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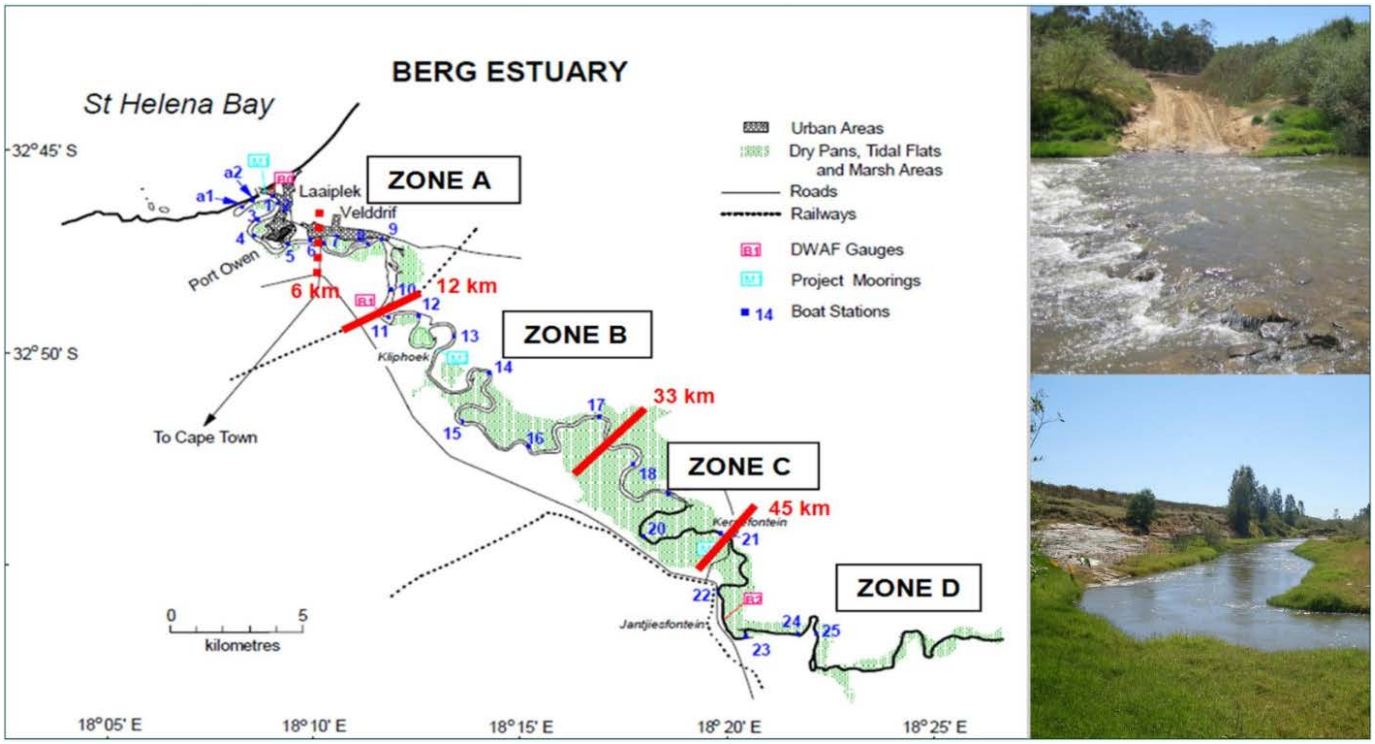
Department of Water Affairs
Directorate: Options Analysis

PRE-FEASIBILITY AND FEASIBILITY STUDIES FOR AUGMENTATION OF THE WESTERN CAPE WATER SUPPLY SYSTEM BY MEANS OF FURTHER SURFACE WATER DEVELOPMENTS

