Ecological Indicators 63 (2016) 209–218

Contents lists available at ScienceDirect

Ecological Indicators

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

Fatty acids' profiles as indicators of stress induced by of a common herbicide on two marine bivalves species: *Cerastoderma edule* (Linnaeus, 1758) and *Scrobicularia plana* (da Costa, 1778)

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ARTICLE INFO

Article history:
Received 26 September 2015
Received in revised form 1 December 2015
Accepted 3 December 2015

Keywords:
Marine bivalves
Fatty acids
Herbicide effects
Food quality

ABSTRACT

In Europe, mainly the Mediterranean region, intensive use of fertilizers and pesticides has been recorded over the past 30 years, exceeding, in some cases, the limits of contamination authorized by the European Union. The intensive use of pollutants in fields near ecological coastal wetlands has led to implementation of pesticide monitoring programs to recover aquatic systems such as the Mondego estuary (Figueira da Foz, Portugal). According to information from the agricultural cooperatives of the Mondego valley, Primextra® Gold TZ is the most-used herbicide in corn crop fields. Biomarkers, such fatty acids (FAs), proved to be new and potentially powerful tools to detect, illustrate, and evaluate exposure to and the effects of contamination hazards. They play important roles in establishing neural levels in organisms' biochemical and physiological responses and are considered good bio-indicators of stress and potential indicators of ecosystem health. Bivalves are currently used in ecotoxicological bioassays because of their ecological importance, wide geographic distribution, ease of handling in the laboratory and in the field, and their ability to filter and ingest large volumes of water and sediment particles. Thus, the main goal of this work was to determine the toxic and biochemical (namely fatty acid profiles) responses of two size classes (small and big) of the two marine bivalve species *Cerastoderma edule* and *Scrobicularia plana* to the herbicide Primextra® Gold. Furthermore, we aimed to compare the fatty acid contents, and thus the nutritive values, of both species and size classes collected in the field with those under laboratory conditions. Results show *S. plana* is more sensitive to the herbicide than *C. edule*. In general, among the larger-sized specimens in the field, *S. plana* is more nutritive than *C. edule*, but among the smaller-sized specimens, the opposite tendency is seen, where *C. edule* presents a greater abundance of FAs.

1. Introduction

In Europe, mainly in the Mediterranean region, there is an overexploitation of farmland, and when combined with fertilizer and pesticide overuse, has adverse effects on surrounding aquatic systems. Because many estuaries are surrounded by farmland, residential, and industrial areas, they are subject to various anthropogenic pressures and behaviors that cause ecological stresses, affecting not only the water quality, but also the biological communities of these ecosystems (McCarthy et al., 2007; Cardoso et al., 2008; Gonçalves et al., 2010a, 2010b; Sameling et al., 2013; Verdelhos et al., 2005, 2014).

As in other estuaries, Mondego estuary, located near Figueira da Foz city, Portugal, is under strong anthropogenic pressures. The main stressors are related to port, beach, and industrial activities as well as the exploitation of marine resources. The eutrophication process is caused primarily by discharges of pollutants (e.g., fertilizers and inorganic compounds) from agricultural fields, particularly those used to grow rice and corn, where production is more intensive (Cardoso et al., 2008; Duarte et al., 2008). Pesticides used in agricultural practices have been found in surface

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http://dx.doi.org/10.1016/j.ecolind.2015.12.006
1470-160X/

and ground waters; this contamination may have ecotoxicological effects on aquatic flora and fauna with consequences on human health (Carrasco et al., 2003; Macedo et al., 2005). According to information from the agricultural cooperatives of the Mondego valley, Primextra® Gold TZ is the most-used herbicide in corn fields. It is a selective and systemic herbicide that controls weeds, which are primarily grasses and *Cyperus esculentus*, with residual action and the major annual weeds found in corn crops. The herbicide is absorbed by the leaf and root, preventing growth; plants die before emerging or shortly after emergence (Syngenta®, 2014). Indeed, herbicides and fertilizers used in agricultural practices affect biologics at several organizational levels, from molecular to ecosystem and may be affective from early germination onward, leading to biochemical and physiological alterations and differences in enzymatic and non-enzymatic antioxidants, resulting in residues in plants, legumes, fruit, and non-target organisms (Parween et al., 2014). Primextra® Gold TZ, produced by Syngenta AG, is composed of two active ingredients (a.i.), terbuthylazine and S-metolachlor, which are also used by Syngenta AG in other commercial formulations used worldwide. Terbuthylazine is a selective systemic herbicide that acts as a photosynthesis inhibitor and is used as a large spectrum herbicide in maize, sorghum, vines, citrus, coffee, potatoes, vegetables, and forestry (Roberts et al., 1998). Nowadays, it is the second-most frequently used *s*-triazine (Velisek et al., 2014) and is adsorbed through roots and leaves and distributed throughout the plant, enabling it to be used in both pre- and post-emergent treatment. S-metolachlor, the major component of the herbicide, is potentially dangerous to environmental and aquatic systems. Metalochlor, and consequently Primextra® Gold TZ, was developed to control grass weeds following pre-emergent application (Karam et al., 2003). Its mode of action consists of inhibiting several biosynthesis processes, namely of lipids, fatty acids (FAs), leaf wax, terpenes, flavonoids, and protein synthesis, in addition to inhibition of cell division and interference with hormonal regulation (Weed, 1994; Liebl, 1995). Metolachlor is classified as an inhibitor of very long chain fatty acid formation. It interferes with normal cell development and inhibits both cell division and cell enlargement (Liu and Xiong, 2009). The mode of action of this xenobiotic suggests that it affects the lipid (FA) profile of aquatic species.

