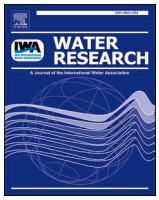
Water Research 234 (2023) 119793

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres



High Hg biomagnification in North Atlantic coast ecosystems and limits to the use of $\delta^{15}N$ to estimate trophic magnification factors

M. Teresa Boquete [a,*], Jesús R. Aboal [a], Rubén Villares [b], Uxía Dorado-García [a], J. Ángel Fernández [a]

[a] CRETUS Institute, Department of Functional Biology, Ecology Unit, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain
[b] Department of Functional Biology, Ecology Unit, Escola Politécnica Superior de Enxeñaría, Universidade de Santiago de Compostela, 27002 Lugo, Spain

ARTICLE INFO

Keywords:
Dogwhelk
Food chains
Mussel
Nitrogen isotope ratio
Marine pollution

ABSTRACT

Mercury contamination is a global environmental problem. This pollutant is highly toxic and persistent which makes it extremely susceptible to biomagnify, i.e. increase its concentrations as it moves up the food chain, reaching levels that threaten wildlife and, ultimately, ecosystems' function and structure. Mercury monitoring is thus crucial to determine its potential to damage the environment. In this study, we assessed the temporal trends of the concentrations of Hg in two coastal animal species closely connected by a predator-prey interaction, and evaluated its potential transfer between trophic levels using the $\delta^{15}N$ signatures of the two species. For this, we performed a multi-year survey of the concentrations of total Hg and the values of $\delta^{15}N$ in the mussel *Mytilus galloprovincialis* (prey) and the dogwhelk *Nucella lapillus* (predator) sampled along ~1500 km of the North Atlantic coast of Spain over a 30-year period (five surveys between 1990 and 2021). Concentrations of Hg decreased significantly between the first and the last survey in the two species studied. Except for the 1990 survey, the concentrations of Hg in mussels were amongst the lowest registered in the literature for the North East Atlantic Ocean (NEAO) and the Mediterranean Sea (MS) between 1985 and 2020. Nonetheless, we detected Hg biomagnification in almost all surveys. Worryingly, trophic magnification factors obtained here for total Hg were high and comparable to the found in the literature for methylmercury, the most toxic and readily biomagnified form of this element. The $\delta^{15}N$ values were useful to detect Hg biomagnification under normal circumstances. However, we found that nitrogen pollution of coastal waters differentially affected the $\delta^{15}N$ signatures of mussels and dogwhelks limiting the use of this parameter for this purpose. We conclude that Hg biomagnification could constitute an important environmental hazard even when found at very low concentrations in the lower trophic levels. Also, we warn that use of $\delta^{15}N$ in biomagnification studies when there is some underlying nitrogen pollution problem might lead to misleading conclusions.

1. Introduction

Mercury is one of the most toxic elements on Earth having important effects at neural, hormonal, and reproductive levels within individuals (Boening, 2000; Fernandes Azevedo et al., 2012; Tan et al., 2009) with potential demographic consequences at the population level (Goutte et al., 2014). This element occurs naturally and can be released to the environment through volcanic eruptions, rock weathering, and evaporation from the ocean (Boening, 2000). Human activities like coal combustion and mining, however, have mobilized mercury from the lithosphere deposits and increased its concentrations in the atmosphere by 450% above natural levels (UN Environment Programme, 2019). Mercury is foremost emitted to the atmosphere in elemental form that is highly volatile and has an atmospheric lifetime of about one year, making it remarkably susceptible to long-range atmospheric transport (Fitzgerald and Lamborg, 2003). Eventually, Hg released into the atmosphere will be deposited directly into the oceans or onto land from where it can be washed into water bodies. This Hg can be re-emitted to the atmosphere and deposited again (Selin et al., 2008). Deposited Hg on oceans and land will take about 2000 years to return to more stable terrestrial and ocean reservoirs (Selin et al., 2008; Sunderland and Mason, 2007).Thus, anthropogenic Hg emissions can negatively impact the environment for hundreds to thousands of years from their first release (UN Environment Programme, 2019).

* Corresponding author: M. Teresa Boquete, Rúa Lope Gómez de Marzoa, s/n, 15705, Santiago de Compostela, Spain.
E-mail address: teresa.boquete@usc.es (M.T. Boquete).

https://doi.org/10.1016/j.watres.2023.119793
Received 5 November 2022; Received in revised form 31 January 2023; Accepted 21 February 2023
Available online 23 February 2023

It is undeniable that mercury contamination is a serious and global environmental problem. Inger Andersen, the United Nations Deputy Secretary General and Executive Director of the United Nations Environment Program, has recently defined it as "*one of the greatest challenges to the environment and human health*" (UNEP, 2019). The magnitude of this problem is such that, partly driven by the Minamata Convention on Hg (the first globally legally binding instrument establishing the measures to be undertaken to protect human health and the environment from Hg and its compounds; (UNEP, 2013)), organizations like the United Nations (UN), the U.S. Environmental Protection Agency (EPA), and the European Union (EU) have developed coordinated programs to specifically protect the environment from the negative impacts of Hg released into the air, water, and soil (UN Environment Program Global Mercury Partnership -https://www.unep.org/globalmercurypartnership/-, EPA Mercury -https://www.epa.gov/mercury-, and EU Global Mercury Observation System -https://www.gmos.eu/). These programs highlight the necessity to develop long-term spatiotemporal observations of Hg in the environment to better predict its impact as well as to evaluate the effectiveness of the protective measures undertaken.

Inorganic Hg that reaches aquatic systems can be biomethylated by microorganisms to form methylmercury, the main form in which this element is accumulated in both humans and wildlife (D'Itri, 1991). Once incorporated into organisms' tissues directly from the water or through food consumption, this organic form binds strongly to sulfhydryl groups in proteins, resulting in low rates of excretion (Bernhard and Andreae, 1984). Thus, Hg biomagnification is one of the most concerning issues related to Hg contamination. Biomagnification is defined as the progressive buildup of pollutants in organisms through the food web (Zweig, 1973). Accordingly, the concentrations of pollutants in organisms of higher trophic levels will be higher than those in their diet. Biomagnification is thus a special case of pollutant bioaccumulation process in which the main exposure route is the organism's diet (Connell, 1989; Gray, 2002). Mercury biomagnification in marine food webs has been reported before (Kidd et al., 2011), with the most classical and tragic example being the deaths of the Minamata population as a result of Hg poisoning from the consumption of contaminated fish and shellfish (D'Itri, 1991). Detecting and quantifying biomagnification processes in natural ecosystems is thus crucial to assess how the presence of Hg in aquatic environments might translate into local to global exposure risk.

Pollutant biomagnification processes can be identified through the estimation of the trophic magnification factor (TMF) as the slope of the regression between the concentrations of the pollutant on each member of the trophic chain/web against its trophic level. A significant and positive TMF would be indicative of biomagnification whereas a significant but negative TMF would point to biodilution. The use of stable nitrogen isotope ratio ($\delta^{15}N = {}^{15}N{:}^{14}N$) to investigate pollutant biomagnification processes has been proposed as a more quantitative approach than traditional methods like food composition analyses. (Broman et al., 1992; Chouvelon et al., 2018; Foster et al., 2012; Hong et al., 2013; Kehrig et al., 2013; Lavoie et al., 2013). The reliability of this method, however, depends upon the quality of the $\delta^{15}N$ signature as a good food web descriptor (Cabana and Rasmussen, 1994). It is well known that nitrogen contamination processes can alter the $\delta^{15}N$ signatures of organisms (Heaton, 1986; McClelland and Valiela, 1998). Under such circumstances, the $\delta^{15}N$ signature could not be a reliable tool for TMF estimation. Yet, to the best of our knowledge, no single study has addressed this issue so far.

Considering all of the above, we present here a dataset of the concentrations of total Hg (that includes both organic – methylmercury – and inorganic forms of this element) and the $\delta^{15}N$ values in two common dwellers of the intertidal rocky shore of the Northeast Atlantic Ocean closely connected by a predator-prey interaction: the Mediterranean mussel *Mytilus galloprovincialis* (prey) and the Atlantic dogwhelk *Nucella lapillus* (predator). These species were collected along ~1500 km of the North Atlantic coast of Spain during five surveys spanning 30 years between 1990 and 2021. This study this aimed at (i) investigating the temporal trends of the concentrations of Hg in these two animal species; (ii) evaluating its potential transfer between trophic levels as well as potential changes in trophic transfer over time; and (iii) assessing the utility of $\delta^{15}N$ to identify and monitor Hg biomagnification patterns.

