



## Short Communication

## Can copper isotope composition in oysters improve marine biomonitoring and seafood traceability?



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## ABSTRACT

This study provides the first geographic and temporal large-scale analysis of Cu stable isotope compositions in indigenous oysters to biomonitor anthropogenic Cu inputs in aquatic systems. It includes oyster samples from French marine environments with distinct Cu-pollution levels and histories and extends over several decades of biomonitoring. Sample series composed of oysters from the same season and similar size/age intended to avoid biological biases. Oysters in macrotidal estuaries (Loire and Gironde, Western France) display fluctuating Cu concentrations without clear temporal trends, challenging to infer anthropogenic influence. Conversely, the time series of their Cu isotope ratios indicate a constant proportion of natural and anthropogenic Cu sources since the 1980s. In contrast, Cu isotope compositions in oysters from the progressively developing locales (urbanization and recreational boating) of Arcachon and Vilaine bays shift positively over time, along with increasing Cu concentrations. This finding suggests a gradual augmentation in the bioaccumulation of anthropogenic Cu over time. Thus, Cu isotope compositions in oysters is an unambiguous and robust approach to pinpoint anthropogenic Cu contamination in marine biomonitoring. Furthermore, Cu concentrations combined with isotope compositions yield site-specific fingerprints allowing geographic discrimination among oysters. This two-dimensional Cu signature is promising as a tool for seafood traceability.

## 1. Introduction

Systematic sampling of bivalve mollusks across time and space at target sites combined with elemental analysis of their soft tissues have been employed in environmental and seafood safety monitoring programs (“Mussel Watch programs”) of anthropogenic metals in coastal environments (Bennion et al., 2021; Briant et al., 2017; Farrington et al., 2016; Goldberg, 1975; Schöne and Krause, 2016). Such programs take advantage of various features of their bivalve monitors: sessile, abundant, tissues enriched with metals. They are used to scan for metal pollution hotspots along the coastline and to detect temporal changes in metal bioavailability (Beyer et al., 2017; Briant et al., 2017, 2021b; Cossa and Tabard, 2020; Jeong et al., 2021b; Lu et al., 2019; Zhou et al., 2008). However, their use is limited when attempting to ascertain the origins of metals and to deconvolve anthropogenic from natural factors like body size, age, season, etc., especially when metal concentrations fluctuate close to their natural baseline, or when their bioavailability

changes due to varying geochemical conditions (Baines and Fisher, 2008; Belivermiş et al., 2020; Cossa and Tabard, 2020; Griscom and Fisher, 2004; Hédouin et al., 2010; Pourmozaffar et al., 2019; Wright and Zamuda, 1987).

In the last decade, the isotope variability of metals (Cu, Zn, Fe, Ni, Cr, Ag, etc.) in natural and anthropic matrices has been used for environmental forensics purposes in diverse metal pollution contexts: mining (Araújo et al., 2017a, 2017b; Araújo et al., 2018; Ratié et al., 2016), industry (Jeong et al., 2020; Tonhá et al., 2021), urban land use (Nitzsche et al., 2021; Souto-Oliveira et al., 2018), and agriculture (Imseng et al., 2019). Metals become anthropogenic after being initially extracted from natural sources, such as ore deposits and coal, and subsequently processed into manufactured materials that make up electronic goods, urban structures, alloys, biocides, foodstuffs, pharmaceuticals, etc., and their related byproducts including mining wastes, industrial aerosols, and wastewaters (Araújo et al., 2022b). This cycling into the “anthroposphere” imprints specific isotope compositions on anthropogenic

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metals that enables them to be distinguished from their natural isotope baseline (Broczka et al., 2019; Desauty and Petelet-Giraud, 2020; Liu et al., 2020; Souto-Oliveira et al., 2019; Sun, 2019; Yin et al., 2016). This latter, in turn, is determined mainly by weathering processes and biological processes and may vary geographically (Guinoiseau et al., 2018; Huh et al., 1998; Lv et al., 2020; Mulholland et al., 2015; Ratié et al., 2015; Vance et al., 2016; Vance et al., 2008). The differentiation of isotope compositions between anthropogenic and naturally occurring metals allow for the identification of their respective sources, pathways and dispersion in the surface environment (Araújo et al., 2017a, 2017b; Araújo et al., 2019a, 2019b; Choi et al., 2019; Millot and Négrel, 2021; Reinfelder and Janssen, 2019; Wang et al., 2021; Zhong et al., 2020). In the case of copper (Cu), the variability on the abundances of its two stable isotopes ( $^{65}\text{Cu}$ ,  $^{63}\text{Cu}$ ) may result from mixing different sources or isotope fractionation effects due to biogeochemical processes, including partial leaching, redox processes, adsorption, chemical complexation and biological uptake (Bigalke et al., 2010; Coutaud et al., 2018; Komárek et al., 2021; Li et al., 2015; Li and Liu, 2022; Moynier et al., 2017). Therefore, Cu isotope ratios have been applied to constrain Cu anthropogenic inputs and transfer mechanisms at the water-sediment interface of aquatic systems (Babcsányi et al., 2016; Blotvogel et al., 2018; El Azzi et al., 2013; Peel et al., 2009; Petit et al., 2013).

