles (Tonhá et al., 2020; Brocza et al., 2019; Li
354 et al., 2021; Shiel et al., 2010; Zhong et al., 2020).

355 It is noted that anthropogenic fingerprints in natural sample archives like sediments,
356 exceed in amplitude the isotope range from the natural Cu baseline. The latter normally centered
357 around 0 ‰ (Fig. 4). This isotope pattern is illustrated when comparing sediment from almost
358 pristine to highly contaminated sites, like those of the Loire estuary, Port Camargue, and Toulon
359 Bay (Fig. 4). The latter is characterized by lighter isotope signatures related to warfare and
360 shipbuilding contamination legacies that ranges from -0.79 to $+0.34$ ‰ (Araújo et al., 2019a). In
361 contrast, Port Camargue sediments range from -0.13 to $+0.44$ ‰ (Briant, 2014), tending in overall,
362 to more positive values related to Cu-based antifouling paints. In turn, the relatively
363 uncontaminated Loire estuary sediments have a narrower isotope range, with $\delta^{65}\text{Cu}$ values
364 between -0.24 and $+0.09$ ‰, with an average of -0.04 ± 0.18 ‰ (2s, n = 31). This average value is
365 close to that of UCC (~ 0 ‰) and likely reflect the variability of its natural sources, such as
366 weathered particles derived from soils and rocks of the Loire river watershed (Araújo et al.,
367 2019b).

368 These studies and ours demonstrate that Cu isotopes can be useful to discriminate
369 anthropogenic and natural sources in despite of possible isotope modifications of anthropogenic
370 metals after their release into the environment. While identifying specific coastal sources remain
371 a challenge task because these post-depositional isotope changes, they still carry isotope signals
372 that can be traced and apportioned in particulate and dissolved phases, and ultimately, into the
373 organisms.

374

375 *Conclusions*

376 This study confirmed, for the first time, the applicability of a “non-traditional” metal stable
377 isotope system in transplanted oysters to monitor metal bioaccumulation. Our findings
378 demonstrate that Cu isotopes can constrain the continental Cu fraction bioaccumulated in oysters
379 and infer its natural or anthropogenic origin. The present methodology of transplanting oysters
380 into an environmental contamination gradient can be extended to other metal isotope systems and
381 then yield an apportionment of anthropogenic contributions to the metal body burden of these
382 organisms. Furthermore, it does not hinge on the availability of sample banks of costly
383 environmental monitoring networks operating for long time series.

384 Unfortunately, source pinpointing remains elusive and further studies are sorely needed.
385 Indeed, the biogeochemical reactions of anthropogenic metal-based substances released into
386 aquatic environments may potentially induce fractionation of metal isotopes between particulate
387 and dissolved phases. This results mainly from changes in the metal atom’s coordination, strength
388 of bonds, ligand complexation (inner-sphere vs. outer-sphere formations), and adsorbent features
389 on the water column (Balistrieri et al., 2008; Dong and Wasylenki, 2016; Ducher et al., 2016;
390 Guinoiseau et al., 2016; Li et al., 2015; Lu et al., 2016; Moynier et al., 2017). Thus, further
391 laboratory and field experiments are required to observe and model how anthropogenic metals
392 from a range of compounds known to contaminate marine environment yield different isotope
393 signals in particulate and dissolved phases. For Cu isotopes, in the context of marine pollution,
394 Cu-based anti-fouling paints are an obvious first experimental target.

395 Nevertheless, the combined use of several isotope systems, like Zn, Cd, Ag, and Pb in the
396 so-called “multi-isotope approaches”, which have been successfully applied to individual pollutant
397 tracking (Araújo et al., 2021c; Li et al., 2019; Shiel et al., 2012), could enhance their power of
398 discrimination for different metal pollutant sources. This multi-isotope approach is timely for
399 environmental forensic applications addressing pollution source identification in marine
400 environments, since concentrations of trace metals, notably Cu, Zn, and Ag, are on the rise in
401 urbanized marine coasts (Barletta et al., 2019; Zalasiewicz, 2018), or still present in mobile and
402 bioavailable forms in legacy inventories (Araújo et al., 2019a; Briant et al., 2013; Caplat et al.,
403 2005; Dang et al., 2015b, 2015a; Resongles et al., 2014; Tonhá et al., 2020).