2. Material and methods

2.1. Sample collection and processing

We collected samples of two coastal animal species belonging to two different trophic levels and using different feeding strategies: the bivalve mollusc *Mytilus galloprovincialis* (Lamarck, 1819), a filter-feeder (Deudero et al., 2009); and the gastropod mollusc *Nucella lapillus* (Linnaeus, 1758), a predator (Crothers, 1985) that preferentially feeds on mussels (Hughes and Drewett, 1985). Samples were collected in summer during four extensive (1990, 2001, 2003 and 2021) and one intensive (2017) sampling surveys at 89 different sampling sites (SS) spread across the Galician coast (NW Spain; Fig. 1). In 1990, the survey included a total of 58 SS with *M. galloprovincialis* collected at 49 SS and *N. lapillus* at 16 SS (7 SS in common). In 2001, we sampled 24 SS with *M. galloprovincialis* collected at 22 SS and *N. lapillus* at 12 SS (10 SS in common). In 2003, we sampled 37 SS with *M. galloprovincialis* collected at 37 SS and *N. lapillus* at 25 SS (25 SS in common). In 2017, we intensified the sampling in two rias (tidal inlets) that were most seriously affected by Hg contamination (Prego and Cobelo-García, 2003): i) the Ría de Ferrol (Zone E in Fig. 1); and ii) the Ría de Pontevedra (second southernmost ria in Zone D, Fig. 1). Both rias are affected by different pollution sources, such as highly populated urbanized zones, industrial facilities (i.e. shipyards and steel works in Ferrol and a chlor-alkali in Pontevedra) and busy commercial ports. Therefore, in this survey we sampled a total of 8 SS, with *M. galloprovincialis* collected in all 8 SS and *N. lapillus* in 6 SS (6 SS in common). Finally, in 2021 we conducted the last extensive survey which included 32 SS, with *M. galloprovincialis* collected in all 32 SS and *N. lapillus* in 23 SS (23 SS in common). All sites sampled per survey and species are shown in Table S1.

All SS were located more than 300 m from waste pipes, ports and industrial installations, and more than 150 m from the mouths of primary or secondary effluents. The sampling was always conducted at low tide in the intertidal zone following a zigzag path between points on the coastline separated by a linear distance of 50 m. At each SS, we collected at least 25 specimens of *M. galloprovincialis* (shell length $\geq$ 6 cm) and 30 specimens of *N. lapillus* (shell length $\geq$ 2 cm). All the specimens were washed in situ with seawater and combined to form a composite sample of each species per site before being transported in cool boxes (ca. 4 °C) to the laboratory.

In the laboratory, all the specimens were purged for 48 to 72 h in continuously flowing, aerated and filtered (active carbon) seawater. The samples were then stored frozen at −30 °C. Prior to sample preparation/processing, the material was thawed at room temperature. Then, we separated each specimen's meat from its shell with the aid of high-density polythene dissection tools. These samples were homogenized (Waring Blender 34BL99), dried in a forced air oven at 40 °C, and again homogenized in a mill (Retsch MM400 Ultra Centrifugal Mill). After processing, all samples were stored at room temperature in darkness until they were analyzed.

2.2. Chemical analysis

We determined the concentrations of Hg in an elemental analyzer (DMA 80 Milestone). During the analysis, we included samples of two certified reference material (TORT-1, lobster hepatopancreas; and ERM-CE278k, Mussel) and analytical blanks for quality control. Also, all samples were analyzed in duplicate. The recovery rate of the reference material was highly satisfactory (106 and 90% respectively), while the overall error for the analysis of duplicate samples was very low (3%). The limit of quantification, calculated from the values obtained from the

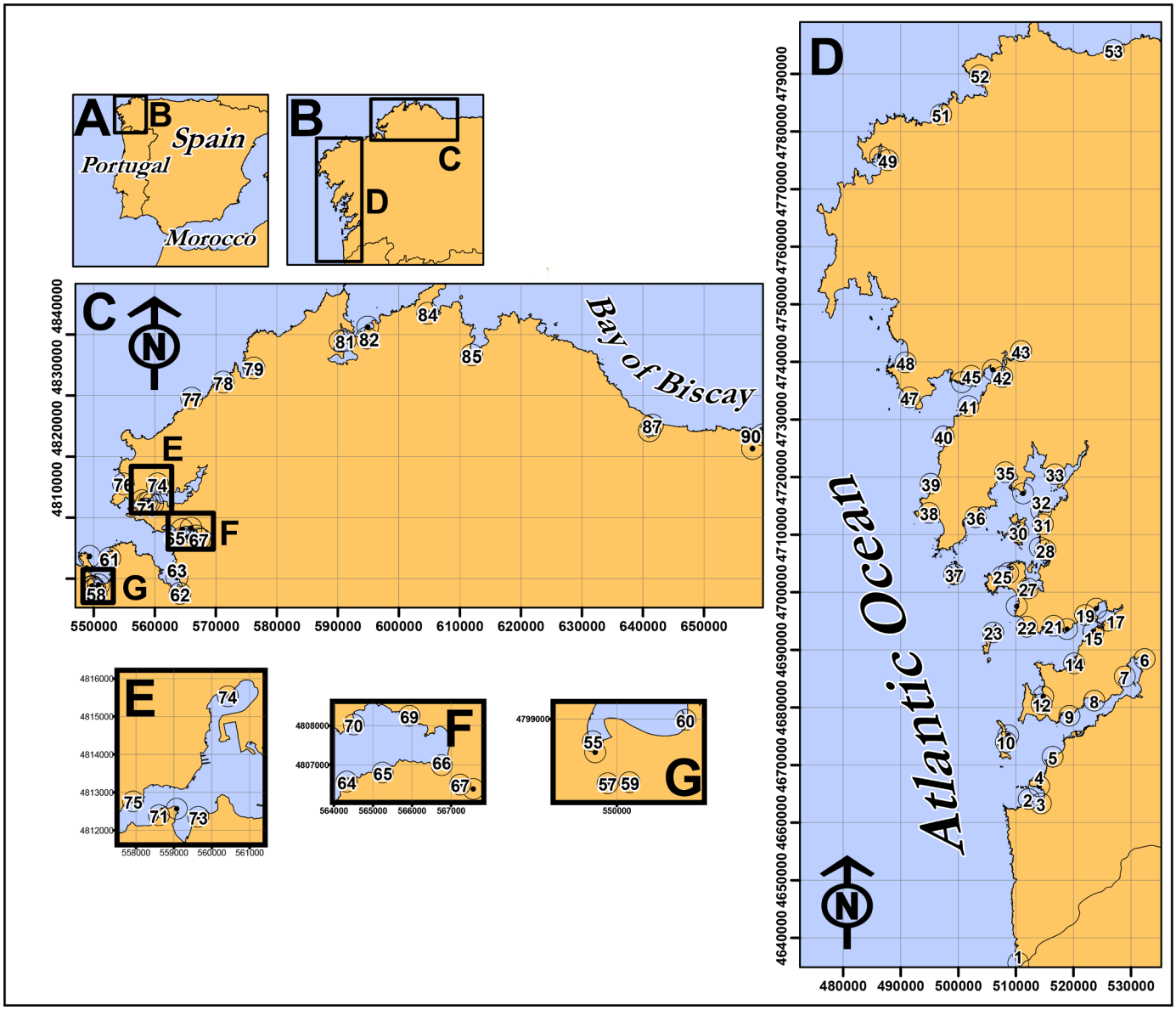


Fig. 1. Map showing the location of the study region in the NW of Spain (A), the rias where the sampling was carried out within the study region (B), and a detailed view of the sampling sites located in the northern (C, E, F, G) and western (D) rias.

analytical blanks (as $Mean_{blanks} + 10*StdDev_{blanks}$), was 0.4 ng g^{-1}.

To determine $\delta^{15}N$ values, we weighed aliquots of 3.00 ± 0.1 mg of each sample in tin capsules (Elemental Microanalysis). The analytical determinations were conducted by the Research Support Services at the University of A Coruña (Spain), using an element analyzer (FlashEA1112, ThermoFinnigan) coupled to an isotope ratio mass spectrometer (DeltaV Advantage, Thermo Scientific) via an interface unit (ConfloIV). The accuracy of the procedure was determined by analysis of acetanilide as a calibration standard. Calibration of the reference gas (N_2) for atmospheric ^{15}N was carried out with IAEA-N-1 ($(NH_4)_2SO_4$), IAEA-N-2 ($(NH_4)_2SO_4$) and IAEA-NO-3 (KNO_3) as standards. The isotopic ratio (^{15}N /^{14}N) in the samples was determined by comparison with a standard (atmospheric N_2). The relative abundance of ^{15}N in the sample ($\delta^{15}N$) was calculated using the formula $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$, where R is the $^{15}N/^{14}N$ ratio. The overall error calculated for the replicate samples was very low (3%).

