

# Response of microphytobenthos to flow and trophic variation in two South African temporarily open/closed estuaries

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## Abstract

Variations in microphytobenthic biomass were investigated in relation to major environmental factors in the Mdloti and Mhlanga temporarily open/closed estuaries (TOCEs), South Africa, from March 2002 to March 2003. Both estuaries receive different volumes of treated sewage waters. This has caused eutrophication and an increase in the frequency of mouth opening, particularly at the Mhlanga. No significant differences were found in microphytobenthic chl *a* concentrations between the two estuaries. At the Mdloti, microphytobenthic biomass varied considerably, with values ranging from 1.33 to 131 mg chl *a* m<sup>-2</sup> during the open phase, and from 18 to 391 mg chl *a* m<sup>-2</sup> during the closed phase. At the Mhlanga, microphytobenthic biomass ranged from 7.0 to 313 mg chl *a* m<sup>-2</sup> during the open phase, and from 1.7 to 267 mg chl *a* m<sup>-2</sup> during the closed phase. Unlike in the Mdloti, the higher microphytobenthic biomass values at the Mhlanga were not always associated with the closed mouth state. For the duration of the study, the Mdloti functioned as a typical temporarily open/closed system, with prolonged open and closed phases, while the Mhlanga behaved more like a permanently open estuary than a TOCE (it lacked a period of prolonged mouth closure).

**Keywords:** freshwater inflow; microphytobenthic biomass; nutrients; South Africa; temporarily open/closed estuary.

## Introduction

Microphytobenthos is an important autotrophic component of estuarine systems. This is due primarily to its role in the trophodynamics of estuarine food webs, not only as a carbon source, but also as an important component of nutrient cycles, as well as a sediment stabiliser via the

secretion of exopolymers (Delgado et al. 1991, Rizzo et al. 1992, MacIntyre et al. 1996, Miller et al. 1996, Underwood et al. 2004). In these ways, microphytobenthos exerts its effect over a range of different scales going from the thin sediment layer, where it is active, to the scale of whole estuaries, where it is able to influence the morphology of the coast.

There are approximately 250 functional estuaries on the South African coastline of which 71% are temporarily open/closed systems (Whitfield 1992, Cooper et al. 1999). Here, a functional estuary is defined as a partially enclosed coastal system that receives freshwater input from at least one river and exchanges its water with the marine environment, although not necessarily on a frequent basis (*sensu* Whitfield 1992, 1995). Temporarily open/closed estuaries (TOCEs) are greatly influenced by highly seasonal river flow, wave conditions and rainfall patterns (Schumann et al. 1999). This results in an intermittent opening to the sea that varies in terms of frequency, timing and duration. In KwaZulu-Natal, TOCEs are generally closed in winter (May to October) and open in summer (November to April) (Perissinotto et al. 2003). The closed phase is characterised by relatively low freshwater inflow from the catchment area and the absence of tidal influences, ultimately reducing both sediment disturbance and turbidity, and increasing water clarity. As a result of the increased water clarity, a large percentage of surface irradiance is able to reach the sediment, providing a favourable environment for microphytobenthic growth (Wooldridge and McGwynne 1996, Nozais et al. 2001, Perissinotto et al. 2002, Perissinotto et al. 2003).

At the onset of the rainy season, the water level inside the estuary may rise suddenly or gradually until it exceeds the height of the sandbar at the mouth (Whitfield 1992). When the height of the water column exceeds that of the sandbar, breaching occurs, releasing large volumes of water into the ocean. As a result, large areas of substratum, which may have been submerged for long periods and colonised by a rich community of algae and animals, are exposed (Perissinotto et al. 2000). This process is often very dramatic and can empty virtually the entire estuary within hours to a few days (Begg 1984, Cooper et al. 1999).

With a growing human population, TOCEs are now under unprecedented pressure and are susceptible to environmental degradation. For instance, increases in sewage discharge rate, retention of freshwater by dams for industrial, agricultural and domestic purposes (i.e., both leading to unnatural inflows) and artificial breaching are thought to impact most heavily on the ecosystem structure and functioning of TOCEs (Morant and Quinn 1999). There is, therefore, an urgent need to increase our knowledge of these peculiar estuarine systems.

Until recently, most information on TOCEs dealt with taxonomic, recruitment and stock assessment studies of

macro-organisms, essentially for economic and conservation purposes (Begg 1978, de Villiers et al. 1999). During the past few years, however, there has been a shift towards a deeper understanding of the trophic functioning of these complex ecological systems. Aspects of the dynamics of phytoplankton, microphytobenthos, zooplankton and meiofauna inhabiting TOCEs have been documented (Nozais et al. 2001, Kibirige et al. 2003, Kibirige and Perissinotto 2003a,b, Mundree et al. 2003, Perissinotto et al. 2003, 2004, Nozais et al. 2005).

In particular, it has been reported that microphytobenthic biomass is higher in TOCEs than in permanently open estuaries (Adams and Bate 1999, Snow et al. 2000a,b). A general trend in TOCEs seems to suggest an association of high microphytobenthic biomass with the closed phase of the estuary (Adams and Bate 1999, Nozais et al. 2001, Perissinotto et al. 2003). Microphytobenthos is capable of taking advantage of favourable environmental conditions which prevail in TOCEs, particularly during the closed phase. These include low turbidity and current speed, a more stable sediment and salinity environment, as well as a larger potentially available nutrient pool within the substratum (Adams and Bate 1999, Schumann et al. 1999, Nozais et al. 2001, Perissinotto et al. 2003). A decline in microphytobenthic biomass is generally observed during the open phase. This is likely due to the high turbidity and frequent flushing/scouring that occurs, particularly at the onset of the open phase (Nozais et al. 2001). In TOCEs, it is not unusual for the microphytobenthic standing stock to equal that of the vertically integrated phytoplankton biomass in the overlying water, often exceeding it by 1–3 orders of magnitude (Nozais et al. 2001, Perissinotto et al. 2002, Thomas et al. 2005).

This study originated from preliminary assessments of the ecological state and freshwater requirements of the Mdloti and the Mhlanga estuaries, two typical TOCEs situated on the north coast of KwaZulu-Natal (Figure 1). With a catchment area of 560 km<sup>2</sup> (Grobler 1987), the Mdloti is about five times larger than the Mhlanga (118 km<sup>2</sup>, Cooper 1991). There are no dams present in the catchment of the Mhlanga, while at the Mdloti, the Hazelmere Dam is located approximately 20 km upstream from the mouth (Begg 1978). Presently, the eThekweni Municipality is running sewage plants, which discharge 28 000 m<sup>3</sup> d<sup>-1</sup> treated sewage effluents into these systems (W. Pfaff, eThekweni Municipality, pers. comm.). The much smaller Mhlanga receives three times more sewage effluent (20 000 m<sup>3</sup> d<sup>-1</sup>) than the Mdloti (8 000 m<sup>3</sup> d<sup>-1</sup>). This increases the natural flow of the two estuaries by 0.23 m<sup>3</sup> s<sup>-1</sup> and 0.092 m<sup>3</sup> s<sup>-1</sup>, respectively (W. Pfaff, eThekweni Municipality, pers. comm.). Being under pressure from urban development in their catchments, it is expected that the sewage discharge rate at both estuaries will increase drastically in the future.

The alterations in flow regime and trophic state caused by these discharges in the two estuaries have already been linked to substantial changes in the dynamics of their pelagic subsystem. For instance, much denser phytoplankton blooms but lower zooplankton biomass have been observed at the Mhlanga, compared to the Mdloti (Thomas et al. 2005, Kibirige et al. in press). Shifts in the

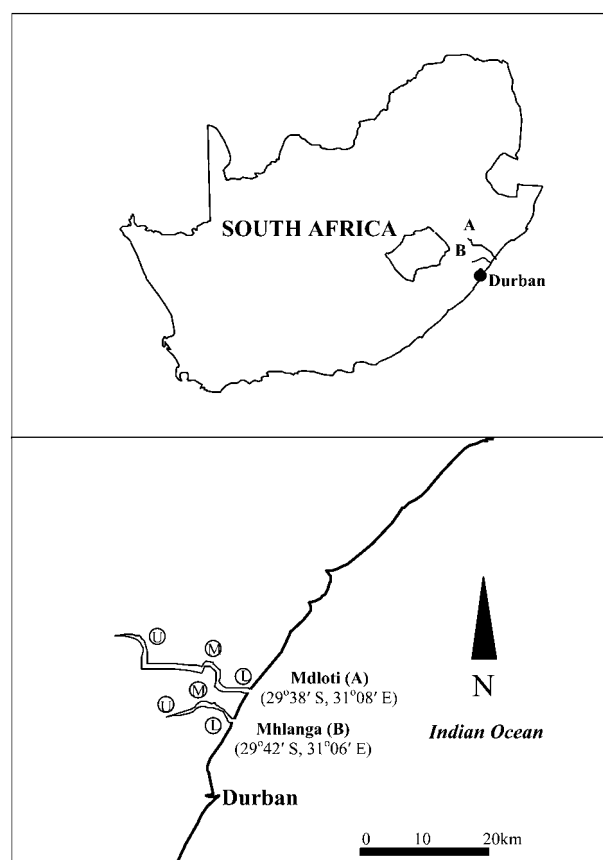
pelagic community structure have also been shown, with a decrease in the smaller size fractions in the most heavily impacted Mhlanga Estuary (Kibirige et al. in press).

