

Rapid dead-end ultrafiltration concentration and biosensor detection of enterococci from beach waters of Southern California

Stephaney D. Leskinen, Valerie J. Harwood and Daniel V. Lim

ABSTRACT

Same-day microbial water quality assessments are not possible with standard methods, which increases the possibility of public exposure to fecal pathogens. This study examined the efficacy of high-volume hollow fibre ultrafiltration coupled to biosensor detection for enterococci in marine waters to allow same-day public notification of poor water quality. Fifty-six 100 l ultrafiltered samples and 100 ml grab samples were collected weekly from May to July 2007. Post-ultrafiltration processing included sonication and micron sieve passage to remove interfering particulates, followed by centrifugation for secondary concentration. Levels of enterococci in grab and ultrafiltration samples were determined by a standard method (EPA method 1600) for calculation of recovery efficiencies and concentration factors. Each final retentate was analysed with the RAPTOR evanescent wave biosensor. Enterococci levels increased over 26,000-fold in final retentates. Enterococci were detected when ambient concentrations exceeded the regulatory standard for a single sample (≥ 105 CFU/100 ml), and detection was highly correlated with breaches of the single-sample regulatory limit. The combined procedure required 2.5 h for detection compared with 24 h for EPA method 1600. This field study achieved rapid detection of enterococci by ultrafiltration, secondary concentration and biosensor analysis, and demonstrates its potential usefulness for water quality monitoring.

Key words | beach water, biosensor detection, dead-end ultrafiltration, enterococci, recreational water quality

Stephaney D. Leskinen (corresponding author)

Daniel V. Lim

Division of Cell Biology,
Microbiology and Molecular Biology,
Department of Biology,
University of South Florida,
4202 E. Fowler Ave., SCA 110,
Tampa FL 33620-5200,
USA

Tel.: +1 813-974-1316

Fax: +1 813-974-5175

E-mail: sleskine@cas.usf.edu

Valerie J. Harwood

Division of Integrative Biology,
Department of Biology,
University of South Florida,
4202 E. Fowler Ave., SCA 110,
Tampa FL 33620-5200,
USA

INTRODUCTION

The indicator paradigm states that certain microorganisms, including enterococci, act as sentinels for the possible presence of human pathogens of fecal origin. Standard monitoring techniques for fecal contamination involve culturing indicator organisms, which requires an incubation period of 24 hours or more (US Environmental Protection Agency 2000, 2002). Recreational water users may be exposed to fecal contamination of those waters prior to the posting of beach advisories during the sample processing period. Rapid approaches for assessing microbial water quality are needed to identify fecal pollution events to prevent this exposure.

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Several technologies have recently been developed for rapid detection of microbial targets in complex matrices, including nucleic acid-based and antibody-based procedures (Lim *et al.* 2005). In general, nucleic acid-based detection systems are more sensitive; PCR assays detect as few as 30 cells in a short period (Fode-Vaughan *et al.* 2003; Ibekwe & Grieve 2003; Haugland *et al.* 2005). However, PCR requires samples to be relatively free of inhibitors commonly found in complex matrices, such as environmental waters. Another possible weakness of PCR is its high sensitivity and the chance of amplifying the DNA of other organisms present in the sample, which can result in

false-positive results (Lim *et al.* 2005). Antibody-based biosensor assays have been successfully performed on complex matrices, such as ground beef, apple juice, sprout irrigation waters, talc, chlorinated and chloraminated potable water, river water and beach water (Narang *et al.* 1997; DeMarco *et al.* 1999; DeMarco & Lim 2002; Kramer & Lim 2004; Tims & Lim 2004; Kearns *et al.* 2008; Leskinen & Lim 2008). The following microorganisms have been detected within one or more of these matrices: *Escherichia coli* O157:H7, *Salmonella enterica* Typhimurium, *Bacillus anthracis*, *Bacillus atrophaeus* and enterococci. Limits of detection for these studies were approximately 10^5 CFU ml⁻¹ without pre-enrichment of the samples. Compared with the standard, culture-based methods for these organisms, biosensor-based techniques can accurately detect their presence in hours compared with days or weeks (Feng & Weagant 2002; US Environmental Protection Agency 2002; Andrews & Hammack 2003; Szabo *et al.* 2007).