REPORT No.1 – VOLUME 3
Berg Estuary Environmental Water Requirements

APPENDIX No.E

Specialist Report - Microalgae



June 2012

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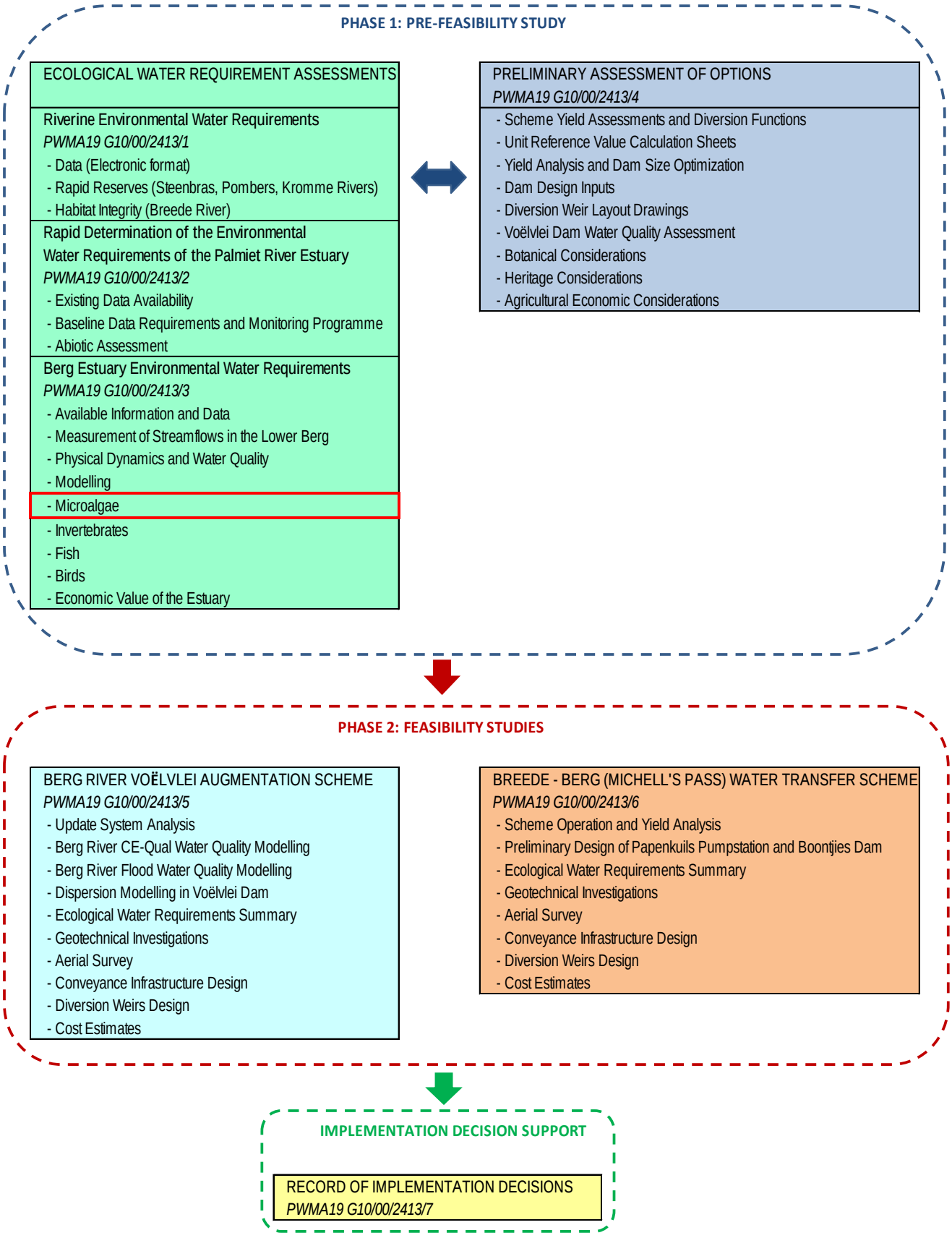
STUDY REPORT MATRIX DIAGRAM



ACKNOWLEDGEMENTS

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1. INTRODUCTION

Research on estuarine microalgal ecology has been neglected in South Africa, with the main effort on fish and invertebrate ecology. The result is that our understanding of the microalgal component is small and rather fractured. It has been realised that the phytoplankton and benthic microalgae are important contributors to primary production with the result that there has been an increased research effort in this area in the last two decades. Comprehensive studies relating microalgal biomass to freshwater flow is limited to a few Eastern Cape estuaries and there has been no such study in the Western Cape. As a result, data from once- or twice-off studies will be included for comparative purposes but relating biomass to flow in the Berg will be based on predictions only.