Therefore, the overarching aim of this study is to investigate the extent to which the benthic subsystem of the two estuaries may also have diverged in response to the different inflows of treated waters. Specific aims of this study are: (1) to compare the microphytobenthic biomass of the Mdloti and the Mhlanga, with emphasis on freshwater inflow, alternation of closed and open phases and trophic status; and (2) to determine spatial and temporal trends in microphytobenthic biomass within each estuary in response to key physico-chemical factors.

## Materials and methods

### Study area

This study was conducted in the Mdloti (29°38' S, 31°08' E) and the Mhlanga (29°42' S, 31°06' E) subtropical TOCEs. At each estuary, three stations were sampled at regular monthly intervals, from March 2002 to March 2003. These included the lower reaches, on the seaward boundary, the middle reaches, approximately mid-way estuary and upper reaches, at the river boundary (Figure 1). Survey dates were predetermined to coincide with the middle of a neap tidal cycle, in order to minimise tidal effects during the open phase.



**Figure 1** Location of Mdloti (A) and Mhlanga (B) estuaries showing the positions of the sampling stations. L: lower, M: middle and U: upper reaches.

## Physico-chemical variables

On each occasion and at each station, vertical profiles of salinity (psu) and temperature (°C), were recorded using a YSI 6920 Water Logger (YSI, Yellow Springs, USA). A vertical profile of downwelling irradiance (PAR, 400 to 700 nm) was also measured with a LI-COR LI-189 underwater spherical quantum sensor (LI-COR, Lincoln, USA). Light levels were measured every 50 cm down to the bottom. The diffusive attenuation coefficient,  $K_d$  ( $m^{-1}$ ), an inherent optical property of the water, was then estimated using the relation:  $K_d = -\ln(I_z/I_{z_1})/Z_2 - Z_1$ , where  $I_z$  = irradiance ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at depth  $Z_2$  (m),  $I_{z_1}$  = irradiance at depth  $Z_1$  ( $Z_2 > Z_1$ , i.e.,  $z$  increases positively downwards) (Kirk 1994). Additionally, irradiance at the sediment surface,  $E_z$  (i.e., the light available to the microphytobenthos) was approximated using the Lambert-Beer law:  $E_z = E_0 \exp^{-K_d z}$ , where  $E$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) is the light intensity at a given depth  $z$  (m),  $E_0$  is the light intensity at the surface and  $K_d$  is the diffusive attenuation coefficient ( $m^{-1}$ ) (Kirk 1994).

Daily precipitation data (mm) were provided by the South African Sugar Association Experiment Station, Mount Edgecombe. Ezemvelo KZN Wildlife and independent residents in the area provided daily observations on the state of the mouth.

Pore water samples for the measurement of dissolved inorganic nitrogen (DIN; nitrate+ammonium), and dissolved inorganic phosphorus (DIP; orthophosphate), were collected using a hand operated vacuum pump extractor connected to a stainless steel closed pipe inserted in the sediment (5–10 cm below the sediment-water interface). At the lower tip of the pipe, tiny holes of 500  $\mu\text{m}$  in diameter were drilled to ensure the inward flow of pore water, while minimising the passage of fine sand and silt particles. Pore water samples were then placed in 500 ml acid pre-washed polyethylene bottles. Concentrations of pore water DIN and DIP were determined in triplicate samples from each site by the Analytical Laboratory of the CSIR-Durban, using a Technicon autoanalyzer II system (Technicon, New York, USA) and following the methods of Mostert (1983).

## Microphytobenthic biomass

Microphytobenthic biomass was estimated as chlorophyll *a* (chl *a*) concentration. Triplicate core samples were taken on each occasion at each station using a clear acrylic plastic twin-corer of 20 mm internal diameter. The upper 1 cm of the sediment was collected and placed into a 100 ml polyethylene bottle with 30 ml of 90% acetone. Pigments were extracted over 24–48 h at 4°C in the dark. After extraction, all chl *a* concentrations readings were taken using a Turner 10-AU fluorometer (Turner Designs, Sunnyvale, USA) fitted with the narrow band, non-acidification system of Welschmeyer (1994). This system allows for precise measurements (maximum 10% error) of chl *a* without interference from other photosynthetic pigments or their degradation products, using a filter combination of 680 nm for emission and 436 nm for excitation. Phaeopigments were also measured separately using the combination of 680 nm for emission and 405 nm for excitation (www.turnerdesigns.com).

**Table 1** A summary of the analytical design, with replication and independent and dependent variables used in the study of the Mdloti and Mhlanga estuaries, March 2002 to March 2003.

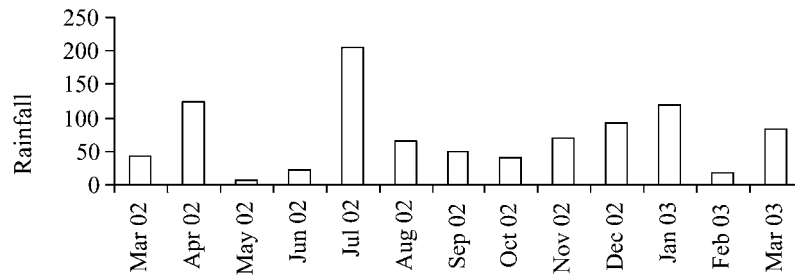
Independent variables	Dependent variables
Time (sampling date)	Microphytobenthic biomass*
Space (lower, middle and upper reaches)	Zooplankton biomass*
Mouth state (open and closed phases)	Depth $K_d$ $E_z$ Rainfall Temperature Salinity Pore DIN* Pore DIP* Pore N:P*

\* Triplicate measurements.

The ratio of phaeopigments to chl *a* concentration was also determined using triplicate sample data. This ratio has previously been used as an indicator of the physiological or grazing state of microalgal communities (Shuman and Lorenzen 1975). High ratios (>0.5) represent a stressed or declining community, while low ratios (<0.5) indicate an actively growing community relatively free of grazing pressure (Bidigare et al. 1986, Light and Beardall 1998, Brotas and Plante-Cuny 2003).

## Statistical analyses

A summary of the analytical design, with a full list of dependent and independent variables used in the study, is provided in Table 1. Prior to analysis, data normality and variance homogeneity were assessed using the Kolmogorov-Smirnov goodness of fit and Cochran's tests, respectively (Zar 1999). When required, data were  $\log_{10}$ -transformed to comply with the assumptions of the various parametric tests employed. Whenever data could not be normalised and/or variance homogenised through transformation, non-parametric tests were employed. Temporal and spatial variations in physico-chemical variables at each estuary were determined using a 1-way ANOVA. An ANOVA was also performed to compare microphytobenthic chl *a* concentrations between the Mdloti and the Mhlanga estuaries. Differences in microphytobenthic chl *a* concentrations between the open and closed phases and between the different reaches of each estuary were examined using a 2-way ANOVA. Where significant differences were detected, a Tukey HSD *post-hoc* test was applied to determine the extent of the variation. Mann-Whitney *U*-tests were performed to investigate potential differences in the physico-chemical and biological variables between estuaries and within each estuary, during its open and closed phase. These analyses were done using the Statistica 5.5 (StatSoft Inc, Tulsa, OK, USA) package. Multivariate correlation analysis between microphytobenthic biomass and physico-chemical and biological variables were performed using the BIOENV routine of the PRIMER v 5 (Plymouth Routines in Multivariate Ecological Research, Plymouth, UK) statistical package. Similarity matrices were generated from Bray-Curtis similarities on untransformed and non-



**Figure 2** Monthly rainfall data (mm) for the Mdloti and Mhlanga estuaries during the study period (March 2002 to March 2003).

standardised data. Zooplankton biomass data used in the correlation analysis were obtained from Kibirige et al. (in press).

## Results

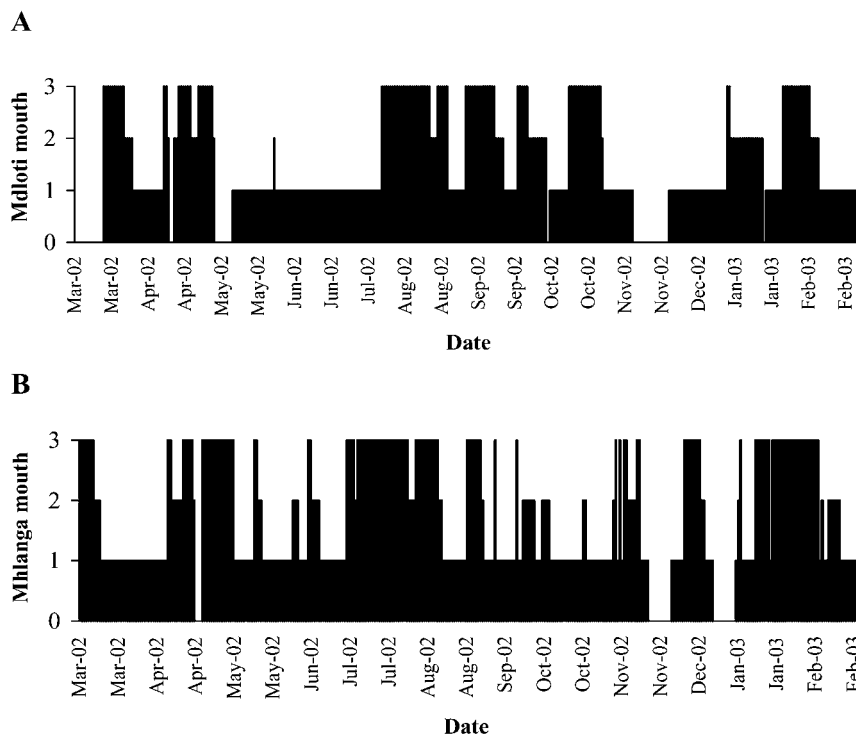
### Physico-chemical environment

An unusual erratic rainfall pattern occurred during the study period (Figure 2). The total rainfall for the year of sampling amounted to 938 mm, with an unusual peak in winter (291 mm), a steady decline in spring (160 mm), a sudden increase in summer (228 mm), and again a decrease in autumn (174 mm). The greatest amount of rain fell in July 2002 (205 mm), accounting for approximately 21% of the total. The lowest figures were recorded in May 2002 (6.3 mm), June 2002 (22 mm) and February 2003 (18.1 mm). The state of the mouth of both estuaries was affected by this unseasonal rainfall pattern. The Mdloti and the Mhlanga experienced prolonged periods of open phase during the winter and unusual

