Chemosphere
April 2024, Volume 353 Pages 141572 (9p.)
https://doi.org/10.1016/j.chemosphere.2024.141572
https://archimer.ifremer.fr/doc/00880/99177/

Seasonal and multi-decadal zinc isotope variations in blue mussels from two sites with contrasting zinc contamination levels

Ferreira Araujo Daniel [1, *], Ponzevera Emmanuel [1], Jeong Hyeryeong [1], Briant Nicolas [1], Le Monier Pauline [1], Bruzac Sandrine [1], Sireau Teddy [1], Pellouin-Grouhel Anne [1], Knoery Joël [1], Brach-Papa Christophe [2]

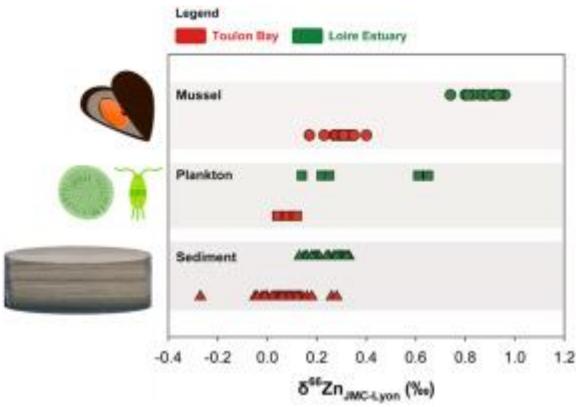
[1] Ifremer, CCEM- Unité Contamination Chimique des Écosystèmes Marins, F-F-44300, Nantes, France
[2] Ifremer, LERPAC- Unité Littoral- Laboratoire Environnement Ressources Provence-Azur-Corse, F-83507, La Seyne-sur-Mer, France

* Corresponding author : Daniel Ferreira Araujo, email address : daniel.ferreira.araujo@ifremer.fr

Abstract :

Zinc (Zn) isotope compositions in soft mussel tissues help identify internal biological processes and track coastal Zn sources in coastal environments, thus aiding in managing marine metal pollution. This study investigated the seasonal and multi-decadal Zn isotope compositions of blue mussels (genus Mytilus) from two French coastal sites with contrasting Zn environmental contamination. Concurrently, we characterized the isotope ratios of sediments and plankton samples at each site to understand the associations between organisms and abiotic compartments. Our primary objective was to determine whether these isotope compositions trace long-term anthropogenic emission patterns or if they reflect short-term biological processes. The multi-decadal isotope profiles of mussels in the Loire Estuary and Toulon Bay showed no isotope variations, implying the enduring stability of the relative contributions of natural and anthropogenic Zn sources over time. At seasonal scales, Zn isotope ratios were also constant; hence, isotope effects related to spawning and body growth were not discernible. The multi-compartmental analysis between the sites revealed that Toulon Bay exhibits a remarkably lower Zn isotope ratio across all studied matrices, suggesting the upward transfer of anthropogenic Zn in the food web. In contrast, the Zn isotope variability observed for sediments and organisms from the Loire Estuary fell within the natural baseline of this element. In both sites, adsorptive geogenic material carrying significant amounts of Zn masks the biological isotope signature of plankton, making it difficult to determine whether the Zn isotope ratio in mussels solely reflects the planktonic diet or if it is further modified by biological homeostasis. In summary, Zn isotope ratios in mussels offer promising avenues for delineating source-specific isotope signatures, contingent upon a comprehensive understanding of the isotope fractionation processes associated with the trophic transfer of this element through the plankton.

Graphical abstract



Highlights

► Pristine and contaminated sites show samples with distinct isotope fingerprints. ► Systematic lighter $\delta^{66}Zn$ values identify anthropogenic Zn in contaminated samples. ► Zn isotopes trace upward transfer of anthropogenic Zn in the food web. ► Isotope biomonitoring indicates no alterations in Zn sources over four decades. ► Zn isotope ratio constancy at seasonal scale discards biological isotope effects.

Keywords : metal stable isotopes, isotope tracer, biomonitoring, marine pollution, trace metal bioaccumulation, zinc contamination

1. Introduction

Zinc (Zn) is a trace metal essential to life with a complex marine biogeochemical cycle involving diverse processes (biological uptake, adsorption, ligand complexation, etc.) through sediment, water, and biological interfaces (Rainbow, 2007). However, human activities have disturbed its natural cycle by increasing Zn inputs in bioavailable forms into the marine environment. These disruptions have led to the bioaccumulation of Zn in many marine species at elevated levels, negatively impacting biota health and marine seafood quality (de Souza Machado et al., 2016; Sen and Peucker-Ehrenbrink, 2012).

In nature, Zn primarily exists as five stable isotopes: ^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn, and ^{70}Zn (Moynier et al., 2017). These isotopes possess different atomic masses and form bonds with varying quantum energies, resulting in their uneven distribution through biogeochemical processes, and significant variability in isotope compositions across natural materials (Wiederhold, 2015). These isotope compositions, expressed as the ratio between the abundances of two isotopes, can change in time and space as they are modulated by different factors such as geochemical sources, the reversibility of chemical processes, temperature, coordination chemistry (geometry and bond length), and biological regulation (Albarede et al., 2016; Andronikov et al., 2021; Aucour et al., 2015; Juillot et al., 2023; Komárek et al., 2021; Sullivan et al., 2022; Wang et al., 2022). Thus, natural samples have their own Zn isotope "fingerprints" that provide integrated information on the environmental trajectory of this metal, including sources and the processes leading up to its uptake and bioaccumulation (Albarède et al., 2011; Jaouen et al., 2018; McCormack et al., 2021; Schilling et al., 2021; Toubhans et al., 2020).

Continuous analytical and methodological improvements in multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS) have facilitated the isotope characterization of various marine matrices in diverse applications (Araújo et al., 2022b; Little et al., 2021, 2014). For example, the metal isotope compositions of marine sediments reflect source mixing processes and can help verify records of anthropogenic sources, primary activities, and sedimentary material mixing (Araújo et al., 2019b; Little et al., 2018, 2016; Zhang et al., 2018). In addition, they can shed light on different feeding strategies for Zn uptake in deep-sea marine sponges (i.e., Zn associated with dissolved *vs.* particulate phases; Hendry and Andersen, 2013). Zn isotopes in marine carbonates and corals have shown promise for tracking metal pollution and ancient seawater conditions in paleoceanography (Little et al., 2021; Liu et al., 2023; Xiao et al., 2020; Zhang et al., 2022). Unicellular organisms, such as diatoms, have also been shown to fractionate Zn isotopes depending on transport mechanisms across cell membranes via active and passive mechanisms (John et al., 2007; Köbberich and Vance, 2019). These examples underscore the capacity of Zn isotopes to provide insights regarding the metal itself and the physiological mechanisms associated with its cellular transport and processes. Moreover, such insights can be garnered across various levels of biological organization, from molecular chemistry to unicellular organisms to populations of a particular species and ultimately to complete ecosystems.

As a globally abundant bivalve group inhabiting marine environments, mussels have been a staple in human seafood consumption for centuries (Beyer et al., 2017). Today, mussels are a marine commodity, accounting for nearly one-third of all aquaculture products sold in the European Union (FAO, 2024). Given that the majority (90%) of commercial of marine bivalves, including mussels, oysters, and clams, comes from aquaculture, the environmental quality and sanitary conditions of coastal marine environments are critical to ensure sustainable aquaculture practices (Wijsman et al., 2019). France is the third-largest European producer of mussels, following Spain and Italy (Rodríguez-Rodríguez and Bande Ramudo, 2017). While regulations on metal emissions have reduced metal release in coastal environments in developed countries, new technological uses of metals and remobilization from legacy contaminated sediments require constant monitoring (Barreira et al., 2024; Thibon et al., 2021; Thibon et al., 2021).

Mussels, like many other bivalves, feed on phytoplankton by pumping and filtering large volumes of water over their ciliated gills; thus, they incorporate metals dissolved in the water and through the ingestion of filtered particles (Gosling, 2015). Thus mussel "soft tissues" are good biomarker of metal exposure, suitable for biomonitoring anthropogenic contamination in aquatic systems (Cai and Wang, 2019; Goldberg, 1975; Krishnakumar et al., 2018; Lu and Wang, 2018). Several mussel biomonitoring networks, the so-called "Mussel Watch Programs" (MWP), have been implemented in North America, Europe, and Asia (Briand et al., 2023; Briant et al., 2021b; Cantillo, 1998; Claisse, 1989; Cossa and Tabard, 2020; Couture et al., 2010; Jeong et al., 2021b; Lu et al., 2020; Lu and Wang, 2018; Shiel et al., 2012; Wang et al., 2022). The French MWP is a successful example of a perennial biomonitoring project that has operated uninterrupted since the late 1970s, filling a substantial bivalve soft tissue sample bank covering the country's shoreline. This sample collection is highly valuable to test new analytical techniques, including "non-traditional" stable metal isotopes (Araújo et al., 2021b; Shiel et al., 2013). Combined with isotope approaches, such environmental samples help reconstruct the contributions of metal pollution sources or to infer environmental changes that impact marine biota, especially in farm aquaculture under intense anthropic pressure from legacy contamination (Araújo et al., 2021b; Barreira et al., 2024). Subsequent spatial analysis conducted on mussels from the Korean coast show that they reflect the natural and anthropogenic source apportionment of Zn in the surrounding environment (Jeong et al., 2021b).

In this study, we investigated multi-decadal and seasonal variations in the Zn isotope compositions of blue mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) from two distinct environmental locations in France, each characterized by unique pollution histories: the Loire Estuary and Toulon Bay. Our primary objective was to determine whether these isotope compositions trace long-term anthropogenic emission patterns or if they reflect short-term biological processes. To achieve this, we contextualized mussel isotope patterns with those of local sediments and plankton samples to evaluate Zn transfer mechanisms between the studied compartments.

2. Material and methods

2.1 Study sites

The macrotidal Loire Estuary (~106 km long) is located on the Atlantic coast at the mouth of the Loire River, a major European river with a mean annual water flow of ~900 m^3 s^{-1}. The well-mixed and highly turbid sedimentary plume of this estuary extends over a large part of the northern Bay of Biscay continental shelf (Dulaquais et al., 2020; Waeles et al., 2004). The maximum turbidity zone (MTZ) is about 20–50 km long and extends over the Bay of Biscay continental shelf (Briant et al., 2021a; Jalón-Rojas et al., 2016). The Loire Estuary traverses an industrialized and urbanized watershed that comprises the metropolitan areas of Nantes and Saint-Nazaire with over 800,000 inhabitants (Coynel et al., 2016; Grosbois et al., 2012). Concentrations of trace metals in sediments from the Loire Estuary and its nearby coastal environment show an overall and significant decline in metal contaminant levels (lead [Pb], Zn, and copper [Cu]), probably due to a drop in metal emission subsequent to deindustrialization and emission regulations since the 1980s (Briant et al., 2024). There is also an apparent increase in the relative importance of metals related to diffuse urban sources (Araújo et al., 2019b; Briant et al., 2021a; Coynel et al., 2016).

