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An integrated appraisement of multiple faecal indicator bacteria and sterols in the detection of sewage contamination in subtropical tidal creeks

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ABSTRACT

The quality of water bodies has been regulated by national environmental agencies and based on faecal indicator bacteria, such as thermotolerant coliforms *Escherichia coli* and *Enterococcus* sp. Additionally, faecal sterols (mainly coprostanol) have been used to corroborate sewage discharge in marine environments. In this study, faecal material input was evaluated in two sampling campaigns in transects of two tidal creeks using bacterial and chemical indicators to both compare and establish the water quality in a South Atlantic subtropical estuary. The Itiberê tidal creek (S1) was classified as "contaminated" by faecal material, while the Peças tidal creek (S2) presented variable water quality according to the sampling period and sewage indicators considered in this evaluation. Then, the integrated application of chemical and bacterial indicators was applied for tidal creeks with different sewage contamination levels and under distinct environmental conditions and confirmed that *Enterococcus* sp. and coprostanol are the most suitable for estuarine environments.

1. Introduction

The disposal of raw sewage is one of the main sources of disturbance in the natural dynamic of estuarine systems, and it contributes to decreased environmental and human health and impaired water quality for various human uses (Cabral et al., 2018). Human activities produce considerable amount of domestic wastewater discharge into marine environments, which are damaged by the introduced pathogens and enteric viruses (UNEP, 2008; He et al., 2018).

Tidal creeks are unique features in estuarine environments that act as drainage bodies responsible for transferring organic matter (OM) and nutrients as well as contaminants and domestic sewage from the continent to the coastal region. These tidal creeks are routes of meandering water, are highly influenced by the tide and are subject to high variations of salinity and depth during the day (Lana et al., 1989; Siqueira et al., 2009). Tidal creeks are widespread and abundant estuarine ecosystems, but their ecological relevance is undervalued, as reflected by the lack of research on these systems relative to estuarine systems (Mallin and Lewitus, 2004). They have distinctly diverse hydrological and watershed characteristics that can heavily influence the ecosystem function and impact the human use of these resources (Lerberg et al., 2000; Mallin et al., 2000).

Historically, the oral-faecal route, which involves the ingestion of food or water contaminated by pathogens, is considered an important cause of diseases associated with low water quality. Thus, the importance of detecting and quantifying faecal OM in the environment has been responsible for the development of targeted techniques in public health (Leeming et al., 2015; Cabral et al., 2018).

In the evaluation of faecal contamination, chemical and/or biological indicators may have characteristics such as wide occurrence in human faeces; not increasing in number outside the human body; strong resistance to human pathogenic bacteria; and degradation under environmental conditions and disinfection; additionally, they may be strongly associated with the occurrence of pathogenic microorganisms and require a simple and relatively low-cost detection technique (Hurst et al., 2002). To address the mentioned characteristics, three globally popular techniques for analysing the coastal water quality were considered in this study: multiple tubes (total and thermotolerant coliform determination), chromogenic substrate (*Escherichia coli* and *Enterococcus* sp. determination) and the identification and quantification of faecal sterols, particularly coprostanol.

E. coli, the main representative of thermotolerant coliforms, lives exclusively in the gastrointestinal tract of homeothermic animals (Kaper et al., 2004; Ishii et al., 2007), and it has been considered a valuable indicator of faecal contamination in drinking water (Edberg et al., 2000). *Enterococcus* sp. is a more resistant organism that is used as an indicator of water quality and is frequently associated with gastrointestinal infections (Trabulsi et al., 2002).

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Coprostanol has been used as complementary tool for assessing sewage contamination since 1960 because of its resistance to biodegradation and direct relation to suspended particulate matter (SPM), waste and sludge from both raw and treated sewage (Murtaugh and Bunch, 1967; Grimalt et al., 1990). This sterol is the main product of cholesterol biohydrogenation by mixed population of specific bacteria such as *Bifidobacterium* spp., *Clostridium* spp., *Bacteroides* spp. and *Eubacterium* spp. (Dutka et al., 1974; Vivian, 1986; Venkatesan and Santiago, 1989) in the intestinal tract of higher mammals and is rarely detected in environments with no sewage input (Takada and Eganhouse, 1998). Thus, it can help elucidate the sewage input when faecal indicator bacteria (FIBs) are not detected, despite evident sewage sources or when FIBs are present at high levels in pristine environments.

In this context, the aim of this study was to evaluate the faecal material input in two transects from tidal creeks of a subtropical estuary in South Atlantic under different contamination levels and distinct climatic conditions (austral summer and winter, usually assumed as 'rainy' and 'dry' seasons, respectively), considering multiple indicators (FIBs and sterols such as coprostanol, epicoprostanol and other biogenic compounds), to determine the relationships among these indicators as well as their possible influences based on the climatic and physico-chemical variables. The evaluation of raw/treated sewage input in the tidal creeks was evaluated based on diagnostic ratios as epicoprostanol/coprostanol.