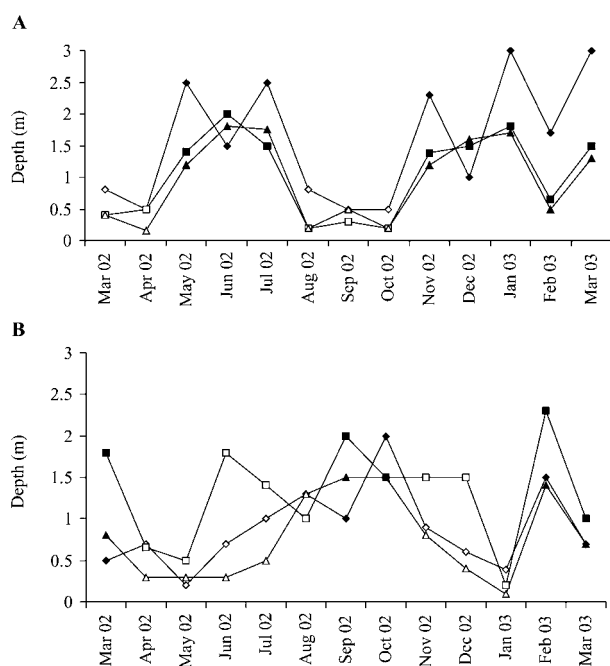
closed phases during part of the summer. The Mhlanga breached at regular (quasi-periodic) intervals, and remained closed for only short periods each time. While the Mdloti was closed 57% of the time and open or partly open 43% (excluding missing data) (Figure 3A), the Mhlanga was closed 49% and open or partly open 51% of the time (Figure 3B).

The depth of the water column ranged from 0.15 to 3.0 m at the Mdloti and from 0.1 to 2.3 m at the Mhlanga (Figure 4). Water depth exhibited significant temporal variations at both the Mdloti and the Mhlanga (Table 2). Significant differences between estuarine phases were observed in both the Mdloti ( $U=6.5$ ,  $p<0.0001$ ) and the Mhlanga ( $U=73.5$ ,  $p<0.05$ ). Water depth generally increased during the closed phase and decreased during the open phase.

Temperature at the sediment-water interface ranged from 14.5 to 29.5°C at the Mdloti and from 17.4 to 30°C at the Mhlanga. Temperature exhibited significant temporal variations at both the Mdloti ( $F_{12, 26}=4.91$ ,  $p<0.0001$ ) and the Mhlanga ( $F_{12, 26}=12$ ,  $p<0.05$ ).



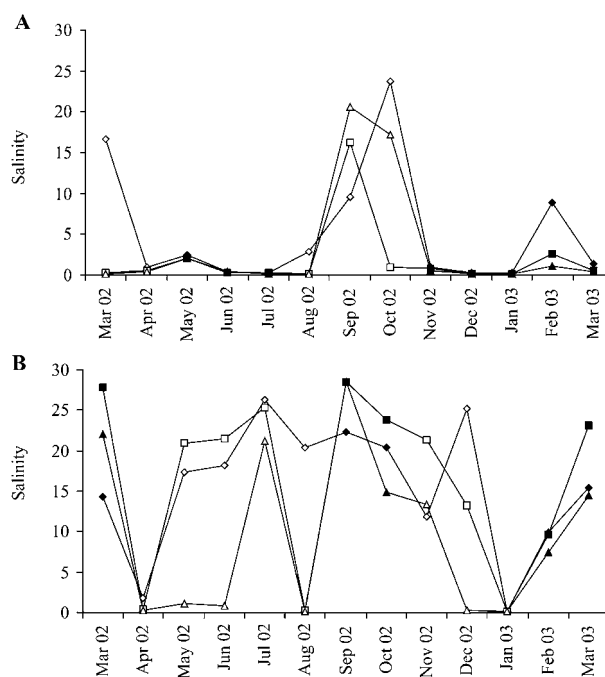
**Figure 3** Mouth conditions at the Mdloti (A) and Mhlanga (B) estuaries during the study period. 0=no data, 1=closed, 2=partly open, 3=open.



**Figure 4** Temporal variations in depth (m), at the lower ( $\diamond$ : open phase;  $\blacklozenge$ : closed phase), middle ( $\square$ : open phase;  $\blacksquare$ : closed phase) and upper reaches ( $\triangle$ : open phase;  $\blacktriangle$ : closed phase) of the Mdloti (A) and Mhlanga (B) estuaries.

Salinity at the sediment-water interface varied significantly from 0.10 to 24 psu at the Mdloti and from 0.1 to 28.5 at the Mhlanga (Figure 5, Table 2). Differences in salinity between estuarine phases were significant only at the Mdloti ( $U=98.5$ ,  $p<0.05$ ).

During the open phase of the Mdloti, the light attenuation coefficient ( $K_d$ ) ranged from 0.08 to 4  $m^{-1}$  (Figure 6A). During the closed phase of the estuary,  $K_d$  values ranged from 1.1 to 3.4  $m^{-1}$ . In this estuary,  $K_d$  values did not exhibit significant differences either temporally, spatially or between estuarine phases (Tables 2 and 3). During the open phase of the Mhlanga,  $K_d$  values ranged from 0.21 to 14  $m^{-1}$ , while during its closed phase these ranged from 0.70 to 2.7  $m^{-1}$  (Figure 6B). At the Mhlanga,  $K_d$  differed significantly temporally and between the open and closed phase ( $U=114$ ,  $p<0.05$ ), but not spatially (Tables 2 and 3). Irradiance at the sediment surface ( $E_z$ ) ranged from 0.44 to 874 mol photons  $m^{-2} d^{-1}$  at the Mdloti and from 0.009 to 543 mol photons  $m^{-2} d^{-1}$  at the Mhlanga (Figure 7). During the Mdloti open phase,  $E_z$  ranged from 5.23 to 874 mol photons  $m^{-2} d^{-1}$  (Figure 7A). During the closed phase of the estuary,  $E_z$  values ranged from



**Figure 5** Temporal variations in salinity (psu) at the lower ( $\diamond$ : open phase;  $\blacklozenge$ : closed phase), middle ( $\square$ : open phase;  $\blacksquare$ : closed phase) and upper reaches ( $\triangle$ : open phase;  $\blacktriangle$ : closed phase) of the Mdloti (A) and Mhlanga (B) estuaries.

0.44 to 376 mol photons  $m^{-2} d^{-1}$ . At the Mhlanga,  $E_z$  ranged from 0.009 to 457 mol photons  $m^{-2} d^{-1}$  during the closed phase, and from 0.013 to 543 mol photons  $m^{-2} d^{-1}$  during the open phase (Figure 7B).

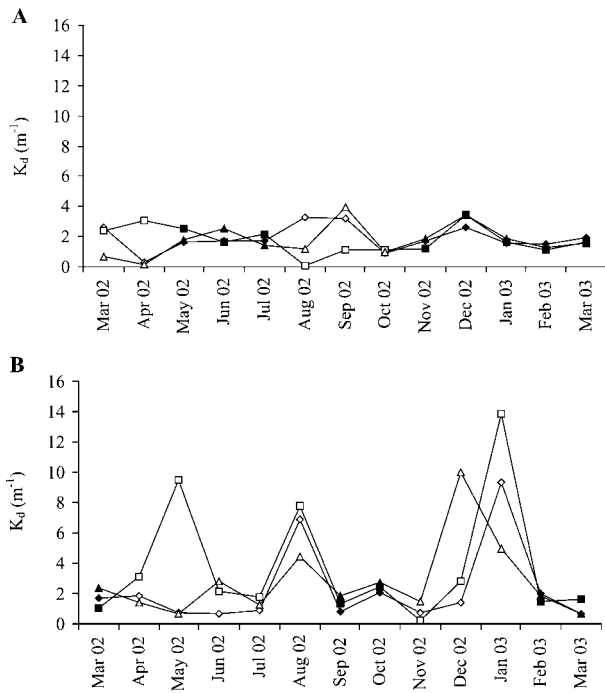
At the Mdloti, the DIN content of pore water ranged from 5.4 to 310  $\mu M$  during the open phase and from 1.5 to 98  $\mu M$  during its closed phase (Figure 8A). Here, pore water DIN concentrations exhibited significant variations temporally, but not spatially (Tables 2 and 3). At the Mhlanga, the DIN content of pore water ranged from 12 to 218  $\mu M$  during the open phase and from 5.3 to 212  $\mu M$  during its closed phase (Figure 9A). Pore water DIN concentrations did not exhibit significant variations temporally (Table 2). However, significant spatial variations were observed (Table 3). A *post-hoc* test (Tukey HSD; Zar 1999) revealed that these significant differences were in the pore water DIN content between lower and upper reaches.

