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Seaweeds and plastic debris can influence the survival of faecal indicator organisms in beach environments

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ABSTRACT

The revised Bathing Water Directive (rBWD) introduces more stringent standards for microbial water quality and promotes more pro-active management of the beach environment through the production of a bathing water profile (BWP). The aim of this study was to determine whether living seaweeds in the littoral zone are colonised by faecal indicator organisms (FIOs), and to quantify the survival dynamics of waterborne *Escherichia coli* in microcosms containing senescing seaweeds. Living seaweed (*Fucus spiralis*) was not associated with FIO colonisation, although could be providing a protected environment in the underlying sand. Senescing seaweeds enhanced waterborne *E. coli* survival compared to plastic debris, with the brown seaweed *Laminaria saccharina* facilitating greater *E. coli* persistence than either *Chondrus crispus* or *Ulva lactuca*. This has important implications for FIO survival on bathing beaches as the majority of beach-cast biomass is composed of brown seaweeds, which could support significant levels of FIOs.

1. Introduction

Escherichia coli and enterococci are faecal indicator organisms (FIOs) commonly used by environmental regulators around the world to provide a measure of microbial pollution in bathing waters (Mansilha et al., 2009). Although FIOs are not pathogenic, compared with quantifying individual waterborne pathogens, their enumeration is relatively straightforward (Quilliam et al., 2011). The primary habitat of *E. coli* and enterococci is the mammalian gut; however, it is becoming clear that FIOs can persist in the environment for significant periods of time (Ferguson and Signoretto, 2011; Byappanahalli et al., 2012; Hernandez et al., 2014).

The environmental pathways for contamination of bathing waters by FIOs can include both diffuse and point-source inputs, e.g. pasture grazed by livestock and sewage discharges. Diffuse source inputs are driven largely by high rainfall, and the subsequent generation of run-off within agricultural catchments is known to contribute to rapid incidental losses of FIOs from land to water via a number of hydrological pathways (Oliver et al., 2005). High flow conditions in streams and rivers may also remobilise stores of FIOs from streambed sediments, to the further detriment of downstream microbial water quality (Muirhead et al., 2004; Yakirevich et al., 2013). While diffuse source contributions of FIOs to bathing waters tend to dominate during wet weather, the risk of point-source microbial inputs via spills from combined sewer overflows (CSOs) can also increase if sewerage infrastructure struggles to cope with potentially high volumes of urban run-off during intense rainfall and flood conditions (Kay et al., 2008).

Maintaining and improving the microbial quality of EU bathing waters is regulated by the Bathing Water Directive (76/160/EEC) and the revised Bathing Water Directive (rBWD; 2006/7/EC). From 2015, the number of designated bathing waters falling below the legally enforceable 'sufficient' standard is likely to rise with the first reporting of classifications linked to the rBWD (Chawla et al., 2005; Oliver et al., 2014). The rBWD introduces more stringent standards for microbial water quality and also promotes pro-active management of the beach environment through the production of a bathing water profile (BWP) for all designated bathing waters. The BWP is intended to provide a qualitative appraisal of potential pollutant sources linked to physical, geographical and hydrological characteristics of the beach environment. Compliance with the (r)BWD also drives designations such as the Blue Flag award, the loss of which has the potential to impact on tourism and local coastal economies (Wyer et al., 2010). The rBWD thus provides significant impetus for EU regulators to identify and manage potential environmental reservoirs of FIOs, in addition to agricultural and sewage related inputs, which could contribute to decreased water quality at bathing beaches.

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The survival of FIOs in beach sand is well documented and certain species of freshwater macroalgae washed up on bathing beaches have been shown to harbour FIOs, including human pathogenic bacteria (Ishii et al., 2006; Skalbeck et al., 2010). The nuisance filamentous green alga *Cladophora* growing in eutrophic regions of the Great Lakes in the US is often associated with significant levels of *Shigella, Salmonella, Campylobacter,* and *E. coli* O157, with evidence for enhanced survival of *Salmonella* and *Shigella* in association with *Cladophora* in freshwater microcosms (Ishii et al., 2006; Byappanahalli et al., 2009). There is also some evidence that marine beach-cast wrack can play a role in enhancing the persistence of FIOs (Imamura et al., 2011). The seaweed surface is an ideal environment for the formation of biofilm, which provides a nutrient-rich habitat for bacterial communities and a niche for protection from harmful UV light and predation (Egan et al., 2013). Living seaweeds can harbour relatively high concentrations of pathogenic species of bacteria, such as *Vibrio*, e.g. *Vibrio vulnificus* and *Vibrio parahaemolyticus* (Mahmud et al., 2007, 2008). However, there remains a significant lack of understanding about the role of living and senescing seaweeds in facilitating the persistence of FIOs in the marine environment, and the effect this has on bathing water quality for the microbial compliance parameters currently used by EU regulators (Anderson et al., 1997; Hannah and Cowey, 2009).

In catchments containing areas of intensive agriculture, increased inputs of FIOs to bathing waters could be coupled with excessive growth of seaweeds due to concomitant eutrophic conditions. Understanding the role seaweeds play in facilitating the persistence of FIOs in bathing waters therefore, is crucial for informing environmental management decisions (and improving BWPs) designed to decrease the risk of exposure to potential human pathogens. Consequently, the aim of this study was to, (i) determine the spatial distribution of FIOs and *Vibrio* spp. colonising living seaweed fronds across the intertidal zone of a beach; and (ii) to quantify the survival dynamics of waterborne *E. coli* in microcosms containing senescing brown, red or green seaweeds, in the presence or absence of an autochthonous bacterial community.

