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1. Introduction

The KwaZulu Natal Bight is formed from a narrow indentation of the south-east coast of South Africa, with a shallow continental shelf extending to the 100m shelf break 45 km offshore at its widest point. The waters of the bight are largely derived from the southward flowing Agulhas Current, composed of aged, highly oligotrophic sub-tropical surface water of the Indian Ocean (Lutjeharms, 2006). The strong southward flow of the current impacting on the shelf results in three hydrological effects which cause episodic upwelling of deep, nutrient rich water onto the bight: a persistent eddy in the south, across-shelf upwelling at the shelf-break and a relatively persistent upwelling cell in the north all advect deep, nutrient rich water to the surface (ibid.). In addition, riverine inputs of allochthonous material are important sources of nutrient, particularly during the summer wet season. World-wide, oligotrophic tropical and sub-tropical waters have been shown to have low primary productivity and food chains in the euphotic are shown to be heavily dependent on recycling of old nutrients through the microbial loop, and also rapidly responsive to new nutrient input (Karl, 2007).

Due to the importance of the link between nutrient status, phytoplankton productivity and heterotrophic bacterial activity it is essential to acquire data on the status of the bacterial populations to truly understand ecosystem functioning. Here, we have quantified bacterial numbers, biomass and productivity across the Bight during the summer (wet) and winter (dry) seasons and correlated them with nutrient status and phytoplankton biomass.

2. Methods

i. Bacterial numbers and biomass.

Two research cruises were undertaken in January and September 2010 during the wet and dry season respectively. For each cruise, sea water samples were taken at pre-determined locations within the Bight during a synoptic survey (see Fig 1) over a period of 10 days. During summer, samples for direct counting were taken at 61 stations. During the winter cruise, these were reduced to 24 stations since (Fig. 2) winter samples served not only to assess seasonal differences but also to optimise and verify flow cytometry samples which were determined across the bight (and are reported elsewhere). Samples were taken with a Rosette sampler at depths determined by in situ fluorimetry: close to the
water surface, at the \( f_{\text{max}} \) and between the bottom and \( f_{\text{max}} \). 20ml samples were preserved with Borax-buffered seawater formaldehyde (4% f.c). In the laboratory, bacterial numbers and biomass were assayed by epifluorescence microscopy using DAPI as a fluorochrome, following standard methods (Porter and Feig, 1980) and an automatic counting assessment based on modification and optimisation of Image-Pro plus digital analysis programs. Numbers and biomass distributions were analysed and displayed using Ocean Data View.

ii. Bacterial productivity.

Bacterial productivity was assessed by uptake and incorporation of \(^3\text{H}\)-Thymidine into macromolecules, following the methods of Smith and Azam, (1992). Four stations were established on each cruise (see Fig 2 a, b.) and heterotrophic bacterial productivity was determined in samples taken at the surface, at the \( f_{\text{max}} \) and between the bottom and the \( f_{\text{max}} \). These were repeated on two days. Three experimental 1.8 ml samples were dispensed into 2ml microfuge tubes preloaded with \(^3\text{H}\)-Thymidine (2.0nM final conc.). A control for each, preloaded with \(^3\text{H}\)-Thymidine and 100µl conc trichloracetic acid, was also established. Samples were incubated, in the dark, at in situ temperature in a running water bath for approximately 5h. Uptake was then stopped by addition of 100µl conc trichloracetic acid, and the samples were stored frozen until analysis. Precipitated macromolecules were pelleted by centrifugation and supernatants discarded. The pellet was washed with 5 % trichloracetic acid. The pellet was then suspended in scintillation fluid and radioactive content determined in a Packard TriCarb scintillation counter.

3. Results

Numbers and biomass distribution for 3 depths are presented in Figs. 3 a-f (Summer wet season) and Figs. 4 a-f (Winter dry season). Bacterial productivity data are presented in Figs. 5 a (summer) and b (winter).

i. Bacterial numbers and biomass: Summer/Wet season.