DIP in pore water ranged from 0.13 to 3.3  $\mu M$  during the open phase of the Mdloti and from 0.10 to 17.3  $\mu M$  during its closed phase (Figure 8B). Here, pore water DIP

**Table 2** Results of a 1-way ANOVA showing temporal variations in physico-chemical variables in the Mdloti and the Mhlanga estuaries, March 2002 to March 2003.

Variable	Mdloti Estuary					Mhlanga Estuary				
	df effect	MS effect	df error	MS error	F	df effect	MS effect	df error	MS error	F
Depth	12	0.06	26	0.01	8.58***	12	0.03	26	0.01	3.97**
Temperature	12	2451	26	500	4.91***	12	0.01	24	0.00	12**
Salinity	12	2499	26	511	4.89***	12	0.59	26	0.14	4.32***
$K_d$	12	0.02	26	0.02	1.33 <sup>ns</sup>	12	0.10	26	0.04	2.28*
DIN	12	0.41	26	0.15	2.77*	12	0.16	26	0.12	1.28 <sup>ns</sup>
DIP	12	0.15	26	0.05	3.31**	12	0.26	26	0.17	1.50 <sup>ns</sup>

<sup>ns</sup>p=not significant; \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.



**Figure 6** Temporal variations in the coefficient of light extinction,  $K_d$ , at the lower ( $\diamond$ : open phase;  $\blacklozenge$ : closed phase), middle ( $\square$ : open phase;  $\blacksquare$ : closed phase) and upper reaches ( $\triangle$ : open phase;  $\blacktriangle$ : closed phase) of the Mdloti (A) and Mhlanga (B) estuaries.

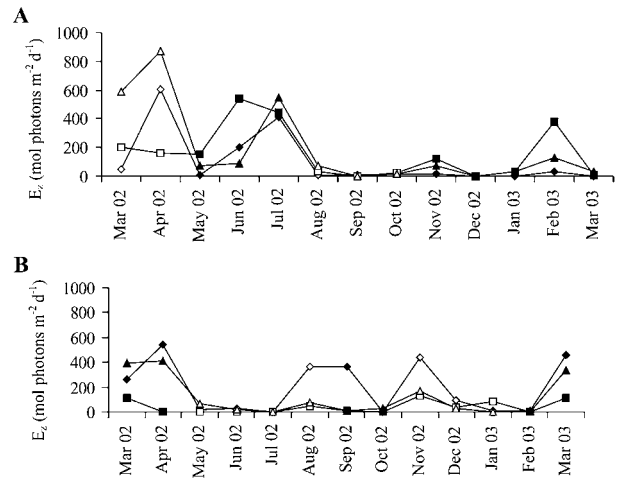
concentrations differed significantly temporally, but not spatially (Tables 2 and 3). At the Mhlanga, pore water DIP concentrations varied between 0.70 and 45.1  $\mu\text{M}$  during the open phase and between 1.4 and 41.3  $\mu\text{M}$  during the closed phase (Figure 9B). At the Mhlanga, pore water DIP did not differ significantly either temporally or spatially (Tables 2 and 3).

DIN:DIP molar ratios were used to test for potentially limiting effects of nutrients on microalgal biomass (Howarth 1988). DIN:DIP molar ratios below the critical value 16:1 are indicative of a potentially limiting effect of nitrogen on microalgal growth, while values above this are suggestive of a potential phosphorus limitation of microalgal growth in estuarine waters. At the Mdloti, the DIN:DIP ratio ranged from 8 to 490 during the open phase and from 5 to 299 during the closed phase (Figure 8C). At the Mhlanga, the DIN:DIP ratio varied between 1.9 and 104 during the open phase and between 0.17 and 71 during the closed phase (Figure 9C).

**Table 3** Results of a 1-way ANOVA showing spatial variations in physico-chemical variables in the Mdloti and Mhlanga estuaries, March 2002 to March 2003.

Variable	Mdloti Estuary					Mhlanga Estuary				
	df effect	MS effect	df error	MS error	F	df effect	MS effect	df error	MS error	F
Depth	2	0.05	36	0.02	2.05 <sup>ns</sup>	2	0.05	36	0.01	3.43 <sup>ns</sup>
Temperature	2	997	36	1123	0.89 <sup>ns</sup>	2	0.00	34	0.00	0.20 <sup>ns</sup>
Salinity	2	1011	36	1146	0.88 <sup>ns</sup>	2	0.55	36	0.27	2.07 <sup>ns</sup>
$K_d$	2	0.003	36	0.02	0.16 <sup>ns</sup>	2	0.11	36	0.06	1.99 <sup>ns</sup>
DIN	2	0.15	36	0.24	0.63 <sup>ns</sup>	2	0.47	36	0.12	4.11*
DIP	2	0.009	36	0.08	0.11 <sup>ns</sup>	2	0.53	36	0.18	2.93 <sup>ns</sup>

<sup>ns</sup>p=not significant; \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

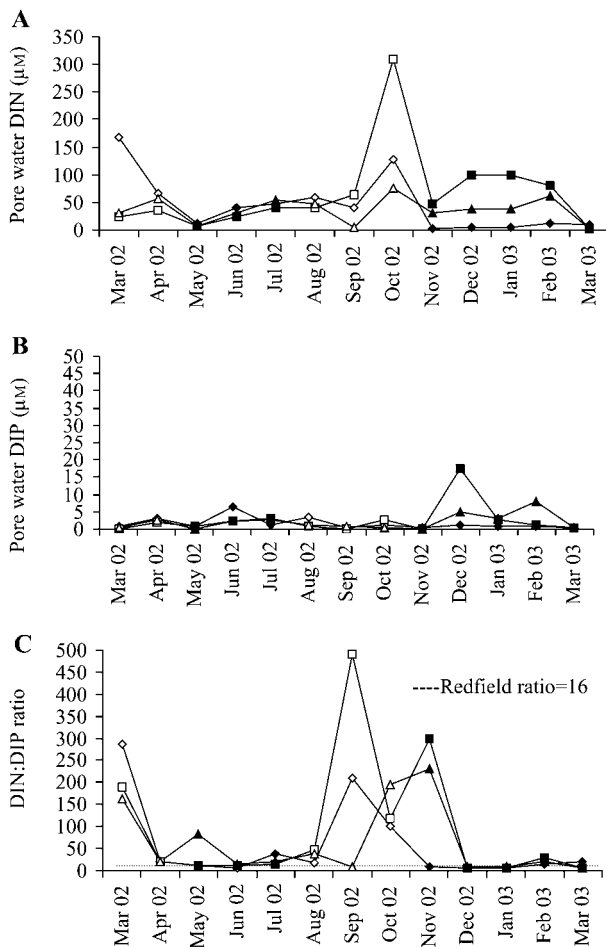


**Figure 7** Temporal variations in irradiance at the sediment surface,  $E_z$ , at the lower ( $\diamond$ : open phase;  $\blacklozenge$ : closed phase), middle ( $\square$ : open phase;  $\blacksquare$ : closed phase) and upper reaches ( $\triangle$ : open phase;  $\blacktriangle$ : closed phase) of the Mdloti (A) and Mhlanga (B) estuaries.

### Microphytobenthic biomass

At the Mdloti, microphytobenthic chl a concentrations varied widely, from 1.33 mg chl a  $\text{m}^{-2}$  (lower reaches, September 2002) to 131 mg chl a  $\text{m}^{-2}$  (upper reaches, September 2002) during the open phase, and from 18 mg chl a  $\text{m}^{-2}$  (lower reaches, February 2003) to 391 mg chl a  $\text{m}^{-2}$  (upper reaches, December 2002) during the closed phase (Figure 10). At the Mhlanga, microphytobenthic chl a concentrations ranged from 7.0 mg chl a  $\text{m}^{-2}$  (lower reaches, December 2002) to 313 mg chl a  $\text{m}^{-2}$  (lower reaches, June 2002) during the open phase, and from 1.7 mg chl a  $\text{m}^{-2}$  (lower reaches, September 2002) to 267 mg chl a  $\text{m}^{-2}$  (middle reaches, February 2003) during the closed phase (Figure 11).

Along the Mdloti, the mean microphytobenthic biomass was lowest in the lower reaches (43.8 mg chl a  $\text{m}^{-2} \pm 66.3$  SD) and highest in the upper reaches of the estuary (96.1 mg chl a  $\text{m}^{-2} \pm 114$  SD). The middle reaches exhibited a mean microphytobenthic biomass of 84 mg chl a  $\text{m}^{-2} \pm 73$  SD. Similarly, at the Mhlanga, mean microphytobenthic biomass was lowest in the lower reaches of the estuary, with 68.1 mg chl a  $\text{m}^{-2} \pm 93.9$  SD. The highest microphytobenthic biomass was observed in the middle reaches with 105 mg chl a  $\text{m}^{-2} \pm 114$  SD, while the upper reaches exhibited a mean microphytobenthic biomass of 96.4 mg chl a  $\text{m}^{-2} \pm 86$  SD.