404

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410

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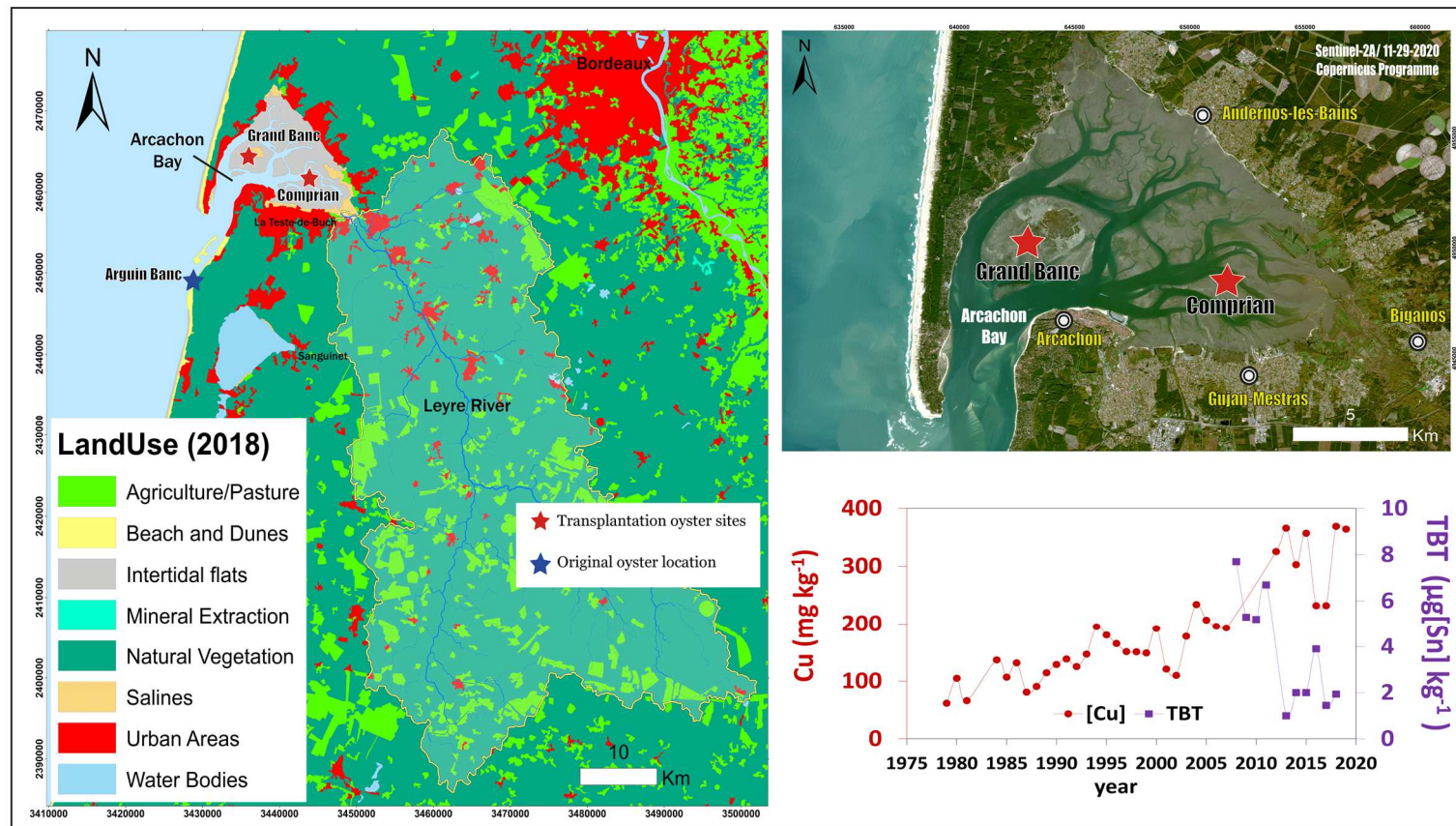


Fig. 1. Study site map and sampling stations. 18-month old oysters harvested from Arguin Banc were transplanted to Comprian and Grand Banc. The historical trends on Cu and TBT concentrations in indigenous oysters from the Comprian site are from ROCCH monitoring network (available at Surval: <https://wwz.ifremer.fr/surval/>).