Coastal environments, such as estuaries, lagoons and bays, trap in their natural compartments (water, sediment, biota) anthropogenic metals emitted from multiple anthropic sources and transported mainly via continental run-off and estuarine discharges (Barletta et al., 2019; Burt et al., 2019; de Souza Machado et al., 2016; Jeong et al., 2020; Tonhá et al., 2020). Anthropogenic metals, including Cu, at concentrations and bioavailable forms that are potentially toxic to biota and human health, may threaten their ecological functioning and viability for economic activities based on marine resources, such as aquaculture (de Souza Machado et al., 2016; Gamain et al., 2017; Mai et al., 2012; Rainbow, 2007; Sussarellu et al., 2018; Wang et al., 2011; Wijsman et al., 2019). Thus, for coastal environments, stable isotopes can be used to constrain metal contaminant transfers between compartments, such as sediment stratification and biota, and emerge as a new tool to better understand the functioning of marine environments (Araújo et al., 2017a, 2017b; Araújo et al., 2021b; Araújo et al., 2018).

Bivalve mollusks are filter-feeders capable of filtering large quantities of seawater to extract their sustenance from seston (e.g., bacteria, phytoplankton, microzooplankton, and organic detritus), and ecological keystone species for nutrient regeneration in the benthic environment (Dame, 2012; Gosling, 2003; Wang and Wang, 2019). As primary consumers, isotope composition of C and N in their tissues are proxies to assess their organic dietary sources (Briant et al., 2018). It is known that bivalves can accumulate Cu through particulate (trophic transfer) and dissolved phases. Thus, Cu isotopes have the potential to infer Cu dietary sources. However, such applications remain untested since Cu isotope compositions of marine food webs (plankton) continue practically unknown (Araújo et al., 2022a; Chifflet et al., 2022).

Conversely, it has been recently demonstrated that Cu isotope composition in bivalve mollusks is sensitive to Cu bioavailability changes related to anthropogenic Cu emissions and therefore, potentially suitable for biomonitoring purposes (Araújo et al., 2021b). Studies have successfully applied Cu isotopes in oysters reared under identical conditions and transplanted in target sites to infer distinguish origins (natural vs. anthropogenic) of the bioaccumulated Cu, and to quantify their respective fractions during a time span of one year (Araújo et al., 2021c; Araújo et al., 2021a).

However, an analysis on a large scale of the spatial and temporal variabilities in Cu isotopes in indigenous oysters has not yet been conducted, and their use for biomonitoring purposes untested. Indeed, it is plausible that isotope records in oysters' tissues may be helpful as a proxy for identifying their habitats, since bioavailable Cu pools tend to vary geographically as a result of combined geochemical factors. To test such hypothesis, this work reports on the Cu isotope variability of

oysters (*Crassostrea gigas*) harvested in French coastal environments contaminated with Cu as a result of their urban setting, or their proximity to agricultural and naval activities. We hypothesize that differences in apportionments of anthropogenic and natural Cu sources would induce spatial and temporal Cu isotope shifts in oysters' soft tissues.

## 2. Methods

### 2.1. Sampling and study sites description

The bivalve mollusks tissue samples were provided by the French National Monitoring Network "ROCCH" (Observation Network of chemical contamination of the marine environment- handled by Ifremer). The chosen sample sets come from sites close to the main estuarine outlets discharging into the Bay of Biscay: Vilaine, Loire, Gironde, and Arcachon. The samples cover a period of one decade for Vilaine Bay (2009–2018) and four decades for the other sites, with temporal series beginning in the early 1980s. All series have sampling intervals varying between 1 and 4 years, with oysters collected from the same season: winter for Gironde and Arcachon and autumn for the Loire estuary. Mediterranean bivalves were represented by two oyster samples from Toulon bay, obtained after transplantation and exposure in situ for six months in 2018. This bay does not present native oysters; therefore, transplanted oysters were used to provide isotope information from this environment. The applicability of isotope compositions in transplanted oysters has been demonstrated previously (Araújo et al., 2021c).

The Loire estuary is the main outlet from the longest French river (Loire Rive), hosting urbanized and industrialized zones from the metropolitan region between Nantes and Saint Nazaire, with a population of almost one million (Fig. 1). It is characterized as a turbid and macrotidal estuary. Concentration profile of trace metals in the sedimentary stratum from the Loire estuary evidence a significant attenuation in their anthropogenic emissions (Pb, Zn, Cu), resulting from deindustrialization and emission regulations (Araújo et al., 2019b; Briant et al., 2021b; Briant et al., 2021a; Coynel et al., 2016; Négrel, 1997).

The Loire estuary delivers a large amount of sedimentary material into the northern continental shelf of the Bay of Biscay. Finer sediment particles are dispersed and accumulate near its southern bank, in Bourgneuf bay where a bivalve sampling station and shellfishing farming are located (Fig. 1). Forty kilometers to the NW, the Vilaine Bay encompasses an area of 200 km<sup>2</sup>, and is the outlet of its main tributary, the 200 km-long Vilaine river (Ratmaya et al., 2019). Secondary fluvial discharges into the northeastern part of Vilaine Bay drain rural areas with small agri-food industries. Resort towns border Vilaine bay, while in the inner bay, prominent local mariculture exists since the end of the 19th century (Treguier and Hitiér, 2013).