Fatty acids are among the main constituents of the cell membrane, occurring in great concentrations in the neural system. They play key roles at the biological level, and are among the most important molecules transferred across the plant-animal interface in aquatic food webs. These compounds are involved in several biochemical pathways and are an important source of energy and constituent of cell membranes, acting on membrane permeability and influencing the traffic of cell compounds and the activity of membrane proteins and signals (Ibarguren et al., 2014; Liu et al., 2015). Therefore, as with other biomarkers, FAs are argued to be good bio-indicators of stress and potentially of ecosystem health (Martinez-Haro et al., 2015). Polyunsaturated fatty acids (PUFAs) are a family of lipids that contain subgroups and are identified by the position of the last double bond in their structures. They include many important compounds, such as essential fatty acids (EFAs). Although the terms *PUFA* and *EFA* are not synonymous, they are often used interchangeably because many biological functions of EFAs are exerted by EFA-derived PUFAs. Bret and Müller-Navarra (1997) pointed out that PUFAs are almost exclusively synthesized by plants, and that animals are able to convert PUFAs by elongation or desaturation. Only a few animals can synthesize this type of FA. The PUFAs play important roles in the organism, regulating cell membrane properties, serving as precursors to important hormones, and being essential to the organism (Neves et al., 2015). Highly unsaturated fatty acids (HUFAs), such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), are linked to growth, reproductive success, and neural development, playing key roles in the health and function of all animals, including plankton invertebrates, benthos, fish, and humans, at all levels. The HUFAs are essential metabolites that cannot be synthesized de novo in sufficient amounts to be taken up via food sources (Ladhar et al., 2014). Indeed, some groups of organisms fed high amounts of HUFAs present greater growth rates, showing the importance of FAs as ecophysiological indicators. Furthermore, lipid components are very sensitive to stressors and environmental changes. The HUFAs, as determined by EFAs such as EPA and DHA, are key nutritional constituents of the bivalves' diets and establish the nutritional value of algae consumed by bivalves (Hendriks et al., 2003). Bivalve species consume nutrients from many material particles, such as phytoplankton, resuspended benthic microalgae, and detritus from both bacterial and myco-heterotrophic sources; however, they are considered herbivorous, and phytoplankton is their primary food source (Pernet et al., 2012). In this study, we used two marine bivalve species, *Cerastoderma edule* and *Scrobicularia plana*, from the Mondego estuary. *C. edule* is a bivalve mollusc from the family *Cardiidae*, and is one of the most abundant shellfish in tidal flats, bays, and estuaries in Northern and Western Europe. It is widely distributed: it is found from North Africa to Northern Norway and Murmansk in the Arctic and on the east coast of the Atlantic, but not in the Mediterranean and Baltic Seas (Freitas et al., 2014). It is an infaunal suspension feeder living in intertidal shallow areas, burrowing just below the sediment surface and playing a key role as a link between primary producers and consumers (Verdelhos et al., 2015). Given its large filtration capacity and ability to accumulate a large amount of environmental pollutants, *C. edule* is widely used as an environmental bio-indicator (Paul-Pont et al., 2010a; Nilin et al., 2012; Cardoso et al., 2013; Freitas et al., 2014). *S. plana*, also a bivalve mollusc, is from the family *Semelidae* and is typically found in brackish waters. It is a dominant species in intertidal soft-substrate estuaries, lagoons, and bays along NE Atlantic seaboard communities from Norway to the Mediterranean and West African regions. It is a deposit filter feeder, inhabiting intertidal and subtidal areas and burrowing on mud to muddy sand sediments at depths up to 25 cm (Verdelhos et al., 2015). Like other bivalves, *S. plana* has a large capacity to filter pollutants that accumulate in the digestive gland (Paul-Pont et al., 2010a, 2010b; Freitas et al., 2014). In general, bivalve species play key roles in the trophic web because they act as links between primary producers and consumers and are the major prey of crustaceans, fish, and wading birds. They have a capacity to filter organic material, clean the freshwater column, and influence the available food and the energy flow in the entire community. Moreover, they can accumulate pollutants and parasite species (Verdelhos et al., 2014). Bivalves are considered standard species in ecotoxicological studies because of their sessile lifestyle, easy handling and collection, and sensitivity to pollutants. Physiological and biochemical responses are used as early indicators of potential ecosystem damage caused by pollutants such as metals and organic contaminants (Nilin et al., 2012). Furthermore, these species are of great economic value because they are significant as a food source (Paul-Pont et al., 2010a). Thus, ecotoxicological studies using bivalve species are extremely important and are crucial to determining their nutritive value as well as their responses to anthropogenic stressors. The main aims of this study were: (1) to determine the ecotoxicological effect of the herbicide Primextra® Gold TZ on two size classes (big [B] and small [S]) of both bivalve species (*C. edule* and *S. plana*), (2) to determine the biochemical response (namely FA profiles) of both size classes of both bivalve species when exposed to the commercial compound, and (3) to compare the nutritive value of both size classes of both bivalve species in the field and under exposure to the contaminant.