Toulon Bay is an urbanized coastal ecosystem that has historically hosted naval activities (ports and shipyards) and approximately 600,000 inhabitants (Tessier et al., 2011; Fig. 1). Two freshwater rivers—the Las and Eygoutier—discharge into Toulon Bay (Fig. 1). A dike built in the 19th century divides Toulon into two sub-basins labeled the "Small Bay" (9.8 km^2, semi-enclosed inner basin) on the northwestern side and the "Large Bay" (42.2 km^2, outer basin) on the southeastern part (Fig. 1). The Small Bay is bordered by continuous urbanized areas, military zones, industries, recreational and commercial ports, and former shipyards (last one closed in 1989). In the same zone at the Saint Lazareth site, shellfish farming developed at the end of the 19th century. The Large Bay has a single military zone, small beaches, and tourist ports.

In November 1942, the French Navy fleet was scuttled and 100 ships were destroyed and sunk, mostly in the Small Bay (Grasset, 2011). The subsequent raising and treatment of the wrecks resulted in strong polymetallic contamination of Cu, Zn, mercury (Hg), cadmium (Cd), Pb, and other trace metals in the bay's sediment (Dang et al., 2015b; Tessier et al., 2011). As a result, the concentrations of metals, including Cu and Zn, are extremely high in the Small Bay. Despite the low hydrodynamic energy that preserves the sedimentary stratum and its legacy of anthropogenic metals, resuspension events in its shallow zone can mix contemporary and legacy sedimentary metals (Araújo et al., 2019a; Pougnet et al., 2014). Indeed, sediment cores reveal that the extreme Zn contamination associated with warfare materials and fleet scuttling has shown only a minor decrease in metal levels in the sedimentary layers since the post-WWII period (Tessier et al., 2011).

2.2 Mussel sampling

The preserved tissue samples of mussels used in this study were obtained from bank samples collected through the French National Monitoring Network 'ROCCH' (Observation Network of Chemical Contamination of the Marine Environment), managed by Ifremer (the French Research Institute for Exploitation of the Sea). The mussel samples selected for this study were obtained from the Chemoulin site in the Loire Estuary and the Saint Lazaret site in Toulon Bay (Fig. 1). The Chemoulin site near the Loire Estuary mouth constitutes a beach system with a coarse deposition of sand grains via high hydrodynamic energy from the sea. The Saint Lazaret site, located in inner Toulon Bay (Small Bay), has very low hydrodynamic energy, possesses a shallow water column (~9m) where high load of fine sediments are often resuspended from the bottom sediments due to seawater currents and intense navy boat traffic.

Two collections of mussel samples spanning four decades of biomonitoring from the early 1980s to the end of the 2010s, were selected from the Loire Estuary (Chemoulin site) and Toulon Bay (Saint Lazaret site), respectively. These multi-decadal bivalve series used samples collected in the same season (autumn) to minimize the potential influence of seasonally varying parameters, such as the organism's spawning cycle, primary production, water biomass, and continental runoff fluxes. Two other collections of mussels from Chemoulin and Saint Lazaret sites representing an entire seasonal period (winter-spring-summer-autumn) in 1997 was also included.

All mussels samples followed the same sampling and chemical preparation protocols (Grouhel, 2023). Briefly, these bivalve mollusks were harvested from beach rocks (Loire Estuary) or aquaculture cages (Toulon Bay) and transported to the laboratory, where they were kept in local seawater for 24 hours to depurate. Each sample represented a pool of at least 50 mussels that were crushed, homogenized, and freeze-dried.

2.3 Plankton

Plankton samples were collected were collected using a polymeric cod-end attached to a plankton net with circular openings of 0.25 m² and a mesh size of 63 µm. The net opening was pulled horizontally at a depth of 5 m below the water surface at a speed of 0.2–1.0 knots. The plankton size fractions were obtained by filtering the contents of the plankton net's cod-end through a vertical array of nylon sieves with decreasing mesh sizes (from 1000 to 63 µm) to obtain three plankton size classes: 1000-500 µm, 500-250 µm, and 250-63 µm. For each sieve, the plankton fraction was collected in acid-cleaned polyethylene tubes, frozen, freeze-dried, and stored for later analysis. Sampling in the Loire Estuary dates back to the spring of 2013 at two stations (LE1 and LE2), and in Toulon Bay, it took place during the spring and autumn of 2018 at two stations (TB1 and TB2). For LE2 station, the 500-1000 µm particle-size class is missing due to insufficient material for chemical analysis.

2.4 Sediments

In the Loire Estuary 17 surface sediments (0-5 cm) collected in 2014 using an Ekman and Reineck crabs were acquired from the sample bank of the ROCCH monitoring framework. In addition, a sediment core labeled "PV1" (300 cm length) was sampled on the inner intertidal mudflat of the Loire Estuary (Fig. 1) during the Paleovase mission in March 2015. The coring used aluminum tubes (6 m long, 74 mm internal diameter, and 1 mm wall), and, was then subsampled into 2-cm-thick sediment slices every 5 cm in the upper meter, and every 10 cm for the rest of the sediment core. The ^{210}Pb dating technique and geochemical metal concentration profiles enabled us to infer the commencement of the postindustrialization period at a depth of 96 cm in the PV1 core, corresponding to approximately the end of the 19th century (Araújo et al., 2021b). In Toulon Bay, 52 sampling sites of surface sediment (0–5 cm) were collected between November 2008 and June 2009 within the framework of the CARTOCHIM project (Fig. 1).

2.5 Sample preparation and chemical analysis

Approximately 200 mg of dry bivalve tissue samples were digested using an HNO_3 solution (50% v/v) in a microwave (MARS 5, CEM®). Dry aliquots of sediments were digested in Teflon® bombs on a Teflon-coated graphite block using multistep additions of high-quality concentrated HF, HCl, and HNO_3 for trace metal analysis. Plankton samples were prepared by following the same protocol as sediments but without HF. Aliquots were taken from final digestion solutions for subsequent elemental and isotope analysis. To ensure accuracy, procedural blanks and certified reference materials were processed in each batch of sediment samples following all procedures (digestion, elemental analysis, ion-exchange chromatography, and isotope determination). Certified reference materials included oyster and mussel tissues (SRM 1566b;NIST®, and ERM-CE278k;ERM®), plankton (BCR-414) and sediments (MESS-3 and PACS-2 from NRC – CNR®).

Elemental Zn was measured by ICP-MS (iCAP, Thermo Scientific), and isotope analyses were conducted using Multicollector (MC-)ICP-MS (Neptune, Thermo Scientific) at the PSO platform (Pôle Spectrométrie Océan, Ifremer, France). The measured Zn concentrations aligned with the certified value of the reference material within ±10%. Isotope sample runs followed the standard bracketing mode combined with external normalization (Cu-doping). The final Zn isotope compositions are expressed using δ-notation as follows:

$$\delta^{66/64}Zn_{JMC-Lyon}\,(‰) = \left(\frac{R\left(\frac{^{66}Zn}{^{64}Zn}\right)_{sample}}{R\left(\frac{^{66}Zn}{^{64}Zn}\right)_{JMC-Lyon}} - 1 \right) x\,1000 \quad \text{(Eq. 1)}$$

To ensure analytical quality control, reference materials of bivalve mollusk tissues, plankton, and sediments were measured at least two times during each analytical session. The average values for $\delta^{66}Zn_{JMC\text{-}Lyon}$ are reported in Table 1. The precision of a given sample measure was two standard deviations (2s) and commonly below 0.1‰. All reagents, labware cleaning, and solution dilutions for elemental and isotope analyses were performed using 18.2 MΩ cm H_2O (Nanop System®) and high-purity acids (PlasmaPure Plus grade, SCP science®).

3. Result and discussions

3.1 Zinc datasets summary

Table 2 summarize all data utilized in the present study, encompassing both newly collected information and compiled data. The plankton data samples from the Loire Estuary were compiled from a prior study (Araújo et al., 2022a) and can be found in Table S1 (Supplementary

Material). Additionally, new plankton data from Toulon Bay are presented this study (Table S1). All bottom sediment data were compiled from previously published studies in Toulon Bay (Araújo et al., 2019a) and in the Loire Estuary (Araújo et al., 2019b), which are detailed in Tables S2 and S3. All mussel isotope data presented here is summarized in the Table S4.

3.2 Elemental and isotope profiles of Zn in mussels at seasonal scale

Both the Loire Estuary and Toulon Bay showed similar seasonal patterns of Zn bioaccumulation, with mussels having higher Zn concentrations in winter and lower levels in summer (Fig. 2). In contrast, the Zn isotope profile in mussel populations of both sites remained seasonally constant over the 1-year biomonitoring period (1997), around +0.89 ±0.05 (n = 4, 2s) and +0.27 ±0.05‰ (n = 4, 2s), for the Atlantic and Mediterreanean sites, respectively. The detailed isotope dataset for mussel samples are included in Table S4.

The seasonal growth and reproductive cycle of mussels strongly influence their bioaccumulation of trace metals at a seasonal scale. *Mytilus* mussels become sexually mature after 1–2 years and tend to spawn in the spring, synchronizing with the main phytoplankton bloom in spring (Beyer et al., 2017). During cold periods (winter and early spring), energy requirements, gonadal development, and gametogenesis increase, leading to higher bioaccumulation of trace metals such as Cu and Zn (Azizi et al., 2018; Benali et al., 2015; Soto et al., 2000). The highest Zn concentrations in the winter samples align with this expected trend. However, it is worth noting that this increase can coincide with anthropogenic inputs via runoff triggered by rainy French winters (Nicolau et al., 2012; Oursel et al., 2014; Schlacher-Hoenlinger and Schlacher, 1998).

In spring, mussel body growth increases with food availability, leading to a biodilution of metal concentrations (Azizi, 2018). In the subsequent spawning period (later spring and summer), the emission of gametes can significantly decrease mussel biomass and metal contents (Adami et al., 2002; Azizi et al., 2018). The timing of *Mytilus* population spawning varies greatly with geographic location (Latouche and Mix, 1981; Lobel and Wright, 1982). The Zn concentrations in Loire Estuary mussels showed drastic concentration decreases of almost 50% between winter and spring, which seems unlikely to be restricted to biodilution effects. We suggest that these mussels start the spawning period earlier, in spring, compared with bivalves from Toulon Bay, which have a summer spawning period. Therefore, organism growth and reproduction cycles likely explain the decreased Cu and Zn concentrations in spring, with the lowest values in summer.