One limitation to the application of antibody-based biosensor assays for detection of biological analytes is the low ambient levels of targets, which are usually much lower than the 10^5 CFU ml⁻¹ detection limit of the instrument. Consequently, sample concentration is necessary to obtain the number of cells required for biosensor analysis in an appropriate volume (Reynolds 2003; Straub & Chandler 2003; Noble & Weisberg 2005; Kearns *et al.* 2008). Hollow fibre ultrafiltration concentrates cells from water with little increase in total assay time. Several recently developed methods have utilized hollow fibre ultrafiltration to concentrate microbes from water samples (Juliano & Sobsey 1997; Kuhn & Oshima 2001, 2002; Simmons *et al.* 2001; Morales-Morales *et al.* 2003; Hill *et al.* 2005, 2007; Kearns *et al.* 2008; Leskinen & Lim 2008). Parasites, bacteria and viruses are retained within the fibres (Morales-Morales *et al.* 2003), and the material that accumulates can be subsequently removed for further analysis. Concentration of indicator microorganisms, such as enterococci, in recreational waters using a hollow fibre ultrafiltration system reduces the time from collection to detection from days to hours, thereby reducing potential exposure to pathogens. Dead-end hollow fibre ultrafiltration concentration of microorganisms in recreational water coupled to biosensor detection was successfully demonstrated in Florida

(Leskinen & Lim 2008). The water sample is forced through the ultrafilter in a single pass instead of being recirculated through the filter in dead-end filtration. The portable concentration system, recreational dead-end concentrator (Rec DEC), was coupled to a biosensor for detection of enterococci within 2.5 h (Leskinen & Lim 2008).

The Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET) in conjunction with the Southern California Coastal Water Resources Project (SCCWRP) evaluated the ability of newly designed rapid methods to assess fecal contamination risks at Doheny State Beach, Dana Point, California, during the summer of 2007. This work was conducted along with a study of the relationship of human health risks from recreational water use with indicators of fecal pollution. Our goal was to assess the ability of the coupled concentrator-biosensor system to concentrate and then detect enterococci in California recreational waters during the evaluation period. The specific aims of this study were threefold: 1) to determine whether the concentration system could increase the levels of enterococci in the collected retentate compared with those of the ambient waters as was observed in Florida; 2) to determine whether the biosensor's limit of detection for enterococci present in the retentates would match that of the assays performed in Florida; and 3) to determine whether a relationship could be established between the ambient concentrations of enterococci and positive signals from the biosensor from samples collected in California.

METHODS

Location and sample collection

Doheny State Beach is an open marine beach located on the Pacific coast in Dana Point, Orange County, California (Griffith *et al.* 2006). Four locations were sampled at this beach. Site A was located at the end of the jetty separating the beach from the southern edge of Dana Point Harbor. Site B was located at the intersection of the jetty and the beach, while site C was approximately 0.09 km south of site B. Site D was nearly 0.8 km south of Site C. Samples were collected weekly (Saturday or Sunday) between May and July 2007 from each site. Samples were collected at 7 am and at 3 pm on each date (weekly $N = 8$, total $N = 56$).

Samples were collected only on the weekends to increase the likelihood of collecting samples during periods when larger numbers of bathers were present. The two sampling times were chosen as a result of the varying ambient concentrations of enterococci present (i.e. higher in the mornings than the afternoons).

Water was collected from each site into five plastic cubitainers (total 100 l) (Thermo Fisher Scientific, Inc., Waltham, Massachusetts) and immediately transported to the SCCWRP laboratory trailer on the beach near site D. Samples were filtered on-site in the SCCWRP laboratory trailer, held for 16–40 h at 4°C, and were shipped by overnight carrier on ice to the USF laboratory. A 1-l surface grab sample from each site was collected concurrently with the concentrated samples. Grab samples of ambient water were processed by the Orange County Sanitation District Environmental Sciences Laboratory within 7 hours of sampling.

Concentration system and procedure

The design and operation of the Rec DEC has been previously described (Leskinen & Lim 2008). The Rec DEC utilized a new F80A Hemoflow Polysulfone High-flux Capillary Dialyzer (Fresenius Medical Care North America, Lexington, Massachusetts) with a molecular weight cut-off

of approximately 15–20 kDa (Hill *et al.* 2005) for each sample (Figure 1). Water was fed into each filter by a Masterflex I/P Precision Brushless peristaltic pump drive with a Masterflex I/P Easy-Load pump head (Cole Parmer Instrument Co., Vernon Hills, Illinois) through Masterflex I/P BioPharm platinum-cured silicone I/P 70 tubing. Filtration of 100 l volumes occurred in approximately 50 minutes while maintaining an internal system pressure of 25–30 psi. Each filter was held at 4°C for 16–40 h after sample collection before shipment on ice to Florida. All Rec DEC components were cleaned between samples with 10% bleach followed by 10% sodium thiosulfate or with 0.8% Lysol followed by deionized water for metal components.