Research by Hilmer & Bate (1990) showed that the highest phytoplankton biomass occurred when flow rate into the estuary was equivalent to a "residence time" of three spring tidal cycles. The term "residence time" has been queried (Van Niekerk and Huizinga, Pers. com. CSIR) and now refers to the flow rate that would fill the estuary in three spring tidal cycles or 42 days. Working in the Gamtoos Estuary, Snow (2000) especially studied the high chlorophyll *a* that is found at the interface between fresh and saline water. This zone, called the "river estuarine interface zone" or REI normally occurs at a salinity of between 10-15 ppt. The relationship between chlorophyll *a* and flow rate is shown in Table 1. As the flow rate increased in the Gamtoos Estuary, the position of the REI zone moved down the estuary towards the mouth and the vertically averaged chlorophyll *a* decreased. The data in Table 1 indicate that above and below the optimum flow rate for maximum biomass, there is a lower phytoplankton biomass.

Table 1 The influence of flow rate on the content of chlorophyll a in the Gamtoos Estuary (after Snow 2000).

Flow	**Chlorophyll *a***
($m^3 \cdot s^{-1}$)	**($\mu g \cdot l^{-1}$)**
0.3	26.7
0.8	47.6
1	35.7
1.2	49.9
1.25	26.7
2.3	13.5
9.7	15.4
30.5	6.4

There are no data in South Africa to indicate whether there is a diatom species seasonal effect in estuaries, however, Bate *et al.* (2002) showed that there was no seasonal effect in the Swartkops River over a 13-month period. This applied especially to the upper reaches at higher altitudes where the quality of the water had the lowest TDS but where temperatures were more varied. By implication there is good reason to suspect that in South African estuaries, diatom species changes are more

likely to be influenced by water quality variables such as salinity and mineral nutrients than by temperature.

This report describes a study conducted prior to the completion of the Berg River Dam and at the time, the dam was expected to affect the quantity and quality of river water entering the estuary. To understand the effects of these changes on microalgae, this study aimed to determine the distribution of phytoplanktonic and benthic microalgal communities in the Berg Estuary. In particular, to determine which groups are numerically dominant in the phytoplankton and which diatoms (generally the dominant microalgal group in estuarine sediment) are numerically dominant in the benthos. In addition, the study aimed to determine the distribution of microalgae in the estuary by using chlorophyll *a* as an index of microalgal biomass. The strength of association between microalgal biomass and environmental variables was tested and the following hypothesis was proposed to test for a significant difference in average biomass between a summer and winter sampling session: Average phytoplankton chlorophyll *a* concentration is highest in winter (~2 $\mu g \cdot l^{-1}$) compared to summer (~0.1 $\mu g \cdot l^{-1}$) due to higher nutrient availability in the fresh water. The results of the study are used to predict the changes in biomass and community composition in response to reduced river input.

