Marine Pollution Bulletin 82 (2014) 117–126

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Trematode communities in cockles (*Cerastoderma edule*) of the Ria de Aveiro (Portugal): Influence of inorganic contamination

R. Freitas [a,*], R. Martins [a], B. Campino [b], E. Figueira [a], A.M.V.M. Soares [a], X. Montaudouin [c]

[a] Departamento de Biologia & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[b] Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[c] Université de Bordeaux, EPOC, UMR 5805 CNRS, 2, rue du Pr Jolyet, F-33120 Arcachon, France

ARTICLE INFO

Keywords:
Western Iberia
Parasitism
Trematodes
Cerastoderma edule
Ecological assessment
Contaminants

ABSTRACT

This work aims to assess the trematode parasites infecting the edible cockle *Cerastoderma edule,* collected in the Ria de Aveiro lagoon, one of the most relevant biodiversity hotspots of the Western Iberia, and evaluate the relationship between the observed patterns and environmental descriptors. A total of 11 of the 16 trematode species known to infect *C. edule* were identified, including *Himasthla continua* and *Psilostomum brevicolle* as new occurrences in this lagoon. *Parvatrema minutum* was the most abundant and dominant species. Species richness and prevalence were high. The relationship between trematode species abundance, intensity and prevalence, and also environmental variables, showed that most parasites preferred muddy sand areas with euhaline conditions in opposition to areas with contamination and/or distant from the lagoon entrance. This study highlighted the good ecological status of the ecosystem and the transitional biogeographic characteristics of the western Portuguese coast where northern and subtropical faunas can coexist.

1. Introduction

In coastal ecosystems, trematodes are frequent macroparasites of many faunal species (Mouritsen and Poulin, 2002; de Montaudouin et al., 2009). They are characterized by a complex life cycle that usually involves three free-living host species. The parasite sexually reproduces inside a vertebrate host, like fishes or birds. Eggs are emitted in the ecosystem and develop into miracidium larvae that will infect a mollusk as first intermediate host. In the host's gonad and/or digestive gland, miracidium transforms into sporocysts/rediae where cercariae larvae are produced (asexual reproduction) before being emitted in the water mass. These cercariae have few hours to penetrate and infect the second intermediate host, where they become metacercariae. The cycle is closed when the final host predates the second intermediate host (e.g. Niewiadomska and Pojmańska, 2011). Thus, there are three important trematode-related processes that are useful for the knowledge of the ecosystem: (1) each trematode parasite species requires three host species to accomplish its lifecycle. Consequently, the presence of many parasite species (i.e. high trematode parasite species richness) is often considered as a proxy of a high general diversity (Hudson et al., 2006; Hechinger et al., 2007,2008) and can even actively boost free-living diversity (Mouritsen et al., 2005); (2) trematodes infect some target species and may impact their host's population dynamics. First intermediate hosts infection is considered severe and may impact host populations, when the prevalence is high (Jensen and Mouritsen, 1992; Jonsson and André, 1992; Thieltges and Reise, 2006; de Montaudouin et al., 2012). As for second intermediate host, the population is affected when individuals undergo a high metacercariae abundance (Desclaux et al., 2004; Gam et al., 2009); (3) trematode life cycle includes two free-swimming stages (i.e. miracidium and cercariae stages). These larvae may be sensitive to pollution (Morley et al., 2003; Pietrock and Marcogliese, 2003) and a lack of infection can be interpreted as the presence of contaminated conditions (MacKenzie et al., 1995; Lafferty, 1997; MacKenzie, 1999). Finally the interpretation of the presence of trematode as an indicator of environmental fitness can be tricky, with contradictory results between effects at the population level and at the community level (Do et al., 2011).

In coastal environments, bivalves are often ecologically and economically important. The edible cockle *Cerastoderma edule*, is a common inhabitant of bays and estuaries that can be found along the Atlantic shores between Barents Sea and Mauritania. In these environments, cockles are known to undergo high trematode infec-

* Corresponding author. Address: Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. Tel.: +351 234370782; fax: +351 234372587.
E-mail address: rosafreitas@ua.pt (R. Freitas).

http://dx.doi.org/10.1016/j.marpolbul.2014.03.012
0025-326X/

tion (de Montaudouin et al., 2000, 2009; Fermer et al., 2011; Thieltges and Reise, 2006) and/or metal contamination (Cheggour et al., 2001; Lobo et al., 2010; Freitas et al., 2012). Experimentally, Paul-Pont et al. (2010) demonstrated that metal contamination (cadmium) did not exert any modification of infection efficiency by trematodes (*Himasthla elongata*). Conversely, infection by *H. elongata* can increase the accumulation of cadmium (Baudrimont and de Montaudouin, 2007). Finally, these studies concerning host-parasite interactions within a given environment highlight many knock-on consequences on the ecosystem, with many complex feed-backs. Thus, it becomes difficult to predict the dynamics of the co-occurrence of trematode parasites and pollutants in the environment. Former studies in other coastal systems monitored metal contamination and parasite load separately. Here, in order to test the hypothesis that a polluted area is also an area deprived of parasitic trematodes, we performed a large survey along the Ria de Aveiro lagoon (Portugal), comparing contamination and trematode infection of the cockle metapopulation. This coastal ecosystem forms an ideal study area to conduct such an investigation since cockles represent the most commercially valuable resource comprising more than 90% of the shellfish harvested (250 licensed professionals) in the lagoon (DGPA, 2011), and also because cockles spread over most of the euhaline area of the Ria de Aveiro, in unpolluted areas as well as in sites near industrial activity where metal contamination is higher (Figueira et al., 2011).