The Arcachon Bay is a French macrotidal coastal lagoon (156 km<sup>2</sup>) surrounded by resorts, suburban centers, and associated marinas. It is one of the main marinas on the French Atlantic coast, accounting for about 12,000 registered boats (Le Berre et al., 2010). The increasing tendency on Cu concentrations observed in Arcachon may be related to the Cu-based antifouling paints used on boat hulls. The inner basin is also affected by the Leyre River and other small rivers that flow into the lagoon (Rimmelín et al., 1998). The Leyre river watershed develops agriculture that can emit pesticides and nutrients for Arcachon Bay (Fauvelle et al., 2018). Farming oysters from Arcachon Bay and those Vilaine Bay, and Bourgneuf Bay respond to one-quarter of the total French oyster harvest.

The Gironde Estuary is about 75 km in length and receives the outflow of the Garonne and Dordogne rivers, which join downstream from Bordeaux and drain a basin of about 80,000 km<sup>2</sup>, hosting a dense population of around ~1 million inhabitants. This major European estuary has had severe Cd contamination from a closed Zn-ore mining metallurgical concern in one of its tributaries (Abdou et al., 2016; Lanceleur et al., 2011; Petit et al., 2015; Petit et al., 2013; Schäfer et al., 2009). The

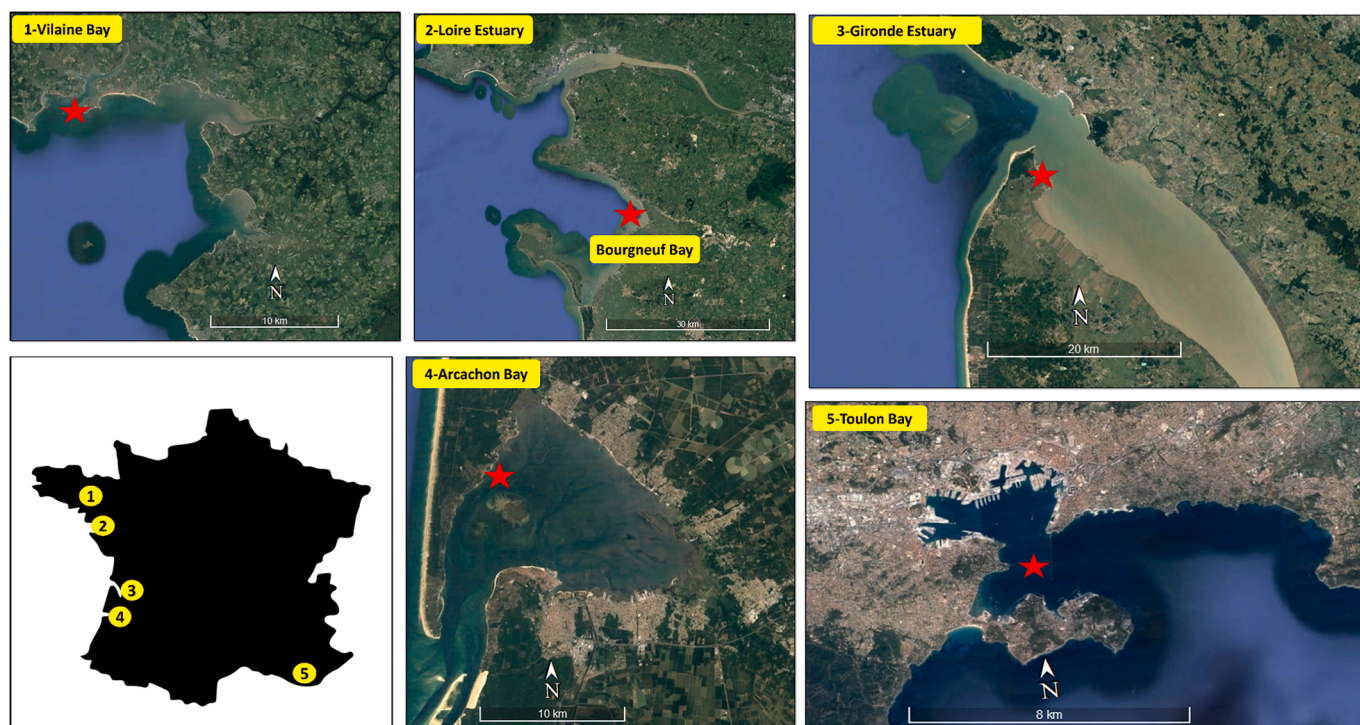


Fig. 1. Oyster sampling station locations (red stars) in French coastal environments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cd levels exceeding the reglementary limits for human consumption lead to the prohibition of oyster farming activities in the estuary (Abdou et al., 2016). As a result, oysters in Gironde present the highest Cu concentrations on the French coastline. The wine-growing industry of the Bordeaux region and its use of copper-based fungicides (“the Bordeaux mixture,” among others) in the Gironde watershed is potentially the primary source of anthropogenic Cu to its estuary.

