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Effect of environmental parameters on pathogen and faecal indicator organism concentrations within an urban estuary

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ABSTRACT

Current World Health Organisation figures estimate that ~2.5 million deaths per year result from recreational contact with contaminated water sources. Concerns about quantitative risk assessments of waterways using *faecal indicator organisms* (FIOs) as surrogates to infer pathogenic risk currently exist. In Melbourne, Australia, the Yarra River has come under public scrutiny due to perceived public health risks associated with aquatic recreation; a characteristic shared with urban estuaries worldwide. A 10-month study of the Yarra estuary investigated the processes that affect FIOs and pathogens within this system. A total of 74 samples were collected from three estuarine and two upstream, freshwater, locations under different climatic and hydrological conditions, and the levels of *Escherichia coli*, enterococci, *Clostridium perfringens*, fRNA coliphages, *Campylobacter* spp. *Cryptosporidium* oocysts, *Giardia* cysts, adenoviruses, and enteroviruses were monitored. Reference pathogenic bacteria, protozoa, and viruses were detected in 81%, 19%, and 8% of samples, respectively. Variations in FIO concentrations were found to be associated with changes in specific climatic and hydrological variables including: temperature, flow, humidity and rainfall. In contrast, pathogen levels remained unaffected by all variables investigated. Limitations of current national and international culture-based standard methods may have played a significant role in limiting the identification of correlative relationships The data demonstrate the differences between FIOs and microbial pathogens in terms of sources, sinks, and survival processes within an urban estuary and provide further evidence of the inadequacy of FIO inclusion in the development of worldwide regulatory water quality criteria and risk assessment models.

1. Introduction

Estuaries are important resources in urban environments and benefit the aesthetics, microclimate, biodiversity, and recreational opportunities of local communities (Pinto et al., 2014). Twenty-two of the world's 32 largest cities are located on estuaries (Ross, 1988), highlighting the importance of these waterways to large sections of the global population. However, water quality is highly variable due to diverse water usage and land management practices, and this often limits how a water system can be used by the community (Findlay and Taylor, 2006). Increased pressure from pollution associated with urbanisation, combined with population growth and climate change, is likely to further impact these estuaries even more in future.

Faecal microorganisms are the leading cause of pollution in urban estuaries (Burton and Pitt, 2002). Contamination of urban waterways from human and non-human excreta can result in the transmission of infectious disease, particularly to individuals partaking in recreational activities. To date, over 150 microorganisms derived from faecal sources are considered waterborne pathogens (McLellan et al., 2013). However, many pathogenic organisms appear intermittently and at low concentrations, making routine detection and quantification difficult (Savichtcheva and Okabe, 2006; Boehm and Soller, 2013).

Faecal indicator organisms (FIOs) have been utilised for the identification of faecal contamination within recreational waters (Chase et al., 2012). In general, FIOs are non-pathogenic microbes that, like faecal pathogens, are typically residents of the

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gastrointestinal tracts of warm and cold-blooded animals and are shed into the environment by defecation (Chase et al., 2012). However, unlike pathogens, FIOs are excreted at high concentrations, so rates of routine detection are better. FIOs are applied to infer the presence of pathogenic organisms in recreational waters (Payment and Locas, 2011; McLellan et al., 2013). To be considered an effective indicator organism, a microbe needs to meet several key criteria (Dufour, 1984; McLellan et al., 2013) including: not growing outside the host environment; having an existing and established relationship with a microbial pathogen; and having a similar growth and survival pattern to the pathogenic organism they represent within the natural environment (Payment and Locas, 2011; McLellan et al., 2013).

The establishment of a quantitative link between FIO concentrations and human health risk in recreational epidemiology studies in marine and fresh waters has resulted in the development of worldwide regulatory water quality criteria (Craun and Calderon, 2006; Chase et al., 2012; Heaney et al., 2012; McLellan et al., 2013). In Australia, these criteria are <150 faecal coliforms $(100\ mL)^{-1}$ per primary contact (e.g. swimming) and <1000 faecal coliforms per secondary contact event (e.g. boating) (Council, 2000). However, studies have proposed that FIOs are unreliable indicators of pathogens in urban estuaries. This is in part due to the complex hydrological and climatic variables that can affect the survival of microorganisms within these systems. These variables include solar radiation, sedimentation, temperature, nutrient availability, antecedent rainfall, tidal variations, salt wedge movements and salinity gradients, and the seasonal and temporal variations in all of these factors (Alkan et al., 1995; Ferguson et al., 1996; Davies and Bavor, 2000; Solo-Gabriele et al., 2000; Lipp et al., 2001; Desmarais et al., 2002; Vogel et al., 2005; Hathaway et al., 2010). The difficulty of such complexity means that only a few studies have related these parameters to FIO concentrations within urban estuaries (Touron et al., 2007; Jayakumar et al., 2013). Even more limited is information about how environmental variables affect the relationship between FIOs and enteric pathogens (Lipp et al., 2001; Nagvenkar and Ramaiah, 2009; Ortega et al., 2009). Thus, further study is necessary to assess the suitability of FIOs as pathogen surrogates in dynamic estuarine systems.

