



Research paper

The isopod *Eurydice spinigera* and the chaetognath *Flaccisagitta enflata*: How habitat affects bioaccumulation of metals in predaceous marine invertebrates



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ABSTRACT

We analyzed the concentrations of 20 trace elements in *Eurydice spinigera* (Isopoda) and *Flaccisagitta enflata* (Chaetognatha), which represent a hyperbenthic and a zooplanktonic marine species respectively, sharing the same predaceous feeding strategy, from a highly productive coastal region (Ligurian Sea, Northwestern Mediterranean). The same elements were also quantified in marine sediments, where geological anomalies were found for arsenic, chromium and nickel. Metal transfer seems to be deeply influenced by different habitats. The carnivorous isopod *E. spinigera*, which spends most of its lifetime on the seabed, was found to have the highest concentrations of most trace elements, such as Al, As, Cd, Ce, Cr, Fe, La, Mn, Ni, Pb, Se, V and Zn, and consequently have the highest bioaccumulation factors (BAFs). Moreover, the estimation of biota sediment accumulation factors (BSAFs) revealed that cadmium, copper and zinc concentrations were higher in the isopod than in the sediment.

Conversely, in the carnivorous chaetognath *F. enflata*, which is not a benthic species, the highest levels of copper and tin were found. Moreover, arsenic speciation analysis confirmed the presence of inorganic As (III + V) in *E. spinigera*. In the context of utilizing a marine organism as a bio-indicator of metal transfer, it is crucial to consider both feeding behavior and feeding habitat.

1. Introduction

In the last decade, many investigations have focused on understanding metal bioaccumulation in marine biota. Aquatic invertebrates are able to accumulate inorganic contaminants such as trace elements from seawater and food, and then participate in the transfer of metals to the upper levels of the marine food web (Fisher and Reinfelder, 1995; Kahle and Zauke, 2003). As the toxicity of inorganic contaminants is strictly related to the bioavailable fraction of trace elements in seawater, biomonitoring plans usually aim to detect the levels of metals incorporated by marine biota (Rainbow, 1993). In fact, the ability of marine invertebrates to bioaccumulate trace elements at concentrations higher than those detected in seawater can be utilized for identifying aquatic environmental hazards (McGeer et al., 2003). The concentration of the contaminant in the aquatic organism results from all possible routes of exposure, such as dietary absorption and transport across the

respiratory surface (Gobas and Morrison, 2000). The BAF (Bioaccumulation Factor) is a model for bioaccumulation that considers the concentration of a contaminant in an exposure medium (seawater) and in biota, and is expressed as the ratio of the two concentrations (McGeer et al., 2003). Then BAF is greatly influenced by the diet of the marine organism. The biota to sediment accumulation factor (BSAF) is a parameter describing bioaccumulation of sediment-associated metals into tissues of ecological receptors, and it is expressed as the ratio of metal concentration in animal tissues and metal in sediment sample (Ju et al., 2016; Li et al., 2016; Ahmed et al., 2017.)

The order Isopoda is a ubiquitous monophyletic taxon that includes around 10,131 species (Boyko et al., 2008). The most significant feature of this group is the diversification into a number of different ecological roles or modes of life (Argano and Campanaro, 2010), and isopod representatives occur in the marine environment from the littoral to abyssal zones (Naylor, 1972).

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Isopods belonging to the genus *Eurydice*, which are highly predaceous carnivores, specifically including copepods and cladocerans (Macquart-Moulin, 1992), display hyperbenthic features (Mees and Jones, 1997). In fact, they have pelagic phases during the night (Macquart-Moulin and Patrì, 1996) and they emerge from the sediment at dusk, i.e. an endogenous light-controlled vertical migration occurs (Macquart-Moulin, 1973; Macquart-Moulin, 1976), while at dawn the animals return to the seabed to hide in the sediment (Macquart-Moulin, 1972; Macquart-Moulin, 1985). The migratory behavior of several *Eurydice* spp., including *E. spinigera*, ensures the transfer of trace elements between the bottom and the surface of seawater.

The relatively small isolated phylum Chaetognatha, also known as arrow worms, comprises 209 species that have been found worldwide. Between them, 20 (16 planktonic and 4 benthic) have been reported to be present in the Mediterranean Sea (Ghirardelli, 2010). Chaetognaths are predaceous and are found in all kinds of marine habitats (Bone et al., 1991), from superficial waters to great depths (Pierrot-Bults and Nair, 1991); they are the second most abundant zoo planktonic phylum after copepods (Feigenbaum, 1991; Shannon and Pillar, 1986; Gibbons, 1992). Ecologically, *Flaccisagitta enflata* predominates in the tropical-subtropical epipelagic waters (Pierrot-Bults and Nair 1991; Duró and Saiz, 2000) and it is the most numerous chaetognath neritic species of the Mediterranean Sea (Batistic, 2003; Ghirardelli and Gamulin, 2004). Recently, bioaccumulation of trace elements in chaetognaths belonging to the family Sagittidae was investigated in the coastal regions of India (Bhattacharya et al., 2014), and in the White Sea (Budko et al., 2015).