## 2.2. Sample preparation and analyses

Detailed sample preparation and analytical procedures can be found elsewhere (Araújo et al., 2021c; Araújo et al., 2021b). Briefly, oysters collected in the field were transported to the laboratory to depurate for 24 h in tanks filled with seawater taken from their locations. Then, individual oysters two-to-three- years old with similar shell lengths were selected to avoid biological biases. Next, the extracted soft tissues of oysters were pooled (at least ten individuals), crushed in a blender, frozen, and finally, lyophilized and stored before sample digestion and analysis.

Sample aliquots around 200 mg, in dry weight, were digested in closed vessels with concentrated nitric acid and with the assistance of a microwave system. Elemental Cu levels were measured by ICP-MS (iCAP, Thermo Scientific), and isotope analyses were performed by Multicollector (MC-)ICP-MS (Neptune, Thermo Scientific) at the PSO platform (Pôle Spectrométrie Océan, Ifremer, France). Before isotope analyses, matrix samples were removed by a chromatography purification step using a column packed with the AG-MP1 resin. The reference material oyster SRM NIST 1566b and blanks were included in batch samples for analytical control. Measured Cu concentrations matched the certified value of the reference material within  $\pm 5\%$ . All sample preparation procedures were carried out with ultrapure water and high-purity acid blends.

Purified Cu samples dissolved in 0.5 M  $\text{HNO}_3$  at concentrations ranging between 200 and 500 ng g<sup>-1</sup> were injected into the instrument using nebulization through a cyclonic chamber. Sample runs followed

the standard bracketing mode, and the final Cu isotope compositions are expressed using  $\delta$ -notation as follows (Eq. 1):

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

The calculated  $\delta^{65}\text{Cu}_{\text{SRM-976}}$  values represent the average and the two standard deviations (2 s) of two or three individual measures run in a single analytical session. The routine precision obtained for individual samples and replicates of reference materials was generally better than  $\pm 0.05 \text{ ‰}$ . The long-term average obtained for SRM NIST 1566b full replicates is  $+0.23 \pm 0.06 \text{ ‰}$  (2 s,  $n = 13$ ). This value concurred with data from other laboratories ( $+0.30 \pm 0.01 \text{ ‰}$ , Jeong et al., 2021a;).

## 2.3. Statistics

Linear regression scatterplots were used to check the correlations between the two variables (Cu concentrations and  $\delta^{65}\text{Cu}_{\text{SRM-976}}$ ). In addition, a Kruskal-Wallis H test was used to verify differences in isotope compositions and concentrations between samples, followed by a post hoc assessment for all possible pairwise combinations. All plots and statistical analyses were performed using the Microsoft Excel® and SPSS® IBM software packages, respectively.

## 3. Results and discussion

### 3.1. Spatiotemporal changes on concentrations and isotope compositions of copper in oysters

The dataset for oysters is summarized in Table 1. Cu concentration ranges in oysters vary spatially among the studied sites and temporally within each of them. As expected, Gironde’s oysters had the highest Cu levels, with an average of  $1535 \pm 430 \text{ µg/g}$  (1 s,  $n = 13$ ). This average is higher than five-fold higher than the average observed for oysters from

**Table 1**

Copper concentrations ( $\mu\text{g/g}$ ) and isotope compositions ( $\text{‰}$ ) of oysters (*Crassostrea gigas*). Arcachon Bay data is compiled from Araújo et al., 2021b.