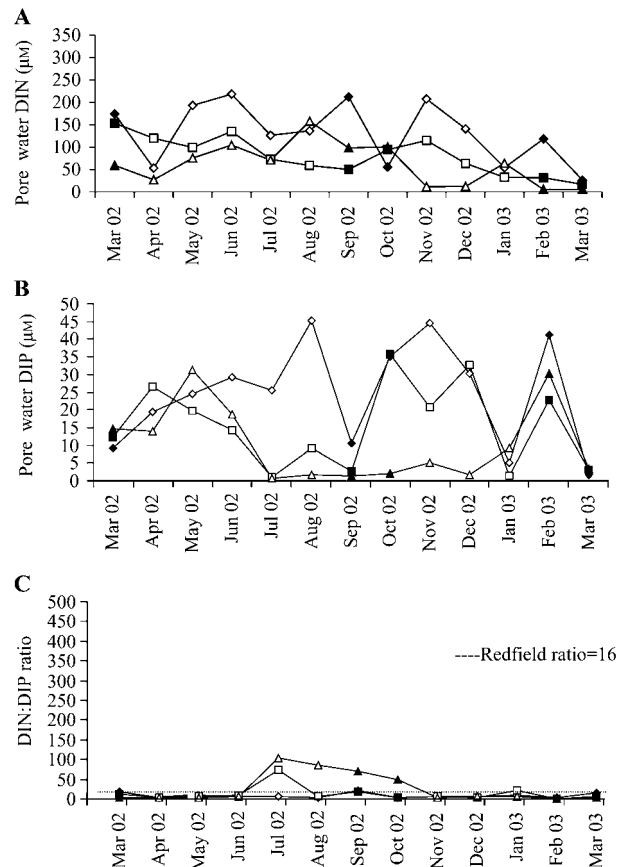


**Figure 8** Temporal variations in pore water dissolved inorganic nitrogen (DIN=nitrate+ammonium) (A); dissolved inorganic phosphorus (DIP: orthophosphate) (B) and DIN:DIP ratios (C) in the Mdloti Estuary.

The DIN:DIP Redfield ratio of 16:1 is indicated by the dashed line. Symbols as in previous Figures.

Over the whole survey, no significant differences were observed in microphytobenthic chl *a* concentrations between the Mdloti and the Mhlanga estuaries. However, when separated into open and closed phases, differences in microphytobenthic chl *a* concentrations were significant between the estuaries during their open phase ( $U=64$ ,  $p<0.05$ ), but not during their closed phase. At the Mdloti, microphytobenthic biomass differed significantly between the open and closed phases ( $U=29$ ,  $p<0.001$ ). However, at the Mhlanga, this was not the case. A 2-way ANOVA on the Mdloti data, performed on  $\log_{10}$ -transformed chl *a* values, revealed significant variations between mouth states (closed vs. open) as well as between reaches, with no significant interaction term (Table 4). At the Mhlanga, however, there was a significant interaction between mouth states and reaches (Table 4). A *post-hoc* test (Tukey HSD; Zar 1999) revealed significant differences between lower and upper reaches as well as between lower and middle reaches, both at the Mdloti and the Mhlanga (Table 5).

The ratio of pheopigments to chl *a* for the entire data set ranged from 0.40 to 0.73 at the Mdloti (Figure 12A), and from 0.43 to 0.70 at the Mhlanga (Figure 12B). At



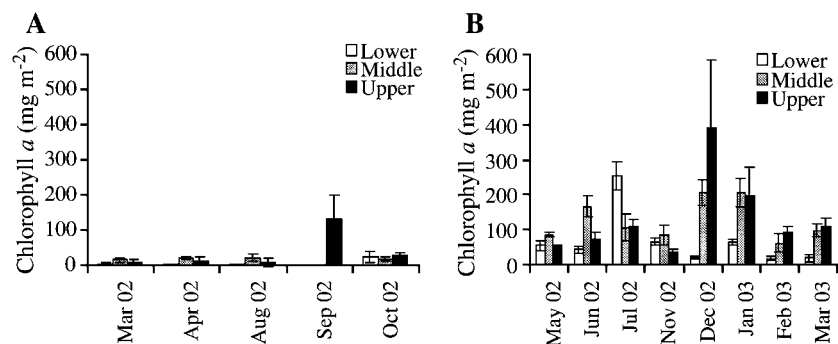
**Figure 9** Temporal variations in pore water dissolved inorganic nitrogen (DIN=nitrate+ammonium) (A); dissolved inorganic phosphorus (DIP: orthophosphate) (B) and DIN:DIP ratios (C) in the Mhlanga Estuary.

The DIN:DIP Redfield ratio of 16:1 is indicated by the dashed line. Symbols as in previous Figures.

the Mdloti, the ratio varied between 0.43 and 0.73 during the open phase and between 0.40 and 0.65 during the closed phase. At the Mhlanga, the ratio varied between 0.50 and 0.70 during the open phase and between 0.43 and 0.70 during the closed phase. Significant temporal differences were observed only at the Mdloti ( $F_{12,26}=2.40$ ,  $p<0.05$ ). Neither estuary exhibited any significant differences between reaches or between estuarine phases.

### Correlation analysis

Results of multiple correlation analysis on the Mhlanga entire data set showed a significant relationship between total microphytobenthic biomass and pore DIN concentration, salinity and  $E_z$  (Table 6). The Mdloti entire data set showed, however, significant correlations between microphytobenthic biomass and mouth state, depth and rainfall. Considering the two phases of each estuary separately, i.e., open and closed, more correlations were observed (Table 6). In particular, during the Mhlanga closed phase, microphytobenthic biomass was correlated with pore DIN concentration,  $K_d$ , salinity and rainfall. During the Mdloti closed phase, microphytobenthic biomass was significantly correlated with mouth state and rainfall (Table 6). Considering the open phase of each estuary, microphytobenthic biomass was correlated to



**Figure 10** Temporal and spatial distribution of microphytobenthic biomass (mean $\pm$ SD) in the Mdloti Estuary during open (A) and closed (B) phase.

pore DIP concentrations at the Mdloti and E<sub>2</sub> at the Mhlanga.

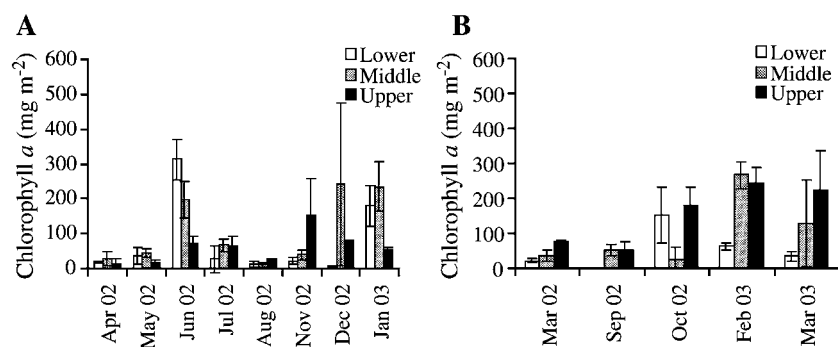
### Discussion and conclusion

Seasonal fluctuations in microphytobenthic standing stocks have been reported in a number of studies (Montagna et al. 1983, Gould and Gallagher 1990, Pinckney and Zingmark 1993), while others have not found any significant differences between seasons (Cadée and Hegeman 1974, Colijn and de Jonge 1984, de Jonge and Colijn 1994). During this study, microphytobenthic biomass differed significantly between the open and closed phases only at the Mdloti (Figure 10). In TOCEs, major changes in microphytobenthic biomass are in fact strongly related to the alternation of the open and closed phase of the mouth (Adams and Bate 1999, Perissinotto et al. 2003). In agreement with previous studies in the area (Nozais et al. 2001, Perissinotto et al. 2002, 2003), the higher microphytobenthic biomass values in the Mdloti Estuary generally coincided with the closed phase (18 to 391 mg chl *a* m<sup>-2</sup>), while the lowest biomass values typically occurred during the open phase (1.33 to 131 mg chl *a* m<sup>-2</sup>) (Figure 10).

Typical of TOCEs, the Mdloti experienced prolonged periods of mouth closure, with a maximum duration of sixty-nine consecutive days and only nine breaching events (Figure 3A). Compared to the 1999–2000 period, a distinct seasonal rainfall pattern was not observed during this study (see Nozais et al. 2001 for further details). In addition, total rainfall recorded for the duration of this

study was much lower than that reported by Nozais et al. (2001). The reduced freshwater inflow into this estuary may be attributed partly to the location of the Hazelmeere Dam in its relatively large catchment area. Furthermore, the low rainfall regime observed during this survey also contributed to the reduction in the volume of freshwater inflow into this estuary, which resulted in the prolonged periods of mouth closure (Figure 3A).

Light and nutrients are the most important proximal factors that influence microalgal production, and therefore biomass accumulations (Droop et al. 1982, Perissinotto et al. 2003). The light extinction coefficient,  $K_d$ , exhibited very low values (1.1 to 3.4 m<sup>-1</sup>) during the Mdloti closed phase (Figure 6A). These values are consistent with those reported for the closed phase of the Mdloti in 1999–2000 (Nozais et al. 2001) and in other TOCEs (Perissinotto et al. 2003). Low  $K_d$  values have the effect of increasing the availability of light and its penetration into deeper layers. Indeed, on most occasions during this study, the depth of the euphotic zone was greater than the total water depth (Thomas et al. 2005). However, because the closed phase was also associated with an increase in the depth of the water-column, the absolute irradiance at the sediment surface,  $E_z$ , during this phase (0.44–376 mol photons m<sup>-2</sup> d<sup>-1</sup>) was less than during the open phase (5.23–874 mol photons m<sup>-2</sup> d<sup>-1</sup>), when shallow water conditions prevailed (Figure 7A). The two factors effectively act in opposite directions and it is, therefore, likely that the larger microphytobenthic biomass observed at the Mdloti during the closed, compared to the open phase may have very little, if anything at all, to do with changes in light availability. This is con-



**Figure 11** Temporal and spatial distribution of microphytobenthic biomass (mean $\pm$ SD) in the Mhlanga Estuary during open (A) and closed (B) phase.