2. MATERIALS AND METHODS

2.1. Study Site

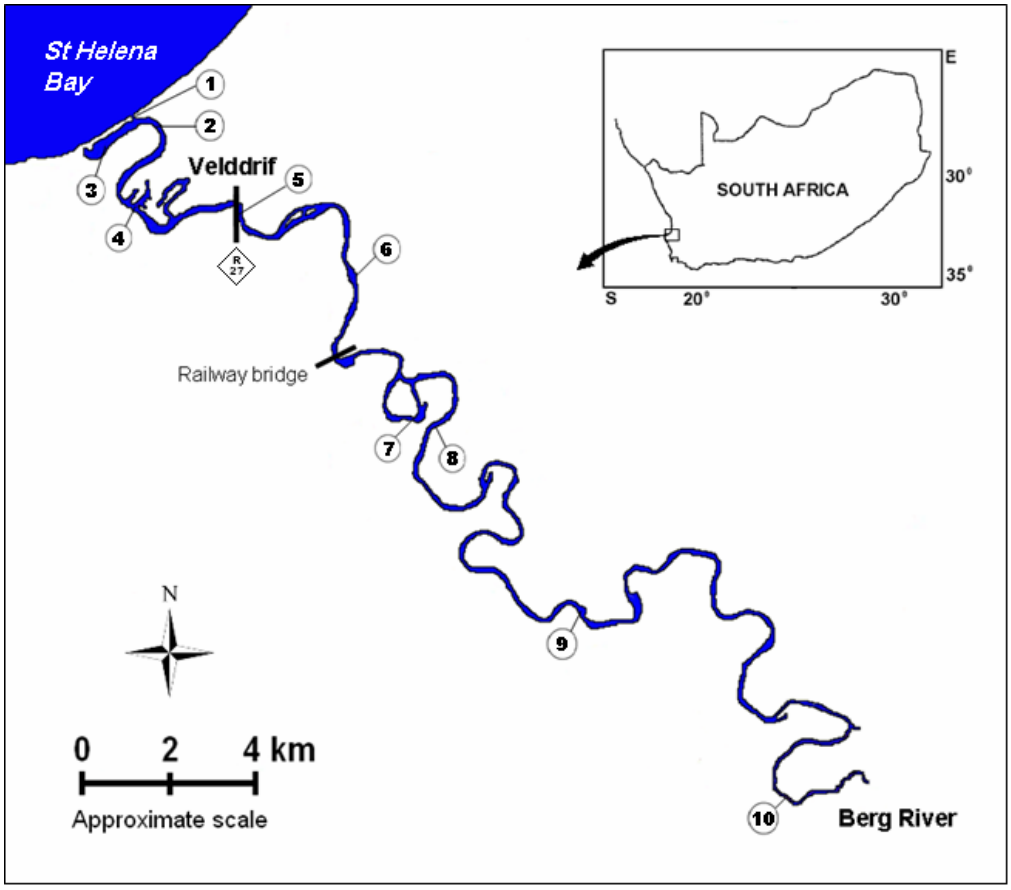

Figure 1 The Berg Estuary indicating locations of the sampling sites.

The Berg is one of only three permanently open estuaries on the west coast of South Africa. It is a river-dominated estuary with tidal influence measurable up to ~70 km from the mouth (Slinger and Taljaard 1994). In 1966 a new estuary mouth was cut through the sand dunes about 1 km north of the original mouth, which was stabilised between concrete walls (Slinger and Taljaard 1994). The original mouth has silted up and the old channel forms a blind arm running parallel to the coast. The lower 4 km of the estuary is frequently dredged to a depth of at least 4 m to allow for boat navigation. The result of the stabilised mouth and deepened channel is a strong tidal current, particularly in the lower and middle reaches of the estuary. Sediment in the lower reaches (sites 1 to 4, Figure 1) were extremely soft, which is usually indicative of a high percentage of fine sediment particles (greater than 30% of <125 µm sediment), high organic content (>3% ash-free dry weight) and a high moisture holding capacity (Snow *unpub. data*). Above the R27 Bridge the intertidal sediment was generally compacted and appeared to have a high fine sand content (125 to 250 µm sediment particle size) and a high detritus content. The upper reaches (>28 km) had a thick fringe of the common reed (*Phragmites australis*) with very little to no exposed intertidal area.

River flow into the estuary was strong in August 2005 and, as a result, sampling was limited to the middle and lower reaches of the estuary (sites 1 to 9, Figure 1). The flow was much weaker in November 2005, hence more sampling effort was placed on the upper reaches of the estuary with an additional two sites 16.5 km (site 8) and 43.2 km from the mouth (site 10) (Figure 1).

2.2. Water Quality

Water quality variables were recorded at each site using an YSI 30-10ft CTD (temperature and salinity), an YSI pH100 pH meter and a WTW Oxi330i oxygen meter. A Secchi disc was used to determine light attenuation. The vertical attenuation coefficient was determined as described by Dawes (1981): K (m^{-1}) = (1.7 / Secchi depth). Filtered water samples (Whatman GF/C) collected in August 2005 were analysed for total oxidised nitrogen (nitrate + nitrite) using the reduced copper cadmium method as described by Bate and Heelas (1975). Ammonium and soluble reactive phosphorus (SRP) were analysed using standard methods (Parsons *et al*. 1984)

2.3. Phytoplankton Identification

Water samples for phytoplankton enumeration were collected at the surface, 0.5 m, 1.0 m and then at 1.0 m intervals to the bottom. The water samples were fixed with 1.5 ml of 1% (v/v) glutaraldehyde solution. Glutaraldehyde was preferred to a 10% neutral formalin solution as formalin can cause flagellates to lose their flagella making identification difficult (Lund et al. 1958, Boney 1989). Samples were then placed in 60 ml settling chambers and allowed to settle for 24 hrs then counted following the Utermöhl method of cell enumeration as modified by Snow et al. (2000). Functional and dominant groups were categorised into flagellates, dinoflagellates, chlorophytes (greens), cyanophytes (blue-greens), diatoms and euglenoids. It is important to note that all flagellates and cyanophytes were included as phytoplankton in this study. Many flagellates do not contain chloroplasts and are more correctly classified as protozoans.

