Marine Pollution Bulletin 69 (2013) 110–115

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

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

Mercury biomagnification in a contaminated estuary food web: Effects of age and trophic position using stable isotope analyses

J.P. Coelho [a,*], C.L. Mieiro [a], E. Pereira [a], A.C. Duarte [a], M.A. Pardal [b]

[a] CESAM (Centre for Environmental and Marine Studies), Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[b] CEF (Centre for Functional Ecology), Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal

ARTICLE INFO

Keywords:
Mercury
Organic mercury
Stable isotopes
Trophic level
Biomagnification
Trophic magnification factor (TMF)

ABSTRACT

The main aim of this study was to ascertain the biomagnification processes in a mercury-contaminated estuary, by clarifying the trophic web structure through stable isotope ratios. For this purpose, primary producers (seagrasses and macroalgae), invertebrates (detritivores and benthic predators) and fish were analysed for total and organic mercury and for stable carbon and nitrogen isotopic signatures. Trophic structure was accurately described by $\delta^{15}N$, while $\delta^{13}C$ reflected the carbon source for each species. An increase of mercury levels was observed with trophic level, particularly for organic mercury. Results confirm mercury biomagnification to occur in this estuarine food web, especially in the organic form, both in absolute concentrations and fraction of total mercury load. Age can be considered an important variable in mercury biomagnification studies, and data adjustments to account for the different exposure periods may be necessary for a correct assessment of trophic magnification rates and ecological risk.

1. Introduction

The first insight on the role of food webs with regard to the chemical fate of mercury in the environment was given by the poisoning outbreak in Minamata Bay, Japan, in the 1950s. This poisoning episode revealed complex processes on the ecosystem scale, such as the concept of biomagnification, the increase in mercury concentrations in successive trophic levels from primary producers to top predators or carnivorous consumers (Boudou and Ribeyre, 1997).

The mercury speciation in the environment (organic and inorganic forms) is essential for biomagnification processes. While both inorganic and methylmercury are accumulated in phytoplankton at equivalent rates, only the methylated form is transferred to zooplankton (Lawson and Mason, 1998). Mason et al. (1995) suggest this mechanism to be related with the greater relative concentration of methylmercury in algal cytoplasm when compared with inorganic mercury, which is mainly associated with cellular membranes, and is excreted rather than absorbed when ingested by consumers. Field data indicate that this difference in the efficiency of transfer between inorganic mercury and methylmercury also applies to other unicellular microorganisms and their predators (Watras and Bloom, 1992). Despite existing evidence on the subject, until recently no direct measure of mercury biomagnification or comparison between systems was possible. The coupling of mercury bioaccumulation data with trophic structure methodologies, such as stable isotope analyses, permitted to ascertain the existence of magnification processes, and also to numerically quantify it, an essential step for ecosystem intercomparisons.

The variation in the ratio of stable isotopes of carbon and nitrogen has been used in ecology as a useful tracer of energy flow, and to clarify the relative trophic position of species as a function of its time-integrated diet history (Deniro and Epstein, 1981; Minagawa and Wada, 1984). Moreover, nitrogen isotopic signatures ($^{15}N/^{14}N = \delta^{15}N$) were found effective at quantifying the trophic position of an organism because enrichment of the heavier isotope (^{15}N) occurs incrementally across trophic levels at a constant rate (2–4‰) (Post, 2002). In contrast, $\delta^{13}C$ ($^{13}C/^{12}C$) values usually display lower trophic fractionation (0.8–1‰), but are valuable biomarkers for identifying different sources of primary production (salt marsh plants, seagrasses, macroalgae) and therefore are effective at distinguishing between benthic and pelagic trophic linkages (France, 1995). Recently, some authors have taken advantage of stable isotope techniques to determine patterns of contaminant trophic transfer in food webs, and hence to estimate the biomagnification of organic and inorganic contaminants in both freshwater and marine ecosystems (Quinn et al., 2003; Hobson et al., 2004; Campbell et al., 2005; Dehn et al., 2006).

The regression slopes between log [Hg] and $\delta^{15}N$ have been used as a measure of biomagnification, and are usually found to be significant and positive when applied to food webs. These slopes are surprisingly consistent throughout different regions and ecosystems (usually ~0.2), but often do not consider organism size

* Corresponding author. Tel.: +351 234 401 527; fax: +351 234 370 084.
E-mail address: jpcoelho@ua.pt (J.P. Coelho).

0025-326X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marpolbul.2013.01.021

or age (e.g. Riget et al., 2007; Campbell et al., 2008; Lavoie et al., 2010). Given that these variables affect both [Hg] and $\delta^{15}N$ (Overman and Parrish, 2001), previous comparisons of log [Hg]–$\delta^{15}N$ slopes among systems may be hindered by variations in fish size and/or age (Swanson and Kidd, 2010). Whilst age/size standardization has been addressed in fish biomagnification studies (Swanson and Kidd, 2010), no information is available concerning a similar approach to invertebrate trophic links.

In the specific case of the Ria de Aveiro, a coastal lagoon in Western Portugal with an historical mercury contamination problem due to chlor-alkali industry discharges, considerable amount of information on mercury bioaccumulation processes is available in the literature for several trophic levels (Coelho et al., 2005, 2006, 2008; Mieiro et al., 2009). Considering the commercial harvest of several estuarine species for human consumption, an holistic approach to mercury biomagnification in the system is essential but still missing, partially as a result of the need to clarify the trophic web struture of the system.

Therefore, the main aims of this study were to: (a) characterise the trophic position of key species of a mercury contaminated coastal lagoon, through stable nitrogen and carbon isotopic ratios; (b) to ascertain and quantify the mercury biomagnification processes occurring in the system, and (c) to evaluate the effect of age standardization on the overall biomagnification pattern and rate.