In contrast to elemental data, a constant seasonal Zn isotope profile indicates that body growth and reproductive cycles do not affect the isotope compositions of soft tissues. It is well known that Zn coordination changes can trigger isotope fractionation through transport, compartmentalization, detoxification, and excretion in living organisms (Balter et al., 2010; Caldelas et al., 2011; Caldelas and Weiss, 2017; Moynier et al., 2020). We can then hypothesize that internal cellular Zn trafficking modulated by ligands with similar electron donors, such as metallothionein proteins (S-donors), leads to similar Zn speciation among cellular and tissue Zn pools, resulting in a negligible isotope fractionation during spawning processes.

3.3 Elemental and isotope profiles of Zn in mussels over decades

The Zn isotope compositions in mussels from both sites have remained almost constant during several decades (Fig. 3). In the Loire Estuary, mussels have $\delta^{66}Zn_{JMC\text{-}Lyon}$ values ranging from approximately +0.7‰ to +0.9‰ and concentrations around 135 ± 77 µg g^{-1} (n = 10, 2s). Toulon Bay mussels have lighter $\delta^{66}Zn_{JMC\text{-}Lyon}$ values, falling in the range of +0.2‰ to +0.4‰, and relatively higher concentrations with an average of 180 ± 97 µg g^{-1} (n = 14, 2s). The lack of discernible temporal trends in elemental and isotope Zn patterns complicates the attribution of isotope shifts to changes in source apportionment. Nevertheless, the remarkable disparity in isotope signatures among sampling stations stands out. In subsequent sections, we employ sediment data to contextualize natural and anthropogenic Zn sources. Additionally, we explore the roles of plankton size classes in the trophic Zn transfer to explain the observed spatial differences.

3.4 Zn systematics in sediments

Metal concentrations in sediments are susceptible to grain-size distributions, especially when comparing sediments from different geological settings. Given the acknowledged significance of analyzing finer fractions in the assessment of trace metals, which demonstrate a high affinity for adsorption onto clay minerals and Fe and Mn-oxyhydroxides, we opted in this study to use aluminum (Al) as a proxy for the clay fraction content and to normalize concentrations based on 5% of this element (Kersten and Smedes, 2002; OSPAR, 2018). Consequently, normalized Zn concentrations in sediments are used henceforth in the following discussion

In the Loire Estuary, surface and core sediments display low to moderate Zn concentrations varying between 43 and 157 µg g^{-1}, an overall average of 116 ± 28 µg g^{-1} (n = 32, 1s, Fig. 4) (Fig. 4). The Zn isotope compositions of sediments are relatively homogeneous (Fig. 4), with $\delta^{66}Zn_{JMC\text{-}Lyon}$ averaging +0.24‰ ± 0.11‰ (2s, n = 26) (Fig. 4). Most $\delta^{66}Zn_{JMC\text{-}Lyon}$ values of Loire sediment fall within the crustal range, indicating dominance of geogenic Zn. This pattern seems consistent considering Zn concentrations are close to the natural background estimated at 90 µg g^{-1} to the North-East Atlantic coast (OSPAR, 2018). Some samples with Zn concentrations above 100 µg g^{-1} fall outside the geogenic isotope range, likely indicating a minor influence of anthropogenic sources. Overall, anthropogenic urban Zn sources tend to have lighter $\delta^{66}Zn_{JMC}$ values than the geological background, such as tire wear (from +0.00‰ to +0.20‰), gasoline (from −0.50‰ to +0.07‰), urban runoff (from +0.13‰ to +0.15‰), wastewaters and sludge (~0.05‰), industrial effluents (chemical and agro-food industry, from +0.10‰ to +0.15‰), and atmospheric industrial emissions (from −0.6‰ to +0.15‰). A detailed compilation of Zn sources for aquatic systems can be found in Desaulty and Petelet-Giraud, (2020). Nevertheless, the relatively close values between natural (~0.3) and anthropogenic Zn (~0.1), along with the low degree of Zn enrichment in the investigated samples, make it challenging to precisely define and compute the anthropogenic Zn's relative influence.

In turn, the levels of Zn contamination in Toulon's Small and Large Bays show a remarkable contrast (Fig. 4). In the Small Bay, sediments are highly enriched in Zn, averaging around 387 ± 320 µg g^{-1} (n = 24, 1s), while in the Large Bay, Zn concentrations remain mostly within 125 ± 55 µg g^{-1} (n = 28, 1s). Nevertheless, Zn isotope compositions in the Small and Large Bays have identical averages: +0.06‰ ± 0.05‰ (1s, n = 24) and +0.06‰ ± 0.11‰ (n = 28, 1s), respectively (Fig. 4). Similarly, the spatial distribution of Pb isotopes in surface sediments are relatively homogeneous between the two sub-bays, despite higher Pb contamination levels in Small Bay (Dang et al., 2015b). The consistent spatial isotope distribution pattern of Zn and Pb may be attributed to the significant export of anthropogenic Zn from the inner bay toward the open sea. Thus, the mussel sampling station located in the Small Bay, with an overwhelming dominance of anthropogenic Zn, provides an opportunity to test whether anthropogenic isotope signals from the mussel's environment can be transduced into mussel tissues or whether they remain traceable after isotope fractionation by biogeochemical processes.

3.5 Zn systematics in plankton

The plankton from Toulon Bay exhibit greater isotope homogeneity across different plankton sizes (Fig. 5a), with $\delta^{66}Zn_{JMC\text{-}Lyon}$ values displaying a remarkably consistent range of around +0.08‰ ± 0.06‰ (n = 9, 2s). We found no significant spring-to-summer change in the Zn isotope compositions (average $\Delta^{66}Zn_{spring\text{-}summer}$ = –0.01‰). Spatial comparison between sites located in the Small and Large bays from Toulon Bay (TB1 *vs*. TB2) were very similar (Fig. 5a). Zinc concentrations vary between 95 and 264 µg g^{-1} (Table S1), and are uncorrelated to isotope ratios. Comparatively, the observed $\delta^{66}Zn_{JMC\text{-}Lyon}$ values in plankton sub-fractions from the Loire Estuary display a broader range, from +0.14 to +0.76‰ (Fig. 5a) and lower Zn concentration levels, with minimum and maximum of 66 to 199 µg g^{-1}, respectively (Table S1). The LE2 stations stand out with the larger plankton sizes showing more positive isotope ratios. These two samples also accompany the lowest Al contents (Fig. 5b).

Geogenic particles carrying substantial amounts of Zn can obscure the biogenic Zn signal. Utilizing Al and iron (Fe) as proxies for terrigenous materials allows us to observe a considerable presence of geogenic material in plankton samples (Fig S1). Consequently, the close isotope values between some plankton size classes and sediments observed at the two sites are likely related to the contamination of geogenic particles in plankton (Fig. 6). A survey on Mediterranean plankton has demonstrated a tendency for plankton samples associated with geogenic particles to enrich in Zn lighter isotopes (Chifflet et al., 2022). In Toulon Bay, the homogeneity of Zn isotope composition is likely explained by the resuspension of sediment particles from the seafloor. This process increases the abundance of geogenic materials in Toulon Bay's shallow water column, masking the small biogenic fraction of the seston. In the Loire Estuary, the balance between geogenic and biogenic pools can vary based on the hydrodynamic particle sorting along the estuary's sediment plume dispersion (Araújo et al., 2022a). Nevertheless, we infer that the low Al contents in the two exceptional samples from the Loire Estuary (LE2 station, 250-500 and 500-1000 µm) suggest minimal geogenic material, indicating biogenic Zn dominance. Notably, these two samples exhibit isotope ratios more in line with those of mussels from the Loire Estuary, even though they do not overlap (Fig. 6).

3.6 Multi-compartmental Zn isotope analysis: summary and final considerations

Multi-compartmental Zn isotope patterns reveal a distinct trend toward a lighter isotope composition for sediment, plankton, and mussels from Toulon Bay compared with the Loire Estuary (Fig. 6). In Toulon Bay, we identified this light isotope signature in sediments as anthropogenic. The high contents of geogenic elements (Al and Fe) in plankton samples indicate an important fraction of mineral material. Thus, the close values between plankton and surface sediments are likely a result of sediment particle resuspension from the bottom to the water column in this shallower area of the inner bay. Consequently, the obscured isotope signals of the ‘true plankton fraction contribute to uncertainty in understanding whether the observed isotope offset in *Mytillus galloprovincialis* at this site stems from a possible biological isotope fractionation process related to the internal Zn regulation. In the Loire Estuary, sediments mostly fall in the natural isotope range of Zn for coastal sediments. The plankton of different size classes exhibits varying isotope variability. However, as noted for Toulon Bay, the isotope signal of the biogenic fraction can be masked depending on the relative presence of geogenic material (Araújo et al., 2022a). The heavy Zn isotope ratio for biogenic particles (identified here with low Al contents, Fig. 5b) falls close to the isotope range of mussels. Nevertheless, conducting additional plankton sampling in the vicinity of bivalve mollusks could enhance the precision of the analysis of biogenic Zn pools.

The presented multi-compartmental analysis shows thus that understanding the isotope composition of plankton is crucial for a more nuanced comprehension of Zn food-web transfers and the associated isotope fractionation. Mussels species have physiologies adapted to feed on specific plankton communities that vary in size and Zn contents (Gosling, 2015). Therefore, it becomes imperative to conduct isotope analyses targeting specific plankton fractions that constitute the specific diets for each species. However, the inherent mineral "contamination" of plankton sampling in turbid coastal environments makes determining its specific isotope compositions analytically challenging. As a result, establishing elusive isotope baselines for the marine food web remains a significant challenge. The temporal isotope profiles of mussels over multi-decadal periods remain relatively constant in both the Loire Estuary and Toulon Bay, suggesting that the relative contributions of natural and anthropogenic Zn sources have remained unchanged. In contrast, in the Loire Estuary, natural zinc is expected to be more dominant. Building upon previous studies, we posit that anthropogenic zinc is likely strongly associated with suspended particles and susceptible to exchange with biotic interfaces (Dang et al., 2015a; Tessier et al., 2011). Thus, we conclude that the elevated concentration of anthropogenic Zn in Toulon Bay has moved up in the food web, bringing lighter isotope ratios in the mussels.

At short seasonal scales, internal biological isotope fractionation linked to spawning and body growth does not significantly affect Zn isotope ratios over seasons. Given the consistent Zn isotope ratios in mussels throughout seasonal and multi-decadal scales, they can offer potential avenues for delineating source-specific isotope signatures, contingent upon understanding the isotope fractionation related to the trophic transfer of Zn via plankton diets.

Acknowledgments

The authors thank the Pays de La Loire and Provence-Alpes-Côte d'Azur for funding the POLLUSOLS and SCOTTI projects.