2.4. Phytoplankton Chlorophyll A

Water samples (500 ml) were gravity filtered through Whatman GF/C filters then stored in the dark of a cooler box until they could be frozen. The chlorophyll *a* was extracted by placing the frozen filters into 10 ml of 95% ethanol (Merck 4111). After extraction for 24 hours, spectrophotometric

determinations of chlorophyll *a* were performed according to Nusch (1980). Absorbance was measured before and after acidification of extracts with 0.1 N HCl.

2.5. Benthic Chlorophyll A

Four replicate intertidal benthic samples were collected from premarked locations (20 mm internal diameter circle) at low tide from each site by scraping a known area of surface sediment (<2 mm depth) just above the estuarine water level. Four subtidal samples were collected from each site using a 20 mm internal diameter corer attached to an extension pole and the surface sediment was scraped from the core. Both intertidal and subtidal samples were stored in the dark of a cooler box until they could be frozen. The samples were freeze-dried, approximately 0.1 g was added to 4 ml of 95% ethanol (Merck 4111) and then stored for 24 hours at 0 °C. Once the chlorophyll *a* had been extracted the samples were whirlimixed, filtered through Whatman GF/C filters and the extract was analysed on a Waters M-45 high performance liquid chromatograph (HPLC). Samples collected in November 2005 were analysed spectrophotometrically according to Nusch (1980) before and after acidification of the extracts with 0.1 N HCl.

2.6. Benthic Diatom Collection and Identification

The epipelon was sampled based on the method described by Round (1981) and the details described in Bate et al. (2004). Samples were taken using a length of aluminium piping (~5 mm I.D.) that was drawn across the sediment and allowed to fill with a mixture of surface sediment and water. This process was repeated up to five times in different positions in order to get a sample that was representative of the different micro-habitats. The mixture was stored in a plastic sample container (50 ml). In a field laboratory, some of the settled material was placed in a Petri dish and five clean degreased cover slips (covering ca. 40% of the sediment surface) were placed on top of the wet sediment. On the same day (ca. 1-2 hours later) the cover slips were carefully removed with as little sediment as possible. In this way only living cells that had attached to the cover slips were sampled. The five cover slips from each sample were placed in glass bottles and transported to the laboratory. There is no time limit at this stage to process the diatoms further. To each glass bottle containing the cover slips, 2 ml of saturated $KMnO_4$ and 2 ml of 10 M HCl was added. This mixture was heated on a hot plate at ca. 60°C until the solution cleared (~20-40 mins) and became straw coloured. All acid cleaned samples were washed with distilled water using five consecutive spins (2000 rpm for 10 mins). Permanent light microscopy slides were made with 1-2 drops of diatom 'digest', placed onto an acid-washed cover slip (previously stored in ethanol) and allowed to dry in air. Cover slips treated in this manner allow the drop of sample to spread more evenly. Once completely dry, a small amount of Naphrax® mounting medium (Northern Biological Supplies, U.K.) was dotted onto a glass microscopy slide and the cover slip placed over it. Air trapped under the slide and the Naphrax were dispersed by heating the slide on a hot plate (~60°C). The Naphrax was allowed to dry for 2-3 days. The slides were logged and stored in a slide library, to form a permanent record.

Diatom frustules were examined under a Zeiss Axioplan light microscope with Differential Interference Contract (DIC) optics. Using a television camera (JVC KY-F3), images of the dominant taxa were visualised using the AnalySIS image analysis programme (©1999, Soft Imaging System GmbH). Diatom valves were counted in each sample using 1000x magnification until the obvious dominant was established. At least one of every taxon was made into a digital image. All the images were then printed and used in the counting procedure. This achieves two important aspects, (1) a digital image of each taxon and (2) a count of the total number of taxa. The nomenclature of Krammer & Lange-

Bertalot (1986-91 and 2000) was used with a few exceptions associated with some taxonomic revisions suggested by Round et al. (1990). Other taxonomic works consulted included Archibald (1983), Hustedt (1976), Lange-Bertalot & Krammer (1989), Simonsen (1987) and various articles by R.E.M. Archibald, B.J. Cholnoky and F.R. Schoeman (e.g. Schoeman and Archibald 1976).