| Site                            | Oyster sample year | season | $\delta^{65}\text{Cu}$<br>SRM-976 | 2s   | [Cu] |
|---------------------------------|--------------------|--------|-----------------------------------|------|------|
| Loire Estuary/<br>Bourgneuf Bay | 1985               | autumn | 0.00                              | 0.01 | 349  |
|                                 | 1990               | autumn | 0.08                              | 0.06 | 328  |
|                                 | 1992               | autumn | 0.07                              | 0.07 | 429  |
|                                 | 1995               | autumn | 0.05                              | 0.05 | 271  |
|                                 | 1997               | autumn | 0.04                              | 0.05 | 296  |
|                                 | 2000               | autumn | 0.09                              | 0.01 | 382  |
|                                 | 2003               | autumn | -0.02                             | 0.00 | 413  |
|                                 | 2005               | autumn | 0.08                              | 0.04 | 346  |
|                                 | 2008               | autumn | 0.09                              | 0.05 | 270  |
|                                 | 2010               | autumn | 0.10                              | 0.04 | 240  |
|                                 | 2011               | autumn | 0.09                              | 0.03 | 308  |
|                                 | 2012               | autumn | 0.05                              | 0.02 | 237  |
|                                 | 2013               | autumn | -0.01                             | 0.07 | 335  |
|                                 | 2014               | autumn | 0.04                              | 0.06 | 234  |
|                                 | 2015               | autumn | -0.01                             | 0.07 | 231  |
|                                 | 2016               | autumn | 0.03                              | 0.02 | 237  |
| Vilaine Bay                     | 2009               | autumn | 0.36                              | 0.02 | 98   |
|                                 | 2010               | autumn | 0.34                              | 0.02 | 74   |
|                                 | 2011               | autumn | 0.26                              | 0.00 | 69   |
|                                 | 2012               | autumn | 0.41                              | 0.04 | 139  |
|                                 | 2013               | autumn | 0.43                              | 0.04 | 182  |
|                                 | 2014               | autumn | 0.43                              | 0.02 | 145  |
|                                 | 2015               | autumn | 0.42                              | 0.02 | 244  |
|                                 | 2016               | autumn | 0.38                              | 0.04 | 160  |
|                                 | 2017               | autumn | 0.35                              | 0.06 | 95   |
|                                 | 2018               | autumn | 0.38                              | 0.01 | 117  |
| Gironde Estuary                 | 1986               | winter | -0.06                             | 0.05 | 1148 |
|                                 | 1990               | winter | 0.02                              | 0.01 | 1574 |
|                                 | 1992               | winter | -0.02                             | 0.04 | 794  |
|                                 | 1996               | winter | -0.03                             | 0.02 | 1697 |
|                                 | 1998               | winter | -0.05                             | 0.05 | 1761 |
|                                 | 2000               | winter | -0.07                             | 0.03 | 1927 |
|                                 | 2002               | winter | 0.11                              | 0.01 | 1536 |
|                                 | 2006               | winter | 0.00                              | 0.03 | 1021 |
|                                 | 2010               | winter | -0.04                             | 0.04 | 1345 |
|                                 | 2012               | winter | -0.05                             | 0.05 | 1574 |
|                                 | 2015               | winter | -0.04                             | 0.03 | 2461 |
|                                 | 2016               | winter | -0.07                             | 0.02 | 1808 |
|                                 | 2017               | winter | -0.07                             | 0.05 | 1308 |
| Arcachon Bay                    | 1984               | winter | 0.06                              | 0.08 | 102  |
|                                 | 1990               | winter | 0.23                              | 0.01 | 139  |
|                                 | 1994               | winter | 0.33                              | 0.01 | 166  |
|                                 | 1996               | winter | -0.32                             | 0.10 | 208  |
|                                 | 1998               | winter | 0.30                              | 0.04 | 100  |
|                                 | 2000               | winter | 0.29                              | 0.12 | 108  |
|                                 | 2002               | winter | 0.32                              | 0.07 | 159  |
|                                 | 2004               | winter | 0.49                              | 0.07 | 243  |
|                                 | 2005               | winter | 0.48                              | 0.03 | 219  |
|                                 | 2006               | winter | 0.13                              | 0.12 | 180  |
|                                 | 2007               | winter | 0.64                              | 0.03 | 219  |
|                                 | 2012               | winter | 0.64                              | 0.01 | 207  |
|                                 | 2013               | winter | 0.63                              | 0.04 | 341  |
|                                 | 2014               | winter | 0.36                              | 0.05 | 354  |
|                                 | 2015               | winter | 0.45                              | 0.02 | 383  |
|                                 | 2016               | winter | 0.52                              | 0.01 | 381  |
|                                 | 2018               | winter | 0.61                              | 0.00 | 351  |
|                                 | 2019               | winter | 0.56                              | 0.01 | 395  |
| Toulon Bay                      | 2018               | spring | -0.13                             | 0.08 | 584  |
|                                 | 2018               | autumn | -0.16                             | 0.01 | 1130 |

Bourgneuf Bay ( $306 \pm 66 \mu\text{g/g}$ , 1 s,  $n = 16$ ), in the vicinity of the Loire Estuary. Arcachon oysters have Cu concentrations at similar magnitudes, while oysters from Vilaine bay presented the lowest levels around  $132 \pm 54 \mu\text{g/g}$  (1 s,  $n = 10$ ). All temporal profiles at these sites show

significant variations on Cu concentrations over time. In Arcachon Bay case, a sharp increase in Cu concentrations is observed (Fig. 2a, b). The two transplanted oysters in Toulon Bay also had relatively high Cu concentrations varying between 584 and 1130  $\mu\text{g/g}$ .

