



## Regrowth of enterococci indicator in an open recycled-water impoundment

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### HIGHLIGHTS

- The supposition that enterococci faecal-indicator organisms do not multiply in open water impoundments was challenged.
- The resultant risk of false-positives leading to downgrading or over-chlorination of stored recycled water was identified.
- Ambient temperature and total dissolved carbon were identified as primary growth factors for the indicator.
- Nitrate and phosphate were not found to be growth-limiting. Rainfall and duck presence also did impact on numbers.
- A formula for the indicator–temperature relationship was derived for food-production and climate-change modelling.

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### ABSTRACT

The purpose of the research was to assess the potential for enterococci faecal-indicator to regrow in recycled water while under environmentally-open storage. Regrowth would result in false-positive indicator results with possible downgrading, rejection or over-chlorination of recycled water. The research setting was the main 93-megalitre storage impoundment of the Hawkesbury Water Recycling Scheme in Sydney's North West, receiving tertiary treated (chlorinated) effluent from the Richmond sewage treatment plant. The water is used to irrigate horticultural food crops, pasture for dairy cattle, sheep, deer and horses, and for the maintenance of lawns and sports fields. Highly significant positive relationships were noted in multivariate analysis between indicator counts and the growth factors atmospheric temperature and UV<sub>254</sub> unfiltered as proxy for total organic carbon ( $p = 0.001$  and  $0.003$  respectively). Nitrate and phosphate did not show significant relationships suggesting that these nutrients may not be growth-limiting at levels found in recycled water. Rainfall and wild duck presence did not appear to have an impact on enterococcal growth in the study. The overall predictive power of the regression model was shown to be highly significant ( $p = 0.001$ ). These findings will assist in recycled water monitoring and the revision of guidelines, with potential for the reduction of the chlorination by-product burden on the environment. A formula derived for the relationship between the indicator and atmospheric temperature could be used in food-production and climate-change modelling.

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### 1. Introduction

Enterococci indicator (ENT) is increasingly being used to assess water quality in complex environmental settings because of its resistance to inactivation when exposed to micro-ecological and chemical challenges, and its ability to accurately reflect the presence of pathogens (Lleò et al., 2005; Wu et al., 2011). It is already in wide use as an indicator of the quality of marine and other recreational waters internationally, and in recent years has become an important indicator in water recycling where safe irrigation of food and fodder crops is to be ensured (Benami et al., 2013; Chevremont et al., 2013; Poma et al., 2012).

A required property of faecal indicator bacteria (FIB), such as total coliform (TC), faecal coliform (FC) *Escherichia coli* (EC) and ENT, is that they do not give false negative or positive results. To avoid false negative results, it is important that FIB remain viable in recycled water so that the original water quality or subsequent contamination event is accurately reflected. This is problematic in environmentally-open water storages, such as large recycled-water storage dams, because microbiological and biochemical competition continues following treatment, which is aimed primarily at the removal of pathogens. Exposure to environmental factors such as low temperature, sunlight, wave action, oxidation and settlement further threatens indicator organism survival (Asano, 1998).

With simplified assessment approaches, EC has become the most widely used indicator for drinking water quality but it is readily inactivated when stored in environmental settings. FC provides a

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broader monitoring alternative but the group includes varying proportions of EC, introducing a reliability issue (Blaustein et al., 2013; Jamieson et al., 2004). For this reason both EC and FC are regarded only as indicators of recent faecal contamination, then in relatively pure water, with ENT being increasingly incorporated into monitoring schedules for non-potable water types, including recycled water.

While there are a number of publications concerning the inactivation of enterococci in the open environment, few studies have dealt with regrowth, which would lead to false positives being recorded in terms of fixed monitoring criteria (Chowdhury, 2012; Post, 1970). This lack of information appears to have resulted in a false sense of security, with a number of influential texts advising that environmental regrowth is unlikely (Bartram and Rees, 1999; Hurst et al., 2007; Mara and Horan, 2003; Nollet, 2007; World Health Organization, 2011).

The occurrence of false positives could give rise to unnecessary downgrading of recyclable water for less productive agricultural uses, such as turf irrigation instead of food crop or pasture irrigation. An alternative response aimed at sustaining the intended use is hyper-chlorination at the source, resulting in the generation of disinfection by-products, with potential ecological and public health impacts (Ritter et al., 2002; Sun et al., 2009).

The aim of the present study was therefore to investigate the potential for ENT regrowth in recycled water stored in an environmentally-open impoundment, in terms of a range of related growth factors.

## 2. Data and methods

### 2.1. Locality and parameters

Water quality data relating to the primary storage dam (Fig. 1) of the Hawkesbury Water Recycling Scheme (HWRS) was retrieved from the

relevant data archive for the period March 2003 to December 2008. The scheme receives tertiary-treated (chlorinated) effluent from the Richmond sewage treatment plant (STP) in terms of a long standing agreement with Sydney Water Corporation, the sewage catchment being primarily residential. The water is used for agricultural and horticultural irrigation including food crops, pasture for dairy cattle, sheep, deer and horses, and for the maintenance of lawns and sports fields. During this period extensive data had been collected for the development of a risk management system, after which data collection was limited to a routine monitoring subset (Derry et al., 2006; Derry, 2011).

The above ground, clay-lined dam is 200 m wide and four metres deep with a 93 megalitre capacity and a 1.35 megalitre median daily through-flow. It is maintained between 80% and 100% capacity under a pumped, automatic control system regulating both input and output.

The environmental parameters based on the literature for FIB growth in potable water reticulations included water temperature ( $Temp_{water}$ ), the related mean ambient air temperature ( $Temp_{air}$ ),  $UV_{254}$  filtered and unfiltered as proxies for dissolved and total organic carbon, total nitrogen (TN), total phosphorus (TP), dissolved oxygen (DO), biochemical oxygen demand ( $BOD_5$ ), total suspended solids (TSS), electrical conductivity (ECond) and rainfall (American Public Health Association (APHA) et al., 2012; Chowdhury, 2012; Gardini et al., 2001).

### 2.2. Method

Two study designs were considered, the first being cross-sectional, based on the correlation of change in ENT between the dam inlet and outlet with growth factors, the second being longitudinal based on the changes in ENT values under the action of growth factors observed in the region of the dam outlet with time.



Fig. 1. Hawkesbury Water Recycling Scheme main storage dam (lower left) and Richmond sewage treatment plant (upper right) [Coordinates – 33.610, 150.763] [©2013 Google].

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