Marine Pollution Bulletin 79 (2014) 211–219

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Effects of sewage discharges on lipid and fatty acid composition of the Patagonian bivalve *Diplodon chilensis*

Iara Rocchetta [a,c,*], María Y. Pasquevich [b], Horacio Heras [b], María del Carmen Ríos de Molina [a,1], Carlos M. Luquet [c,1]

[a] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, INQUIBICEN-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Pab. II, Ciudad Universitaria, 1428 Buenos Aires, Argentina
[b] Instituto de Investigaciones Bioquímicas de la Plata (INIBIOLP), Facultad de Ciencias Médicas, Universidad Nacional de La Plata – CONICET CCT La Plata, Av. 60 y 120, 1900 La Plata, Argentina
[c] Laboratorio de Ecotoxicología Acuática, INIBIOMA (CONICET-UNCo). CEAN, ruta prov. 61 km 3. 8371, Junín de los Andes, Neuquén, Argentina

ARTICLE INFO

Keywords:
Freshwater bivalve
Pollution
Domestic effluents
Oxidative stress
Lipofuscins
Fatty acid markers

ABSTRACT

Lipid and fatty acid (FA) composition and selected oxidative stress parameters of freshwater clams (*Dipolodon chilensis*), from a sewage-polluted (SMA) and a clean site, were compared. Trophic markers FA were analyzed in clams and sediment. Saturated FA (SAFA), and bacteria and sewage markers were abundant in SMA sediments, while diatom markers were 50% lower. Proportions of SAFA, branched FA, 20:5$n-3$ (EPA) and 22:6$n-3$ (DHA) were higher in SMA clams. Chronic exposure of *D. chilensis* to increasing eutrophication affected its lipid and FA composition. The increase in EPA and DHA proportions could be an adaptive response, which increases stress resistance but could also lead to higher susceptibility to lipid peroxidation TBARS, lipofuscins (20-fold) and GSH concentrations were higher in SMA clams. FA markers indicated terrestrial plant detritus and bacteria are important items in *D. chilensis* diet. Anthropogenic input in their food could be traced using specific FA as trophic markers.

1. Introduction

Lipid composition can influence growth, reproduction, defense/detoxification systems and physiology of organisms under environmental stress (Bell et al., 1995; Leveille et al., 1997; Parrish et al., 2000). It is known that lipid and fatty acid composition can be altered in the aquatic medium by environmental pollution caused by anthropogenic activities (Cheung et al., 2010; Kainz et al., 2008; Leveille et al., 1997; Penha-Lopes et al., 2009; Perrat et al., 2013; Rocchetta et al., 2006). Trace metals or metal ions, such as chromium and copper (Rocchetta et al., 2012; Sabatini et al., 2009), pesticides, like glyphosate (Romero et al., 2011) and sewage discharges, containing high levels of fecal coliform bacteria (Sabatini et al., 2011) can damage lipids by oxidative processes. Not all fatty acids (FA) react equally and the susceptibility of individual FA to peroxidation increases exponentially with the number of double bonds on the carbon chain (peroxidation index) (Holman, 1954). In addition, comparative studies have shown that phospholipid composition can be readily modified by the food quality or pollution (Ayala et al., 2007; Leveille et al., 1997).

It is worth noticing that essential FA (not biosynthesized effectively by the animal) are highly conserved in aquatic food chains (Arts et al., 2001). FA have therefore been utilized to identify trophic interactions between the dominant taxa in food webs, mainly due to their biological specificity and their characteristic of being transferred from primary producers to higher trophic levels (Leveille et al., 1997; Parrish et al., 2000). These compounds have recently been used as markers to follow the transfer of organic matter within food webs (Abdulkadir and Tsuchiya, 2008; Meziane and Tsuchiya, 2002). Although FA have been extensively used as trophic markers in marine and estuarine systems (Carreira et al., 2011; Costa et al., 2011; Koussoroplis et al., 2011; Napolitano et al., 1995; Napolitano et al., 1997; Parrish et al., 2000), not much attention has been paid to their use in freshwater studies (Desvilettes et al., 1994; Gomes et al., 2010; Leveille et al., 1997; Perga et al., 2009; Sushchik et al., 2003).