In a previous study, we analyzed the potentiality of marine zooplankton as bioindicators of trace elements in coastal ecosystems (Battuello et al., 2016). We suggested that zooplankton, due to their ability to concentrate trace elements from the surrounding seawater, could represent a suitable bioindicator of metal transfer in the marine food chain. We then focused on the influence of the different feeding modes (herbivorous, omnivorous and carnivorous) in metal bioaccumulation in calanoid copepods (Battuello et al., 2017), and we found that there was a reduced metal accumulation in carnivores compared to herbivores. In fact, the herbivorous species showed the highest concentrations and BAFs for most of the analyzed metals, in particular for the nonessential elements aluminum and cadmium, and for the essential trace elements copper, iron, manganese and zinc. Now, our study focused on the influence of different habitats in metal bioaccumulation in two marine carnivorous species, *Eurydice spinigera* and *Flaccisagitta enflata*. In fact, both these species have the potential to be sentinel species of a marine environment, and can accumulate and concentrate metals to levels above those in the surrounding waters; in addition, they share the same feeding strategies as they are both carnivores, and are both at the top of the zooplankton food web (Rossi and Jamet, 2008). Nevertheless, they have different habitat requirements, and we therefore postulated that the habitat could have a great influence in the bioaccumulation of metals through the marine food chain.

The study area is a highly productive coastal Italian region characterized by commercial and maritime traffic and several industrial plants. Due to its particular geomorphology, the area has been the target of important anthropogenic impacts: the Arno River drains a wide inland area, transporting trace elements at high concentrations towards the sea (Cortecchi et al., 2009) and the three coastal towns of Pisa, Leghorn and Cecina discharge partially treated effluent into the rivers (Renzi et al., 2010). Moreover, a positive arsenic abnormality is widespread in all the Tuscan Coastal Platform. Arsenic origin is due to multiple and diffuses sources of both natural and anthropogenic origins; the maximum levels were recorded in the areas of Leghorn and north of Piombino (ARPAT, 2015; ARPAT, 2017). In this area, the Regional agency for environmental protection of Tuscany region (ARPAT) detected a concentration of 28 mg kg^{-1} of As in marine sediments, higher than the limit of good environmental quality set by the Italian Legislative Decree 152/06 for Coastal Monitoring. This finding was in line

with what observed at most of the monitoring points of the Tuscany coast.

Then, with the present study we aimed to analyze, for the first time, the concentrations of 20 trace elements in two marine species, the hyperbenthic *E. spinigera* (Isopoda) and zooplanktonic *F. enflata* (Chaetognata), to evaluate the relevance of these two predaceous species, living in different compartments of a marine coastal environment, in the bioaccumulation of metals.

Moreover, the sediment quality of the sampling location was evaluated following the US Environmental Protection Agency (USEPA) sediment quality guidelines (SQGs) and the standard of environmental quality (SQA) set by the Italian Legislative Decree 56/2009 for Coastal Monitoring. Since the presence of arsenic in the sediments of the studied area is well known and that the isopod *E. spinigera* live in close association with sediments, arsenic speciation analysis was also performed to assess the arsenical species present in this benthic marine invertebrate.

2. Material and methods

2.1. Study area and sampling site

The sampling site was located off the Italian coast, in the transition zone between the Northern Tyrrhenian Sea and the Southern Ligurian Sea (Fig. 1). The study area currently has one of the highest levels of shipping in the Mediterranean basin, and is a recipient of pollutants coming from the highly developed coastline of Italy (Barrier et al., 2016; Renzi et al., 2010). The sampling station ($43^{\circ}28'10'' \text{ N}$, $10^{\circ}01'55'' \text{ E}$) was located at 12.5 km off the Tuscan coast, above the continental shelf. This site corresponds to one of the stations previously considered in our investigation about marine zooplankton in the Ligurian Sea (Battuello et al., 2016) and is the same station used for our previous study regarding Calanoida copepods (Battuello et al., 2017). The Ligurian Sea is connected to the Tyrrhenian Sea via the Corsica Channel; a permanent basin-wide cyclonic circulation characterizes these water masses (Bozzano et al., 2014).

2.2. Sampling

Zooplankton samples were collected during September 2015 (summer). Surface zooplankton samples were caught with a WP-2 standard net, with a mesh size of $300 \mu\text{m}$ and a diameter of 60 cm as previously described (Battuello et al., 2016). The horizontal sampling time was approximately 15 min at a vessel cruising speed of 2 knots, while during the vertical sampling the net was hauled at 0.7 m s^{-1} . Net hauls were consistently carried out during the night to allow surface sampling of isopods and chaetognaths, involved in nictemeral migrations.

A Folsom Plankton Splitter was utilized to divide samples from each net into two aliquots. One aliquot, after fixing in 4% neutralized formaldehyde was kept in the dark, and then utilized for analyzing the zooplankton composition and abundance (ind. m^{-3}), paying particular attention to identifying and quantifying the dominant isopod and chaetognath species (Boltovskoy, 1981). In the laboratory, a qualitative-quantitative analysis of the two species was performed on subsamples obtained using the Folsom Splitter, ranging from 1/10 to 1/25, depending on the total sample abundance (Brugnano et al., 2010). The second aliquot was washed and transported, under refrigerated conditions, to the laboratory for metal analysis (Fang et al., 2014; Fernández de Puelles et al., 2014). Samples were sorted, and the two selected species were analyzed for determining trace metal concentrations. Regarding the chaetognaths, only adult specimens with empty guts were utilized for analysis.

Depending on the size and abundance of the different target species, about 300–600 specimens of the two target species were selected separately for each of the four samples.

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