2.7. Data Analyses

The Student's t-test was used to test for differences between the means of two variables. Analysis of variance (ANOVA) was used to test for differences between the means of more than two variables using the Tukey's Test for all pairwise multiple comparisons. The Pearson product moment correlation was used to measure the strength of association between two or more variables. The statistical software package Statistica (version 7) was used to perform statistical analyses. Surfer for Windows (Golden Software), version 6, was used to create filled contour profiles.

3. RESULTS

3.1. Water Quality Variables

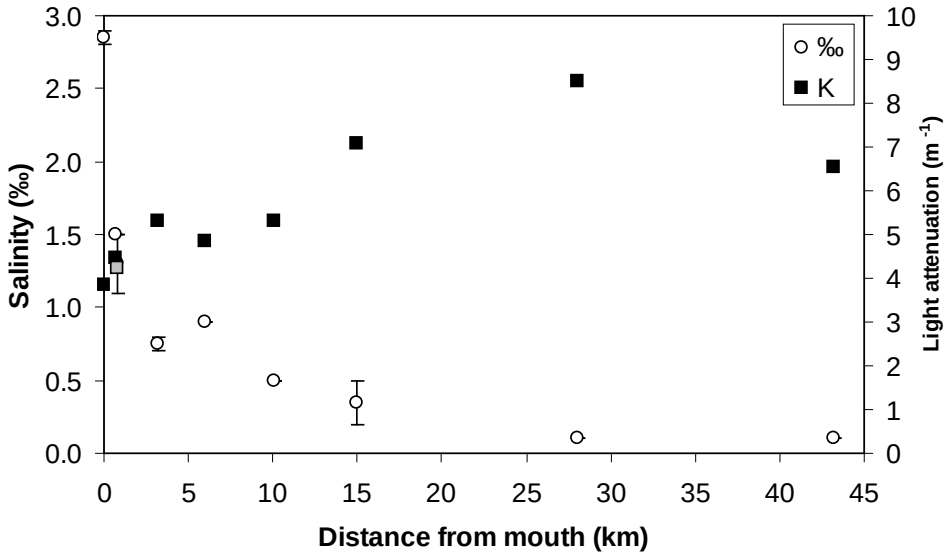

Figure 2 **Vertically averaged salinity (‰) and light attenuation (K) measured along the longitudinal axis in the Berg Estuary, August 2005. Vertical bars represent standard error of the means. Symbols shaded grey represent samples collected in the blind arm, 0.8 km from the mouth of the estuary.**

Salinity profiles measured along the length of the estuary varied considerably from August 2005 to November 2005 (Figures 2 and 3). During August, vertically averaged salinity ranged from 0.1 ± 0.0 ‰ (28 km from the mouth) to 2.9 ± 0.1 ‰ at the mouth of the estuary indicating strong river flow. The turbulent flow resulted in the water column being well mixed and the vertical salinity gradient never exceeded 0.5 ‰. In November, there was a strong longitudinal salinity gradient, indicating a reduced river input relative to August, but the water column remained well mixed. Vertically averaged salinity ranged from 0.4 ± 0.0 ‰ at the head of the estuary (43 km from the mouth) to 32.9 ± 0.0 ‰ at the mouth of the estuary.