The concentrations of Hg and values of $\delta^{15}N$ of all the collected samples (per survey and species) are shown in Table S1.

2.3. Statistical analysis

In the first place, we assessed the amount of intraspecific variation at the site level in the concentrations of Hg and the $\delta^{15}N$ values in mussels and dogwhelk. For this, we estimated the coefficient of variation (ratio of the standard deviation to the mean) in three biological replicates of each of the species consisting of 10 individuals per replicate, in each of 18 SS (for dogwhelks) and 8 SS (mussels) from the 1990 and 2017 surveys.

All statistical analyses were conducted using R v4.2.1 (R Core Team, 2022) implemented under RStudio v1.2.5042 (RStudio Team, 2020). All p-values < 0.05 were considered significant. For all the models, we evaluated the normality and homoscedasticity (homogeneity of variances) of the residuals using the Shapiro and Levene tests respectively, as well as by visually inspecting the plots of the residuals. When these assumptions were not met, we applied transformations to the dependent variables or used alternative, non-parametric tests.

First, using the "full dataset" ($n = 89$ SS in total; 85 SS with 148 data points across surveys for mussels, and 45 SS with 82 data points for

dogwhelks), we tested for significant differences among surveys in the mean concentrations of Hg and $\delta^{15}N$ values separately for each species. For Hg in both species and $\delta^{15}N$ in dogwhelks, we run a one-way ANOVA (analysis of variance) using the function *aov* from the base R package "stats". For $\delta^{15}N$ in mussels, we run the non-parametric equivalent to one-way ANOVA, i.e. Kruskal-Wallis test, with the function *kruskal.test* from the package "stats". When the main ANOVA and Kruskal-Wallis tests resulted significant, we performed all possible pairwise comparisons between surveys using the functions *pairwise.t.test* and *dunn.test* (package "*dunn.test*") respectively, with the Hochberg's GT2 method, designed to address unequal sample sizes. Using the "common dataset", that only included sites where both species were collected together each survey ($n = 41$ SS in total with 77 data points), we implemented similar analyses to test if the ratio between the concentrations of Hg in dogwhelks and in mussels (one-way ANOVA), and the difference between $\delta^{15}N$ values in dogwhelks and in mussels (Kruskal-Wallis test) differed significantly among surveys. Lastly, in order to confirm that the differences observed between surveys reflected reliable temporal trends rather than spatial trends, we performed paired t-tests using only the subset of data points that were common to each pair of surveys.

Second, we looked for unusually high concentrations of Hg and values of $\delta^{15}N$ in the full dataset by estimating the values of the outliers as the sum between the third quartile (Q3) and 1.5-times the interquartile range (IQR) for each element and species. Similarly, we also looked for unusually low values as the first quartile (Q1) minus 1.5-times the interquartile range (Q1 - 1.5*IQR).

Third, we used the "common dataset" to study the relationship between the concentrations of Hg and the values of $\delta^{15}N$ in *N. lapillus* and in *M. galloprovincialis*. For this, we run two separate ANCOVAs with the concentrations of Hg and the $\delta^{15}N$ in *N. lapillus* as response variables, the concentrations of Hg and the $\delta^{15}N$ in *M. galloprovincialis* as predictors, and Year (treated as factor with 5 levels) as a covariate in both models. We tested for the significance of the interaction between the predictor and the covariate to evaluate whether the relationship between the concentration of Hg and $\delta^{15}N$ in mussels and in dogwhelks changed over time. In order to check whether the addition of the interaction term improved the fit of the model, we used the function *anova* (package "stats") to compare the model with and without the interaction. Also, using both the full and the common datasets, we estimated the coefficients of dispersion (the ratio between the median absolute deviation, i.e. the median of the absolute difference between each value and the median, to the median) of the concentrations of Hg and $\delta^{15}N$ values in mussels and dogwhelk for each survey. We used this approach because it is less sensitive to extremely high/low values than the ratio of the mean to the standard deviation.

Fourth, we estimated the trophic magnification factor (TMF) as the slope (b) of the linear regression between the stable nitrogen isotope ratio ($\delta^{15}N$) and the $\log_{10}$ value of the concentration of Hg in both species, ($\log_{10}Hg = a + b\ \delta^{15}N$), to assess Hg biomagnification between trophic levels (Chouvelon et al., 2018; Hong et al., 2013; Kehrig et al., 2013; Lavoie et al., 2010). Additionally, we used ANOVA to evaluate whether the TMF varied among years by testing for the significance of the interaction between $\delta^{15}N$ and Year. Since the interaction was significant, we run individual simple linear regression models to separately estimate the TMF for each survey. Finally, following the arguments by (Isaacs, 1973), who stated that more enclosed sites would be more likely to show pollutant biomagnification than open sea sites due to their differences in the variety of prey available to the predators, we did the same analyses but separately on the sampling sites that were located in the inner parts of the rias (inner sites) and on the sites located in the outermost part of the rias (outer sites).

3. Results

The coefficients of variation calculated from three biological replicates collected per species in a number of SS to estimate the intraspecific and within-site variability of our data were low both for Hg (mean values of 8 and 17% for mussels and dogwhelks respectively) and especially for $\delta^{15}N$ (mean values of 2 and 3% for mussels and dogwhelks respectively). This low within-site variability supports that, overall, our data provide a good representation of the actual concentrations of Hg and the $\delta^{15}N$ values in each species within each site.

Mean concentrations of Hg differed significantly among surveys in both species ($F = 33.4$ and $p < 0.001$ in mussels; $F = 18.3$ and $p < 0.001$ in dogwhelks; Table 1). Although these concentrations showed a 3.4- and a 7.8-fold decline in mussels and dogwhelks respectively between the first (1990) and the last (2021) survey, this decrease was not gradual (Fig. 2A, Table S2, Table S3). Both species showed a strong decrease between 1990 and 2001. Then, mean Hg concentrations in mussels remain rather constant between 2001 and 2017 and declined again significantly in 2021 compared to 2001 and 2017. Mean concentrations in dogwhelks followed a similar pattern except for the fact that the lowest mean concentrations found in 2021 only differed significantly from those in 2017. Mean $\delta^{15}N$ values, on the other hand, remained constant throughout the study period in dogwhelks ($F = 1.99$ and $p = 0.105$; Table 1) and decreased significantly in mussels (Chi sq. = 58.7 and $p < 0.001$; Table 1). This decrease was essentially due to a decline in $\delta^{15}N$ between the first and the second survey (1990 to 2001); after 2001, mean $\delta^{15}N$ values in mussels remained unchanged (Fig. 2C, Table S3).

Total Hg concentrations ranged, across all surveys, between 28.2 and 854 ng g^{-1} in mussels and between 69.7 and 6254 ng g^{-1} in dogwhelks. We did not find any unusually low ($\leq$ Q1 - 1.5*IQR) concentrations of Hg in any of the species. The values of the upper outliers were 385 and 1124 ng g^{-1} of Hg for mussels and dogwhelks respectively. We identified a similar number of outliers for Hg in both species: eight for mussels (seven in 1990 and one in 2003) and seven for dogwhelks (six in 1990 and one in 2003), although none of them coincided in the same collection sites (but they only were collected together in two out of these fifteen sites). The $\delta^{15}N$ values ranged between 4.99 and 23.7 ‰ across all surveys in mussels and between 7.64 and 11.5 ‰ in dogwhelks. As with Hg, we did not find any unusually low $\delta^{15}N$ values in any of the species. The values of the upper outliers were 12.2 and 11.3 ‰ for mussels and dogwhelks respectively and, contrary to what we found for Hg, we identified 24 outliers for $\delta^{15}N$ in mussels (all of them in 1990) and only one in dogwhelks (in 2003). The concentrations of Hg tended to be less variable in mussels than in dogwhelks (coefficients of dispersion ranging between 14 and 38% and 12–42% respectively including both the full and common datasets) whereas the $\delta^{15}N$ values were more variable in mussels compared to dogwhelks (coefficients of dispersion ranging between 3 and 28% and 2–7% for mussels and dogwhelks respectively including both the full and common datasets) (Table S2).