Thus the present study aims to: (1) provide the first extensive survey of trematodes in the Ria de Aveiro; (2) correlate trematode intensity with environmental parameters, with a special focus on metal contamination.

2. Material and methods

2.1. Study area

Ria de Aveiro is a shallow coastal lagoon, with 45 km long and 10 km wide, located on the Northwestern coast of Portugal and connected to the sea by a single channel (Fig. 1). It comprises four main channels (Mira, S. Jacinto/Ovar, Ílhavo and Espinheiro) and extensive intertidal zones, covering a minimum area of approximately 66 km^2 at low spring tide and a maximum of 83 km^2 at a high spring tide (Dias, 2001). In general, water depth is less than 3 m (Dias et al., 1999). The hydrology is essentially dominated by tidal forcing, responsible for a strong mixture of the water masses. The major sources of freshwater inflow are the Vouga River (50 m^3/s) and the Antuã River (5 m^3/s) (Dias et al., 1999). The vertical salinity and temperature gradients are minimal compared to the longitudinal gradients (Dias et al., 1999). Mira and Ílhavo channels present salinities ranging from 30 to 38 near the mouth and 1–10 at the head while in S. Jacinto and Espinheiro channels salinity can range from 0 to 35 depending on the freshwater input (Dias et al., 1999; Dias, 2001; Vaz et al., 2005).

2.2. Biological model, the edible cockle (C. edule)

The cockle *C. edule* is an infaunal suspension-feeder of coastal ecosystems. This species preferably lives in fine to medium sediments and can support low salinities (>11). After a planktonic larval stage, recruitment generally occurs in spring and, in a lesser extent in autumn. Lifespan is about 5–6 years with a 50 mm maximal shell length. Adult density can reach up to 2–3000 ind m^{-2}.

2.3. Sampling strategy

Cockles were sampled in October 2012, during low tide, in twenty eight sheltered intertidal stations, along the Ria de Aveiro (Fig. 1). Stations were selected in order to cover the widest range of the Ria's habitats and different environmental characteristics as possible. Given this, the following six areas were assessed (Esinheiro, Ílhavo, Laranjo, Mira, Ovar and S. Jacinto). At each station, 20 adult cockles of similar shell length were randomly handpicked, 15 of which were used for parasites identification and the remaining 5 for elements quantification. Cockles used for the parasitological survey were transported alive to the laboratory, in seawater, while cockles for element quantification were transported on ice and preserved in the laboratory at −20 °C until analysis. At each sampling station, salinity, pH and conductivity were measured and sediment was collected for sediment grain size analysis, organic matter content determination and elements quantification (Pb, Cr, Cu, Zn, Ni, As, Cd and Hg).

2.4. Sediment grain size and total volatile solids

Sediment grain size analysis was performed by wet and dry sieving, following the procedure described by Quintino et al. (1989). The fines particles fraction (diameter below 0.063 mm) was obtained by wet sieving through a 0.063 mm mesh screen and expressed as a whole, in terms of percentage of the total sediment (dry weight). Sand (diameter from 0.063 to 2 mm) and gravel (diameter above 2 mm) fractions were obtained by dry sieving through a battery of sieves spaced at 1Φ size intervals ($\Phi = -\log_2$ the particle diameter expressed in mm).

The amount of sediment in each sieve was expressed for each site as a percentage of the whole sediment, dry weight. P_{50} is the median value of sediment grain size expressed in phi (Φ) units. The median and the percent content of fines were used to classify the sediment, according to the Wentworth scale: very fine sand (median between 3 and 4 Φ); fine sand (2–3 Φ); medium sand (1–2 Φ); coarse sand (0–1 Φ); very coarse sand (−1 – 0 Φ) (Doeglas, 1968). Samples with more than 50% fines content were classified as mud. Total volatile solids in sediments were determined by loss on ignition at 450 °C (Byers et al., 1978).

2.5. Metal quantification

The concentration of elements (Pb, Cr, Cu, Zn, Ni, As, Cd and Hg) was determined in sediments and cockles (soft tissues) and were expressed in μg g^{-1} wet weight (ww).

Samples were digested overnight (±18 h) at 115 °C in digestion Teflon bombs (sealed chambers) with 10 mL of 65% HNO_3 (Suprapur, Merck) in the case of the wet sediment and 2 mL of 65% HNO_3 in the case of the biological samples (cockles soft-tissue). To prevent the loss of elements by volatilization, chambers were only opened when completely cooled. The cooled digest was made up to 5 ml using 1 M HNO_3, and the concentration of all elements was determined by ICP-MS (Inductively Coupled Plasma-Mass Spectroscopy) in a certified laboratory at the University of Aveiro. Regarding the quality controls, the calibration of the apparatus was made with IV standards, and they were verified with standard reference material (National Institute of Standards and Technology, NIST SRM 1643e). During element analysis, the accuracy observed ranged between 90% and 110% (information given by the laboratory). All samples below this accuracy level were rejected and the analysis repeated. Determinations were performed using 3 replicates.

2.6. Parasite diversity and intensity

Cockle shell length was measured with a calliper, dissected and the tissues were squeezed between two glass slides for trematode observation under a stereomicroscope. All macroparasites found in each cockle were identified and counted. Parasite species were