Marine Pollution Bulletin 74 (2013) 116–124

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

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

Microbiological composition of native and exotic clams from Tagus estuary: Effect of season and environmental parameters

Patrícia Anacleto [a,b,*], Sónia Pedro [a], Maria Leonor Nunes [a], Rui Rosa [b], António Marques [a]

[a] Division of Aquaculture and Upgrading (DivAV), Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.), Av. Brasília, 1449-006 Lisbon, Portugal
[b] Guia Marine Laboratory, Center of Oceanography, Faculty of Sciences, University of Lisbon (FCUL), Campo Grande, 1749-016 Lisbon, Portugal

ARTICLE INFO

Keywords:
Venerupis pullastra
Ruditapes philippinarum
Escherichia coli
Salmonella spp.
Vibrio spp.
Total viable counts

ABSTRACT

The influence of seasonal and environmental parameters on the occurrence of bacteria was investigated in two clam species (*Venerupis pullastra* and *Ruditapes philippinarum*), water and sediment from the Tagus estuary. Total viable counts (TVC), *Escherichia coli*, *Salmonella* spp. and *Vibrio* spp. were evaluated during one-year. Overall, significant seasonal variations were found in both sampling sites, especially for *E. coli* and *Vibrio* spp. levels. In summer, significantly higher *Vibrio* spp. levels were found in *R. philippinarum* and sediment samples, but not in *V. pullastra* clams and water samples. In contrast, significantly higher TVC and *E. coli* levels were observed in winter months in water and sediment samples. *Salmonella* spp. was generally isolated when higher levels of *E. coli* were detected, particularly in *R. philippinarum*. This study is useful for authorities to develop monitoring strategies for coastal contamination and to estimate human health risks associated with the consumption of bivalves.

1. Introduction

The harvesting bivalve molluscs usually occurs in inshore estuaries with high primary productivity and has been an important component of Southern European fisheries related activities since ancient times (Lees, 2000). In 2010, the European annual production reported for this group accounted for 26.2% of total aquaculture production (FAO, 2012). Bivalve molluscs, like pullet carpet shell clam (*Venerupis pullastra*) and Japanese carpet shell clam (*Ruditapes philippinarum*), are the most important resources commercially exploited in Tagus estuary, playing a crucial socio-economic role to riparian human communities because they are easily collected and have high nutritional value (Oliveira et al., 2011). Since these species have a filter-feeding activity, they can accumulate pathogens from seawater (Cook, 1991).

The number and type of microorganisms present in marine or estuarine waters depend on seasonal, climatic and anthropogenic factors (Vernocchi et al., 2007). The microbiota found in shellfish can be divided into three groups (Reilly and Käferstein, 1997): (i) indigenous bacteria that naturally occur in marine or estuarine environments (e.g., *Vibrio* spp., *Listeria monocytogenes*, *Clostridium botulinum* and *Aeromonas hydrophila*); (ii) non-indigenous/enteric bacteria that occur due to faecal contamination (e.g., *Salmonella* spp., *Escherichia coli*, *Shigella* spp., *Campylobacter* spp. and *Yersinia enterocolitica*); and (iii) bacteria from contamination during food preparation and processing by the distribution industry or consumers (e.g., *Bacillus cereus*, *Stapylococcus aureus* and *Clostridium perfringens*). The routes of transmission from the environment to humans include the consumption of raw, uncooked or lightly cooked shellfish, representing a significant health risk (Lees et al., 2010). In 2011, bivalve molluscs represented 4.2% of all European food contaminated with potentially pathogenic bacteria (RASFF, 2012).

Microbiological pollution in shellfish-growing waters is a common problem in almost all the coastal areas (Almeida and Soares, 2012). In this context, the production, harvesting and commercialization of bivalve molluscs as well as the classification of the overlying waters are regulated by the EC Regulations 854/2004 (EC, 2004) and 1441/2007 (EC, 2007). These regulations establish limits for indicator microorganisms (less than 300 fecal coliforms or less than 230 *E. coli* per 100 g of flesh and intravalvular liquid) and pathogens (absence of *Salmonella* spp. in 25 g of flesh) for a production area suitable for direct human consumption (EU class A). However, *Vibrio* species are excluded from the European applicable microbiologic requirements for shellfish-harvesting areas and are not included in the European Network for Epidemiologic Surveillance and Control of Communicable Diseases and from the Microbiological Surveillance System for Infectious Gastroenteritis (EC, 2001).

The presence and concentration of microorganisms in bivalves vary temporarily and spatially in estuarine regions within the same habitat (Lindstrom, 2001), as well as between habitats (Yannarell and Triplett, 2004), due to environmental factors (Hahn, 2006).

* Corresponding author at: Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.), Av. Brasília, 1449-006 Lisbon, Portugal. Tel.: +351 213 027 025; fax: +351 213 015 948.
E-mail address: panacleto@ipma.pt (P. Anacleto).

0025-326X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marpolbul.2013.07.019

Several authors assessed the seasonal microbiological structure of bivalve molluscs from moderately to highly polluted waters, such as clams *Egeria radiata* from Great Kwa estuary in Nigeria (Eja et al., 2008) and oysters *Crassostrea madrasensis* from estuaries along the southwest coast of India (Deepanjali et al., 2005). Additionally, many studies addressed the effects of environmental factors like salinity, temperature, turbidity, pH, tides, etc. (e.g., Martinez-Urtaza et al., 2004; Campos and Cachola, 2007) on levels of microbiological contamination in coastal areas used for the commercial production of bivalves. Yet, few studies addressed the seasonal environmental factors influencing the bacteriological contamination of clams. In this context, this study aim to evaluate the influence of environmental parameters, such as temperature, salinity, pH and dissolved oxygen, on bacterial levels (*E. coli*, *Salmonella* spp., *Vibrio* spp. and total viable counts – TVC) found in water, sediment and clam species (i.e., the native *V. pullastra* and the exotic *R. philippinarum*) from Tagus estuary (Portugal).