The ratio between the concentrations of Hg in dogwhelks and in mussels, corresponding to the SS in which both species were collected at the same time, had a minimum value of 1.5 and a maximum of 16.4 across all surveys whereas the median ratio per survey ranged between 2.3 - in 2001- and 4.5 - in 2017 (Fig. 2B). The overall model testing for differences across surveys in the mean values of this ratio was marginally significant ($F = 2.55$; $p = 0.047$; Table 1); yet, the multiple pairwise comparisons showed no significant differences between surveys (Table S3). Finally, the difference between the $\delta^{15}N$ values in *N. lapillus* and in *M. galloprovincialis* ranged between −10.7 and 4.8 across all surveys whereas the median difference per survey ranged between 0.74 (in 1990) and 1.89 (n 2003; Fig. 2D). The results of the model showed significant differences among surveys in the mean value of this difference (Chi sq. = 16.6; $p = 0.002$). This effect was due to the low values reported in 1990, driven by the unusually high $\delta^{15}N$ values in mussels in this survey as compared to all other surveys (Fig. 2C and 2D; Table S3). All these results were supported by the paired-t-tests despite their lower number of data points per survey.

We found a significant relationship between the concentrations of Hg in mussels and dogwhelks as well as between the $\delta^{15}N$ values in these two organisms (Table 2). The interaction between Year and the

Table 1
Results of the one-way ANOVA (upper table) and Kruskal-Wallis test (bottom table) carried out to test for significant differences among surveys in the mean concentrations of Hg (ng g^{-1}) and the $\delta^{15}N$ (‰) values in each of the species studied. *M.g.: Mytilus galloprovincialis; N.l.: Nucella lapillus; N.l. / M.g.*: ratio between the concentration of Hg in *N. lapillus* and in *M. galloprovincialis; N.l. - M.g.*: difference between the $\delta^{15}N$ values in *N. lapillus* and in *M. galloprovincialis*; Df: degrees of freedom; Sum Sq: sum of squares; Mean Sq: mean squares; F value: F statistic; Pr(>*F*): p-value; ^{ns}: not significant; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Species	Response	Predictor	Df	Sum Sq	Mean Sq	F value	Pr(>*F*)
M.g.	Hg	Year	4	0.061	0.015	33.36	<2E-16***
		Residuals	143	0.066	0.000		
N.l.	Hg	Year	4	0.017	0.004	18.34	1.3E-10***
		Residuals	77	0.018	0.000		
	$\delta^{15}N$	Year	4	0.011	0.003	1.988	0.105ns
		Residuals	77	0.108	0.001		
N.l. / M.g.	Ratio Hg	Year	4	0.141	0.035	2.552	0.0471*
		Residuals	66	0.914	0.014		
Species	Response	Predictor	Df			K-W Chi-sq	p-value
M.g.	$\delta^{15}N$	Year	4			58.73	5.4E-12***
N.l. - M.g.	Diff. $\delta^{15}N$	Year	4			16.62	0.0023**

predictor was not significant in any of the models indicating that the effect of these two variables in mussels on the values of these variables in dogwhelk did not vary among years. Indeed, adding the interaction term did not significantly improve the fit of the models ($F = 0.494$, $p = 0.741$ for Hg; $F = 2.424$, $p = 0.058$ for $\delta^{15}N$). In the model without the interaction, the variation in the concentrations of Hg in mussels significantly explained ~60% of the variation in the concentrations of Hg in dogwhelks whereas that of $\delta^{15}N$ only explained 15% (Table 2).

Finally, we found a significant interaction between Year and $\delta^{15}N$ values in the model used to estimate the TMFs for the dataset including all sampling sites, the dataset including only the sites from the inner part of the rias, and the dataset including sites from the outer part of the rias (Table S4). Therefore, we separately estimated the TMF for each survey in each dataset (Table S5) obtaining the following values: (i) dataset including all sampling sites: 1.4, 1.7, 2.7 and 1.4 respectively for the 2001, 2003, 2017 and 2021 surveys (Fig. 2); (ii) dataset including only the sites in the inner part of the rias: 3.1, 1.6, 2.5, 1.3 respectively for the 1990, 2003, 2017 and 2021 surveys; and (iii) dataset including only the sites in the outer part of the rias: 1.5, 1.8, 1.5 respectively for the 2001, 2003, and 2021 surveys (Fig. S2). TMFs for the 1990 survey in the full and outer sites datasets are not presented because the models were not significant (Table S5).

4. Discussion

In this study, we performed a multi-year survey of the concentrations of Hg in two animal species from different trophic levels sampled along ~1500 km of the Northeast Atlantic coastline over a 30-year time span. We investigated the temporal trends of the concentrations of Hg on the coast and evaluated its potential transfer between trophic levels. Additionally, we assessed the utility of $\delta^{15}N$ to identify and monitor Hg biomagnification patterns. First, we showed that Hg pollution decreased significantly within the study region between 1990 and 2021 (with the most drastic decline happening between 1990 and 2001). This temporal pattern was observed regardless of the trophic level of the organism investigated and despite that one of the latest surveys (2017) only included samples from the two-most contaminated rias in the region. Second, we found evidence of Hg biomagnification in this trophic system that was sustained over time despite the temporal decrease in Hg contamination. This was demonstrated by: (i) the consistently higher concentrations of Hg in the predator (*N. lapillus*) compared to the prey (*M. galloprovincialis*) along with the temporal and spatial stability of the ratio between the concentrations of Hg in the predator and in the prey; (ii) the high amount of variance in the concentrations of Hg in the predator explained by its concentrations in the prey; and, (iii) the fact that the significant TMFs were all greater than one.

Overall, the concentrations of Hg in *M. galloprovincialis* found in this study were low. Following the Mussel Watch program criteria for bivalves including mussels of the genus *Mytilus* (Kimbrough et al., 2008), 66% of our data points would be classified as having low levels of Hg (0–170 ng g^{-1}), 25% as having medium levels (170–350 ng g^{-1}), and only 9% as having high levels (350–1280 ng g^{-1}). Most of the data points within the medium or high levels belonged to the 1990 survey (37 out of 50, i.e. 74%) which showed the highest concentrations of Hg in both study species. Also, our data lay in the lower end of the range of concentrations reported for *M. galloprovincialis* in a literature review that included studies from nine regions spread across the North East Atlantic Ocean (NEAO) and the Mediterranean Sea (MS) between 1985 and 2020 (Fig. S1A,B). Together, all these data points showed no overall increasing/decreasing trend in the concentrations of Hg over time ($t = -1.941$, $p > 0.05$ in a generalized linear model testing for the change in concentrations over time; Fig. S1A). However, when split into smaller and more detailed regional datasets, there were significant temporal trends as the overall decline observed in our data. This agrees with the findings of the Mussel Watch program, which showed no significant country-wise level trends, but significant increases and decreases in the concentrations of Hg in specific regions of the USA coast (Kimbrough et al., 2008; Melwani et al., 2013). Altogether, these results suggest that, to obtain a more reliable view of the problem of Hg contamination in coastal systems, the levels of this element need to be monitored from different spatiotemporal perspectives.

Here we identified a significant transfer of total Hg between trophic levels in all surveys (except 1990 in the complete and outer sites datasets). The TMFs estimated for 2001, 2003 and 2021 were pretty similar to each other whereas those estimated for 1990 and 2017 were comparatively higher. Interestingly, the first corresponded to surveys in which most data points were classified as having low Hg in mussels whereas the latter corresponded to the survey with the highest historical Hg levels (1990) and the one that only included sites from the two-most polluted rias of the region (2017). Reported TMFs for total Hg in the literature are comparable to the obtained in this study for 2001, 2003, and 2021. For example, (Meng et al., 2015) found values of 1.2 in mollusks, including different species of bivalves and gastropods, (Qu et al., 2022) reported values of 1.4 and 1.7 for invertebrates and fish respectively, and (Lavoie et al., 2010) reported a value of 1.4 for a food web including particulate organic matter, zooplankton, gastropods and bivalves. Some of the above studies also reported TMFs for methylmercury, the most toxic and efficiently biomagnified fraction of Hg. These, were higher compared to those of total Hg, and comparable to the obtained here for 1990 and 2017. Thus, (Meng et al., 2015) reported values of 1.9, whereas (Qu et al., 2022) reported values of 2.6 and 2.2 for invertebrates and fish respectively and (Lavoie et al., 2010) reported values of 1.6. Works reporting the fraction of methylmercury to total Hg in mussels of the genus Mytilus showed that, for total Hg concentrations within the range of the found in our study (35–830 ng g^{-1}), methylmercury could account for, at least, almost half of the total concentrations of Hg in mussels (33–98%; (Ipolyi et al., 2004; Jędruch et al., 2019)); for lower total concentrations (2–30 ng g^{-1}), the percentage of