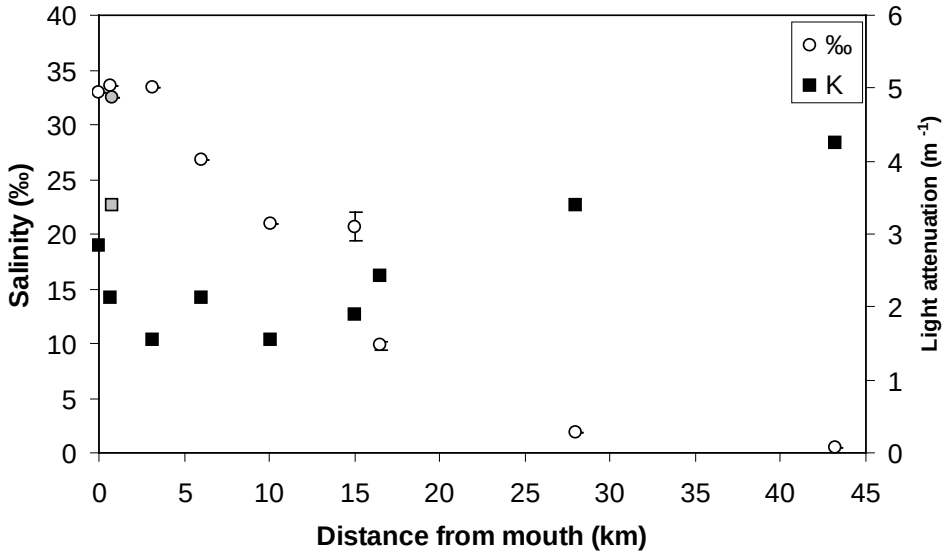

Figure 3 **Vertically averaged salinity and light attenuation (K) measured along the longitudinal axis in the Berg Estuary, November 2005. Vertical bars represent standard error of the means. Symbols shaded grey represent samples collected in the blind arm, 0.8 km from the mouth of the estuary.**

Turbidity, expressed as light attenuation (K), was highest at sites nearest to the head of the estuary in August and November (Figures 2 and 3 respectively) where vertically averaged salinity was less than 10 ‰. Average K in August (5.58 ± 0.50 m^{-1}) was significantly higher than in November (2.56 ± 0.28 m^{-1}) ($t = 5.36$; $p < 0.001$; $n = 10$) as a result of the more turbulent flow. Light attenuation was negatively correlated to salinity in August ($r = -0.79$; $p < 0.05$; $n = 9$) indicating a decrease in turbidity as salinity increases. A similar trend, although not significant, was found in November.

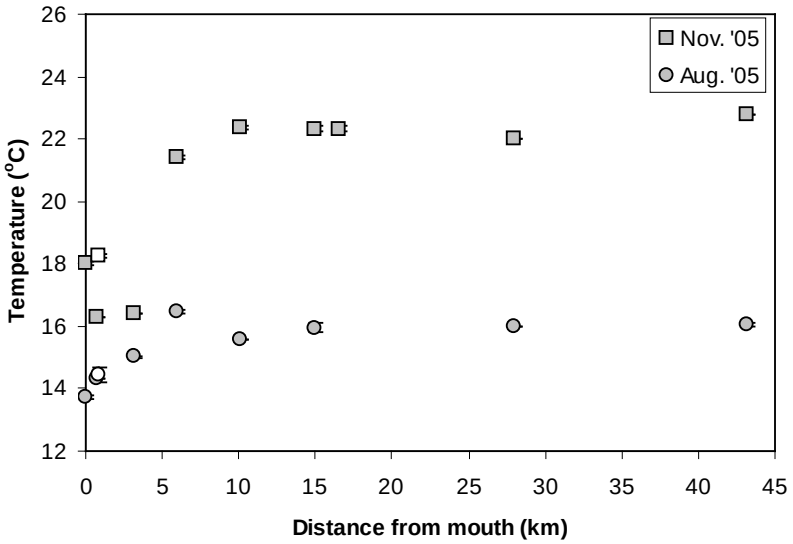

Figure 4 **Vertically averaged temperature measured along the longitudinal axis in the Berg Estuary, August and November 2005. Vertical bars represent standard error of the means. The unshaded symbols represent measurements in the blind arm, 0.8 km from the mouth of the estuary.**

Temperature in the estuary was generally uniform above 5 km from the mouth, with a slight decrease near to the mouth of the estuary (Figure 4). Average temperature in August (15.3 ± 0.3 °C) was significantly lower than in November (20.2 ± 0.8 °C) (t = -5.28; p < 0.001; n = 10). Vertically averaged temperature ranged from 13.7 ± 0.1 °C at the mouth of the estuary to 16.5 ± 0.1 °C (6 km from the mouth) in August, and from 16.3 ± 0.0 °C 0.7 km from the mouth of the estuary to 22.4 ± 0.0 °C (10.1 km from the mouth) in November.