Filter elution and post-filtration processing

Elution of each filter was performed immediately upon receipt in Florida (24–44 h after collection) as previously described (Leskinen & Lim 2008). Elution was performed using 250 ml of elution buffer (4 M urea/50 mM lysine (pH 9.0)) that was fed into the filter by a Masterflex L/S peristaltic pump through Masterflex L/S 36 gauge Tygon tubing (Figure 1) and allowed to interact with the filter fibres for 2 min. The initial retentate was collected from the filter and sonicated for 60 s at 14 W with the Sonic Dismembrator Model 100 (Fisher Scientific, Pittsburgh,

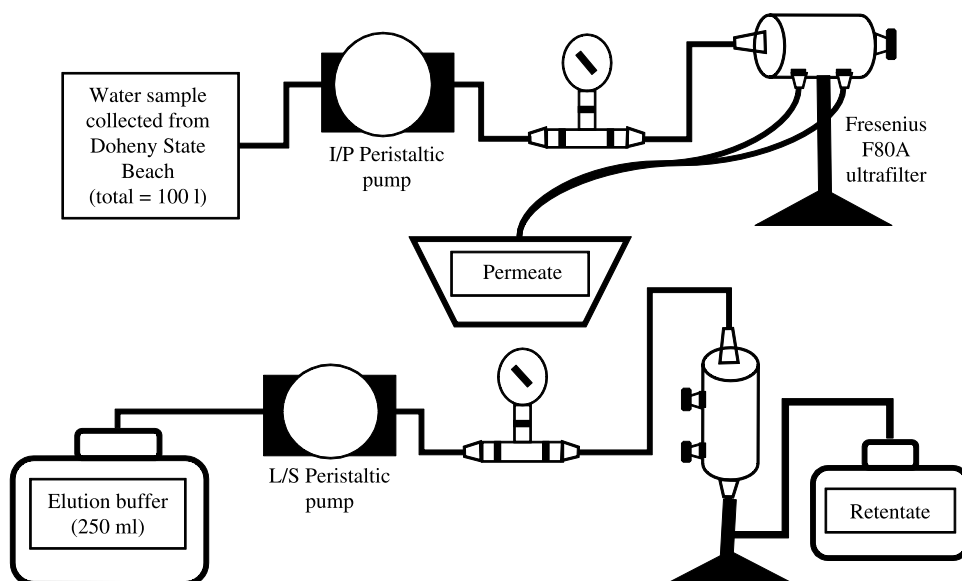


Figure 1 | Schematic of the recreational dead-end concentrator (Rec DEC) in sample collection (top) and sample elution (bottom) modes (adapted from Leskinen & Lim 2008).

Pennsylvania) to disrupt cell attachments to sediment particles. The retentate was poured through stacked stainless steel sieves (pore sizes: 75 μm , 53 μm and 38 μm) to remove particles that could obstruct the microfluidics of the RAPTOR biosensor (Olszewski *et al.* 2005). The flow-through volume was subjected to secondary concentration by centrifugation at $15,180 \times g$ for 5 min at 4°C on a Sorvall Evolution RC Superspeed Refrigerated Centrifuge (Thermo Electron Corporation, Asheville, North Carolina). The supernatant fluid was decanted and the pellet resuspended in 4 ml of 0.1 M carbonate bicarbonate (CBC; 10.59 g Na_2CO_3 and 8.40 g NaHCO_3 per l) buffer (pH 9.3) to produce the final retentate.

Membrane filtration

An aliquot of the final retentate (1 ml) was reserved for membrane filtration to allow comparison with the standard method for recreational water (EPA method 1600), as well as to assess recovery efficiencies and concentration factors. Serial dilutions (1:10) of the final retentates were performed in Dulbecco's phosphate-buffered saline (pH 7.4) and 1 ml of each dilution was filtered through Gelman GN-6 Meticel 47 mm diameter, 0.45 μm pore size membrane filters (Pall Corporation, East Hills, New York). Membranes were placed onto mEI agar and incubated at 41°C for 24 h (US Environmental Protection Agency 2002). All resultant colonies with blue halos were counted as enterococci.