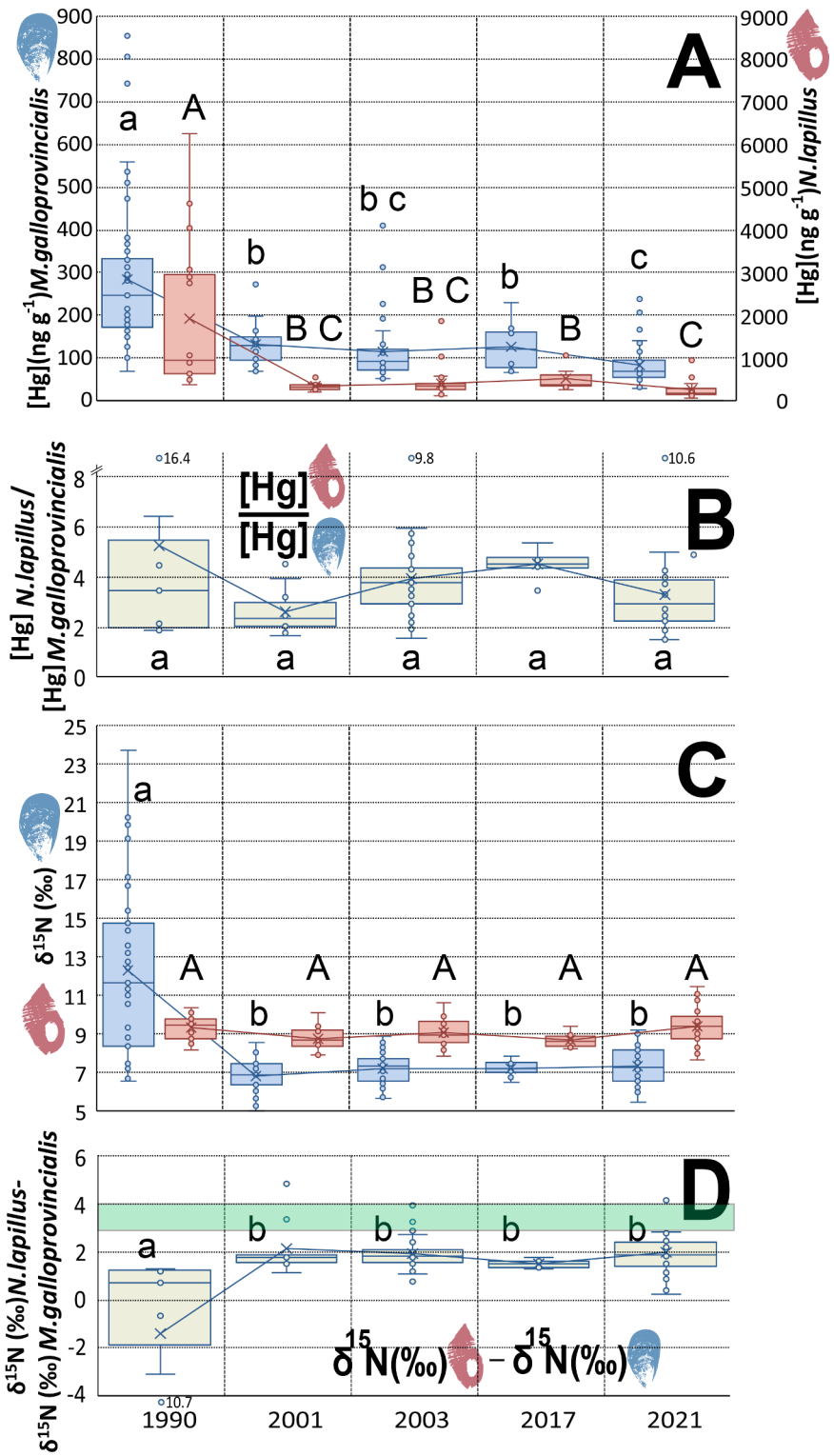


Fig. 2. Boxplots of the concentrations of Hg (ng g^{-1}; A) and the values of $\delta^{15}N$ (‰; C) in samples of *Mytilus galloprovincialis* and *Nucella lapillus* collected during five different sampling surveys (1990, 2001, 2003, 2017, 2021), as well as the ratio between the concentration of Hg in *N. lapillus* and in *M. galloprovincialis* (B) and the difference between the $\delta^{15}N$ values in *N. lapillus* and in *M. galloprovincialis* (D), are also presented. The letters on top of the boxplots represent the results of the post hoc tests carried out to look for significant differences between surveys in the mean values of each variable. In graph A, lower-case letters correspond to the results of the multiple pairwise comparisons in *M. galloprovincialis* and upper-case letters correspond to the results of these comparisons in *N. lapillus*. Shaded horizontal band in graph D: expected difference in $\delta^{15}N$ values between consecutive trophic levels, i.e. theoretical value of the trophic-level fractionation factor.

methylmercury to total Hg was much lower (12–55%; (Knopf et al., 2020)). Taken together, these results suggest that total Hg can biomagnify even when found at relatively low concentrations (2001, 2003 and 2021 surveys) and that the fraction of methylmercury to total Hg might increase under higher Hg contamination levels leading to higher TMFs.

The extent to which a pollutant is biomagnified does not only depend on its physicochemical characteristics but also on biological, ecological and environmental factors. According to (Reinfelder et al., 1998), biological factors include ingestion rate (determined by the weight of the consumer, food availability, growth rate, etc.), pollutant assimilation efficiency (determined by the composition of the food, the concentration of the element in the food source, the gut residence time, etc.), pollutant efflux/elimination through egestion (removal of undigested matter) or excretion (removal of metabolic waste products), and growth rate (potential dilution effect). Ecological factors include the structure and complexity of the food web (Cabana et al., 1994) and the feeding habit (Lavoie et al., 2013), among others. Finally,

Table 2
Results of the ANOVA test carried out to assess the relationship between the concentrations of Hg (ng g^{-1}) and the $\delta^{15}N$ values in *Nucella lapillus* (response variable) and in *Mytilus galloprovincialis* (predictor), including "Year" of the sampling survey as a covariate (first two models) or not (last two models). Df: degrees of freedom; Sum Sq: sum of squares; Mean Sq: mean squares; F value: F statistic; Pr(>*F*): p-value; ^{ns}: not significant; **: $p<0.01$; ***: $p<0.001$; Adj. R^2: adjusted R^2 (%).

Predictor	Df	Sum Sq	Mean Sq	F value	Pr(>*F*)	Adj. R^2
[Hg] in M.g.	1	0.014	0.014	91.8	8.9E-14 ***	57.5
Year	4	0.001	0.000	2.44	0.056 ^{ns}	
[Hg] in M.g.: Year	4	0.000	0.000	0.493	0.741ns	
Residuals	61	0.009	0.000			
$\delta^{15}N$ in M.g.	1	0.010	0.010	8.87	0.004 **	22.1
Year	4	0.011	0.003	2.52	0.051 ^{ns}	
$\delta^{15}N$ in M.g.: Year	4	0.011	0.003	2.42	0.058 ^{ns}	
Residuals	60	0.068	0.001			
[Hg] in M.g.	1	0.014	0.014	94.7	2.6E-14 ***	58.8
Year	4	0.001	0.000	2.52	0.050 ^{ns}	
Residuals	65	0.009	0.000			
$\delta^{15}N$ in M.g.	1	0.010	0.010	8.14	0.006 **	15.2
Year	4	0.011	0.003	2.31	0.067 ^{ns}	
Residuals	64	0.079	0.001			

environmental factors (e.g. pH, salinity or temperature) can influence both pollutant availability and the biological and ecological processes mentioned above. In this work, variability in the concentrations of total Hg in each of the species studied and, consequently, in Hg biomagnification patterns is likely driven by all these factors to some extent but especially by environmental factors. The Galician coast is a highly complex system consisting of a series of open sea areas interspersed with more closed estuaries (i.e. rias). The latter are even more complex as they are subject to both oceanic and continental influences determining water circulation patterns, sedimentation processes, organic matter and nutrient contents, as well as physicochemical parameters like temperature, pH, and salinity (Figueiras et al., 2002; Lorenzo and Taboada, 2005). This environmental complexity predominates in our study with sampling sites located inside and outside of the rias leading to, potentially, site-specific growth rates and metabolic activity of our study species, Hg dispersion patterns, activity of microorganisms transforming inorganic Hg into methylmercury, and characteristics of the sediments and their capacity to remove Hg from the water column and recirculate it again. The different pollution levels and sources to which mussels and dogwhelks were exposed across these sites add yet another layer of complexity to this system, as the normal functioning of these organisms could be significantly altered by pollutant exposure.