**Table 4** Results of a 2-way ANOVA showing differences in microphytobenthic chl *a* concentrations between the mouth state and reaches of the Mdloti and Mhlanga estuaries, March 2002 to March 2003.

Source of variation	Mdloti Estuary					Mhlanga Estuary				
	df effect	MS effect	df error	MS error	F	df effect	MS effect	df error	MS error	F
Mouth state	1	23.58	111	0.14	160.32***	1	0.37	111	0.26	1.40
Reaches	2	2.61	111	0.14	17.80***	2	1.57	111	0.26	5.92*
Mouth state x reach	2	0.19	111	0.14	1.35 <sup>ns</sup>	2	0.84	111	0.26	3.18*

<sup>ns</sup>p=not significant; \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

firmed by the lack of any significant weighting of irradiance parameters in the multiple correlation output (Table 6).

The closed phase was also characterised by lower pore water nutrient values, compared to those of the open phase. In particular, pore water DIN values were approximately three times lower during the Mdloti closed phase, as opposed to its open phase. Microphytobenthic biomass was consistently high during the closed phase, a period of decreased nutrient loading. This inverse relation is probably the typical result of consumption of the finite pool of nutrients available in such a closed system, with its conversion into microphytobenthic biomass. Multiple correlation analysis showed only one significant relationship between microphytobenthic biomass and pore water DIP, during the Mdloti open phase, but none with DIN (Table 6). This could be regarded as an indication of potential, intermittent phosphorus limitation to microalgal growth, as previously reported for this estuary (Nozais et al. 2001). Growth of shallow-water microphytobenthos, like that found in the Mdloti is, however, generally not limited by inorganic nutrients, due to the large particulate pool available and the high rates of remineralisation that occur within the sediment (Sundbäck et al. 1996).

Significant spatial variations in microphytobenthic biomass were observed in the Mdloti. Microphytobenthic biomass was often higher in the upper reaches (96 mg chl *a* m<sup>-2</sup>±114 SD), closest to the freshwater input, than in the lower (44 mg chl *a* m<sup>-2</sup>±66 SD) or middle reaches (84 mg chl *a* m<sup>-2</sup>±73 SD) (Figure 10). This result is consistent with that obtained in a previous study in the Mdloti (Nozais et al. 2001). The upper reaches of the Mdloti are typically sheltered by vegetation and sugar cane plantations, while the mouth region of the estuary is relatively unsheltered and exposed to a great deal of physical disturbance (Mundree 2001). Also, in the upper estuarine reaches sediment is likely more muddy than in the lower reaches (Adams and Bate 1999), and generally microphytobenthic biomass is higher in muddy sheltered habitats, as compared to relatively sandy exposed hab-

itats (MacIntyre et al. 1996). Sediment structure, and particularly the drastic changes it undergoes during the breaching of the estuary's mouth (scouring of the top layer and subsequent deposition of coarse beach sand) is also likely to explain many of the differences observed in the biomass of microphytobenthos from the open to the closed phase of the Mdloti. The much higher levels recorded during the closed phase could be mainly the product of re-deposition of fine silt and sand and the formation of a surface biofilm, following the closure of the mouth (cf. Guarini et al. 2004).

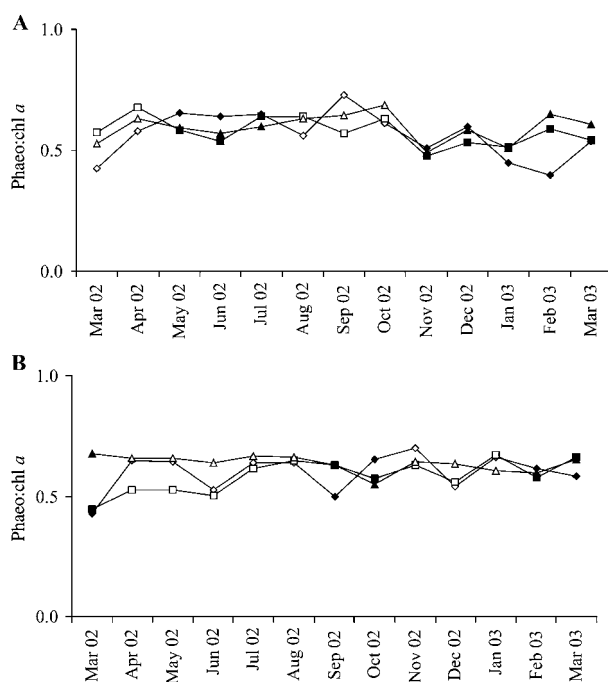
In contrast to previous findings in the Mdloti (Nozais et al. 2001) and in the Mpenjati (Perissinotto et al. 2002), no significant differences were observed in microphytobenthic biomass between the open and the closed phase at the Mhlanga. Unlike typical TOCEs, the higher biomass values at the Mhlanga were not always associated with the closed mouth state. In fact, the highest microphytobenthic biomass value observed at the Mhlanga (313 mg chl *a* m<sup>-2</sup>) was recorded in June 2002, during an extended period (approximately 7 days) of a partly open mouth condition (Figure 11). High biomass levels during the open phase may be related to two factors: decreased scouring efficiency due to frequent breaching (Reddering and Esterhuysen 1987, Cooper et al. 1999), and steady supply of high nutrient levels due to the discharge of treated sewage waters into the estuary. The first factor would ensure that substantial microphytobenthic biomass is still present during and after each breaching event (Figure 11), while the second factor would contribute to a rapid growth response of benthic microalgae following the disturbance (Underwood 2004, Guarini et al. 2004).

During the study period, pore water (Figures 8 and 9) and water column nutrient concentrations (DIN and DIP) were often higher (2–5 times) at the Mhlanga than at the Mdloti (Perissinotto et al. 2004). The high nutrient values observed at the Mhlanga can be explained in terms of the large volume of treated sewage effluent received by this estuary on a daily basis. The Mhlanga receives 20 000 m<sup>3</sup> d<sup>-1</sup> resulting in a capping flow of 0.23 m<sup>3</sup> s<sup>-1</sup>

**Table 5** Results of a Tukey HSD *post-hoc* test showing significant differences in microphytobenthic chl *a* concentrations between reaches at the Mdloti and Mhlanga estuaries, March 2002 to March 2003.

Reaches	Mdloti Estuary			Reaches	Mhlanga Estuary		
	Lower	Middle	Upper		Lower	Middle	Upper
Lower	1			Lower	1		
Middle	0.0001***	1		Middle	0.04*	1	
Upper	0.0001***	0.85	1	Upper	0.002**	0.59	1

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001.



**Figure 12** Ratio of pheopigment to chl a concentration for the Mdloti (A) and Mhlanga (B) estuaries. Symbols as in Figure 4.

(W. Pfaff, eThekweni Municipality, pers. comm.). Through this capping flow, eutrophication is enhanced at the Mhlanga and periods of mouth opening are also increased and prolonged (Perissinotto et al. 2004, Thomas et al. 2005).

Although rainfall was low throughout the study period, thirteen breaching events were recorded at the Mhlanga (Figure 3B). This is possibly due to the large volume of sewage effluent received by this estuary, which never experienced closure of more than fifteen consecutive days. Thus, it is clear that the natural cycles of mouth opening and closure of the Mhlanga are being altered by the high capping flow from the sewage treatment works (Perissinotto et al. 2004). Unlike the Mdloti, which seems to be significantly impacted by the prevailing drought conditions in the region, the Mhlanga seems to be responding strongly to the discharge of treated water effluents into its upper reaches. It was initially expected that the drought conditions observed in this region would result in a reduction in the frequency and duration of mouth opening, especially in the much smaller Mhlanga. However, the opposite was actually the case as a result of this steady capping flow.

During the closed phase of the Mhlanga, microphytobenthic biomass was most strongly correlated with light extinction, DIN concentration, salinity and rainfall (Table 6). During the closed phase in a TOCE, salinity and rainfall are inversely linked through a balance of evaporation and precipitation (Perissinotto et al. 2004). Specific adaptations of microphytobenthos to light, nutrients and salinity have often been suggested in correlative field studies (Cadée and Hegeman 1974, Rasmussen et al. 1983, Blanchard et al. 1996, Barranguet et al. 1998, Underwood and Kromkamp 1999). However, during the Mhlanga open phase, the only factor with substantial weighting on microphytobenthic biomass in the multiple correlation output was light availability (Table 6).