Antibodies

A polyclonal antibody directed against group D streptococci was obtained from American Research Products, Belmont, Massachusetts. The IgG was affinity purified as described previously (Leskinen & Lim 2008). Affinity-purified rabbit polyclonal antibody to *Mycobacterium tuberculosis* was purchased from Meridian Life Science, Inc. (Cincinnati, Ohio) for use in baseline measurements in RAPTOR assays. Lyophilized affinity-purified Cy5-labelled goat anti-rabbit secondary antibody was obtained from Jackson ImmunoResearch (West Grove, Pennsylvania). The lyophilized antibody was rehydrated as described by DeMarco *et al.* (1999).

Bacteria

Enterococcus faecalis ATCC 19433 was utilized in the biosensor assay as a positive control. Cultures were grown for 18 h at 37°C on tryptic soy agar (TSA; BBL, Sparks, Maryland). Cells were removed from the agar surface with sterile swabs and suspended in CBC buffer. Direct counts were performed using a Cellometer (Nexcelom Bioscience, Lawrence, Massachusetts), and the concentration of each cell suspension was adjusted to 1.0×10^8 cells ml^{-1} in CBC buffer for use in biosensor assays.

RAPTOR instrument and waveguide preparation

The RAPTOR evanescent wave biosensor (Research International, Monroe, Washington) was developed by the Naval Research Laboratory (Washington, DC) for lateral-flow detection of biological targets from small sample volumes (i.e. ≤ 2 ml per sample port) (Anderson *et al.* 1994). Target analytes on fibre optic polystyrene waveguides are bound by fluorophore-labelled reporter antibodies, and fluorescent molecules within 100 to 1,000 nm of the waveguide surface are excited by the evanescent field of the laser. A portion of the emission energy recouples into the waveguide and is quantified by the photodiode of the RAPTOR as picoamperes (pA).

Waveguides were cleaned in a sonicating bath containing isopropanol, rinsed with deionized water, and the distal tip was masked with black paint to provide a light dump for the biosensor's laser (Kramer & Lim 2004). Four waveguides were fixed into a coupon with optical glue for each sample. Each coupon containing four waveguides was inserted into the RAPTOR for sample interrogation.

RAPTOR assay of final retentates

Final retentates were analysed for the presence of enterococci with the biosensor assay as described by Leskinen & Lim (2008). Each retentate (125 μl) was incubated in duplicate on waveguides for 10 min at 37°C. Positive and negative control waveguides were included in each assay. The positive control consisted of an aliquot of each final retentate (62.5 μl) mixed with an equal volume of *E. faecalis* 19,433 (1×10^8 cells ml^{-1}). The negative control was sterile

CBC buffer. Each waveguide in a coupon was rinsed ten times with 125 μl of 0.01 M Dulbecco's PBS with 0.1% Tween 20 (PBST) to remove any unbound cells after sample incubation. Each coupon was sealed and inserted into the biosensor.

The RAPTOR ran the following program for baseline readings: an antibody directed against a non-target bacterium was applied to each of the four waveguides within the coupon for 3 min (Leskinen & Lim 2008). The antibody used in this step was the anti-*M. tuberculosis* antibody (1 ml at 10 μgml^{-1} in blocking buffer in 1% BSA and casein (wt/vol) in 0.01 M Dulbecco's PBS). It was chosen for the baseline readings because *M. tuberculosis* cannot grow in marine waters (Jamieson *et al.* 1976), so non-specific binding to the concentrated samples would be limited. Detection antibody (Cy5-labelled antibody at 5 μgml^{-1} in blocking buffer) was incubated on each waveguide within the coupon for 3 min. A baseline reading (pA) was then taken of each waveguide within the coupon. Four baseline readings were taken of each assay coupon and were used as described in the following section.

Detection of enterococci was performed by addition of the group D streptococci antibody, 1 ml at 10 μgml^{-1} in blocking buffer, using the same RAPTOR program to generate the sample readings (pA). A second application of the target antibody was applied to each waveguide to confirm any positive sample readings from the first application.

Calculation of RAPTOR results

A waveguide normalization factor was generated by dividing the emission value for each baseline reading (pA) from the four waveguides within a coupon by the lowest baseline reading within that coupon (Simpson-Stroot *et al.* 2008). This factor was used to minimize variability between waveguides in each immunoassay. Signal normalization was necessary to account for the inherent variability of the fibre optic waveguides that are individually moulded and can vary widely in their baseline readings (Simpson-Stroot *et al.* 2008). Limits of detection (LOD) were calculated for each waveguide after normalization by adding the average of its baseline readings plus three times the standard deviations of the baseline for each waveguide in a coupon.