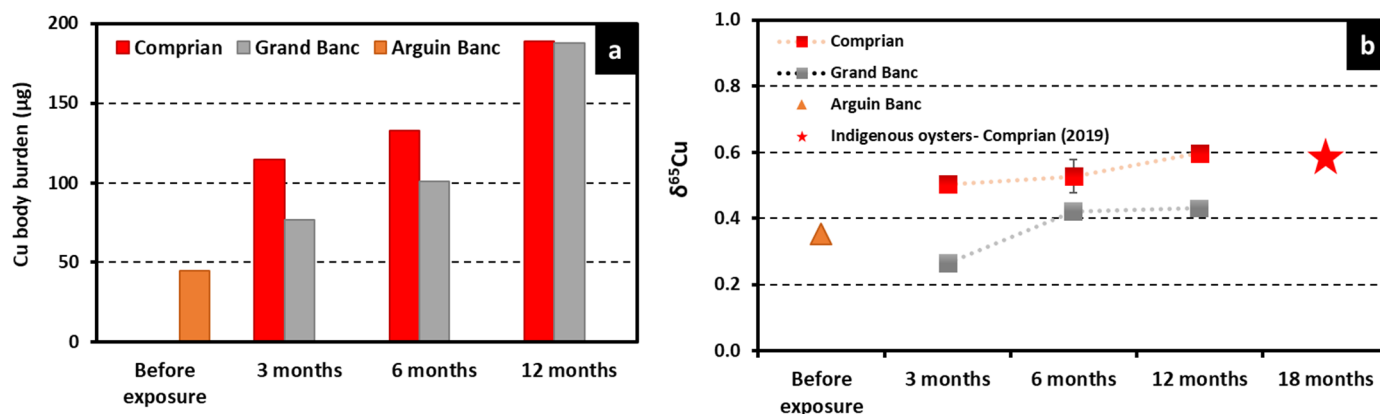


Fig. 2. (a) Temporal evolution of Cu body burden levels (Cu µg/per individual) and (b) $\delta^{65}\text{Cu}$ values (‰) for oysters before (Arguin Banc) and after their transplantation in Arcachon Bay (AB), at Comprian and Grand Banc. The data refer to pools of oysters collected after three-, six-, and twelve-month long transplantation periods. An additional 2019 sample composed of 18-month old indigenous oysters was acquired from the French Mussel Watch program (ROCCH) environmental sample bank.