Numbers and biomass are in general higher the euphotic zone, and there is a marked decrease in numbers below the \( f_{\text{max}} \). To the north-east and south-west, there are large areas distinguished by low bacterial numbers (<5 x 10^4 cells.ml\(^{-1}\)) and biomass which extend from the surface waters to depth. There are small, similar patches along the shelf-break. These low numbers and biomass are typical of the deepest waters sampled, where numbers may fall to < 1 x 10^3 cells.ml\(^{-1}\).

Areas of higher bacterial numbers (>5 x 10^5 cells.ml\(^{-1}\)) and biomass are noted in association with river mouths the coast, particularly close to the inflow of the Mdvoti and Tukhela rivers, and also over the Tukhela Banks. A small area of high numbers/biomass also occurred immediately off Cape Vidal.
ii. Bacterial numbers and biomass: Winter/Dry season.

Numbers and biomass in the euphotic zone were lower than those recorded in summer and similar at all depths sampled (< 0.5-1 x 10^5 cells.ml^-1). Bottom samples remained similar to those determined earlier.

iii. Heterotrophic bacterial productivity: Summer/Wet season

The highest bacterial productivity recorded (>7.5µgC.L^-1.d^-1), was associated with a transient mid-shelf phytoplankton bloom (Fig. 5a). It was evident that this was an ephemeral event: productivity at the same location the following day was much lower (2-4 µgC.L^-1.d^-1) and typical of productivity recorded at other areas of the bight (Richard’s Bay north, Tukhela Mouth: Fig. 5 a). Waters of the Durban Eddy (Fig.5 a) were characterised by lower productivity (<0.5 µgC.L^-1.d^-1).

iv. Heterotrophic bacterial productivity: Winter/Dry season

Heterotrophic productivity was, overall, low during the winter/dry season (Fig.5 b), and across the bight did not exceed 0.4 µgC.L^-1.d^-1 even in surface waters, which is similar to that found in Durban Eddy waters and at depths below the Pmax during the summer.

4. Discussion

These numbers and biomass data are similar to those recorded for comparable waters elsewhere, such as the tropical Pacific (Landry and Kirchman, 2002; sub-tropical Pacific (Jones et al., 1996) and Kenya (Goosen et al., 1997). Productivity, however, is low compared to other systems, and appears most similar to highly oligotrophic systems such as the North Pacific gyre (Jones et al., 1996) and the northwest Indian Ocean (Wiebinga et al., 1997).

We noticed that patterns of distribution and activity were strongly influenced by hydrodynamic events: in summer, for example, lowest numbers, biomass and activity occurred at the Durban Eddy site, along the shelf break and offshore of Cape Vidal, all of which may be influenced by newly upwelled water. Bacterial activity is also strongly influenced by riverine allochthonous inputs (high numbers, biomass and productivity associated with the Mvoti-Tukhela coastal strip) and phytoplankton activity (higher productivity associated with plankton blooms on the mid-shelf and over the Tukhela Banks).

It is evident that there is also a strong seasonal influence on the heterotrophic bacterial populations, particularly in the euphotic zone where numbers and biomass fell during the winter dry season across the bight, and productivities were universally low, equalling the lowest recorded during the summer wet season. These decreases are probably correlated with lower phytoplankton biomass and productivity, as well as with reduced allochthonous inputs from the rivers.
5. References


Figure 1: Sampling stations, summer (synoptic and focus)

Figure 2: Sampling stations, winter (synoptic and focus)
Fig. 3. Bacterial numbers (a-c, cells.ml$^{-1}$) and biomass (d-f, gC.ml$^{-1}$), summer (wet season). 

a=surface, b=F$^{\text{max}}$, c=intermediate (between F$^{\text{max}}$ and bottom).
Fig 4. Bacterial numbers (a-c, cells.ml\(^{-1}\)) and biomass (d-f, gC.ml\(^{-1}\)), winter (dry season). a=surface, b=F\(^{\text{max}}\), c= intermediate (between F\(^{\text{max}}\) and bottom).
Fig. 5. Bacterial productivity (cells.l$^{-1}$.d$^{-1}$; µgC.l$^{-1}$.d$^{-1}$) at named sites measured by uptake and incorporation of $^3$H-thymidine. A: Summer (wet season) B: winter (dry season). - - - =Day 1, ■ ■ ■ = Day 2.
PAPER 2. Brief overview.