The LOD was subtracted from the emission value for each sample reading (pA) to achieve the signal above the limit of detection (SALOD). If the SALOD value was greater than zero and larger than the SALOD value from the negative control waveguide (CBC buffer), the sample was considered positive for enterococci. If the SALOD value was less than zero or less than or equal to that of the negative control, the sample was considered negative.

Statistical analysis

Differences in ambient cell concentrations and those in the final retentates, as well as enterococci concentrations from each post-filtration stage, were analysed using nonparametric analysis with the Wilcoxon matched pairs test (GraphPad InStat 3.0, GraphPad Software, Inc., San Diego, California). Log_{10} -transformations of the data were attempted, but did not resolve the non-normal distribution of the data, thus necessitating the use of nonparametric analyses. Statistical significance was accepted at the 95% confidence level ($\alpha \leq 0.05$).

Concentration factors for the final retentates were calculated to quantify the increase in cell concentration compared with ambient grab samples. Similar ratios have been calculated to quantify changes in cell counts following concentration (Carlucci & Williams 1965). The concentration factor for hollow fibre filtration and post-filtration processing is defined as the ratio of the enterococci concentration in the final retentate to that in the ambient water. Differences in log_{10} -transformed concentration factors between sites were analysed using one-way ANOVA with statistical significance accepted at the 95% confidence level ($\alpha \leq 0.05$) (GraphPad InStat 3.0). The recovery efficiency (percentage recovery) of the ultrafiltration procedure was calculated by determining the number of enterococci in the ambient water and that in the final retentate (Hill *et al.* 2005). After the values were determined, the recovery efficiency was calculated by dividing the total number in the final retentate by the total number in the ambient water. The percentage recovery was determined by multiplication of the fraction by 100.

Binary logistic regression was used to compare the signal from the RAPTOR (SALOD values were analysed as 1 for any positive samples and 0 for any negative samples)

with ambient concentrations relevant to regulatory thresholds in California marine waters (≥ 105 CFU ml⁻¹) (SPSS 16.0 for Windows, SPSS Inc., Chicago, Illinois). Nagelkerke *R* square, which can range from 0.0 to 1.0, denotes the effect size (the strength of the relationship); stronger associations have values closer to 1.0. Relationships were considered significant when the *p* value for the model chi square was < 0.05 and the confidence interval for the odds ratio did not include 1.0. Greater odds ratios indicate a higher probability of change in the dependent variable with a change in the independent variable.

RESULTS

Enterococci concentrations in ambient water samples versus cells recovered following ultrafiltration and post-filtration processing

Fifty-six surface grab and 100l concentrated samples from Doheny State Beach were analysed for enterococci by US EPA method 1600. Culturable enterococci concentrations in ambient water samples ranged from 0 to $2.6 \log_{10}$ CFU/100 ml (Table 1; concentrations presented in terms of total CFU/100l). Many of the ambient samples had enterococci concentrations below the limit of detection for method 1600 and were assigned a value of 1 CFU/100 ml

for calculations of concentration factors. Hollow fibre filtration and backflushing increased the concentrations of enterococci by an average of 322-fold compared with levels in the ambient samples (Wilcoxon matched pairs, $P < 0.0001$). Enterococci concentrations following sonication and sieve passage were similar to those in the initial retentate (Wilcoxon matched pairs, $P = 0.14$). Secondary concentration by centrifugation further increased the levels of enterococci by an average of two orders of magnitude (Wilcoxon matched pairs, $P < 0.0001$). The concentration of enterococci in the final retentates ranged from 1.0 to $5.4 \log_{10}$ CFU/100 ml. The levels of enterococci in the final retentate were at least four orders of magnitude higher than those in ambient samples (Wilcoxon matched pairs, $P < 0.0001$).