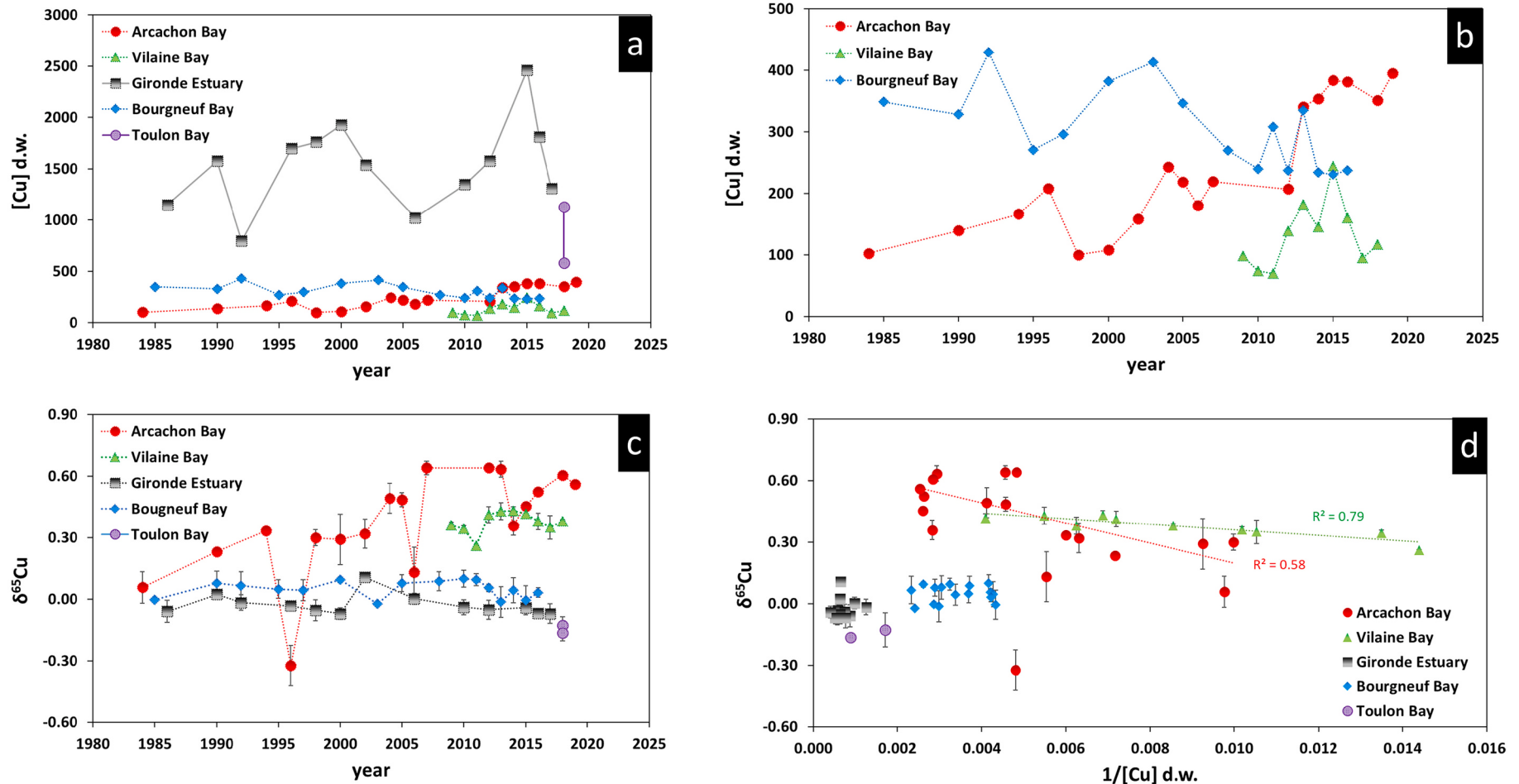
In turn, Cu isotope temporal profiles of the biomonitoring sites were stable, and practically without significant isotope changes (Fig. 2c). Again, only Arcachon Bay oysters had a significative isotope variation over time, accompanying a non-linear tendency toward positive isotope values with Cu concentration increasing. Distinctive negative peaks appear in 1996 and 2006 (Fig. 2c) with no clear explanation. From a spatial perspective, both oyster series harvested close to Gironde and Loire estuary have  $\delta^{65}\text{Cu}$  values centered around 0‰, contrasting with the more positive average values about +0.4‰ for Vilaine Bay and Arcachon Bay. Combining isotope and elemental data in a dispersion plot groups the oyster samples according to their collection sites (Fig. 2d). In Arcachon Bay ( $R^2 = 0.6$ ,  $p < 0.05$ ) and Vilaine Bay ( $R^2 = 0.8$ ,  $p < 0.05$ ), Cu isotope ratios and concentrations correlate positively in the regression analysis (Fig. 2d). For the Arcachon regression, we excluded the 1996 and 2006 samples with  $\delta^{65}\text{Cu}$  values of  $-0.32\text{‰}$  and  $0.13\text{‰}$ , respectively (Fig. 2d). The two transplanted oysters in Toulon Bay contrast with native oysters from Atlantic shore, showing negative  $\delta^{65}\text{Cu}$  values of  $-0.13$  and  $-0.16\text{‰}$ . As noted, the scatterplot of isotope and elemental data distinguishes the oyster samples from the different sampling stations. Kruskal-Wallis H tests showed a statistically significant difference in concentrations and isotope compositions between the other oyster sampling stations ( $p < 0.05$ ). Post hoc tests on each pair of sample groups show significative isotope differences ( $p < 0.05$ ), except between oysters from Loire, Toulon, and Gironde estuaries. In terms of Cu concentrations, the high values found in oysters from macrotidal estuaries (Loire and Gironde) differ significantly ( $p < 0.05$ ) from the relatively lower values in those from coastal bays (Arcachon, Vilaine, and Toulon).

### 3.2. Source and biogeochemical processes as main factors controlling geographic isotope variability of copper in oysters

Cu concentration and isotope ratio Cu ranges of French estuarine oysters show significant inter-sites differences that exceed intra-site temporal variations. The specific geochemical Cu fingerprints observed for each site is ascribed to the combined influence of sources and biogeochemical processes occurring in the sediment-water-biota interface that culminate in the Cu trophic bioaccumulation. Oysters from Arcachon are a particular case displaying a substantial change in elemental and isotope variations.