We argue, on the other hand, that the effect of the ecological factors on the variability of the concentrations of Hg found in this study is minor because both the dietary inferences extracted from the results of the $\delta^{15}N$ values in these species and the data extracted from the literature, point towards a tight predator-prey relationship between dogwhelks and mussels across space and time. Based on the assumption that high intraspecific variation in $\delta^{15}N$ values among habitats is indicative of differences in the relative trophic position occupied by a species in those habitats (Cabana and Rasmussen, 1994), the low levels of intraspecific variation of $\delta^{15}N$ in dogwhelk in this study suggest that dogwhelks' dietary habits are consistent across space and time in the study region (despite the wide range of habitats studied and the long time span analyzed). Also, even though the low values of the differences in $\delta^{15}N$ between dogwhelks and mussels found here (compared to the theoretical 3.4–4 ‰ difference expected for consecutive trophic levels (Minagawa and Wada, 1984)) would point towards a prevalent omnivorous diet in dogwhelks, results from a previous study in two coastal sites located within the same region found that suspension feeders, including *M. galloprovincialis*, could make up to 70% of the prey biomass of *N. lapillus* (Bode et al., 2006). Other studies found that dogwhelks tend to preferentially feed on mussels over barnacles, especially the larger (shell length $\geq$ 1.4 cm) individuals (Hughes and Drewett, 1985; Morton, 2010). Thus, even though both barnacles and mussels were present in many of our study sites, we sampled large dogwhelks (shell length $\geq$ 2 cm) which, according to this, would mostly feed on mussels. Finally, the difference in lifespan between mussels (~15–25 years; (Ceccherelli and Rossi, 1984; Sukhotin et al., 2007) and dogwhelks (between 4 and 6 years; (Crothers, 1985) could also explain some of the variation in their Hg concentrations and lead to the underestimation of the TMFs.

It is well known that, in addition to being an indicator of the relative trophic position of species, $\delta^{15}N$ signatures of organisms can be utilized to trace anthropogenic nitrogen inputs into coastal areas (McClelland et al., 1997; Viana et al., 2011; Viana and Bode, 2013). Nitrogen inputs derived from agriculture are often depleted in ^{15}N compared to seawater (leading to lower $\delta^{15}N$ values), while inputs from urban sewage, terrestrial runoff, or fish farm waste are enriched in ^{15}N (leading to increased $\delta^{15}N$ values) (Heaton, 1986; McClelland and Valiela, 1998). The $\delta^{15}N$ values reported here for *M. galloprovincialis* in the 1990 survey included 24 outliers with values ranging between 12.5 – 23.7 ‰ (median = 14.7 ‰). These values were the highest among the reported for this species in a literature review that included studies from five regions spread across the North East Atlantic Ocean (NEAO) and the Mediterranean Sea (MS) between 1998 and 2018 (Fig. S1C,D), which suggests that the significant enrichment in $\delta^{15}N$ observed in multiple sites during the 1990 survey was the result of anthropogenic sources in the study region. This enrichment in 1990 was also observed in macroalgae of the same region (Viana et al., 2011) but not in dogwhelk. The abnormal peaks of $\delta^{15}N$ in mussels thus led to the alteration of the relationship between $\delta^{15}N$ and the log transformed Hg concentrations in mussels and dogwhelks resulting in the lack of a significant relationship between these two variables. Consequently, we did not find evidence of Hg biomagnification in our system in 1990, except in the inner sites dataset which did not include outliers. Hence, for the first time to the best of our knowledge, we showed that anthropogenically-derived nitrogen sources may differently alter the $\delta^{15}N$ signatures of different marine species. This has important implications for the use of $\delta^{15}N$ to identify Hg biomagnification processes: under natural circumstances, $\delta^{15}N$ could be used as a reliable tracer of Hg biomagnification; when nitrogen contamination processes like the observed in the 1990 survey, differentially alter the $\delta^{15}N$ signature of different organisms, however, this parameter should not be used to infer nor quantify pollutant biomagnification unless additional data are gathered to account for the confounding effect of pollution on $\delta^{15}N$ signatures.

Finally, the difference between mussels and dogwhelks in the sensitivity of their $\delta^{15}N$ signature to nitrogen contamination could be due to differences in their feeding mode. As filter feeding species, mussels' diet consists mainly of plankton as well as particulate and dissolved organic matter present in the water. Indeed, (Bode et al., 2006) estimated that phytoplankton accounted for 40 to 100% of the diet of suspension feeder species, including *M. galloprovincialis*, in three coastal areas of Galicia. Theoretically, the capacity of species to reflect on their isotopic signature changes in the inputs of nitrogen to their environment increases in species with faster growth and nutrient uptake rates (Gartner et al., 2002). Small algae (i.e. microalgae), for example, showed significantly faster nitrogen uptake rates than macroalgae (Hein et al., 1995). Even so, some macroalgae can display altered $\delta^{15}N$ values after only seven days of exposure to sewage-derived dissolved inorganic nitrogen (Gartner et al., 2002). According to this, we would expect greater and faster changes in $\delta^{15}N$ resulting from nitrogen contamination in primary producers (phytoplankton and macroalgae), followed by primary consumers (e.g. mussels) and finally, by secondary consumers (e.g. predators like the dogwhelks).

5. Conclusions

In this study, we found a significant decline in the concentrations of Hg in two common dwellers of the intertidal rocky shore of the Northeast Atlantic Ocean between 1990 and 2021. In addition, using the $\delta^{15}N$ signatures of the two species, we provided robust evidence of Hg biomagnification even when this element was found at relatively low concentrations in the prey. Biomagnification factors were highest, comparable to the estimated in the literature for methylmercury, for the survey with the highest historical Hg levels and the one only including sites from the two-most polluted rias of the region suggesting that the fraction of methylmercury to total Hg increases under higher Hg contamination levels leading to increased biomagnification. Finally, we found that nitrogen contamination may have different effects on the $\delta^{15}N$ signatures of the different species. In this case, anthropogenically-derived nitrogen inputs to the coastal system caused significant shifts in the $\delta^{15}N$ values of the prey (mussels) but not in its predator (dogwhelks) making this parameter useless to detect pollutant biomagnification in natural food chains under these circumstances. In light of these results, we conclude that despite the overall decrease in Hg emissions to the environment, Hg contamination and biomagnification might still be an important environmental problem in some coastal regions and that the $\delta^{15}N$ signature is a reliable tracer of Hg biomagnification as long as there is no interfering nitrogen contamination process.(Fig. 3)

CRediT authorship contribution statement

M. Teresa Boquete: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Jesús R. Aboal:** Conceptualization, Methodology, Validation, Data curation, Writing – review & editing, Visualization, Supervision, Funding acquisition. **Rubén Villares:** Conceptualization, Writing – review & editing, Funding acquisition. **Uxía Dorado-García:** Writing – review & editing, Visualization. **J. Ángel Fernández:** Conceptualization, Methodology, Validation, Data curation, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