Remarkable differences were observed in the dynamics of the two estuaries. The Mdloti experienced periods of prolonged mouth closure, whereas the Mhlanga experienced frequent breaching. These differences were reflected in the phytoplankton and zooplankton stocks, which differed significantly between the two estuaries (Thomas et al. 2005, Kibirige et al. in press). Contrary to these findings, microphytobenthic biomass did not exhibit significant differences between the two estuaries. A possible reason for this may be found in the rapid recovery ability that microphytobenthic communities exhibit in response to both physical and chemical disturbances. Recent studies suggest that the recovery time scale of microphytobenthos exposed to physico-chemical disturbances may be extremely short (Underwood 2004). This is consistent with model results of tidally-induced disturbance on microphytobenthos, which show that even during the intertidal short time-scales microphytobenthic communities exhibit great resilience (Guarini et al. 2004). Results from this study show that at the Mhlanga the microphytobenthic community was able to attain biomass levels equal to those exhibited prior to breaching, within periods of only 5–10 days after breaching with the mouth still open (Figures 3B and 11).

In the pelagic subsystem, the ratio of pheopigment to chl a gives a general indication of the physiological or grazing state of a microalgal community (Shuman and Lorenzen 1975). Similar ratios have been used in intertidal sediments (Brotas and Plante-Cuny 1988, Lucas and Holligan 1999). However, the use of phaeophorbide *a* as a marker of macrofaunal grazing intensity in intertidal sediment has recently been shown as inappropriate (Ford and Honeywill 2002). It is not clear yet if and to what extent this may also apply to other types of sediments and to micro- and mesograzers activities. However, in the absence of more comprehensive tests and results, any

**Table 6** Multiple correlation analysis between microphytobenthic biomass and physico-chemical and biological variables in the Mdloti and Mhlanga estuaries, March 2002 to March 2003.

	Mdloti Estuary			Mhlanga Estuary		
	Open+closed (n=39)	Open (n=15)	Closed (n=24)	Open+closed (n=39)	Open (n=15)	Closed (n=24)
Coefficient	0.316	0.240	0.293	0.238	0.270	0.323
Variables	1, 2, 9	5	1, 9	4, 8, 10	10	4, 6, 8, 9

Key to variable numbers: 1: mouth state, 2: depth, 3: zooplankton biomass, 4: pore DIN concentration, 5: pore DIP concentration, 6:  $K_d$ , 7: temperature, 8: salinity, 9: rainfall, 10:  $E_z$ .

use of the phaeopigment:chl ratio must be treated with caution. The ratios observed in our study may be indicative of stressed/declining (pheopigment:chl  $a > 0.5$ ) microphytobenthic communities in both estuaries, irrespective of their open or closed phase (Figure 12). Exceptions to this at the Mdloti were the lower reaches during March 2002 (pheopigment:chl  $a = 0.43$ ), January 2003 (pheopigment:chl  $a = 0.45$ ) and February 2003 (pheopigment:chl  $a = 0.40$ ). At the Mhlanga, there was only one exception, the middle reaches in March 2002 (pheopigment:chl  $a = 0.45$ ).

Grazing pressure exerted on microphytobenthos by the demersal zooplankton community may also have influenced the ratio of pheopigment to chl  $a$ . Although zooplankton was not amongst the factors exhibiting the highest association with microphytobenthic biomass (Table 6), simple correlation analysis showed that microphytobenthic biomass was positively and significantly correlated with zooplankton biomass, both at the Mdloti and the Mhlanga (not shown). This confirms that microphytobenthos represents an important food source to these primary consumers (see also Kibirige and Perissinotto 2003a,b, Kibirige et al. 2003). Preliminary studies in TOCEs suggest that these systems may at times exhibit very low phytoplankton biomass in conjunction with high zooplankton standing stocks (Perissinotto et al. 2000, Kibirige and Perissinotto 2003a,b). In the Mpenjati TOCE, the dominant zooplankton grazers, such as the mysid *Gastrosaccus brevifissura* Tattersall and the copepods, *Pseudodiaptomus hessei* (Mrázek) and *Acartia natalensis* Connell et Grindley, were able to utilise microphytobenthos as an alternative energy source to phytoplankton and detritus (Kibirige et al. 2002, Kibirige and Perissinotto 2003). Since *G. brevifissura* and *P. hessei* live in close association with the sediment, at least during the daytime, it seems reasonable that substantial proportions of the microphytobenthos may be used as an alternative autotrophic food source by the mysids and copepods in this and similar estuaries (Kibirige et al. 2003). Meiofaunas also have a potential to affect the phaeopigment:chl  $a$  ratio through their grazing on microphytobenthos (Miller et al. 1996), but Nozais et al. (2005) have shown that in these estuarine systems this is on average  $< 11\%$  of the total microphytobenthic biomass.

In a typical TOCE, like the Mdloti, microphytobenthic biomass is clearly associated with the alternation of open/closed phases (Nozais et al. 2001, Perissinotto et al. 2002, Mundree et al. 2003). In such systems, it is not unusual for microphytobenthic biomass to increase during the closed phase and decline during the open phase. The Mhlanga system, on the other hand, seems to lack a prolonged period of mouth closure, which is a distinct characteristic of TOCEs, and therefore did not exhibit any significant differences in microphytobenthic biomass between the open and the closed phase. Also, the Mhlanga appears much more complicated than the Mdloti, primarily due to very frequent breaching, to the point that the whole system has become disturbed. Thus, for the duration of this study, the Mhlanga appeared to be functioning more like a permanently open estuary than a TOCE, as it lacked a period of prolonged mouth closure. The response of microphytobenthos to the

increased flow and nutrient addition from the sewage works demonstrates that the Mhlanga is on a trajectory of drastic change. The vigilant management and monitoring of the volume and quality of sewage effluent discharged into the Mhlanga Estuary is crucial to ensure the ecological integrity of this system.

## Acknowledgements

We wish to thank the South African National Research Foundation (NRF), Marine and Coastal Management (MCM) and the Water Research Commission (WRC) for funding this project. The South African Sugarcane Research Institute (SASRI, Dr D. Watt) is thanked for providing rainfall and other climatic data, and Ezemvelo KZN Wildlife for providing data on the state of the mouth of the Mdloti and Mhlanga estuaries. The assistance of Peter Upfold, Israel Kibirige, Cheryl Thomas and Xolani Thwala with fieldwork and laboratory analyses is also greatly appreciated. Finally, we wish to thank two anonymous referees for their constructive comments on an earlier version of the manuscript.

## References

- Adams, J.B. and G.M. Bate. 1999. Primary producers. In: (B.R. Allanson and D. Baird, eds) *Estuaries of South Africa*. Cambridge University Press, Cambridge. pp. 91–99.
- Barranguet, C., J. Kromkamp and J. Peene. 1998. Factors controlling primary production and photosynthetic characteristics of intertidal microphytobenthos. *Mar. Ecol. Prog. Ser.* 173: 117–126.
- Begg, G.W. 1978. *The estuaries of Natal*. Natal Town and Regional Planning Report 41. Pietermaritzburg. pp. 657.
- Begg, G.W. 1984. *The estuaries of Natal. Part 2*. Natal Town and Regional Planning Report 55. Pietermaritzburg. pp. 631.
- Bidigare, R.R., T.J. Frank, C. Zastrow and J.M. Brooks. 1986. The distribution of algal chlorophylls and their degradation products in the South Ocean. *Deep Sea Res.* 33: 923–937.
- Blanchard, G.F., J.M. Guarini, P. Richard, P.H. Gros and F. Mornet. 1996. Quantifying the short-term temperature effect on light saturated photosynthesis of intertidal microphytobenthos. *Mar. Ecol. Prog. Ser.* 134: 309–313.
- Brotas, V. and M.R. Plante-Cuny. 1988. Spatial and temporal patterns of microphytobenthic taxa of estuarine tidal flats in the Tagus estuary (Portugal) using pigment analysis by HPLC. *Mar. Ecol. Prog. Ser.* 171: 43–57.
- Brotas, V. and M.R. Plante-Cuny. 2003. The use of HPLC pigment analysis to study microphytobenthos communities. *Acta Oecol.* 24: S109–S115.
- Cadée, G.C. and J. Hegeman. 1974. Primary production of the benthic microflora living on the tidal flats in the Dutch Wadden Sea. *Neth. J. Sea Res.* 8: 260–291.
- Colijn, F. and V.N. de Jonge. 1984. Primary production of microphytobenthos in the Ems-Dollard estuary. *Mar. Ecol. Prog. Ser.* 14: 185–196.
- Cooper, J.A.G. 1991. *Sedimentary models and geomorphological classification of river mouths on a subtropical wave dominated coast, Natal, South Africa*. Ph.D thesis, University of Natal, South Africa. pp. 401.
- Cooper, J.A.G., C.I. Wright and T.R. Mason. 1999. Geomorphology and sedimentology. In: (B.R. Allanson and D. Baird, eds) *Estuaries of South Africa*. Cambridge University Press, Cambridge. pp. 5–25.
- de Jonge, V.N. and F. Colijn. 1994. Dynamics of microphytobenthic biomass in the Ems estuary measured as chlorophyll  $a$  and carbon. *Mar. Ecol. Prog. Ser.* 104: 185–196.