2. Study area

The Paranaguá estuarine system (PES; 25°30′S - 48°25′W) (Fig. 1) is a humid subtropical environment located on the southwest Atlantic coast of Brazil in one of the most highly preserved areas of Atlantic rainforest in South America, and it has been classified as a biosphere reserve by the United Nations Educational, Scientific and Cultural Organisation (UNESCO) (Martins et al., 2010). The PES has approximately 612 km^2 of total area and can be divided into two main water bodies: (i) the bays of Paranaguá and Antonina, where human activities are more intensive (Lana et al., 2001; Combi et al., 2013), and (ii) the bays of Laranjeiras, Guaraqueçaba and Pinheiros, which are located in the northern section and are considered a more pristine area (Martins et al., 2012).

Approximately 200,000 people occupy the PES shorelines, with the largest human settlements concentrated in the cities of Pontal do Paraná, Antonina and Paranaguá (IBGE, 2016). Paranaguá, the most populated city in the region, has a precarious system of sewage collection and treatment, with part of its wastewater being dumped directly into the tidal creeks surrounding the city or directly into the estuary (Kolm et al., 2002).

Two tidal creeks were selected for study: (i) Itiberê tidal creek (25°32′50″S - 48°30′80″W) borders the city of Paranaguá and flows into Paranaguá Bay (Fig. 1). This area is approximately 5 km^2 and has approximately 22,000 inhabitants (Babu et al., 2002). Historically, this area is recorded as being contaminated by faecal material (Abreu-Mota et al., 2014; Cabral and Martins, 2018). (ii) Peças tidal creek (25°27′60″S - 48°20′00″W) is located near the entrance of Laranjeiras Bay, which surrounds the homonymous island. A small village of approximately 300 inhabitants is near its mouth, resulting in an apparently "pristine" area (Martins et al., 2012).

3. Material and methods

3.1. Sample collection and physico-chemical water column parameters

Sampling campaigns were conducted in January, 28th (austral summer) and September, 05th (austral winter) 2013, simultaneously in both tidal creeks by use of two boats, during the same period of the day.m..m. and the ebbing spring tide. Subsurface water samples (0–1 m) were collected at three sites along a transect in Itiberê (S1) and Peças (S2) tidal creeks. In total, 36 water samples were taken for microbiological identification (18 per sampling campaign, triplicate in each transect site in each tidal creek), and 12 water samples (4 L) were

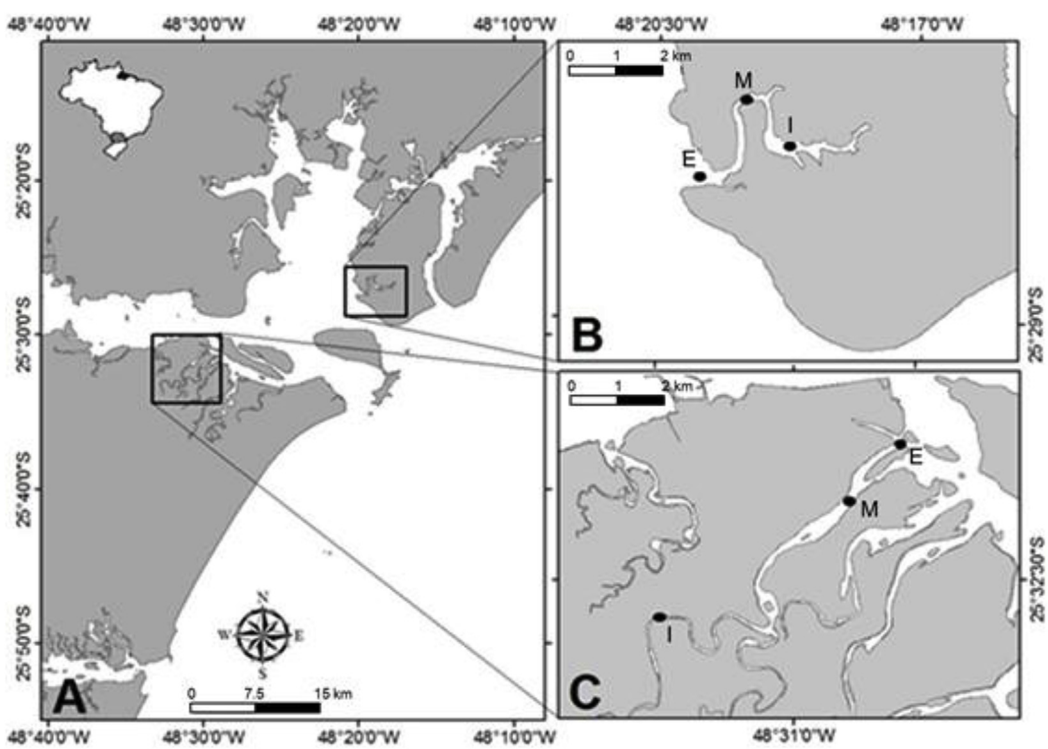

Fig. 1. Map of the study area and sampling region, highlighting the Paranaguá Estuarine System (PES) (A), the Peças (S2, B) and Itiberê (S1, C) tidal creeks, with an indication of the sampling site transect (internal – I, middle – M, and external - E).