To begin with the geological source rocks, felsic and ultramafic rocks have homogenous Cu isotope compositions yielding an isotope estimation to the Bulk Silicate Earth (BSE) around  $0.07 \pm 0.10 \text{‰}$  (2 s, Savage et al., 2015). Since weathering processes induce small or negligible Cu isotope fractionation in temperate systems, the detrital material exported from soils to rivers and estuaries has Cu isotope signatures close to the Bulk Silicate Earth (Vance et al., 2016; Vance et al., 2008). Therefore, regional geological features and weathering processes are likely to have a minor role in the diversity of the Cu isotope variations observed here. The correlations between labile metal forms present in sediments and oyster concentrations (Chakraborty et al., 2015; Griscom and Fisher, 2004) suggests that the incorporation of Cu in soft tissues of these bivalves depends on metal speciation in their food and water.

Elemental transfers of Cu can occurs between reactive phases of sediments and the water column depending on several geochemical factors controlling Cu speciation, such as pH, salinity, temperature, organic matter, etc. (Bianchi, 2007; Rainbow, 2018; Schäfer et al., 2009; Tessier and Turner, 1995; Turner and Millward, 2002). The dynamic changes on the estuarine geochemical parameters, like pH and ionic strength, during mixing of freshwater and seawater may favor the release of Cu via desorption, followed by its complexation by organic matter (Bianchi, 2007). Speciation also determines Cu scavenging from



**Fig. 2.** Temporal isotope (‰) and elemental ( $\mu\text{g/g}$ ) profiles of oyster sample collections and dispersion plots: a) Temporal profile of Cu concentration in selected oyster sample collections; b) Zoom in temporal profile of Cu concentrations, excluding Gironde Estuary; c) Temporal profile of Cu isotope compositions; d) dispersion plot between Cu isotope compositions and Cu concentrations.

the water column and deposition on bottom sediments after flocculation of suspended particles (Briant et al., 2021a; Petit et al., 2015). In both cases, elemental transfers associated with Cu speciation changes that modify the chemical nature of Cu ligands, their bond lengths, geometry, and oxidation state of copper, will induce significant isotope fractionations (Moynier et al., 2017). For example, the mineralization of organic matter and the associated Cu releases explained the gradual dissolved Cu increases along the river-estuarine continuum of Gironde (Petit et al., 2013). In this case, the preferential transfer of a Cu pool enriched in the light isotope was used to explain the shift of  $\delta^{65}\text{Cu}$  values for dissolved Cu from the Gironde estuarine waters toward lighter values (Petit et al., 2013).

The subsequent isotope fractionation steps of Cu incorporation into biomass and trophic transfers in estuaries are poorly understood, since no field or laboratory studies have reported Cu isotope fractionation by plankton. In this sense, isotope signals in oysters' soft tissues are advantageous because they reflect bioaccumulated metal via either or both dietary inputs (food) and dissolved phases (surrounding waters), and hence, they act as "integrative isotope recorders" of metal sources and biogeochemical processes controlling the Cu bioavailability in the surrounding environment (Araújo et al., 2021a). As noted in Fig. 2d, the combined information of Cu concentrations and isotope compositions oyster clusters related to their provenance, and as presumed initially, they can be helpful as a proxy for identifying their habitats.

### 3.3. Temporal profiles isotope patterns of oysters reveal copper source apportionment tendencies

Our sampling scheme based on oysters with similar size and collected in the same season reduces isotope variability associated with changes in estuarine biogeochemical conditions, and makes variability related to source apportionment relatively more apparent. In a previous study, the same approach conducted in the Loire estuary revealed a similar co-evolution in Zn isotope time series between oysters samples, and a dated sediment core collected nearby (Araújo et al., 2021c). Usually, metal isotope compositions in sediments essentially reflect mixing source processes (Araújo et al., 2019a; Guinoiseau et al., 2018). Then, temporal sedimentary isotope signals matching those from oyster tissues are strong evidence to validate the experimental strategy of using the latter to detect source apportionment changes using these organisms.

The oyster samples were collected over the last four decades, which is short to account for all the environmental, chemical contamination history across the Industrial Revolution and the "Great Acceleration" at the beginning of the 1950s. Therefore, the recent period recorded in oyster tissue isotope composition may not coincide with hypothetical isotope shifts from natural isotope baselines to anthropogenically sourced ones. For example, the traditional vineyard treatment with Cu compounds (the so-called "*Bordeaux Mixture*") dating from the late 19th century heavily impacts soils in this region, resulting in chronic leaching of Cu into Gironde the estuary (Michel et al., 2000). The extremely high Cu concentrations and the homogenous isotope compositions in oyster tissues collected from the Gironde estuary since the 1980's shows that no source apportionment changes occurred within this period. Thus, we suggest that legacy Cu contamination accumulated in watershed soils continues to be the principal Cu source for this estuary. It is worth noting that urban emissions and emerging anthropogenic Cu sources, like Cu-based antifouling paints, with their distinct signatures (Briant, 2014) seem to be negligible compared to viticulture's. Considering the elemental profile does not show a robust upward or downward trend, Cu concentrations are rather related to estuarine influxes of Cu. They ultimately depend on river flow and winter floods (Michel et al., 2000).