2. Materials and methods

2.1. Field site and transect sampling

Bracken Bay in Ayr, Scotland, consists of a 0.84 km stretch of sand and shingle beach with a bedrock foreshore. This site is adjacent to a popular tourist holiday park and is situated approximately 800 m south–west of a designated bathing water site (Heads of Ayr beach). The rural catchment draining into this part of the coast is just over 4 km^2 with intensive dairy farming being the main agricultural activity. Average summer rainfall for the region is 392 mm compared to 331 mm across the rest of Scotland. Transect sampling was carried out in a single day in May 2013 during a low tide. Two transects were used to provide environmental and spatial data in relation to coverage by the tide. The first transect ran from the strandline at the top of the beach down to the water's edge; the second transect equidistantly cut across the middle of the first transect at the 40 m point and remained parallel to the sea. Samples of living and attached *Fucus spiralis* together with the sand directly beneath this brown seaweed were collected at 10 m intervals along each of the transects, transferred to zip-lock plastic bags, stored in a cool box for return to the laboratory and processed within 12 h. In addition, samples of seawater were collected in sterile plastic bottles at approximately 1 m below the surface and a selection of plastic rubbish debris was collected from the beach for further microbiological analysis.

2.2. Microbiological quantification and physico-chemical analysis

Following EU guidelines, all seawater samples were stored at 4 °C and processed within 6 h of collection. Each water sample was briefly shaken and 100 mL was vacuum-filtrated through a 0.45 μm cellulose acetate membrane (Sartorius Stedim Biotech., Gottingen, Germany). The membrane was aseptically transferred to the surface of a plate containing membrane lactose glucuronide agar (MLGA) (CM1031, Oxoid, Basingstoke, UK); the plate was inverted, incubated at 37 °C and enumerated 24 h later. Sand samples (5 g) were added to 10 ml of sterile seawater and shaken for 15 min at 225 rev min^{-1}, vortexed four times in 30 s bursts, and allowed to settle for 5 min. Seaweed samples (25 g) were added to 100 ml sterile seawater (sterilised by autoclaving), and plastic debris (4 g) added to 10 ml sterile seawater, and shaken for 15 min at 225 rev min^{-1} and vortexed four times in 30 s bursts. The seaweed wash solution, the plastic debris wash solution, and the sand supernatant were subsequently serially diluted with sterile seawater and used to simultaneously culture loosely attached epiphytic bacteria. Selective media were used to quantify *E. coli* (MLGA; Oxoid), total *Vibrio* spp. (TCBS cholera agar; CM0330, Oxoid, UK), enterococci (Slanetz & Bartley agar; CM0377, Oxoid), and heterotrophic bacterial cells (R2A agar; CM0906, Oxoid). All plates were inverted and incubated at 37 °C (*E. coli*, *Vibrio* spp. and enterococci) for 24 h, or 25 °C (heterotrophic bacteria) for 48 h, and bacterial Colony Forming Units (CFU) enumerated. Electrical conductivity (EC) and pH were measured directly using standard electrodes, and the seawater turbidity measured with an LP2000 turbidity meter (Hanna Instruments, Bedfordshire, UK) and expressed as nephelometric turbidity units (NTU). An isolate of *E. coli* colonising the seaweed was further cultured for use in subsequent microcosm experiments.

2.3. Senescing seaweed microcosms

Microcosms were created by adding either 100 ml of seawater, or 100 ml of sterile seawater, to a sterile 180 ml polypropylene container with a polyethylene screw cap (Fisher, UK). Seawater microcosms included the native microbial community (including bacterial grazers), which would be competing with *E. coli* for both resources and space. Comparable sterile seawater microcosms were included to assess how well *E. coli* competed with this autochthonous community. Representative red (*Chodrus crispus)*, brown (*Laminaria saccharina*) and green (*Ulva lactuca*) seaweeds, that were freshly detached and with no obvious signs of disease or damage, were collected from the surf zone at the field site described above. Fronds from each species were cut into strips (2.0 cm by 7.8 cm) and vigorously rinsed in sterile seawater three times to remove loosely attached epiphytic *E. coli* cells. A single strip of seaweed was added to each replicate ($n = 4$) microcosm. Control microcosms contained a piece of polythene freezer bag cut to the same size as the seaweed and rinsed in sterile seawater as described above. Each microcosm was inoculated with 1 ml of 3.4×10^9 *E. coli* CFU generated from a fresh overnight liquid culture (18 h, 37 °C, 80 rev min^{-1}, grown in LB broth, washed three times in sterile seawater and re-suspended in sterile seawater) of an environmental isolate of *E. coli* capable of colonising seaweed (isolated from the beach sampling survey), and all microcosms were incubated at 10 °C in the dark. At 1, 2, 3, 4, 5, 6, 7, 9, 13, 21, 27 and 38 days, 1 ml of water was removed from each microcosm, and the concentration of *E. coli* and total heterotrophic bacteria quantified as described above. On day 38 the seaweed and plastic samples were removed from the microcosms and following vigorous washing in sterile seawater, loosely attached epiphytic *E. coli* were quantified. Finally, the seaweed tissue was rinsed several times in sterile seawater and then ground in a pestle and

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