The extrapolated number of culturable enterococci present in 100l of ambient water ranged from 3.2 to $4.9 \log_{10}$ CFU (Table 1). The total number in 4 ml of final retentate ranged from 2.6 to $4.5 \log_{10}$ CFU. Differences in the concentration factors between sites were not found (ANOVA, $P = 0.75$). The values for the total CFU in 100l of ambient water are calculated extrapolations of concentrations obtained by membrane filtration of 100 ml according to EPA method 1600. Variability in enterococci concentrations is possible between the large (100l) and small sample (100 ml) volumes. Variation in actual concen-

Table 1 | Mean ultrafiltration recovery efficiencies and concentration factors of enterococci from ambient samples

Site*	Time	CFU (\log_{10}) \pm SD Ambient (100 l) [†]	Final retentate (4 ml) [‡]	Recovery (%) [§] \pm SD	Concentration factor (\log_{10})
A	7 a.m.	4.9 \pm 0.50	4.5 \pm 1.10	119 \pm 201	4.5
	3 p.m.	4.4 \pm 0.65	4.0 \pm 0.65	170 \pm 245	4.6
B	7 a.m.	3.9 \pm 0.91	3.5 \pm 0.69	104 \pm 154	4.4
	3 p.m.	3.8 \pm 0.83	2.9 \pm 0.44	37 \pm 103	4.2
C	7 a.m.	3.7 \pm 0.90	3.4 \pm 1.16	195 \pm 290	4.7
	3 p.m.	3.2 \pm 0.49	2.8 \pm 0.45	59 \pm 48	4.1
D	7 a.m.	4.3 \pm 0.73	3.5 \pm 0.59	109 \pm 157	4.4
	3 p.m.	3.3 \pm 0.49	2.6 \pm 0.66	71 \pm 107	4.2

CFU, colony-forming units; SD, standard deviation.

**N* = 7 for each time point.

[†]Extrapolated values from culturable counts as determined by EPA method 1600 (CFU/100 ml) represent the number of culturable enterococci present in the 100 l sample. Example: $100 \text{ CFU}/100 \text{ ml} \times 100,000 \text{ ml}$ (equivalent to 100 l) = 100,000 CFU.

[‡]Extrapolated values from culturable counts of samples processed after shipment represent the number of culturable enterococci present in the 4 ml sample. Example: $1 \times 10^4 \text{ CFU ml}^{-1} \times 4 \text{ ml} = 4 \times 10^4 \text{ CFU}$.

[§]Recovery percentage and concentration factors calculated as described in Leskinen & Lim (2008).

trations of enterococci present in the different sample volumes can lead to samples that are positive by biosensor analysis yet are on the edge of the statutory limit, which is based on small volume samples. Mean recovery efficiencies for each site and time point ranged from 37% to 195% with an overall average of 107%. Recoveries over 100% have been reported by others performing hollow fibre ultrafiltration of known spiked amounts of the target microbes (Hill *et al.* 2005, 2007; Polaczyk *et al.* 2008), as well as previously in Florida using this procedure (Leskinen & Lim 2008).

Detection of enterococci in final retentates

An aliquot of each final retentate was examined with the RAPTOR to determine its ability to detect enterococci in the concentrated California water samples. Positive SALOD values (>0) were obtained when the ambient concentrations of enterococci were higher than the regulatory threshold for a single sample maximum for California marine waters (≥ 105 CFU/100 ml) (California State Assembly 1998). These samples had concentrations of enterococci within the final retentates of at least 10^5 CFU ml $^{-1}$, which is the detection limit of the sensor

assay based on the linear relationship established between signals generated by the RAPTOR when interrogating various concentrations of enterococci suspended in buffer (Leskinen & Lim 2008). SALOD values ranged from 7 to 280 pA when the single sample maximum was exceeded. One sample with an ambient concentration (100 CFU/100 ml) slightly below the single sample maximum produced a positive SALOD value in the RAPTOR assay (20 pA). The final retentate of this sample had the highest cell concentration during the study (150,500 CFU ml $^{-1}$). The concentration process for this sample may have been unusually efficient or the ambient concentration may have been somewhat different from that collected in the cubitainers.

The microbial water quality of the ambient waters was divided into categories based on enterococci concentrations to simplify comparison between RAPTOR assay results and method 1600 results. Poor quality waters were defined as having concentrations of ≥ 105 CFU/100 ml, while moderate and good quality waters were defined as having 36–104 CFU/100 ml and ≤ 35 CFU/100 ml, respectively. No positive signals were recorded for the 43 samples with good water quality, while one moderate water sample was positive as previously mentioned (Figure 2). The ambient