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

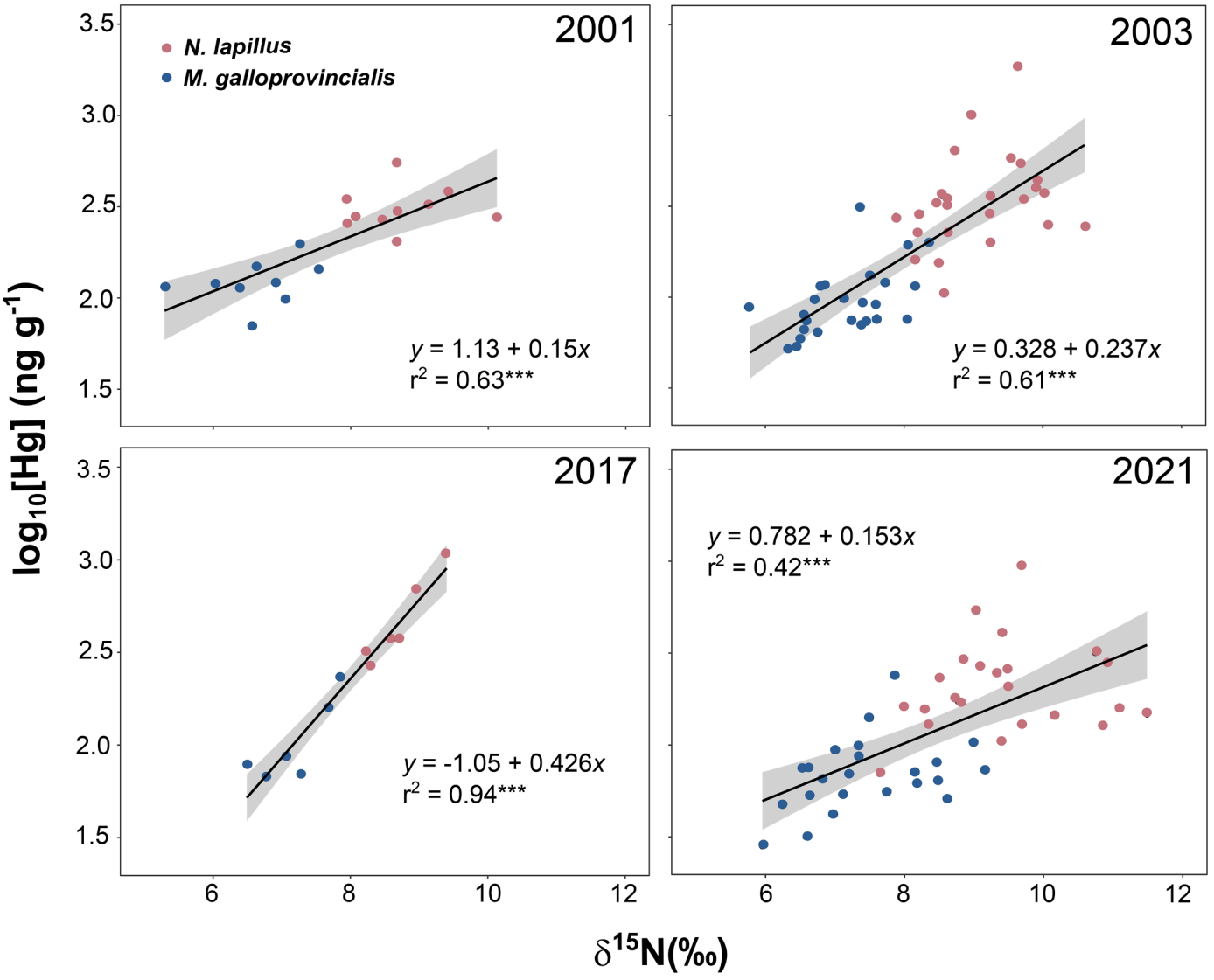


Fig. 3. Scatterplots depicting the relationship between the stable nitrogen isotope ratio ($\delta^{15}N$; ‰) and the $\log_{10}$ value of the concentration of Hg (ng g^{-1}) in *Nucella lapillus* and *Mytilus galloprovincialis* for the dataset including all common sampling sites within each survey (except the 1990 survey in which the relationship was not significant). The equation of the simple linear regression model and the coefficient of determination with its significance are also shown. ***: $p<0.001$.

Data availability

The data are available within the electronic supplementary materials.

Acknowledgements

Authors belong to the Grupo de Referencia Competitiva GRC GI-1252/GPC2020–23 (ED431C 2020/19) which is co-funded by Xunta de Galicia and ERDF (EU). Dr. M. Teresa Boquete is supported by the Maria Zambrano program from the Spanish Ministry of Science, Innovation and Universities.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2023.119793.

References

Bernhard, M., Andreae, M.O., 1984. Transport of Trace Metals in Marine Food Chains. In: Nriagu, J.O. (Ed.), Changing Metal Cycles and Human Health, Dahlem Workshop Reports, Life Sciences Research Report. Springer, Berlin, Heidelberg, pp. 143–167. https://doi.org/10.1007/978-3-642-69314-4_8.

Bode, A., Alvarez-Ossorio, M.T., Varela, M., 2006. Phytoplankton and macrophyte contributions to littoral food webs in the Galician upwelling estimated from stable isotopes. Mar. Ecol. Prog. Ser. 318, 89–102. https://doi.org/10.3354/meps318089.

Boening, D.W., 2000. Ecological effects, transport, and fate of mercury: a general review. Chemosphere 40, 1335–1351. https://doi.org/10.1016/S0045-6535(99)00283-0.

Broman, D., Rolff, C., Näf, C., Zebühr, Y., Fry, B., Hobbie, J., 1992. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in two food chains from the Northern Baltic. Environmental Toxicology and Chemistry 11, 331–345. https://doi.org/10.1002/etc.5620110307.

Cabana, G., Rasmussen, J.B., 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372, 255–257. https://doi.org/10.1038/372255a0.

Cabana, G., Tremblay, A., Kalff, J., Rasmussen, J.B., 1994. Pelagic Food Chain Structure in Ontario Lakes: a Determinant of Mercury Levels in Lake Trout (Salvelinus namaycush). Can. J. Fish. Aquat. Sci. 51, 381–389. https://doi.org/10.1139/f94-039.

Ceccherelli, V.U., Rossi, R., 1984. Settlement, growth and production of the mussel Mytilus galloprovincialis. Mar. Ecol. Prog. Ser. 16, 173–184.

Chouvelon, T., Cresson, P., Bouchoucha, M., Brach-Papa, C., Bustamante, P., Crochet, S., Marco-Miralles, F., Thomas, B., Knoery, J., 2018. Oligotrophy as a major driver of mercury bioaccumulation in medium-to high-trophic level consumers: a marine ecosystem-comparative study. Environmental Pollution 233, 844–854. https://doi.org/10.1016/j.envpol.2017.11.015.

Connell, D.W., 1989. Biomagnification by aquatic organisms — A proposal. Chemosphere 19, 1573–1584. https://doi.org/10.1016/0045-6535(89)90501-8.

Crothers, J.H., 1985. Dog-whelks: An Introduction to the Biology of Nucella Lapillus (L.). Field Studies Council.

Deudero, S., Cabanellas, M., Blanco, A., Tejada, S., 2009. Stable isotope fractionation in the digestive gland, muscle and gills tissues of the marine mussel Mytilus galloprovincialis. J Exp Mar Biol Ecol 368, 181–188.

D'Itri, F.M., 1991. Mercury contamination-what we have learned since Minamata. Environ Monit Assess 19, 165–182. https://doi.org/10.1007/BF00401309.

Fernandes Azevedo, B., Barros Furieri, L., Peçanha, F.M., Wiggers, G.A., Frizera Vassallo, P., Ronacher Simões, M., Fiorim, J., Rossi de Batista, P., Fioresi, M., Rossoni, L., Stefanon, I., Alonso, M.J., Salaices, M., Valentim Vassallo, D., 2012. Toxic effects of mercury on the cardiovascular and central nervous systems. J Biomed Biotechnol 2012, 949048. https://doi.org/10.1155/2012/949048.

Figueiras, F.G., Labarta, U., Fernández Reiriz, M.J., 2002. Coastal upwelling, primary production and mussel growth in the Rías Baixas of Galicia. Hydrobiologia 484, 121–131. https://doi.org/10.1023/A:1021309222459.

Fitzgerald, W., Lamborg, C., 2003. Treatise On Geochemistry, Chapter 4. Pergamon.

Foster, K.L., Stern, G.A., Pazerniuk, M.A., Hickie, B., Walkusz, W., Wang, F., Macdonald, R.W., 2012. Mercury Biomagnification in Marine Zooplankton Food Webs in Hudson Bay. Environ. Sci. Technol. 46, 12952–12959. https://doi.org/10.1021/es303434p.

Gartner, A., Lavery, P., Smit, A.J., 2002. Use of d15N signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. Mar. Ecol. Prog. Ser. 235, 63.

Goutte, A., Bustamante, P., Barbraud, C., Delord, K., Weimerskirch, H., Chastel, O., 2014. Demographic responses to mercury exposure in two closely related Antarctic top predators. Ecology 95, 1075–1086. https://doi.org/10.1890/13-1229.1.

Gray, J.S., 2002. Biomagnification in marine systems: the perspective of an ecologist. Mar. Pollut. Bull. 45, 46–52. https://doi.org/10.1016/S0025-326X(01)00323-X.

Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chemical Geology: isotope Geoscience section. Calibration of the Phanerozoic Time Scale 59, 87–102. https://doi.org/10.1016/0168-9622(86)90059-X.

Hein, M., Pedersen, M.F., Sand-Jensen, K., 1995. Size-dependent nitrogen uptake in micro- and macroalgae. Mar. Ecol. Prog. Ser. 118, 247–253.