- de Villiers, C.J., A.N. Hodgson and A.T. Forbes. 1999. Studies on estuarine macroinvertebrates. In: (B.R. Allanson and D. Baird, eds) *Estuaries of South Africa*. Cambridge University Press, Cambridge. pp. 167–207.
- Delgado, M., V. de Jonge and H. Peletier. 1991. Experiments on the resuspension of natural microphytobenthos populations. *Mar. Biol.* 108: 321–328.
- Droop, M.R., M.L. Mickelson, J.M. Scott and M.F. Turner. 1982. Light and nutrient status of algal cells. *J. Mar. Biol. Ass. UK.* 62: 403–434.
- Ford, R.B. and C. Honeywill. 2002. Grazing on intertidal microphytobenthos by macrofauna: is pheophorbide a useful marker? *Mar. Ecol. Prog. Ser.* 229: 33–42.
- Gould, D. and E. Gallagher. 1990. Field measurement of specific growth rate, biomass, and primary production of benthic diatoms of Savin Hill Cove, Boston. *Limnol. Oceanogr.* 35: 1757–1770.
- Grobber, N.G. 1987. *Sedimentary environments of Mdloti, u-Mgababa and Lovu lagoons, Natal, South Africa*. MSc thesis, University of Natal, South Africa. pp. 171.
- Guarini, J.M., P. Richard and G.F. Blanchard. 2004. Quantification of the dynamics of the intertidal microphytobenthos in semi-enclosed littoral ecosystems: regulation of the resuspension by the biomass. In: Abstract book of the ASLO 2004 Summer Meeting, Savannah 13–18 June 2004. American Society of Limnology and Oceanography. p. 27.
- Howarth, R.W. 1988. Nutrient limitation of net primary production in marine ecosystems. *Annu. Rev. Ecol. Syst.* 19: 89–110.
- Kibirige, I. and R. Perissinotto. 2003a. *In situ* feeding rates and grazing impact of zooplankton in a South African temporarily open estuary. *Mar. Biol.* 142: 357–367.
- Kibirige, I. and R. Perissinotto. 2003b. The zooplankton community of the Mpenjati Estuary, a South African temporarily open/closed system. *Estuar. Coast. Shelf Sci.* 58: 727–741.
- Kibirige, I., R. Perissinotto and C. Nozais. 2002. Alternative food sources of zooplankton in a temporarily open estuary: evidence from  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . *J. Plankt. Res.* 24: 1089–1095.
- Kibirige, I., R. Perissinotto and C. Nozais. 2003. Grazing rates and feeding preferences of the mysid shrimp *Gastrosaccus brevifissura* in a temporarily open estuary in South Africa. *Mar. Ecol. Prog. Ser.* 251: 201–210.
- Kibirige, I., R. Perissinotto and X. Thwala. In press. A comparative study of zooplankton dynamics in two subtropical temporarily open/closed estuaries, South Africa. *Mar. Biol.*
- Kirk, J.T.O. 1994. *Light and photosynthesis in aquatic ecosystems*. 2nd edition. Cambridge University Press, Cambridge. pp. 509.
- Light, B.R. and J. Beardall. 1998. Distribution and spatial variation of benthic microalgal biomass in a temperate, shallow-water marine system. *Aquat. Bot.* 61: 39–54.
- Lucas, C.H. and P.M. Holligan. 1999. Nature and ecological implications of algal pigment diversity on the Molenplaat tidal flat (Westerschelde estuary, SW Netherlands). *Mar. Ecol. Prog. Ser.* 180: 51–64.
- MacIntyre, H.L., R.J. Geider and D.C. Miller. 1996. Microphytobenthos: the ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries* 19: 186–201.
- Miller, D.C., R.J. Geider and H.L. MacIntyre. 1996. Microphytobenthos: the ecological role of the “secret garden” of unvegetated, shallow water marine habitats. II. Role in sediment stability and shallow water food webs. *Estuaries* 19: 202–212.
- Montagna, P., B. Coull, T. Herring and B. Dudley. 1983. The relationship between abundances of meiofauna and their suspected microbial food (diatoms and bacteria). *Estuar. Coast. Shelf Sci.* 17:381–394.
- Morant, P. and N. Quinn. 1999. Influence of man and management of South African estuaries. In: (B.R. Allanson and D. Baird, eds) *Estuaries of South Africa*. Cambridge University Press, Cambridge. pp. 289–320.
- Mostert, S.A. 1983. Procedure used in South Africa for the automatic photometric determination of micronutrients in seawater. *South Afric. J. Mar. Sci.* 1: 189–198.
- Mundree, S. 2001. *Dynamics of the microphytobenthic community of a temporarily open estuary: Mdloti, KwaZulu-Natal*. MSc thesis, University of Durban Westville, South Africa. pp. 126.
- Mundree, S., R. Perissinotto and C. Nozais. 2003. Seasonal variations in the vertical distribution of benthic microalgae in the upper sediment of the Mdloti Estuary, South Africa. *Bot. Mar.* 46: 323–331.
- Nozais, C., R. Perissinotto and S. Mundree. 2001. Annual cycle of microalgal biomass in a South African temporarily open estuary: nutrient versus light limitation. *Mar. Ecol. Prog. Ser.* 223: 39–48.
- Nozais, C., R. Perissinotto and G. Tita. 2005. Temporal dynamics of meiofauna in a South African temporarily open estuary (Mdloti Estuary, Indian Ocean). *Estuar. Coast. Shelf Sci.* 62: 325–338.
- Perissinotto, R., D.R. Walker, P. Webb, T.H. Wooldridge and R. Bally. 2000. Relationships between zoo- and phytoplankton in a warm-temperate, semi-permanently closed estuary, South Africa. *Estuar. Coast. Shelf Sci.* 51: 1–11.
- Perissinotto, R., C. Nozais and I. Kibirige. 2002. Spatio-temporal dynamics of phytoplankton and microphytobenthos in a South African temporarily open estuary. *Estuar. Coast. Shelf Sci.* 54: 363–374.
- Perissinotto, R., C. Nozais, I. Kibirige and A. Anandraj. 2003. Planktonic food webs and benthic pelagic coupling in three South African temporarily open estuaries. *Acta Oecol.* 24: S307–S316.
- Perissinotto, R., D. Stretch, A.T. Forbes, A. Connell, A. Blair, N.T. Demetriades, I. Zietsman, I. Kibirige, X. Thwala, C.M. Thomas, K. Iyer, H. Simpson and M.J. Joubert. 2004. *Responses of the biological communities to flow variation and mouth state in two KwaZulu-Natal temporarily open/closed estuaries*. Water Research Commission report K5/1247. Pretoria, South Africa. pp. 166.
- Pinckney, J. and R.G. Zingmark. 1993. Biomass and production of benthic microalgal communities in estuarine habitats. *Estuaries* 16: 887–897.
- Rasmussen, M.B., K. Henriksen, K. and A. Jensen. 1983. Possible causes of temporal fluctuation in primary production of microphytobenthos in the Danish Wadden Sea. *Mar. Biol.* 73: 109–114.
- Reddering, J.S.V. and K. Esterhuysen. 1987. The effects of river floods on sediment dispersal in small estuaries: a case study from East London. *South Afric. J. Geol.* 90: 458–470.
- Rizzo, W.M., G.L. Lackey and R.R. Christian. 1992. Significance of euphotic subtidal sediments to oxygen and nutrient cycling in a temperate estuary. *Mar. Ecol. Prog. Ser.* 86: 51–61.
- Schumann, E.H., J.L. Largier and J.H. Slinger. 1999. Estuarine hydrodynamics. In: (B.R. Allanson and D. Baird, eds) *Estuaries of South Africa*. Cambridge University Press, Cambridge. pp. 27–52.
- Shuman, F.R. and C.J. Lorenzen. 1975. Quantitative degradation of chlorophyll by a marine herbivore. *Limnol. Oceanogr.* 20: 580–586.
- Snow, G.C., J.B. Adams and G.C. Bate. 2000a. Effect of river flow on estuarine microalgal biomass and distribution. *Estuar. Coast. Shelf Sci.* 51: 255–266.
- Snow, G.C., G.C. Bate and J.B. Adams. 2000b. The effects of a single artificial freshwater release into the Kromme Estuary. 2. Microalgal response. *Water S.A.* 26: 301–310.
- Sundbäck, K., L. Carlson, C. Nilsson, B. Jönsson, A. Wulff and S. Odmark. 1996. Response of benthic microalgal mats to drifting green algal mats. *Aquat. Microb. Ecol.* 10: 195–205.
- Thomas, C.M., R. Perissinotto and I. Kibirige. 2005. Phytoplankton biomass and size structure in two South African eutroph-

- ic, temporarily open/closed estuaries. *Estuar. Coast. Shelf Sci.* 65: 223–238.
- Underwood, G.J.C. 2004. Recovery rates and responses of intertidal benthic microalgae to disturbance. In: Abstract book of the ASLO 2004 Summer Meeting, Savannah 13–18 June 2004. American Society of Limnology and Oceanography. p. 65.
- Underwood, G.J.C. and J. Kromkamp. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. *Adv. Ecol. Res.* 29: 93–153.
- Underwood, G.J.C., M. Boulcott, C.A. Raines and K. Waldron. 2004. Environmental effects on exopolymer production by marine benthic diatoms – dynamics, changes in composition and pathways of production. *J. Phycol.* 40: 293–304.
- Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol. Oceanogr.* 39: 1985–1992.
- Whitfield, A.K. 1992. A characterization of southern African estuarine systems. *South Afric. J. Aquat. Sci.* 18:89–103.
- Whitfield, A.K. 1995. Available scientific information on individual South African estuarine systems. Water Research Commission report 577/1/95. Pretoria, South Africa. pp. 204.
- Wooldridge, T.H. and L. McGwynne. 1996. The estuarine environment. Institute for Coastal Research report C31. Port Elizabeth, South Africa. pp. 91.
- Zar, J.H. 1999. *Biostatistical analysis*. Prentice Hall International, Upper Saddle River, New Jersey. pp. 663.

Received 18 May, 2005; accepted 6 December, 2005

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