In the vicinity of the Loire estuary, oyster tissues from the sampling station in Bourgneuf Bay have a stable Cu isotope composition over time. Like that was discussed for Gironde estuary, the trend in the Cu isotope profile of Bourgneuf oysters shows that relative contributions of natural and anthropogenic sources remained stable along the

biomonitoring period. However, establishing the predominant source based on Cu concentrations is difficult because they vary over time with no clear trend. Since the oyster tissue were collected at the same season and thus under similar geochemical conditions, and like for the Gironde estuary, we attribute the varying Cu concentrations to changes in Cu fluxes reaching the Bourgneuf Bay rather anthropic factors. A Cu deposition profile from a sediment core in the Loire Estuary shows concentrations and isotope signatures in the same natural range of the Upper Continental Crust, coherent with an overwhelming natural Cu dominance in the elemental budget of this element in this study site (Araújo et al., 2019b). We consider the same to be true for oysters harvested there.

In contrast to constant isotope signals recorded in oysters from Bourgneuf and Gironde, those from Arcachon and Vilaine bays show isotope variations that correlate positively with Cu concentrations. We attribute these to apparitions of new anthropogenic Cu sources. The rapid joint elevation of Cu concentrations and isotope variations in indigenous oysters of Arcachon correspond to the increased use of Cu as an alternative biocide after the ban of tributyltin (TBT) in the 1980's, today estimated to surpass 4500 l per year at this site (Auby et al., 2004). The delta values of Cu-based antifouling paints reported in the literature ( $+0.54 \pm 0.03$ , Briant, 2014) are near those of the most recent indigenous oysters, and thus, reinforce our suspicion that these compounds are a major Cu sources in Arcachon. However, local end-members (including pesticides and urban influents) should be characterized to confirm it. Interesting to note the general isotope and elemental Cu pattern observed for Arcachon's oysters is almost identical to that observed in transplanted oysters in the same sampling station and with a one-year exposure duration (Araújo et al., 2021a). Exceptionally, in the decadal native series, two outliers data appear in 1996 and 2006 (Table 1). These outliers could be attributed to punctual emissions from unknown sources. An alternative hypothesis would be a large change in isotope signature of antifouling paints. At present it is difficult to narrow down the possibilities.

Compared to Arcachon Bay, isotope and elemental Cu variations in oysters from Vilaine are less clear-cut, maybe due to the reduced anthropogenic activities in the smaller marinas and ports of this bay. While the identification of specific sources at this site are premature, this preliminary isotope information detected a new category of anthropogenic Cu emissions that the monitoring of elemental levels would have missed.

## 4. Conclusion

We have conducted a survey of a series of oyster tissues from the French marine monitoring network to examine the usefulness of isotope information in biomonitoring studies and seafood traceability. By normalizing size and age, isotope records of these organisms shown be helpful to detect anthropogenic sources changes over time in sites suspected of anthropogenic Cu (Arcachon and Vilaine bays). Characterization of local end-members (antifouling paints, urban sewage and pesticides) can help pinpoint the relative contributions of these sources. A multi-isotope approach combining Zn, Pb and other isotope systems can also improve traceability of coastal anthropogenic emissions.

In macrotidal estuaries (Loire and Gironde), oyster samples showed irregular Cu concentration profiles that were difficult to interpret in terms of anthropogenic influence, while stable isotope time series indicated constant proportion of natural and anthropogenic Cu sources over time. These cases illustrate the limitations of elemental data to deconvolve natural and anthropic influences on bioaccumulated levels of Cu and the advantages of Cu isotope composition to eliminate environmental "noise" and describe anthropogenic Cu contamination tendencies. Otherwise, the similar isotope signatures of oysters from estuaries with contrasting pollution contexts and levels, as for the Gironde and Loire, suggest that natural and anthropic isotope compositions of Cu can overlap. Therefore, mixing isotope models for source

apportionment purposes will probably be based on locally specific end-members, consequently not allowing for screening and quantifying anthropogenic contamination in large geographic areas.

Lastly, the observed site-specific Cu concentrations and isotope compositions in biomonitoring oysters allow us to discriminate their provenance and thus are promising to add the Cu isotope tool to the seafood traceability toolkit. This finding also opens new opportunities to test the combined use of metal stable isotope systems (Ag, Zn, Fe, Cd, etc.) for such purpose. Several studies have proposed the trace element fingerprints in soft tissues and shells as a potential universal traceability tool for mollusk production (Bennion et al., 2021; Morrison et al., 2019). Our study is the first to report Cu isotope ratios in oysters covering decadal time windows in a large geographic zone, and it reveals that metal isotope information is helpful for marine biomonitoring and promising for seafood traceability.

## CRediT authorship contribution statement

**Daniel F. Araújo:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration. **Emmanuel Ponzevera:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Project administration. **Joël Knoery:** Conceptualization, Methodology, Investigation, Writing – review & editing, Funding acquisition. **Nicolas Briant:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Sandrine Bruzac:** Methodology, Formal analysis, Investigation. **Teddy Sireau:** Methodology, Formal analysis, Investigation. **Anne Pellouin-Grouhel:** Methodology, Formal analysis, Investigation, Resources, Project administration. **Christophe Brach-Papa:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Project administration, Funding acquisition.

## Declaration of Competing Interest

None.

## Data availability

Data will be made available on request.

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