2. Materials and methods

2.1. Sampling

The study was conducted in the Ria de Aveiro coastal lagoon, north western coast of Portugal, that for 50 years received continuous mercury discharges from a chlor-alkali industry. Such discharges resulted in the storage of ca. 25×10^3 kg of this contaminant in the sediments of the system (Pereira et al., 1998). Even though discharges ceased more than 10 years ago, mercury contamination problems still occur as a result of internal loading from the sediments.

Sampling was conducted in the most contaminated area of the system, the Laranjo Basin, in low tide in late spring/early summer, when macroalgal presence is common. Primary producers (macroalgae *Ulva* spp. and *Fucus vesiculosus*, seagrass *Zostera noltii*) were randomly collected (three replicates consisting of composite samples) by hand from different patches. Sediment dweller (*Hediste diversicolor*, a polychaete, and *Scrobicularia plana*, a bivalve) samples were collected from the shoreline, in the same site, and consisted of five individual replicates for total mercury and five composite samples for organic mercury analyses. Samples of *H. diversicolor* were adult individuals (18 months average age), while sampling of *S. plana* focused on 3-year-old adults (about 3 cm shell width), the smallest commercially available individuals. Bias related to reproduction was avoided by sampling outside the recruitment periods of both species. Individuals were left to empty gut content in aerated seawater (24 h), freeze-dried and analysed whole (bivalve shells removed) for mercury and stable isotope ratios.

Samples of the green crab *Carcinus maenas* ($n = 6$) of 3.5–4.5 cm carapace width, corresponding to 2+ year-old individuals, were captured in low water conditions, using baited circular drop nets. In the laboratory, specimens were measured (cephalothorax width), weighed (total fresh weight), and foreleg muscle tissue samples collected. Immature (approximately 1 year old, total length 11.6 ± 1.25 cm) golden grey mullet (*Liza aurata*) and juvenile (of the same age, total length 11.8 ± 1.4 cm) European sea bass (*Dicentrarchus labrax*) were caught respectively using a beach seine net and by fishing rod ($n = 5$ for each species). Fish were transported to the laboratory, measured, weighed, dissected and muscle tissue samples taken.

All samples were freeze-dried at −50 °C and 0.06 bars, and homogenised for analysis.

2.2. Trophic position and trophic magnification factor

Stable isotope analyses were performed on freeze-dried samples using a stable isotope ratio mass spectrometer Flash EA for IRMS Delta V advantage Thermo. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) ratios were expressed as the relative difference (‰) between the sample and conventional standards (Pee Dee Belemnite carbonate and N_2 in air, respectively) according to:

$$\delta X = [R_{sample}/R_{standard} - 1] \times 1000 \ (‰)$$

where $X = \delta^{13}C$ or $\delta^{15}N$, and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Relative percent difference between duplicate subsamples was always below 8%.

Trophic position was estimated through $\delta^{15}N$ ratios, as follows:

$$TL = [(\delta^{15}N_{species} - \delta^{15}N_{base})/\Delta\ \delta^{15}N] + TL_{base}$$

where $\delta^{15}N_{species}$ is the $\delta^{15}N$ signature of the species in question, $\delta^{15}N_{base}$ is the $\delta^{15}N$ signature of a representative baseline for the system and TL_{base} is the trophic level of that baseline. $\Delta\ \delta^{15}N$ represents the trophic fractionation of $\delta^{15}N$, estimated at 3.4‰ (Post, 2002). In this work, the average primary producer $\delta^{15}N$ of long lived species *F. vesiculosus* and *Z. noltii* was assumed as representative baseline, and thus $TL_{base} = 1$.

The trophic magnification factor (TMF) of the estuarine food web was calculated for both total and organic mercury, through the equation of the regression between $\delta^{15}N$ and log-transformed mercury concentrations:

$$Log_{10}\ [Hg] = a + (b \times \delta^{15}N)$$

From where the slope b was used to calculate the trophic magnification factor using the equation $TMF = 10^b$. Trophic magnification studies and TMF calculations were initially developed for organic pollutants (Fisk et al., 2001; Kelly et al., 2007; Houde et al., 2008), which usually follow an exponential accumulation curve in food webs, and hence data logaritmization was performed to provide a linear regression with trophic level.

2.3. Mercury quantifications

Biological samples were analysed for total mercury content by thermal decomposition atomic absorption spectrometry with gold amalgamation, using a LECO AMA-254 (Advanced Mercury Analyser), with no pre-treatment of samples. Organic mercury determinations in biological tissues were determined after sample digestion with a mixture of 18% KBr in 5% H_2SO_4, followed by extraction of organic mercury into toluene (for further details see (Válega et al., 2006)), and also measured by thermal decomposition atomic absorption spectrometry after gold amalgamation.

Accuracy and precision of both total and organic mercury determinations were assessed by analysing aliquots of certified reference materials (CRMs) with matrix similar to real samples. The CRMs used for primary producer samples were BCR-60 (*Lagarosiphon major*, recovery: 90.7% ± 3% at 0.05 significance level for total mercury) and IAEA-140TM (*Fucus* sp. homogenate, recovery: 85.0% ± 9% at 0.05 significance level for organic mercury), for sediment dwellers NIST-2976 (mussel homogenate, efficiency: 98.0% ± 8% at 0.05 significance level for total and 92.6% ± 4% at 0.05 significance level for organic mercury), while for *C. maenas* and fish samples TORT-2 was used (lobster hepatopancreas, efficiency: 99.8% ± 7% at 0.05 significance level for total and