References

Adami, G., Barbieri, P., Fabiani, M., Piselli, S., Predonzani, S., Reisenhofer, E., 2002. Levels of cadmium and zinc in hepatopancreas of reared Mytilus galloprovincialis from the Gulf of Trieste (Italy). Chemosphere 48, 671–677. https://doi.org/10.1016/S0045-6535(02)00196-0

Albarede, F., Télouk, P., Balter, V., Bondanese, V.P., Albalat, E., Oger, P., Bonaventura, P., Miossec, P., Fujii, T., 2016. Medical applications of Cu, Zn, and S isotope effects. Metallomics 8, 1056–1070. https://doi.org/10.1039/C5MT00316D

Albarède, F., Telouk, P., Lamboux, A., Jaouen, K., Balter, V., 2011. Isotopic evidence of unaccounted for Fe and Cu erythropoietic pathways. Metallomics 3, 926. https://doi.org/10.1039/c1mt00025j

Andronikov, A.V., Novak, M., Oulehle, F., Chrastny, V., Sebek, O., Andronikova, I.E., Stepanova, M., Sipkova, A., Hruska, J., Myska, O., Chuman, T., Veselovsky, F., Curik, J., Prechova, E., Komarek, A., 2021. Catchment Runoff in Industrial Areas Exports Legacy Pollutant Zinc from the Topsoil Rather than Geogenic Zn. Environ. Sci. Technol. 55, 8035–8044. https://doi.org/10.1021/acs.est.1c01167

Araújo, D., Machado, W., Weiss, D., Mulholland, D.S., Boaventura, G.R., Viers, J., Garnier, J., Dantas, E.L., Babinski, M., 2017. A critical examination of the possible application of zinc stable isotope ratios in bivalve mollusks and suspended particulate matter to trace zinc pollution in a tropical estuary. Environmental Pollution 226, 41–47. https://doi.org/10.1016/j.envpol.2017.04.011

Araújo, D.F., Knoery, J., Briant, N., Ponzevera, E., Chouvelon, T., Auby, I., Yepez, S., Bruzac, S., Sireau, T., Pellouin-Grouhel, A., Akcha, F., 2021a. Metal stable isotopes in transplanted oysters as a new tool for monitoring anthropogenic metal bioaccumulation in marine environments: The case for copper. Environmental Pollution 290, 118012. https://doi.org/10.1016/j.envpol.2021.118012

Araújo, D.F., Knoery, J., Briant, N., Ponzevera, E., Mulholland, D.S., Bruzac, S., Sireau, T., Chouvelon, T., Brach-Papa, C., 2022a. Cu and Zn stable isotopes in suspended particulate matter sub-fractions from the Northern Bay of Biscay help identify biogenic and geogenic particle pools. Continental Shelf Research 104791. https://doi.org/10.1016/j.csr.2022.104791

Araújo, D.F., Knoery, J., Briant, N., Vigier, N., Ponzevera, E., 2022b. “Non-traditional” stable isotopes applied to the study of trace metal contaminants in anthropized marine environments. Marine Pollution Bulletin 175, 113398. https://doi.org/10.1016/j.marpolbul.2022.113398

Araújo, D.F., Ponzevera, E., Briant, N., Knoery, J., Bruzac, S., Sireau, T., Brach-Papa, C., 2019a. Copper, zinc and lead isotope signatures of sediments from a mediterranean coastal bay impacted by naval activities and urban sources. Applied Geochemistry 111, 104440. https://doi.org/10.1016/j.apgeochem.2019.104440

Araújo, D.F., Ponzevera, E., Briant, N., Knoery, J., Sireau, T., Mojtahid, M., Metzger, E., Brach-Papa, C., 2019b. Assessment of the metal contamination evolution in the Loire estuary using Cu and Zn stable isotopes and geochemical data in sediments. Marine Pollution Bulletin 143, 12–23. https://doi.org/10.1016/j.marpolbul.2019.04.034

Araújo, D.F., Ponzevera, E., Weiss, D.J., Knoery, J., Briant, N., Yepez, S., Bruzac, S., Sireau, T., Brach-Papa, C., 2021b. Application of Zn Isotope Compositions in Oysters to Monitor and Quantify Anthropogenic Zn Bioaccumulation in Marine Environments over Four Decades: A “Mussel Watch Program” Upgrade. ACS EST Water 1, 1035–1046. https://doi.org/10.1021/acsestwater.1c00010

Aucour, A.-M., Bedell, J.-P., Queyron, M., Magnin, V., Testemale, D., Sarret, G., 2015. Dynamics of Zn in an urban wetland soil–plant system: Coupling isotopic and EXAFS approaches. Geochimica et Cosmochimica Acta 160, 55–69. https://doi.org/10.1016/j.gca.2015.03.040

Azizi, G., 2018. The use of Mytilus spp. mussels as bioindicators of heavy metal pollution in the coastal environment. Journal of Materials Science & Technology 9(4), 1170–1181.

Azizi, G., Layachi, M., Akodad, M., Yáñez-Ruiz, D.R., Martín-García, A.I., Baghour, M., Mesfioui, A., Skalli, A., Moumen, A., 2018. Seasonal variations of heavy metals content in mussels (Mytilus galloprovincialis) from Cala Iris offshore (Northern Morocco). Marine Pollution Bulletin 137, 688–694. https://doi.org/10.1016/j.marpolbul.2018.06.052

Balter, V., Zazzo, A., Moloney, A.P., Moynier, F., Schmidt, O., Monahan, F.J., Albarède, F., 2010. Bodily variability of zinc natural isotope abundances in sheep. Rapid Commun. Mass Spectrom. 24, 605–612. https://doi.org/10.1002/rcm.4425

Barreira, J., Araújo, D.F., Knoery, J., Briant, N., Machado, W., Grouhel-Pellouin, A., 2024. The French Mussel Watch Program reveals the attenuation of coastal lead contamination over four decades. Marine Pollution Bulletin 199, 115975. https://doi.org/10.1016/j.marpolbul.2023.115975

Benali, I., Boutiba, Z., Merabet, A., Chèvre, N., 2015. Integrated use of biomarkers and condition indices in mussels (Mytilus galloprovincialis) for monitoring pollution and development of biomarker index to assess the potential toxic of coastal sites. Marine Pollution Bulletin 95, 385–394. https://doi.org/10.1016/j.marpolbul.2015.03.041

Beyer, J., Green, N.W., Brooks, S., Allan, I.J., Ruus, A., Gomes, T., Bråte, I.L.N., Schøyen, M., 2017. Blue mussels (Mytilus edulis spp.) as sentinel organisms in coastal pollution monitoring: A review. Marine Environmental Research 130, 338–365. https://doi.org/10.1016/j.marenvres.2017.07.024

Briand, M.J., Herlory, O., Briant, N., Brach-Papa, C., Boissery, P., Bouchoucha, M., 2023. The French Mussel Watch: More than two decades of chemical contamination survey in Mediterranean coastal waters. Marine Pollution Bulletin 191, 114901. https://doi.org/10.1016/j.marpolbul.2023.114901

Briant, N., Chiffoleau, J.-F., Knoery, J., Araújo, D.F., Ponzevera, E., Crochet, S., Thomas, B., Brach-Papa, C., 2021a. Seasonal trace metal distribution, partition and fluxes in the temperate macrotidal Loire Estuary (France). Estuarine, Coastal and Shelf Science 262, 107616. https://doi.org/10.1016/j.ecss.2021.107616

Briant, N., Knoery, J., Araújo, D.F., Ponzevera, E., Chouvelon, T., Bruzac, S., Sireau, T., Thomas, B., Mojtahid, M., Metzger, E., Brach-Papa, C., 2024. Vanishing lead in the Loire River estuary: An example of successful environmental regulation. Environmental Pollution 340, 122860. https://doi.org/10.1016/j.envpol.2023.122860

Briant, N., Le Monier, P., Bruzac, S., Sireau, T., Araújo, D.F., Grouhel, A., 2021b. Rare Earth Element in Bivalves' Soft Tissues of French Metropolitan Coasts: Spatial and Temporal Distribution. Arch Environ Contam Toxicol. https://doi.org/10.1007/s00244-021-00821-7

Cai, C., Wang, W.-X., 2019. Inter-species difference of copper accumulation in three species of marine mussels: Implication for biomonitoring. Science of The Total Environment 692, 1029–1036. https://doi.org/10.1016/j.scitotenv.2019.07.298

Caldelas, C., Dong, S., Araus, J.L., Jakob Weiss, D., 2011. Zinc isotopic fractionation in Phragmites australis in response to toxic levels of zinc. Journal of Experimental Botany 62, 2169–2178. https://doi.org/10.1093/jxb/erq414

Caldelas, C., Weiss, D.J., 2017. Zinc Homeostasis and isotopic fractionation in plants: a review. Plant Soil 411, 17–46. https://doi.org/10.1007/s11104-016-3146-0

Cantillo, A.Y., 1998. Comparison of results of Mussel Watch Programs of the United States and France with Worldwide Mussel Watch Studies. Marine Pollution Bulletin 36, 712–717. https://doi.org/10.1016/S0025-326X(98)00049-6

Chifflet, S., Briant, N., Freydier, R., Araújo, D.F., Quéméneur, M., Zouch, H., Bellaaj-Zouari, A., Carlotti, F., Tedetti, M., 2022. Isotopic compositions of copper and zinc in plankton from the Mediterranean Sea (MERITE-HIPPOCAMPE campaign): Tracing trophic transfer and geogenic inputs. Marine Pollution Bulletin 185, 114315. https://doi.org/10.1016/j.marpolbul.2022.114315

Claisse, D., 1989. Chemical contamination of French coasts: The Results of a Ten Years Mussel Watch. Marine Pollution Bulletin 20, 523–528. https://doi.org/10.1016/0025-326X(89)90141-0

Cossa, D., Tabard, A.-M., 2020. Mercury in Marine Mussels from the St. Lawrence Estuary and Gulf (Canada): A Mussel Watch Survey Revisited after 40 Years. Applied Sciences 10, 7556. https://doi.org/10.3390/app10217556

Couture, R.-M., Chiffoleau, J.-F., Auger, D., Claisse, D., Gobeil, C., Cossa, D., 2010. Seasonal and Decadal Variations in Lead Sources to Eastern North Atlantic Mussels. Environ. Sci. Technol. 44, 1211–1216. https://doi.org/10.1021/es902352z

Coynel, A., Gorse, L., Curti, C., Schafer, J., Grosbois, C., Morelli, G., Ducassou, E., Blanc, G., Maillet, G.M., Mojtahid, M., 2016. Spatial distribution of trace elements in the surface sediments of a major European estuary (Loire Estuary, France): Source identification and evaluation of anthropogenic contribution. Journal of Sea Research 118, 77–91. https://doi.org/10.1016/j.seares.2016.08.005