Hong, Y.-S., Hull, P., Rifkin, E., Bouwer, E.J., 2013. Bioaccumulation and Biomagnification of Mercury and Selenium in the Sarasota Bay Ecosystem. Environmental Toxicology and Chemistry 32, 1143–1152. https://doi.org/10.1002/etc.2169.

Hughes, R.N., Drewett, D., 1985. A COMPARISON OF THE FORAGING BEHAVIOUR OF DOGWHELKS, NUCELLA LAPILLUS (L.), FEEDING ON BARNACLES OR MUSSELS ON THE SHORE. Journal of Molluscan Studies 51, 73–77. https://doi.org/10.1093/oxfordjournals.mollus.a065884.

Ipolyi, I., Massanisso, P., Sposato, S., Fodor, P., Morabito, R., 2004. Concentration levels of total and methylmercury in mussel samples collected along the coasts of Sardinia Island (Italy). Anal. Chim. Acta 505, 145–151. https://doi.org/10.1016/S0003-2670(03)00174-0. Papers presented at the 3rd Aegean Analytical Chemistry Days.

Isaacs, J.D., 1973. Potential trophic biomasses and trace-substance concentrations in unstructured marine food webs. Mar. Biol. 22, 97–104. https://doi.org/10.1007/BF00391775.

Jędruch, A., Bełdowska, M., Kwasigroch, U., 2019. Forms of mercury in the Baltic mussel (Mytilus trossulus): human and ecosystem health risk assessment. Environ. Res. 179, 108755 https://doi.org/10.1016/j.envres.2019.108755.

Kehrig, H.A., Seixas, T.G., Malm, O., Di Beneditto, A.P.M., Rezende, C.E., 2013. Mercury and selenium biomagnification in a Brazilian coastal food web using nitrogen stable isotope analysis: a case study in an area under the influence of the Paraiba do Sul River plume. Mar. Pollut. Bull. 75, 283–290. https://doi.org/10.1016/j.marpolbul.2013.06.046.

Kidd, K., Clayden, M., Jardine, T., 2011. Bioaccumulation and Biomagnification of Mercury through Food Webs, in: environmental Chemistry and Toxicology of Mercury. John Wiley & Sons, Ltd 453–499. https://doi.org/10.1002/9781118146644.ch14.

Kimbrough, K., Johnson, W., Lauenstein, G., Christensen, J., Apeti, D., 2008. Assessment of two Decades of Contaminant Monitoring in the Nation's Coastal Zone. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 74, 105.

Knopf, B., Fliedner, A., Radermacher, G., Rüdel, H., Paulus, M., Pirntke, U., Koschorreck, J., 2020. Seasonal variability in metal and metalloid burdens of mussels: using data from the German Environmental Specimen Bank to evaluate implications for long-term mussel monitoring programs. Environmental Sciences Europe 32, 7. https://doi.org/10.1186/s12302-020-0289-7.

Lavoie, R.A., Hebert, C.E., Rail, J.-F., Braune, B.M., Yumvihoze, E., Hill, L.G., Lean, D.R. S., 2010. Trophic structure and mercury distribution in a Gulf of St. Lawrence (Canada) food web using stable isotope analysis. Science of The Total Environment 408, 5529–5539. https://doi.org/10.1016/j.scitotenv.2010.07.053.

Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L.M., 2013. Biomagnification of Mercury in Aquatic Food Webs: a Worldwide Meta-Analysis. Environ. Sci. Technol. 47, 13385–13394. https://doi.org/10.1021/es403103t.

Lorenzo, M.N., Taboada, J.J., 2005. Influences of atmospheric variability on freshwater input in Galician Rías in winter. Journal of Atmospheric & Ocean Science 10, 377–387. https://doi.org/10.1080/17417530601127472.

McClelland, J.W., Valiela, I., 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnol. Oceanogr. 43, 577–585. https://doi.org/10.4319/lo.1998.43.4.0577.

McClelland, J.W., Valiela, I., Michener, R.H., 1997. Nitrogen-stable isotope signatures in estuarine food webs: a record of increasing urbanization in coastal watersheds. Limnol. Oceanogr. 42, 930–937. https://doi.org/10.4319/lo.1997.42.5.0930.

Melwani, A., Davis, J., Gregorio, D., Jin, J., Stephenson, M., Maruya, K., Crane, D., Lauenstein, G., 2013. Mussel Watch Monitoring in california: long-terM Trends in Coastal Contaminants and Recommendations For Future Monitoring. San Francisco Estuary Institute, Richmond, California. SFEI Contribution #685.

Meng, M., Shi, J., Liu, C., Zhu, N., Shao, J., He, B., Cai, Y., Jiang, G., 2015. Biomagnification of mercury in mollusks from coastal areas of the Chinese Bohai Sea. RSC Adv 5, 40036–40045. https://doi.org/10.1039/C5RA02919H.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of 15 N along food chains: further evidence and the relation between δ15N and animal age. Geochim. Cosmochim. Acta 48, 1135–1140. https://doi.org/10.1016/0016-7037(84)90204-7.

Morton, B., 2010. Predator–prey interactions between a population of Nucella lapillus (Gastropoda: muricidae) recovering from imposex and Mytilus galloprovincialis (Bivalvia: mytilidae) on the south-east coast of England. Journal of the Marine Biological Association of the United Kingdom 90, 671–681. https://doi.org/10.1017/S0025315409991275.

Prego, R., Cobelo-García, A., 2003. 20th century overview of heavy metals in the Galician rías (NW Iberian Peninsula). Environ. Pollut. 121, 425–452.

Qu, P., Pang, M., Wang, P., Ma, X., Zhang, Z., Wang, Z., Gong, Y., 2022. Bioaccumulation of mercury along continuous fauna trophic levels in the Yellow River Estuary and adjacent sea indicated by nitrogen stable isotopes. J. Hazard. Mater. 432, 128631 https://doi.org/10.1016/j.jhazmat.2022.128631.

R Core Team, 2022. R: A language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reinfelder, J.R., Fisher, N.S., Luoma, S.N., Nichols, J.W., Wang, W.-X., 1998. Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. Science of The Total Environment 219, 117–135. https://doi.org/10.1016/S0048-9697(98)00225-3.

RStudio Team, 2020. RStudio: Integrated Development Environment for R.

Selin, N.E., Jacob, D.J., Yantosca, R.M., Strode, S., Jaeglé, L., Sunderland, E.M., 2008. Global 3-D land-ocean-atmosphere model for mercury: present-day versus preindustrial cycles and anthropogenic enrichment factors for deposition. Global Biogeochem Cycles 22. https://doi.org/10.1029/2007GB003040.

Sukhotin, A.A., Strelkov, P.P., Maximovich, N.V., Hummel, H., 2007. Growth and longevity of Mytilus edulis (L.) from northeast Europe. Marine Biology Research 3, 155–167. https://doi.org/10.1080/17451000701364869.

Sunderland, E.M., Mason, R.P., 2007. Human impacts on open ocean mercury concentrations. Global Biogeochem Cycles 21. https://doi.org/10.1029/2006GB002876.

Tan, S.W., Meiller, J.C., Mahaffey, K.R., 2009. The endocrine effects of mercury in humans and wildlife. Crit. Rev. Toxicol. 39, 228–269. https://doi.org/10.1080/10408440802233259.

UN Environment Programme, 2019. Global Mercury Assessment 2018. UN Environment Programme, Chemicals and Health Branch, Geneva Switzerland. Geneva, Switzerland.

UNEP, 2019. Minamata Convention on Mercury (Text and Annexes) | Minamata Convention On Mercury [WWW Document]. URL. https://www.mercuryconvention.org/en/documents/minamata-convention-mercury-text-and-annexes. accessed 10.27.22.

UNEP, 2013. Minamata Convention On mercury: Text and Annexes.

Viana, I.G., Bode, A., 2013. Stable nitrogen isotopes in coastal macroalgae: geographic and anthropogenic variability. Science of The Total Environment 443, 887–895. https://doi.org/10.1016/j.scitotenv.2012.11.065.

Viana, I.G., Fernández, J.A., Aboal, J.R., Carballeira, A., 2011. Measurement of δ15N in macroalgae stored in an environmental specimen bank for regional scale monitoring of eutrophication in coastal areas. Ecol Indic 11, 888–895. https://doi.org/10.1016/j.ecolind.2010.12.004.

Zweig, G., 1973. Pesticide residues in food: with special reference to toxicology and environmental problems. Plant Food Hum Nutr 23, 77–112. https://doi.org/10.1007/BF01092244.