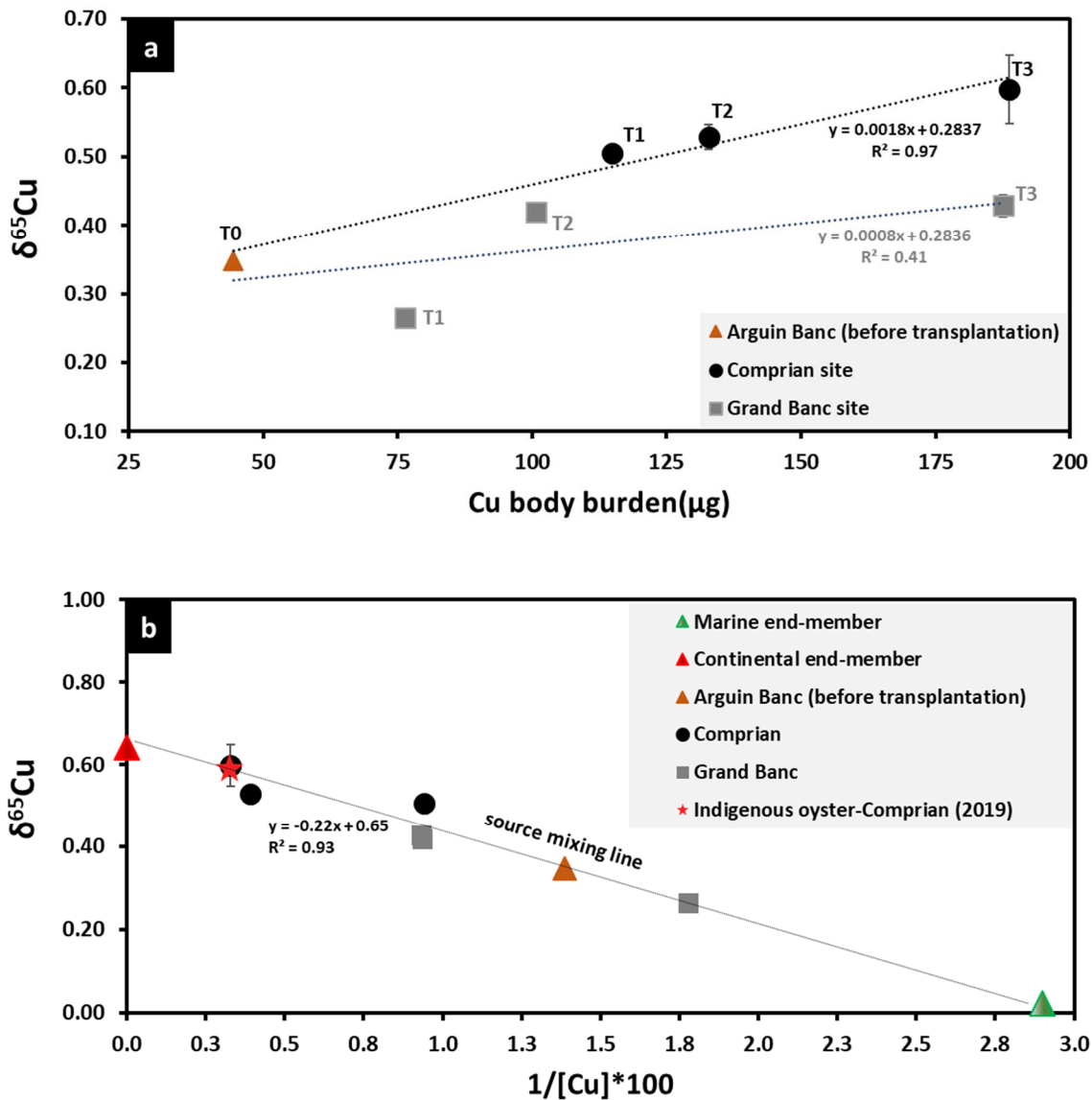


Fig. 3. (a) Plot of $\delta^{65}\text{Cu}$ values (‰) against Cu body burden levels (Cu $\mu\text{g}/\text{individual bivalve}$) for oysters transplanted from Arguin Banc to the Comprian and Grand Banc sites; T1, T2, T3 labels refers to three-, six-, and twelve-month long transplantation periods. (b) Binary isotope source mixing model based on the regression analysis of oyster samples. The 2019 sample composed of indigenous oysters was acquired from the ROCCH sample bank. The natural bioaccumulated end-member is estimated at +0.02‰ using worldwide concentration baseline for oysters (Lu et al., 2019). The continental Cu end-member (+0.65‰) is estimated using the highest Cu concentration in the ROCCH database for Pacific oysters ($2,5000 \text{ mg}\cdot\text{kg}^{-1}$). Error bars represent the analytical precision (2s) obtained for two measurements performed for each sample.