Dang, D.H., Lenoble, V., Durrieu, G., Omanović, D., Mullot, J.-U., Mounier, S., Garnier, C., 2015a. Seasonal variations of coastal sedimentary trace metals cycling: Insight on the effect of manganese and iron (oxy)hydroxides, sulphide and organic matter. Marine Pollution Bulletin 92, 113–124. https://doi.org/10.1016/j.marpolbul.2014.12.048

Dang, D.H., Schäfer, J., Brach-Papa, C., Lenoble, V., Durrieu, G., Dutruch, L., Chiffoleau, J.-F., Gonzalez, J.-L., Blanc, G., Mullot, J.-U., Mounier, S., Garnier, C., 2015b. Evidencing the Impact of Coastal Contaminated Sediments on Mussels Through Pb Stable Isotopes Composition. Environ. Sci. Technol. 49, 11438–11448. https://doi.org/10.1021/acs.est.5b01893 de Souza Machado, A.A., Spencer, K., Kloas, W., Toffolon, M., Zarfl, C., 2016. Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity. Science of The Total Environment 541, 268–281. https://doi.org/10.1016/j.scitotenv.2015.09.045

Desaulty, A.-M., Petelet-Giraud, E., 2020. Zinc isotope composition as a tool for tracing sources and fate of metal contaminants in rivers. Science of The Total Environment 728, 138599. https://doi.org/10.1016/j.scitotenv.2020.138599

Druce, M., Stirling, C.H., Rolison, J.M., 2020. High-Precision Zinc Isotopic Measurement of Certified Reference Materials Relevant to the Environmental, Earth, Planetary and Biomedical Sciences. Geostandards and Geoanalytical Research 44, 711–732. https://doi.org/10.1111/ggr.12341

Dulaquais, G., Waeles, M., Breitenstein, J., Knoery, J., Riso, R., 2020. Links between size fractionation, chemical speciation of dissolved copper and chemical speciation of dissolved organic matter in the Loire estuary. Environ. Chem. 17, 385. https://doi.org/10.1071/EN19137

FAO, 2024. The European market for mussels [WWW Document]. URL https://www.fao.org/in-action/globefish/fishery-information/resource-detail/es/c/338588/ (accessed 2.2.24).

Goldberg, E.D., 1975. The mussel watch — A first step in global marine monitoring. Marine Pollution Bulletin 6, 111. https://doi.org/10.1016/0025-326X(75)90271-4

Gosling, E.M., 2015. Marine bivalve molluscs, Second edition. ed. Wiley Blackwell, Chichester, West Sussex, UK ; Hoboken, NJ, USA.

Grasset, D., 2011. Histoire d'une tragédie navale Le sabordage de la flotte française Toulon- 27 novembre 194.

Grosbois, C., Meybeck, M., Lestel, L., Lefèvre, I., Moatar, F., 2012. Severe and contrasted polymetallic contamination patterns (1900–2009) in the Loire River sediments (France). Science of The Total Environment 435–436, 290–305. https://doi.org/10.1016/j.scitotenv.2012.06.056

Grouhel, A., 2023. Prescriptions techniques pour l'échantillonnage de mollusques du réseau national d'observation des contaminants chimiques (ROCCH).

Hendry, K.R., Andersen, M.B., 2013. The zinc isotopic composition of siliceous marine sponges: Investigating nature's sediment traps. Chemical Geology 354, 33–41. https://doi.org/10.1016/j.chemgeo.2013.06.025

Jalón-Rojas, I., Schmidt, S., Sottolichio, A., Bertier, C., 2016. Tracking the turbidity maximum zone in the Loire Estuary (France) based on a long-term, high-resolution and high-frequency monitoring network. Continental Shelf Research 117, 1–11. https://doi.org/10.1016/j.csr.2016.01.017

Jaouen, K., Colleter, R., Pietrzak, A., Pons, M.-L., Clavel, B., Telmon, N., Crubézy, É., Hublin, J.-J., Richards, M.P., 2018. Tracing intensive fish and meat consumption using Zn isotope ratios: evidence from a historical Breton population (Rennes, France). Sci Rep 8, 5077. https://doi.org/10.1038/s41598-018-23249-x

Jeong, H., Ra, K., Choi, J.Y., 2021a. Copper, Zinc and Lead Isotopic Delta Values and Isotope Ratios of Various Geological and Biological Reference Materials. Geostandards and Geoanalytical Research 45, 551–563. https://doi.org/10.1111/ggr.12379

Jeong, H., Ra, K., Won, J.-H., 2021b. A nationwide survey of trace metals and Zn isotopic signatures in mussels (Mytilus edulis) and oysters (Crassostrea gigas) from the coast of South Korea. Marine Pollution Bulletin 173, 113061. https://doi.org/10.1016/j.marpolbul.2021.113061

John, S.G., Genevieve Park, J., Zhang, Z., Boyle, E.A., 2007. The isotopic composition of some common forms of anthropogenic zinc. Chemical Geology 245, 61–69. https://doi.org/10.1016/j.chemgeo.2007.07.024

Juillot, F., Noël, V., Louvat, P., Gelabert, A., Jouvin, D., Göttlicher, J., Belin, S., Müller, B., Morin, G., Voegelin, A., 2023. Can Zn isotopes in sediments record past eutrophication of freshwater lakes? A pilot study at Lake Baldegg (Switzerland). Chemical Geology 620, 121321. https://doi.org/10.1016/j.chemgeo.2023.121321

Kersten, M., Smedes, F., 2002. Normalization procedures for sediment contaminants in spatial and temporal trend monitoring. J. Environ. Monit. 4, 109–115. https://doi.org/10.1039/B108102K

Köbberich, M., Vance, D., 2019. Zn isotope fractionation during uptake into marine phytoplankton: Implications for oceanic zinc isotopes. Chemical Geology 523, 154–161. https://doi.org/10.1016/j.chemgeo.2019.04.004

Komárek, M., Ratié, G., Vaňková, Z., Šípková, A., Chrastný, V., 2021. Metal isotope complexation with environmentally relevant surfaces: Opening the isotope fractionation black box. Critical Reviews in Environmental Science and Technology 0, 1–31. https://doi.org/10.1080/10643389.2021.1955601

Krishnakumar, P.K., Qurban, M.A., Sasikumar, G., 2018. Biomonitoring of Trace Metals in the Coastal Waters Using Bivalve Molluscs, in: Saleh, H.E.-D.M., El-Adham, E. (Eds.), Trace Elements - Human Health and Environment. InTech. https://doi.org/10.5772/intechopen.76938

Latouche, D., Mix, M.C., 1981. Seasonal variation in soft tissue weights and trace metal burdens in the bay mussel,Mytilus edulis. Bulletin of Environmental Contamination and Toxicology 27, 821–828.

Little, S.H., Archer, C., Milne, A., Schlosser, C., Achterberg, E.P., Lohan, M.C., Vance, D., 2018. Paired dissolved and particulate phase Cu isotope distributions in the South Atlantic. Chemical Geology 502, 29–43. https://doi.org/10.1016/j.chemgeo.2018.07.022

Little, S.H., Vance, D., McManus, J., Severmann, S., 2016. Key role of continental margin sediments in the oceanic mass balance of Zn and Zn isotopes. Geology 44, 207–210. https://doi.org/10.1130/G37493.1

Little, S.H., Vance, D., Walker-Brown, C., Landing, W.M., 2014. The oceanic mass balance of copper and zinc isotopes, investigated by analysis of their inputs, and outputs to ferromanganese oxide sediments. Geochimica et Cosmochimica Acta 125, 673–693. https://doi.org/10.1016/j.gca.2013.07.046

Little, S.H., Wilson, D.J., Rehkämper, M., Adkins, J.F., Robinson, L.F., van de Flierdt, T., 2021. Cold-water corals as archives of seawater Zn and Cu isotopes. Chemical Geology 578, 120304. https://doi.org/10.1016/j.chemgeo.2021.120304

Liu, Y., Chen, M., Zhang, T., Zhang, R., Cao, F., Sun, S., Zheng, W., Sun, R., Chen, J., 2023. Isotope Compositions of Century-Long Corals Reveal Significant Dissolved Cu, Zn Fluxes From Human-Accelerated Weathering Into the Ocean. Geophysical Research Letters 50, e2022GL102482. https://doi.org/10.1029/2022GL102482

Lobel, P.B., Wright, D.A., 1982. Gonadal and Nongonadal Zinc Concentrations in Mussels. Marine Pollution Bulletin 13, 320–323.

Lu, G., Pan, K., Zhu, A., Dong, Y., Wang, W.-X., 2020. Spatial-temporal variations and trends predication of trace metals in oysters from the Pearl River Estuary of China during 2011–2018. Environmental Pollution 264, 114812. https://doi.org/10.1016/j.envpol.2020.114812

Lu, G.-Y., Wang, W.-X., 2018. Trace metals and macroelements in mussels from Chinese coastal waters: National spatial patterns and normalization. Science of The Total Environment 626, 307–318. https://doi.org/10.1016/j.scitotenv.2018.01.018

Ma, L., Li, Y., Wang, W., Weng, N., Evans, R.D., Wang, W.-X., 2019. Zn Isotope Fractionation in the Oyster *Crassostrea hongkongensis* and Implications for Contaminant Source Tracking. Environ. Sci. Technol. 53, 6402–6409. https://doi.org/10.1021/acs.est.8b06855

Ma, L., Wang, W.-X., Evans, R.D., 2021. Distinguishing multiple Zn sources in oysters in a complex estuarine system using Zn isotope ratio signatures. Environmental Pollution 289, 117941. https://doi.org/10.1016/j.envpol.2021.117941

McCormack, J., Szpak, P., Bourgon, N., Richards, M., Hyland, C., Méjean, P., Hublin, J.-J., Jaouen, K., 2021. Zinc isotopes from archaeological bones provide reliable trophic level information for marine mammals. Commun Biol 4, 683. https://doi.org/10.1038/s42003-021-02212-z

Moynier, F., Borgne, M.L., Lahoud, E., Mahan, B., Mouton-Liger, F., Hugon, J., Paquet, C., 2020. Copper and zinc isotopic excursions in the human brain affected by Alzheimer's disease. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 12, e12112. https://doi.org/10.1002/dad2.12112

Moynier, F., Vance, D., Fujii, T., Savage, P., 2017. The Isotope Geochemistry of Zinc and Copper. Reviews in Mineralogy and Geochemistry 82, 543–600. https://doi.org/10.2138/rmg.2017.82.13

Nicolau, R., Lucas, Y., Merdy, P., Raynaud, M., 2012. Base flow and stormwater net fluxes of carbon and trace metals to the Mediterranean sea by an urbanized small river. Water Research, Special Issue on Stormwater in urban areas 46, 6625–6637. https://doi.org/10.1016/j.watres.2012.01.031

OSPAR, 2018. CEMP guidelines for monitoring contaminants in sediments. Revised in 2018. Agreement 2002-16. https://www.ospar.org/documents?d=32743.