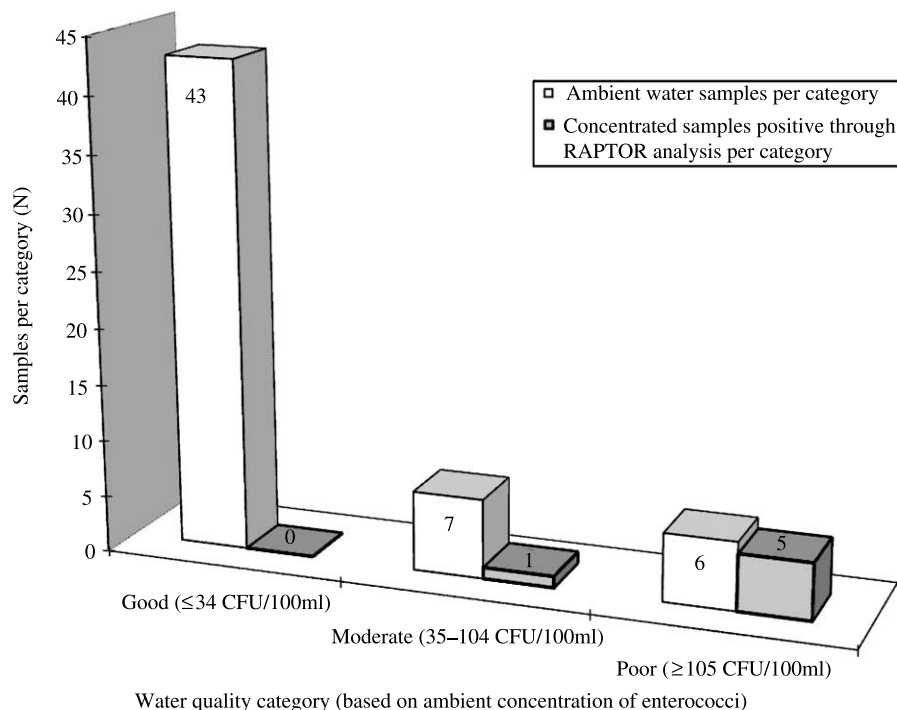


Figure 2 | RAPTOR signals above the assay limit of detection (SALOD) vs. water quality categories (based on the ambient concentrations of enterococci) at Doheny State Beach.

concentration of enterococci for this sample was 100 CFU/100 ml, however. The false positive sample was collected from site C at 7 am. Five of six poor water quality ambient samples were positively identified as such within the concentrated sample by the RAPTOR assay. Ambient samples that exceeded the single sample maximum and were designated as positive by the RAPTOR assay were collected at 7 am from sites A (5) and B (1).

The sensitivity (calculated as the percentage of true positives versus all positive samples) and specificity (calculated as the percentage of true negatives versus all negative samples) of the assay were determined by comparing the RAPTOR data with the number of samples in breach of the regulatory standard (Figure 2). The specificity of the RAPTOR assay (positive and negative samples adjusted to 1 or 0, respectively) following the concentration procedure was 98%. Removal of the one false positive sample from the moderate water quality category (100 CFU ml⁻¹) would increase the specificity of the assay to 100%. Approximately 11% (6) of the ambient water samples exceeded the California monitoring standard and the sensitivity of the RAPTOR assay for those few samples was 83%.

No relationship was observed between the actual SALOD values and concentrations of enterococci in the ambient water as was observed previously with concentrated samples (Leskinen & Lim 2008). A relationship was established between detection of enterococci with the RAPTOR and ambient concentrations relevant to regulatory thresholds for California waters using binary logistic regression (Nagelkerke *R* square = 0.819, *P* < 0.001). A similar relationship was observed in Florida (Leskinen & Lim 2008).

DISCUSSION

There is a need for rapid methods for identification of fecal pollution events to minimize exposure of the public to microbial pathogens in recreational waters. Various procedures have been developed recently in an attempt to meet this need that would permit 'same-day' warning systems. Ultrafiltration of California marine water was linked to an immunoassay-based biosensor in this study. Detection with the combined procedure occurred when enterococci

concentrations in the ambient water exceeded the regulatory standard for a single sample (≥ 105 CFU/100 ml), corroborating results from the Gulf of Mexico samples (Leskinen & Lim 2008). The main advantage of this technology over culture-based methods is the speed at which the results are provided. Detection of enterococci required 2.5 h compared with 24 h for traditional membrane filtration methods (US Environmental Protection Agency 2002). Detection of target analytes in complex matrices, such as the marine water sampled in this study, without interference from background materials, has been accomplished in previous biosensor immunoassays (Lim *et al.* 2005). The same cannot be said for many of the rapid molecular techniques, including PCR, that require a sample free from inhibitors. Furthermore, unlike the molecular techniques, RAPTOR analysis following hollow fibre ultrafiltration does not rely on amplifying captured targets (i.e. replication of DNA). This aspect of the procedure is unique with respect to the monitoring of recreational waters for indicator bacteria. The ultrafiltration-biosensor procedure measures captured organisms directly from large volumes of water, rather than relying on amplification of a small number of targets.