The freshwater clam *Diplodon chilensis* (Bivalvia: Hyriidae, Gray, 1828) plays an important role in maintaining the aquatic ecosystems equilibrium in Andean water bodies of Southern Argentina and Chile, by its ability to reduce chlorophyll and nutrient loads, due to its remarkable filter-feeding capacity (Lara et al., 2002; Parada et al., 2008; Sabatini et al., 2011; Valdovinos and Pedreros, 2007).

* Corresponding author. Address: Functional Ecology Department, Alfred-Wegener-Institut für Polar-und Meeresforschung, Am Handelshafen 12, 27570 Bremerhaven, Germany. Tel.: +49 (0)471 4831 1430; fax: +49 (0)471 4831 1149.
E-mail addresses: lara.Rocchetta@awi.de, rocchetta@bg.fcen.uba.ar (I. Rocchetta).
[1] These authors contributed equally to this work.

0025-326X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marpolbul.2013.12.011

The lipid class composition of *D. chilensis* shows seasonal variations with no changes in the FA pattern within each class. However, its FA composition is affected by food quality (Pollero and Brenner, 1981).

A population of *D. chilensis* living in the shore of Lacar lake, at San Martín de los Andes Bay (SMA) in North Patagonia (40°10′S, 71°20′W), are exposed to treated and untreated domestic effluents with an important load of fecal bacteria. Adults of this population show an enhanced antioxidant defence system without signs of neither protein oxidative damage nor changes in the total lipid content. However, they present significant oxidative damage to lipids, suggesting that oxidative stress, caused by the increased eutrophication process of the place, is not fully compensated (Sabatini et al., 2011). Under this stressing conditions, lipid composition and the unsaturated/saturated FA ratio may be altered, but this has not been tested yet. Besides, changes in specific FA could contribute to reduce lipid peroxidation (Pamplona, 2008).

We propose to evaluate the effect of sewage pollution on the lipid classes and FA composition in the freshwater clam *D. chilensis* and to elucidate if the anthropogenic input in their food could be traced by using specific FA as trophic markers. Selected morphometric and oxidative stress parameters are also analyzed in order to discuss the results within a metabolic frame.

2. Materials and methods

2.1. Sample collection

Individuals of *D. chilensis* (length 55.4–62.0 mm) and sediment (triplicates) were collected at two different sites in the Lacar lake shore during winter. Control samples were obtained from the area of Yuco (40°10′S, 71°31′30″W), about 20 km from the city of San Martín de los Andes, where no pollution has been detected (Sabatini et al., 2011). The other sampling site, also described in our previous paper, was next to the city of San Martín de los Andes, at approximately 50 m from the discharge of the sewage treatment plant (SMA) (40°10°S, 71°20°60°W). The sampling sites have been characterized before, showing an increase in the total nitrogen and phosphorous content, higher total suspended solid and organic matter content and higher total and fecal coliform bacteria for SMA area, compared to Yuco (control) (Sabatini et al., 2011). Clams and sediments were randomly sampled by a diver from the two banks, located between 5 and 8 m of depth, as was previously describe (Sabatini et al., 2011).