Oursel, B., Garnier, C., Zebracki, M., Durrieu, G., Pairaud, I., Omanović, D., Cossa, D., Lucas, Y., 2014. Flood inputs in a Mediterranean coastal zone impacted by a large urban area: Dynamic and fate of trace metals. Marine Chemistry, Estuarine Biogeochemistry 167, 44–56. https://doi.org/10.1016/j.marchem.2014.08.005

Pougnet, F., Schäfer, J., Dutruch, L., Garnier, C., Tessier, E., Dang, D.H., Lanceleur, L., Mullot, J.-U., Lenoble, V., Blanc, G., 2014. Sources and historical record of tin and butyl-tin species in a Mediterranean bay (Toulon Bay, France). Environ Sci Pollut Res 21, 6640–6651. https://doi.org/10.1007/s11356-014-2576-6

Rainbow, P.S., 2007. Trace metal bioaccumulation: Models, metabolic availability and toxicity. Environment International 33, 576–582. https://doi.org/10.1016/j.envint.2006.05.007

Rodríguez-Rodríguez, G., Bande Ramudo, R., 2017. Market driven management of climate change impacts in the Spanish mussel sector. Marine Policy 83, 230–235. https://doi.org/10.1016/j.marpol.2017.06.014

Schilling, K., Harris, A.L., Halliday, A.N., Schofield, C.J., Sheldon, H., Haider, S., Larner, F., 2021. Investigations on Zinc Isotope Fractionation in Breast Cancer Tissue Using in vitro Cell Culture Uptake-Efflux Experiments. Front Med (Lausanne) 8, 746532. https://doi.org/10.3389/fmed.2021.746532

Schlacher-Hoenlinger, M.A., Schlacher, T.A., 1998. Accumulation, contamination, and seasonal variability of trace metals in the coastal zone – patterns in a seagrass meadow from the Mediterranean. Marine Biology 131, 401–410. https://doi.org/10.1007/s002270050333

Sen, I.S., Peucker-Ehrenbrink, B., 2012. Anthropogenic Disturbance of Element Cycles at the Earth's Surface. Environ. Sci. Technol. 46, 8601–8609. https://doi.org/10.1021/es301261x

Shiel, A.E., Weis, D., Cossa, D., Orians, K.J., 2013. Determining provenance of marine metal pollution in French bivalves using Cd, Zn and Pb isotopes. Geochimica et Cosmochimica Acta 121, 155–167. https://doi.org/10.1016/j.gca.2013.07.005

Shiel, A.E., Weis, D., Orians, K.J., 2012. Tracing cadmium, zinc and lead sources in bivalves from the coasts of western Canada and the USA using isotopes. Geochim. Cosmochim. Acta 76, 175–190. https://doi.org/10.1016/j.gca.2011.10.005

Soto, M., Ireland, M.P., Marigómez, I., 2000. Changes in mussel biometry on exposure to metals: implications in estimation of metal bioavailability in 'Mussel-Watch' programmes. Science of The Total Environment 247, 175–187. https://doi.org/10.1016/S0048-9697(99)00489-1

Sullivan, K.V., Kidder, J.A., Junqueira, T.P., Vanhaecke, F., Leybourne, M.I., 2022. Emerging applications of high-precision Cu isotopic analysis by MC-ICP-MS. Science of The Total Environment 838, 156084. https://doi.org/10.1016/j.scitotenv.2022.156084

Tessier, E., Garnier, C., Mullot, J.-U., Lenoble, V., Arnaud, M., Raynaud, M., Mounier, S., 2011. Study of the spatial and historical distribution of sediment inorganic contamination in the Toulon bay (France). Marine Pollution Bulletin 62, 2075–2086. https://doi.org/10.1016/j.marpolbul.2011.07.022

Thibon, Fanny, Metian, M., Oberhänsli, F., Montanes, M., Vassileva, E., Orani, A.M., Telouk, P., Swarzenski, P., Vigier, N., 2021. Bioaccumulation of Lithium Isotopes in Mussel Soft Tissues and Implications for Coastal Environments. ACS Earth Space Chem. 5, 1407–1417. https://doi.org/10.1021/acsearthspacechem.1c00045

Thibon, F., Weppe, L., Vigier, N., Churlaud, C., Lacoue-Labarthe, T., Metian, M., Cherel, Y., Bustamante, P., 2021. Large-scale survey of lithium concentrations in marine organisms. Science of The Total Environment 751, 141453. https://doi.org/10.1016/j.scitotenv.2020.141453

Toubhans, B., Gourlan, A.T., Telouk, P., Lutchman-Singh, K., Francis, L.W., Conlan, R.S., Margarit, L., Gonzalez, D., Charlet, L., 2020. Cu isotope ratios are meaningful in ovarian cancer diagnosis. Journal of Trace Elements in Medicine and Biology 62, 126611. https://doi.org/10.1016/j.jtemb.2020.126611

Waeles, M., Riso, R.D., Maguer, J.-F., Le Corre, P., 2004. Distribution and chemical speciation of dissolved cadmium and copper in the Loire estuary and North Biscay continental shelf, France. Estuarine, Coastal and Shelf Science 59, 49–57. https://doi.org/10.1016/j.ecss.2003.07.009

Wang, L., Wang, X., Chen, H., Wang, Z., Jia, X., 2022. Oyster arsenic, cadmium, copper, mercury, lead and zinc levels in the northern South China Sea: long-term spatiotemporal distributions, combined effects, and risk assessment to human health. Environ Sci Pollut Res 29, 12706–12719. https://doi.org/10.1007/s11356-021-18150-6

Wang, Z., Kwon, K.D., Peacock, C., Mo, X., Gou, W., Feng, X., Li, W., 2022. Zn stable isotope fractionation during adsorption onto todorokite: A molecular perspective from X-ray absorption spectroscopy and density functional theory. Geochimica et Cosmochimica Acta 327, 116–136. https://doi.org/10.1016/j.gca.2022.04.016

Wiederhold, J.G., 2015. Metal Stable Isotope Signatures as Tracers in Environmental Geochemistry. Environ. Sci. Technol. 49, 2606–2624. https://doi.org/10.1021/es504683e

Wijsman, J.W.M., Troost, K., Fang, J., Roncarati, A., 2019. Global Production of Marine Bivalves. Trends and Challenges, in: Smaal, A.C., Ferreira, J.G., Grant, J., Petersen, J.K., Strand, Ø. (Eds.), Goods and Services of Marine Bivalves. Springer International Publishing, Cham, pp. 7–26. https://doi.org/10.1007/978-3-319-96776-9_2

Xiao, H., Deng, W., Wei, G., Chen, J., Zheng, X., Shi, T., Chen, X., Wang, C., Liu, X., Zeng, T., 2020. A Pilot Study on Zinc Isotopic Compositions in Shallow-Water Coral Skeletons. Geochemistry, Geophysics, Geosystems 21, e2020GC009430. https://doi.org/10.1029/2020GC009430

Zhang, R., Russell, J., Xiao, X., Zhang, F., Li, T., Liu, Z., Guan, M., Han, Q., Shen, L., Shu, Y., 2018. Historical records, distributions and sources of mercury and zinc in sediments of East China sea: Implication from stable isotopic compositions. Chemosphere 205, 698–708. https://doi.org/10.1016/j.chemosphere.2018.04.100

Zhang, T., Sun, R., Liu, Y., Chen, L., Zheng, W., Liu, C.-Q., Chen, J., 2022. Copper and Zinc isotope signatures in scleratinian corals: Implications for Cu and Zn cycling in modern and ancient ocean. Geochimica et Cosmochimica Acta 317, 395–408. https://doi.org/10.1016/j.gca.2021.10.014

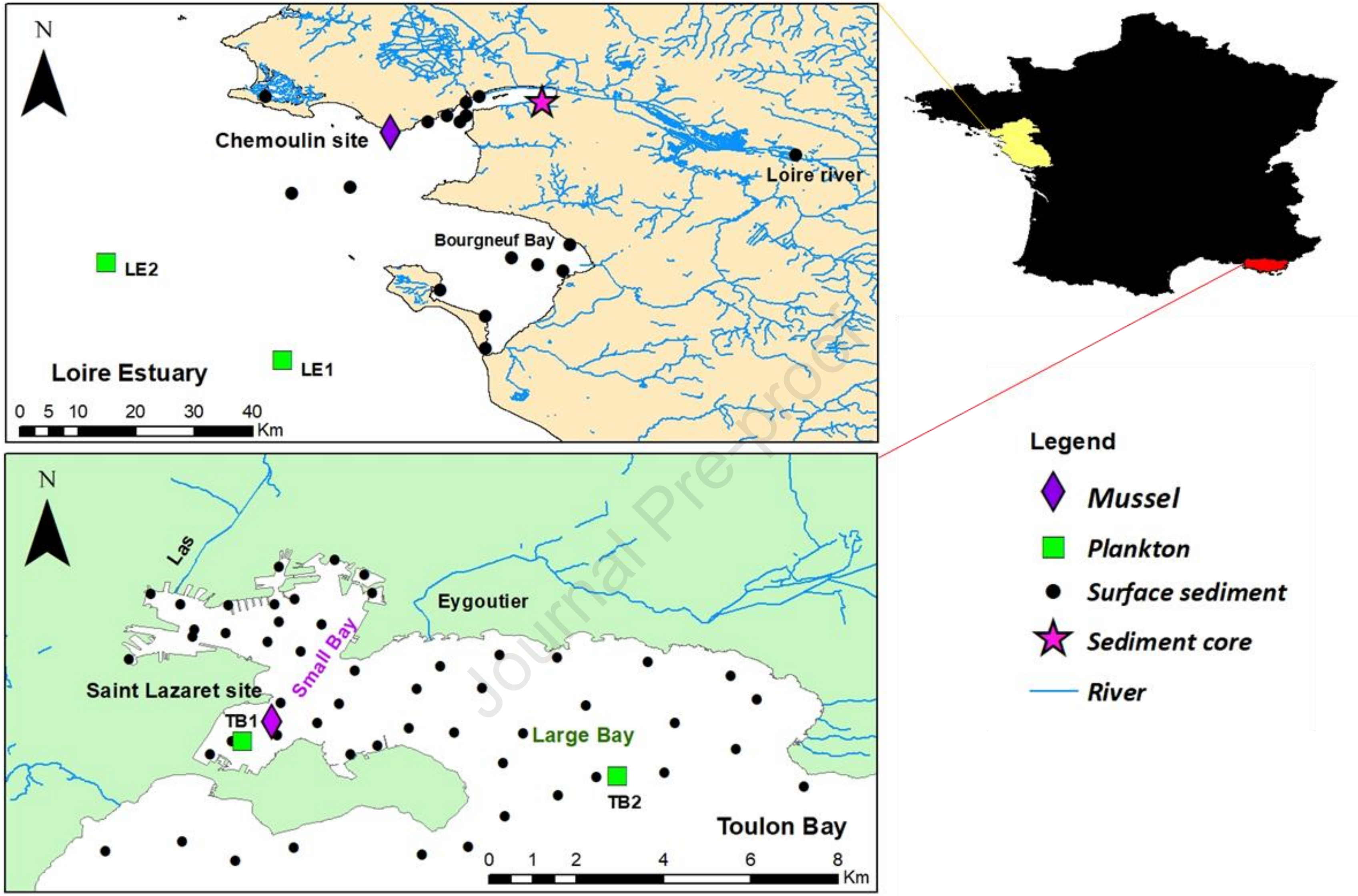


Fig. 1. Geographic distribution of sampling stations: mussel stations (rhombus symbols) are positioned at the Chemoulin site (Loire Estuary) and the Saint Lazaret site (Toulon Bay).