2. Materials and methods

2.1. Study area

Clams were collected in two active bivalve fishing sites of the Tagus estuary, Trafaria (38°67′682″N, 9°24′362″W) and Barreiro (38°65′442″N, 9°09′365″W) (Fig. 1). This estuary, located in the most populated area of Lisbon, Portugal, is one of the largest on the west coast of Europe. It has a broad shallow bay covering an area of about 320 km^2 (Brogueira and Cabeçadas, 2006). The Tagus river is the main source of freshwater to the estuary, representing the second most important hydrological basin in the Iberian Peninsula. Seawater enters the estuary through a deep narrow inlet channel, and is classified as a mesotidal estuary according to the National Oceanic and Atmospheric Administration (NOAA), with semidiurnal tides ranging from 0.4 m at neap tide to 4.1 m at spring tide. The estuary receives effluents from agricultural, industrial and urban sources (Gameiro and Brotas, 2010).

2.2. Sampling of clams, water and sediment

Studies were carried out between July 2011 and June 2012 in the Tagus estuary. Monthly collections of estuarine water, sediment and clams samples were made. Native pullet carpet shell clam (*V. pullastra*) was harvested from Trafaria, located in the mouth of Tagus estuary, and exotic Japanese carpet shell clam (*R. philippinarum*) was harvested from Barreiro, located in the upstream part of the estuary (Fig. 1). These clam species were selected due to their different filtering capacities, adaptability and tolerance to environmental variability, and also due to their colonization of different niches in the aquatic ecosystem. Sampling of *V. pullastra* was performed during the first 6 months (July–December 2011), due the fact that the Tagus estuary has been affected by a clam overexploitation. All clams and sediment samples were collected through diving and immediately stored in sterile plastic bags and bottles, respectively. Water samples were collected using a sterile Niskin bottle near the bottom, i.e., at 27–30 m in Trafaria and 2–6 m in Barreiro.

A total of 54 samples were collected from both sampling sites and from each sample three replicates were made. Water temperature, salinity, pH and dissolved oxygen were measured *in situ* with a WTW handheld Meter Multi 350i (WTW, Germany). Table 1 shows the means of each parameter in the two sites of the Tagus estuary. All samples were transported to the laboratory in thermally insulated boxes in aseptic conditions and then stored at 4 °C until further analysis. Only live clams were used for analysis and around 1 kg of clams were sampled per month in each site, being thoroughly washed with sterile water and a brush to remove any material adhering to the shells and dried with absorbent paper. Subsequently, clams were opened and the muscle and intervalvar liquid were aseptically extracted into a sterile container using a sterile scalpel.

2.3. Microbiological analyses

2.3.1. E. coli and total viable counts (TVC)

2.3.1.1. Clams and sediment samples. The enumeration of *E. coli* was carried out according to the method of the International Organization for Standardisation (ISO 16649-2; ISO, 2001) and the pour plate method was used for total viable counts (TVC). Edible clam meat with intervalvar fluid or sediment was initially diluted in Maximum Recovery Diluent (MRD; Oxoid Ltd., Basingstoke, Hampshire, UK) and homogenized during 60 s in a Stomacher 400 (Seward Laboratory System, London, UK). For *E. coli* quantification, appropriate serial decimal dilutions were performed in MRD,

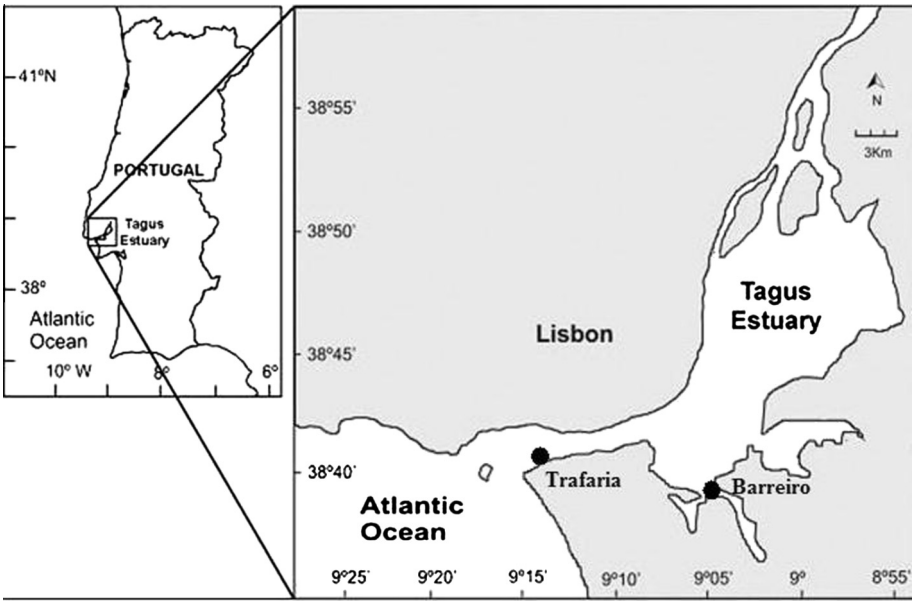

Fig. 1. Map showing Tagus estuary (Portugal) and sampling locations (Trafaria for *Venerupis pullastra* and Barreiro for *Ruditapes philippinarum*).