2.2. Morphometric parameters and sample preparation

Twenty animals from each site were processed. Morphometric parameters were recorded (shell length, width, height and mass, and total and soft tissue mass). Mussels were anesthetized by placing them on ice before sacrifice. The ratios between total soft tissue mass and shell length (STM/SL), STM and shell height (SH), and STM and shell mass (SM) were calculated. Total digestive mass (digestive gland plus digestive tubes) and part of the gonad, which in this species cannot be separated from the digestive tube, was weighed and homogenized in 134 mM KCl solution (ratio 1:5 g tissue mass/mL) containing 0.5 mM PMSF (phenylmethylsulfonyl fluoride) and 0.2 mM benzamidine (protease inhibitors) to study lipid and FA composition, and oxidative stress and antioxidant/detoxifying defense parameters. In order to avoid the effects of reproductive condition, samples were taken during winter (no reproductive activity) and only males were analyzed to avoid sex variability, according to previously observed differences in total lipid content (see data in Results, Section 3). Aliquots of the homogenates were centrifuged at 11,000×*g* for 20 min. Oxidative stress and antioxidant parameters were measured in the supernatant (Sections 2.3 and 2.4). Total soluble protein content was measured by the method of Bradford (1976), using bovine serum albumin as standard. Total glycogen was measured using Van Handel (1965) method. Lipid and FA analyses are described in Section 2.5. Lipofuscins were analyzed on histological preparations (Section 2.4).

2.3. Reduced glutathione and glutathione-S-transferase

Reduced glutathione (GSH) levels were measured following Anderson (1985) procedure. Absorbance at 412 nm was read after 30 min incubation at room temperature with a UV/VIS JAS-CO 7850 spectrophotometer. Results were expressed as nmol GSH/mg proteins.

Glutathione-S-transferase (GST) activity was measured by the technique of Habig et al. (1974). One GST Unit was defined as the amount of enzyme needed to catalyze the formation of 1 μmol of the conjugate of CDNB (1-chloro-2,4-dinitrobenzene) and reduced glutathione, GS-DNB per minute at 25 °C.

2.4. Oxidative damage

Lipid peroxidation was measured by the thiobarbituric acid reactive substances (TBARS) method, according to Fraga et al. (1988). TBARS concentration was estimated using an extinction coefficient of 156 mM^{-1} cm^{-1} and reading absorbance at 535 nm. Results were expressed as μmol TBARS/mg wet mass.

Protein oxidation was quantified as carbonyl groups according to Reznick and Packer (1994). Carbonyl content was calculated from the peak absorbance (355–390 nm) using an extinction coefficient of 22,000 M^{-1} cm^{-1}. Results were expressed as nmol carbonyl/mg proteins.

For lipofuscin analysis, small pieces of digestive gland tissue were transferred to histocettes and fixed in Baker's formalin (100 mL 40% formaldehyde and 20 g $CaCO_3$ in 1 L distilled water). Samples were then dehydrated through an ethanol series, cleared in xylene, and embedded in paraffin. Lipofuscins were detected in 5 μm thick sections by Schmorl-staining as described by Strahl et al. (2007). For lipofuscins identification, we previously checked unstained thin sections for auto-fluorescent lipofuscin-like granules (Lomovasky et al., 2002). The histochemical properties of these granules were assessed by PAS-Alcian blue-hematoxylin (Moore et al., 1980) and by Sudan Black B and Oil Red O techniques (Bluhm et al., 2001). A Leica ICC50 light microscope attached to a digital camera and the Image J 1.43u program were used to detect and quantify lipofuscins. For each individual, 10 digital color images were randomly taken from the region around the digestive gland and the intestine. The mean individual lipofuscin area was obtained by averaging results from these 10 images. The lipofuscin area was measured and expressed as percentage of the total tissue area analysed for each image. Results are expressed as $Lipofuscin_{CT}$ (area of lipofuscin granules per total area fraction, multiplied by 100).

2.5. Lipid and fatty acid composition

Total lipids of digestive tissue and sediments were extracted with chloroform/methanol (2:1, v/v) according to Bligh and Dyer (1959) and measured gravimetrically. Lipid classes were separated by thin-layer chromatography (TLC) on silica gel plates (Merck, Darmstadt, Germany), using a series of two-solvent systems. First, the plate was developed with chloroform: methanol: acetic acid: water (65:25:4:4 v/v/v/v), for polar lipids (PL), followed by development in the same TLC plate with hexane: ethyl ether: acetic acid (80:20:1 v/v/v), for neutral lipids (NL).