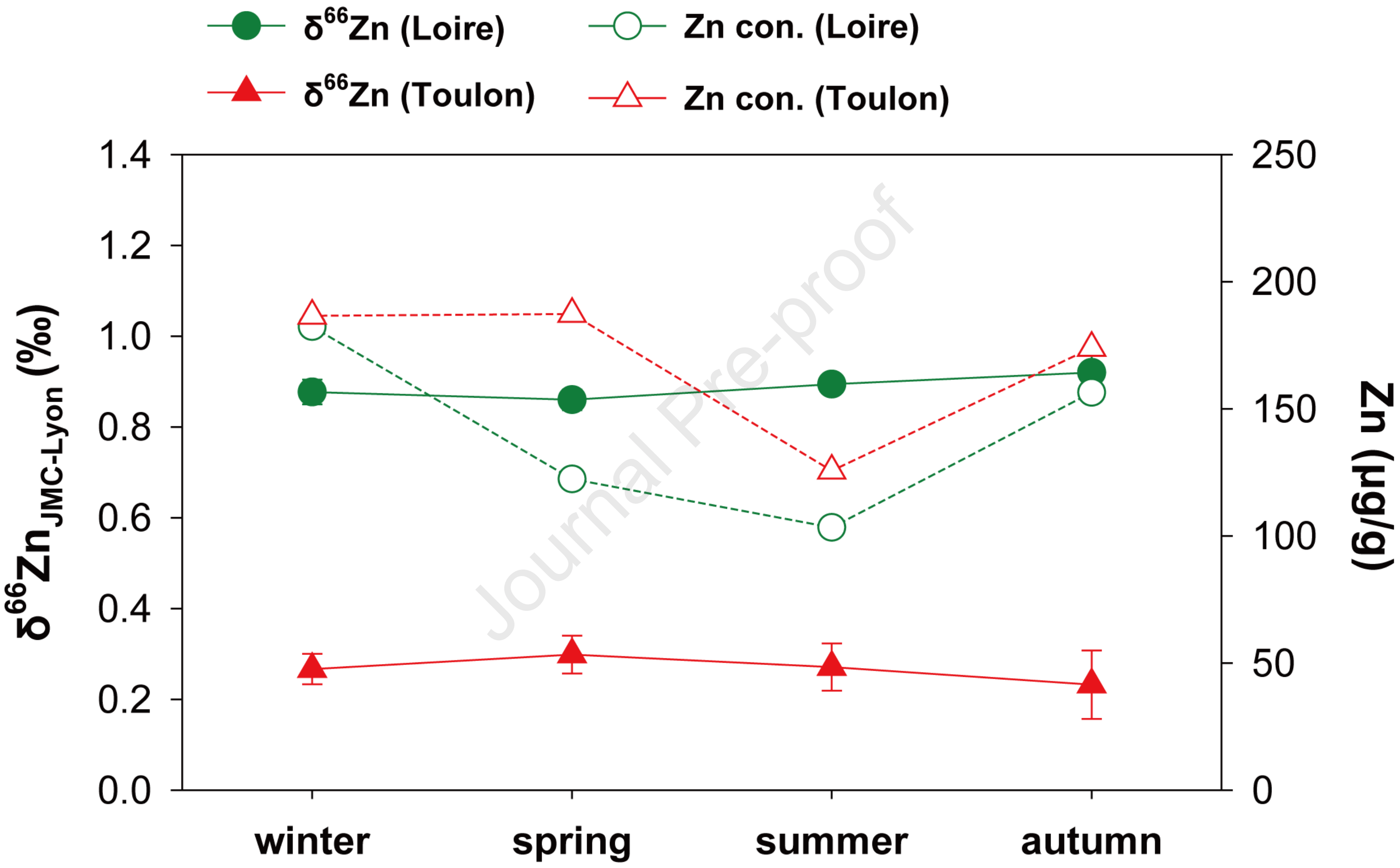


Fig. 2. Seasonal elemental and isotope variations in zinc (Zn) in mussels.

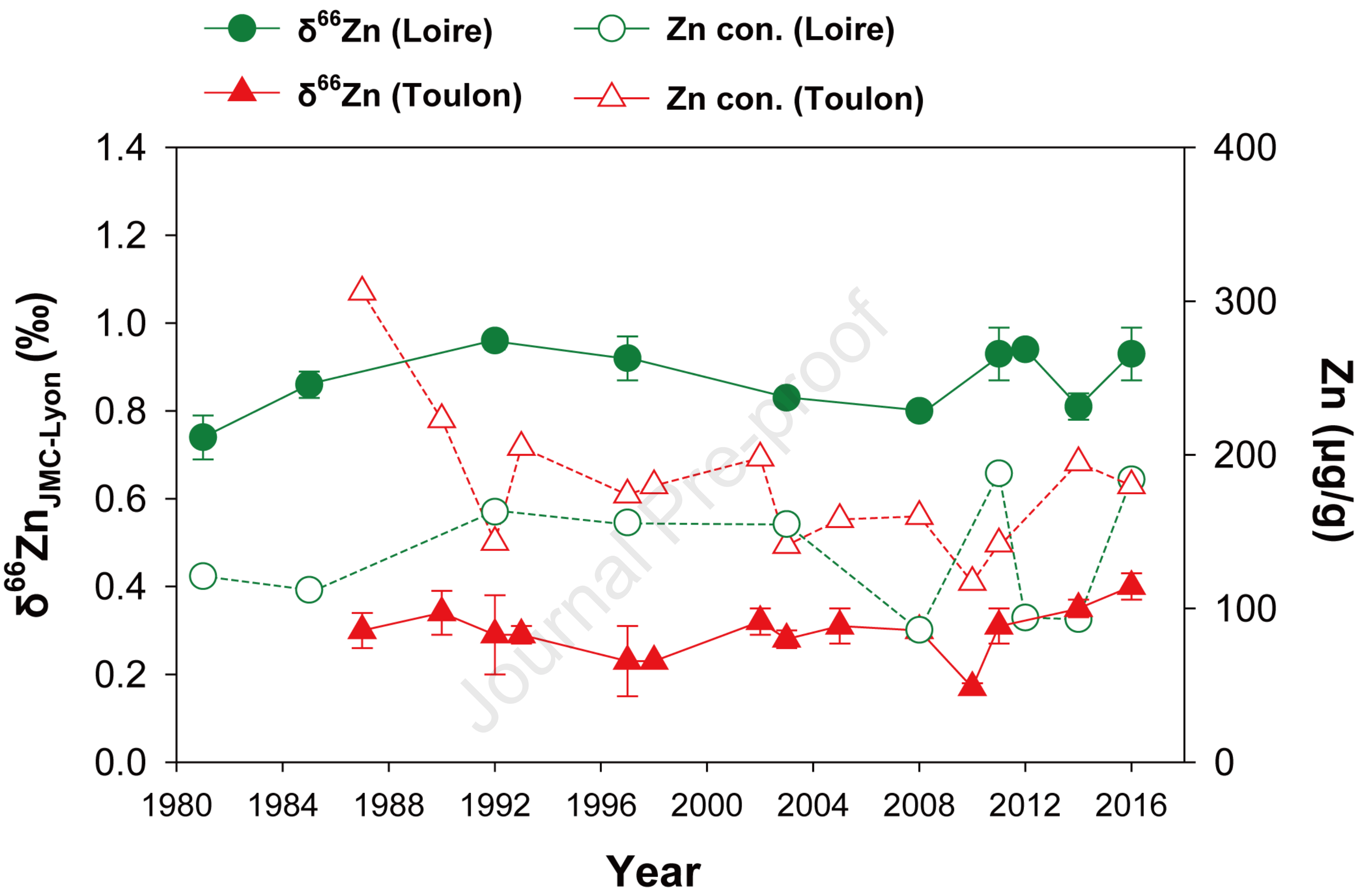


Fig. 3. Elemental and isotope variations in zinc (Zn) in mussels over decades.