Bacterial numbers and biomass distribution across the KwaZulu-Natal Bight during the winter dry season, determined by flow cytometry: a comparison with the standard DAPI epifluorescence technique.

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1. Introduction

Epifluorescent microscopy is a well known method for assessing counts of microscopic material (Porter and Feig, 1980). It is relatively cheap, requires only a basic working knowledge of a light microscope, but it is very time consuming, which limits determinations to single runs of samples. We have used it to determine numbers and biomass of bacteria on the KwaZulu-Natal Bight during the wet season and dry season (data above).

Flow cytometry, however, has elsewhere proved to be a more accurate method of determining bacterial numbers and biomass, and has been used as a means for fast, reliable and accurate counts of microscopic material (Marie et al., 1997; Vives-Rego et al., 2000). The rapidity of the method allows for replicate counts to be made with ease. However, flow cytometry equipment and reagents are expensive and the method requires specialized training, not only in the use of the equipment, but also in the interpretation of the output. Flow cytometry, however has the further advantage that it can discriminate particles to the level of viruses (Duhamel and Jacquet, 2006) and also that it allows a discrimination of heterotrophic and autotrophic prokaroplankton, which direct counting does not. Here, we compare analysis of samples for total bacterial counts and biomass, taken during the dry season ACEP cruise by both methods, and use flow cytometry results to describe the distribution of bacteria across the bight.

2. Methods

Samples were collected at 21 stations during the dry season ACEP cruise as described before for epifluorescent direct counting, but a second set of samples covering 40 stations were collected for analysis by flow cytometry. These were either fixed with formaldehyde for epiflorescent microscopy or with glutaraldehyde and stored in liquid nitrogen for flow cytometric analysis. Direct, epifluorescent count methods are described elsewhere. Flow cytometry has been completed and data are currently in analysis.
3. Results/Expected outcomes

We expect that the flow cytometer will yield higher and more accurate counts of bacterial numbers and biomass with the waters tested, but that distribution patterns already shown by direct count will be very similar. We are also hopeful that we can obtain accurate counts for two sets of marine viruses that are evident in the initial flow data. Please note that this work is coupled to flow cytometry of autotrophic picoplankton (Prochlorococcus, Synechococcus, pico-eukaryotes), which is presented as a separate set of papers.

4. References


An image-analysis based method for automated direct counting of bacteria.

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1. Introduction

Current direct counting methods usually involve staining bacterial cells with a fluorochrome dye (such as DAPI), visualizing them under UV light microscopy and manually counting from digitised images. However, not only is manual counting very subjective, particularly if the operator is assigning cells to size classes for biomass determination, but it is very time consuming, tedious, and monotonous, which militates against accuracy and repeatability. A further problem lies in the need to assess through 3-dimensional space: cells may be missed unless the operator physically scans through all planes of focus (filters seldom lie perfectly flat, for example) and captures a single image for each. Automatic counting methods on the other hand, while relying on the same digitised images, are complex to set up and run and particularly difficult to optimise, but produce standardised, unbiased and repeatable counts very rapidly once they have been properly set up.

2. Methods

Available commercial and web-based macros were reviewed, and a new set of macros were written for Image Pro Plus, to automatically stack images derived from several planes of focus, as well as to count and size bacterial cells depending on the pre-set limits. Macros were also written for MS Excel to automatically analyse the data.

3. Results/Expected outcomes

We will present data comparing counts made by eye and by automatic assessment. We believe the automatic counting method devised above is more accurate, less prone to operator error and less time consuming than manual counting. We also present, as supplementary material, macros which other users of Image-Pro Plus can copy to perform the same operations as we have here.

4. References