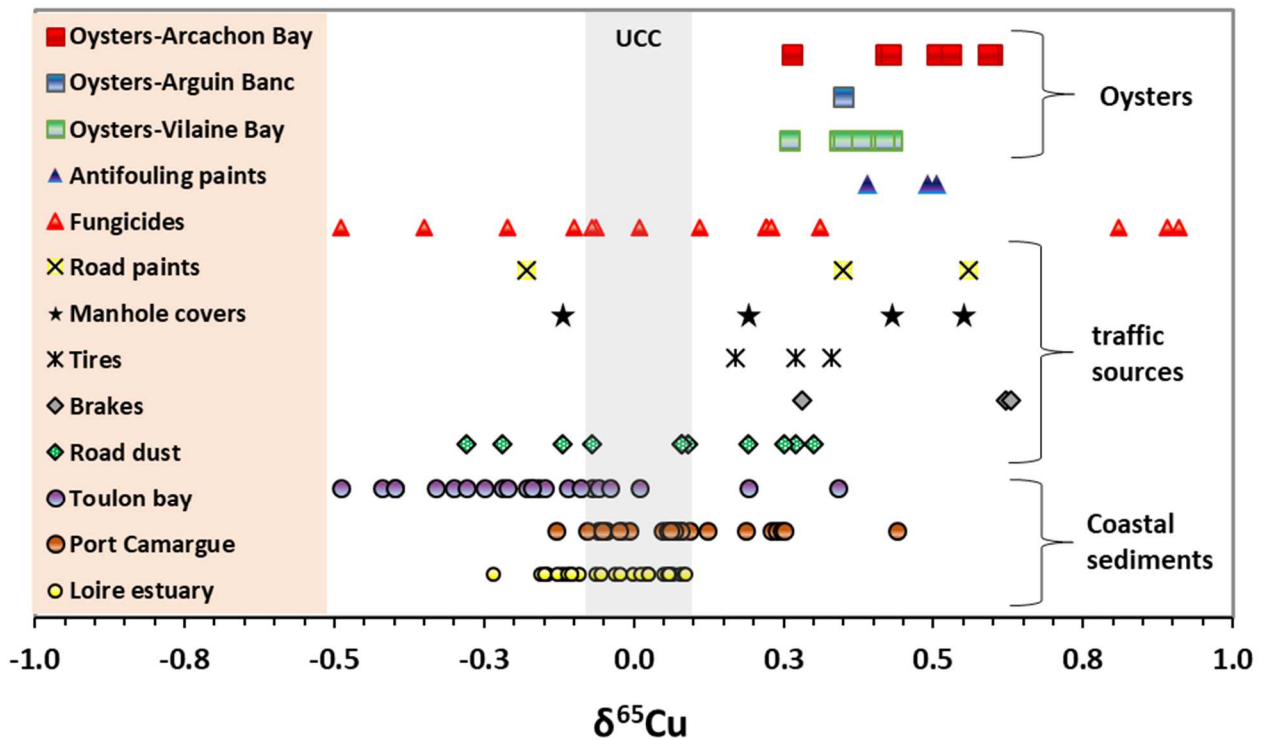


Fig. 4. Copper isotope compositions ($\delta^{65}\text{Cu}$, values in ‰) of oysters (this study) and anthropogenic materials reported in the literature: antifouling paints (Briant, 2014), fungicides (Babcsányi et al., 2016; Blotevogel et al., 2018; El Azzi et al., 2013), vehicle traffic-related sources (Dong et al., 2017; Schleicher et al., 2020; Souto-Oliveira et al., 2019). Oyster isotope data from Vilaine bay (Biscay Bay, Atlantic French shore) were published previously (Araújo et al., 2021a) and are included here for comparison. Coastal sediments include samples from Toulon bay (Araújo et al., 2019a), Port Camargue (French Mediterranean shore, Briant, 2014) and Loire estuary (Araújo et al., 2019c) (Biscay Bay, Atlantic French shore). They represent sediment isotope signatures related to harbor activities mixed to warfare contamination legacy, Cu-based antifouling paints and a low-Cu contaminated system, respectively. Grey band centered around 0 ‰ represents Cu isotope range average reported for Upper Continental Crust (UCC) (Liu et al., 2015).

Table 1. Copper concentrations, Cu body burden, and isotope compositions for dry-pooled oyster samples. The quantification of the continental Cu fraction (%) bioaccumulated in oysters using a binary isotope model is detailed in the text.

Oyster sample ID	Exposure period	[Cu] (mg kg⁻¹)	Cu body burden (µg)	δ⁶⁵Cu (‰)	2s	Bioaccumulated continental Cu fraction (%)
Oysters before exposure experiment (T0)	before exposure	72.1	44	0.35	0.06	52
Indigenous oyster (2019) in Comprian	18 months	305	ND	0.59	0.01	90
Transplanted oysters in Comprian						
Com-T1	3 months	106	115	0.50	0.01	77
Com-T2	6 months	257	133	0.53	0.02	81
Com-T3	12 months	304	189	0.60	0.05	92
Transplanted oysters in Grand Banc						
GB-T1	3 months	56	76	0.26	0.01	39
GB-T2	6 months	107	101	0.42	0.00	63
GB-T3	12 months	107	188	0.43	0.02	65

Graphical abstract