The ability to monitor such large volumes (i.e. 100 l) of recreational water is another advantage of the procedure compared with the standard methodology and the rapid molecular techniques. More representative assessments of recreational microbial water quality are possible with the combined ultrafiltration-biosensor methodology that would better protect public health and the designated uses of recreational waters. Although on-site processing of the samples was not possible in this study because of the logistics involved in the large, multi-organizational CICEET project, the ability to concentrate large samples on location has been demonstrated (Leskinen & Lim 2008). The large volume of water processed with the Rec DEC-RAPTOR procedure provides a more complete picture of the true water quality at the beach at a given time than does a smaller volume (e.g. 100 ml for method 1600 and for membrane filtration followed by QPCR) (Straub & Chandler 2003; Noble & Weisberg 2005; Stewart *et al.* 2008). While the concentration-biosensor system would be more expensive during initial implementation owing to equipment costs, it would

provide a more rapid, representative determination of microbial water quality.

Assessment of recreational waters for pathogens, not simply enterococci or other indicators, could be accomplished through the combined procedure of dead-end ultrafiltration and biosensor detection. Direct detection of microbial pathogens in the environment is possible because of their simultaneous concentration with hollow fibre ultrafiltration (Juliano & Sobsey 1997; Morales-Morales *et al.* 2003; Hill *et al.* 2005, 2007). Direct pathogen detection in recreational waters would alleviate problems associated with indicator organisms, such as false positives from possible regrowth in sands and sediments (Shibata *et al.* 2004). Furthermore, large volumes would be needed for detection of pathogens due to their low concentrations in ambient waters. Other filtration methods for pathogens of fecal origin require the collection of large volumes of water and have been validated by the US Environmental Protection Agency (1999). Recently developed immunoassay-based biosensor technologies have the ability to simultaneously detect the presence of multiple target microbes within a single sample that would allow for multiplex detection of viral, bacterial and protozoan pathogens (Taitt *et al.* 2004; Sapsford *et al.* 2006).

The overall concentration factors for this study are slightly lower than previously reported (Leskinen & Lim 2008). Concentration factors were calculated by comparing culturable enterococci present in the ambient samples as assessed by EPA method 1600 with those in the final retentate. The extended storage times required for this study are most likely to have led to the reduced culturability of the cells within the filter, which would have caused the calculated concentration factors to be lower than they may have been if the retentates could have been recovered sooner. Lang *et al.* (1999) reported that nearly half of the strains of enterococci evaluated lost culturability in apple cider under refrigerated storage after two days. Although the storage medium differs (marine water versus apple cider) the die-off of nearly $3.0 \log_{10} \text{CFU ml}^{-1}$ within the cider over the same storage period as in this study points to the probable loss of enterococci within the marine water remaining in the filter cartridge during storage and transport. Moreover, die-off of enterococci in natural river water

mesocosms maintained at 5°C has been observed (Medema *et al.* 1997). Regardless of the culturability of the enterococci within the final retentate due to extended storage at refrigerated temperatures, the RAPTOR assay was able to detect the presence of the cells in samples with ambient concentrations above California regulatory standards. Immunoassays can detect viable and non-viable cells, so that any variability in culturable counts could be negated to allow detection of enterococci in poor quality water (Stopa 2000; Zhang *et al.* 2006).

CONCLUSIONS

The research described in this study confirms that the dead-end hollow fibre ultrafiltration process can be successfully performed for rapid recreational water quality monitoring in California marine waters just as it was in Florida marine waters (Leskinen & Lim 2008). It confirmed that a specific target of interest, enterococci, could be recovered from the filter in a concentration two orders of magnitude higher than in the ambient water. The concentration of enterococci after ultrafiltration and post-filtration processing was sufficient for rapid detection of the target analyte, which was well-correlated with the regulatory standard for poor water quality. These data, coupled with our Florida study (Leskinen & Lim 2008), demonstrate the potential of the Rec DEC-RAPTOR procedure for rapid water quality monitoring in marine recreational waters.

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