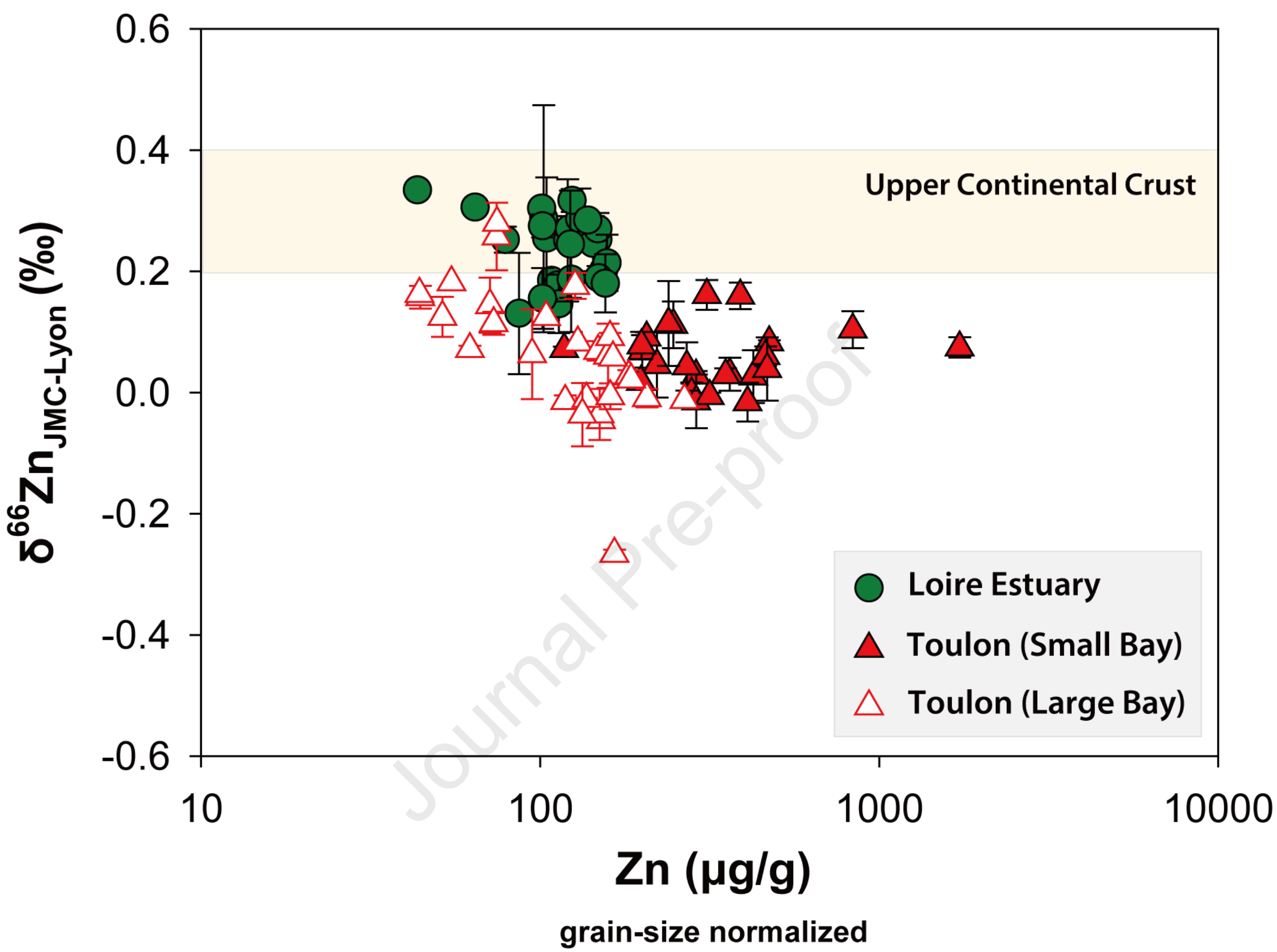


Fig. 4. Zinc (Zn) elemental and isotope data of Loire Estuary and Toulon Bay sediments. The Zn concentrations are normalized to grain size using an aluminum (Al) content of 5% and are displayed on a logarithmic scale. The Upper Continental Crust (UCC) correspond to the aproximative average range reported in the literature (Moynier et al., 2017).

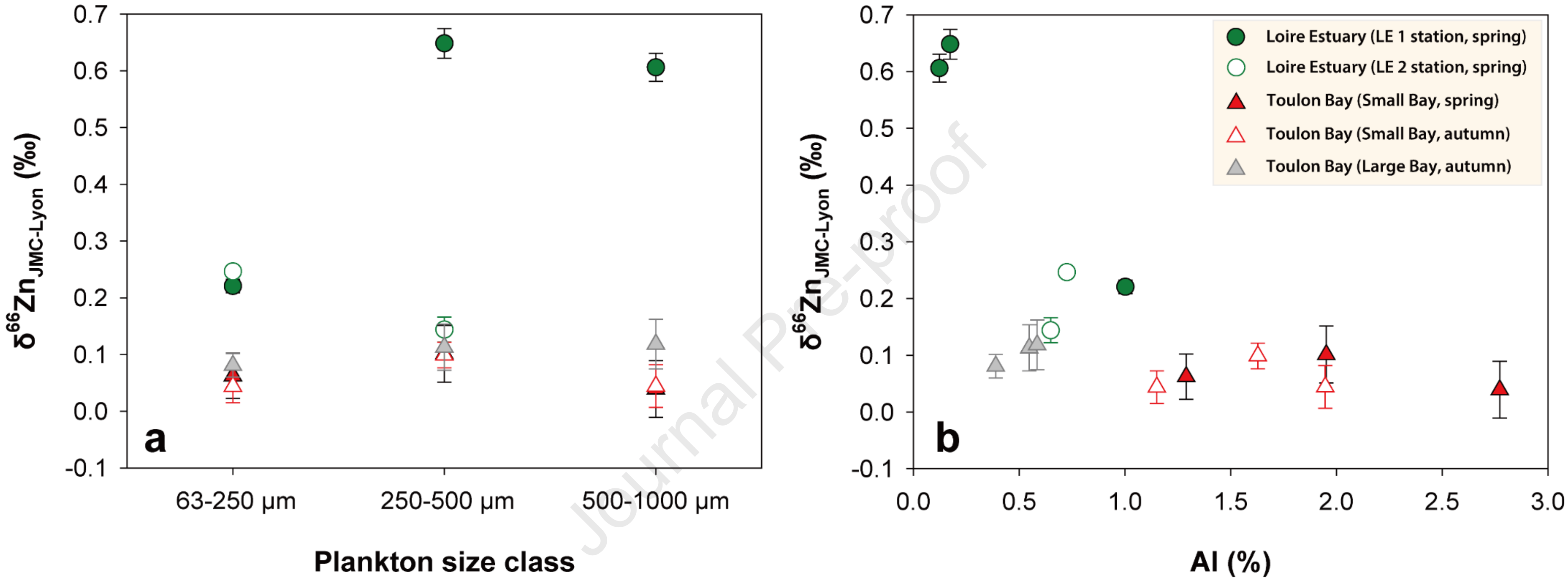


Fig. 5. Zinc (Zn) isotope compositions of plankton samples. a) δ^{66}Zn values for the different plankton size classes; (b) scatter plot of δ^{66}Zn *vs.* aluminum (Al).

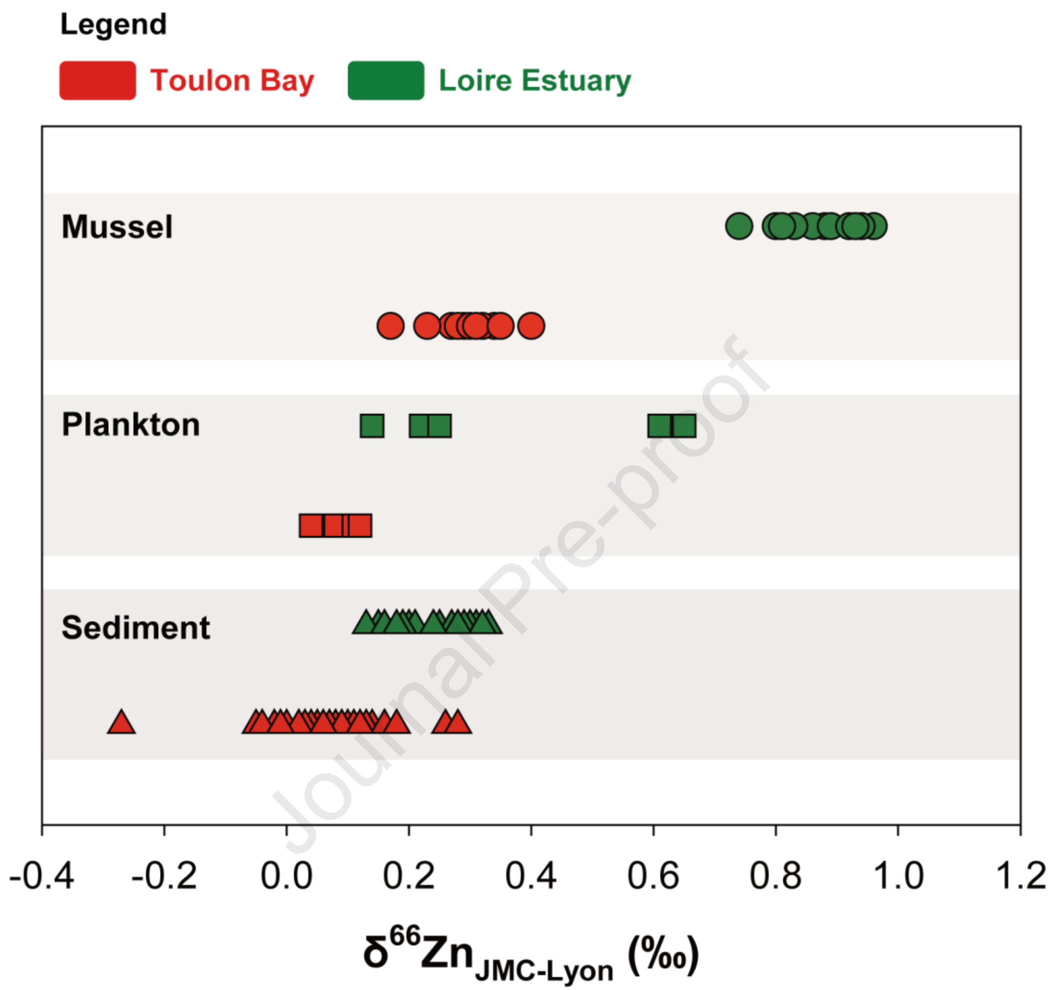


Fig. 6. Multi-compartment zinc (Zn) isotope compositions from the Loire Estuary and Toulon Bay.

Table 1. Isotope compositions of reference materials (RM)s reported against the "JMC-Lyon" standard. The *"n"* refers to number of distinct aliquots procced through all procedure. Each aliquot was analysed twice or thrice.

Matrix	Reference material	δ^{66} Zn (this study)	δ^{66} Zn (Literature)	authors
Oyster	SRM 1566b	0.70 ± 0.05 (*n* = 5)	0.71 ± 0.02 (n = 4)	Jeong et al., 2021
Mussel	ERM-CE278K	0.41 ± 0.05 (*n* = 3)	0.56 ± 0.04 (n = 4)	Jeong et al., 2021
Sediment	MESS-3	0.26 ±0.06 (*n* = 10)	0.28 ± 0.02 (n = 13)	Druce et al., 2020
Plankton	BCR 414	0.23 ± 0.09 (*n* = 1)	0.29 ± 0.04 (n = 4)	Jeong et al., 2021

Table 2. Summary of elemental and isotope datasets of zinc used in this study.

Site	Matrix	Sampling date	Reference	Table
Loire Estuary	Surface sediment/Sediment core	2014 and 2015	Araújo et al., 2019b	**S3**
	Plankton	2013	Araújo et al., 2022a	**S1**
	Mussel	From 1981 to 2016	**This study**	**S4**
Toulon Bay	Surface sediment	2008 and 2009	Araújo et al., 2019a	**S2**
	Plankton	2018	**This study**	**S1**
	Mussel	From 1987 to 2016	**This study**	**S4**

Highlights

- Pristine and contaminated sites show samples with distinct isotope fingerprints.
- Systematic lighter $\delta^{66}Zn$ values identify anthropogenic Zn in contaminated samples.
- Zn isotopes trace upward transfer of anthropogenic Zn in the food web.
- Isotope biomonitoring indicates no alterations in Zn sources over four decades.
- Zn isotope ratio constancy at seasonal scale discards biological isotope effects.

All authors have approved the submission of this manuscript, and we collectively affirm that the content of the manuscript is original and has not been published or is currently under consideration elsewhere.