Globally, waterborne microbial disease outbreaks have been directly associated with faecal pollution of urban estuaries (Jones, 2001; Worth and Biggs, 2003; Pond, 2005; Dale et al., 2010). However, despite perceived risks, limited investigation has been undertaken to assess reference pathogen levels within these systems (Ellaway et al., 1982; Sokolov and Black, 1996; EPAV, 2001; Daly et al., 2013). The Yarra River in Melbourne, Australia, contains a marine-linked urban estuary impacted by faecal contamination. To date, water quality studies pertaining to this river have focused primarily on FIOs and chemical pollutants with observed FIO concentrations similar to those reported in other national and international estuarine systems (Wade, 2002; Jones et al., 2004; Chandran and Mohamed Hatha, 2005; García-Barcina et al., 2006; Touron et al., 2007; Council, 2013; Daly et al., 2013). As with other estuaries, outbreaks of waterborne disease associated with primary contact events have also been reported (Worth and Biggs, 2003). These similarities indicate the suitability of the Yarra River as a representative estuary for this study.

This paper explores ten exogenous parameters and their relationships to FIO and reference pathogen concentrations in freshwater and estuarine locations. The results represent one of the largest datasets on FIO and pathogen behaviour within urban estuaries and provide further evidence of the inadequacy of the use of FIOs for inference of pathogen associated risks in water quality criteria. The application of this data will aid in directing the development of more effective risk assessment models to help preserve, not only the Yarra River, but urban estuaries in general, for future public recreational usage.

2. Materials and methods

2.1. Study locations

The Yarra River flows approximately 242 km from its beginnings in the Yarra Ranges National Park north-east of Melbourne and drains into Port Phillip Bay near central Melbourne (Fig. 1). Annual rainfall across the 4078 km^2 catchment varies from 600 mm near the city centre to over 1000 mm near its upper reaches (Daly et al., 2013). The water column within the estuary ranges from predominantly fresh at the riverine end to a salt-wedge region at the marine boundary, with a stratified zone in the middle. The city relies on the upper reaches of the river to service its drinking water storages, and its water quality is regularly monitored by the utility Melbourne Water. Monitoring data suggest that the upper Yarra has good water quality (low FIO levels; 100–1000 faecal coliforms/100 mL), while in the middle and lower reaches, reduced water quality (>1000 faecal coliforms/100 mL) is associated with urban and rural stormwater run-off, as well as point source pollution from unsewered residential areas and illegal cross-connections between sewerage and stormwater drainage systems (Water and EPA, 2009). It is important to note that, within the designated study area, sewerage and stormwater drainage (the latter discharging into local waterways), are separate systems. The study encompassed the estuarine region, which is used extensively for both active and passive recreation (Fig. 1). All sites were selected from a list of Melbourne Water's routine water quality monitoring program sites so that data could be compared with existing datasets if required.

2.2. Sample collection and microbial analysis

Surface water samples were collected fortnightly between October 2010 and June 2011 from three estuarine sites at Docklands (DK), South Yarra (SY), and Abbotsford (ABT). Upstream sites at Kew and Warrandyte (WD) were sampled, using the same method, from January to June 2011. Due to the number of sites and distances between, samples were collected at variable tide levels and independent of local or regional weather patterns (Table S1). To determine levels of pollution within the estuary, faecal-derived microbial indicators and pathogenic organisms commonly employed in international and national water quality guidelines were selected for evaluation. Every fortnight, 5 L of water was collected 3 m perpendicular from the bank and at an approximate depth of 15 cm, from each of the five sites and analysed for FIOs (*Escherichia coli* and enterococci), *Salmonella* spp. and *Campylobacter* spp. Once a month, an additional 40 L of water was collected at each site to enable water testing for FIOs *Clostridium perfringens* and fRNA coliphages; and for these pathogens: *Cryptosporidium* oocysts, *Giardia* cysts, adenoviruses, and enteroviruses. Samples were collected using a peristaltic pump and reinforced food-grade tubing to channel the water into sterile plastic containers. Between sites, all sampling apparatus were cleaned (using deionised water) and rinsed with river water before sample collection. Once collected, samples were placed on ice and taken for analysis to ALS Environmental, a testing laboratory in Melbourne (accredited by the National Association of Testing Authorities, NATA).

All microbial analyses were performed by ALS Environmental using the methods listed in Table 1. Technical replicates were undertaken as specified in each of the methods (Table 1). Cultural analysis of faecal indicators, reference pathogenic bacteria and viruses were undertaken within 24 h of receiving the sample. *Giardia* and *Cryptosporidium* analyses was conducted within 72 h. Due to