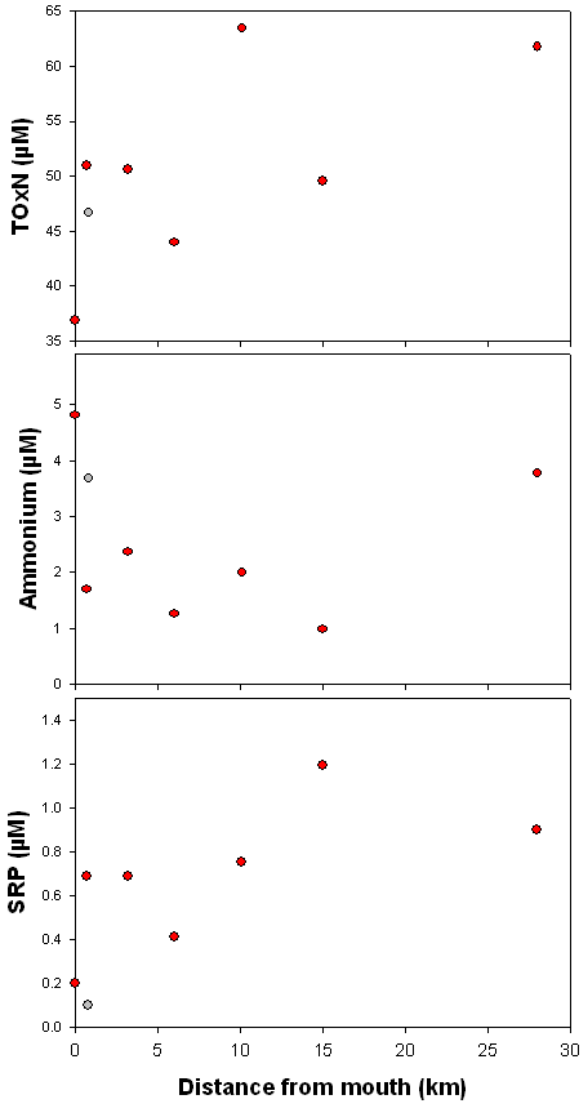

Figure 5. **Total oxidised nitrogen (TOxN), ammonium and soluble reactive phosphorus (SRP) concentrations measured along the longitudinal axis in the Berg Estuary, August 2005. Concentrations are the average of 0.5 m and bottom samples. The symbols shaded grey represents measurements in the blind arm, 0.8 km from the mouth of the estuary.**

In August, nutrients were measured in samples collected at 0.5 m and the bottom and the concentrations averaged. TOxN (nitrate + nitrite) and soluble reactive phosphorus showed gradual increases in concentrations with distance from the mouth (Figure 5). TOxN and SRP ranged in concentrations from 36.9 to 63.5 µM and 0.1 to 1.2 µM respectively. Ammonium was highest at the

mouth (4.8 µM) and closest to the head of the estuary (3.8 µM), and was lowest (1.0 µM) 15 km from the mouth (Figure 5).

In November, water samples were collected and analysed by Anchor Environmental Consultants (unpub. data). Both TOxN and SRP increased as salinity increased, which indicates a marine source. of nutrients into the Berg Estuary during this period of low flow (Figure 6).

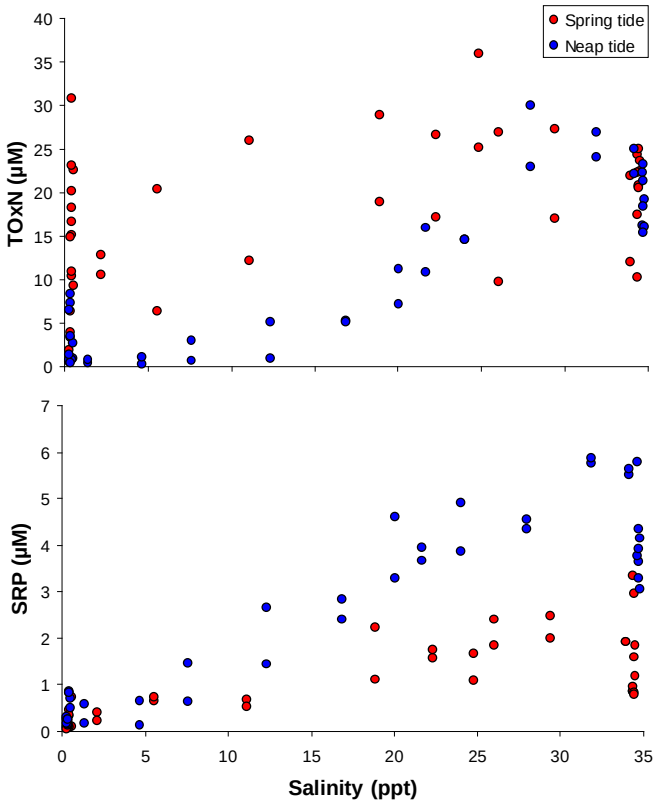

Figure 6. Total oxidised nitrogen (TOxN) and soluble reactive phosphorus (SRP) concentrations in relation to salinity in the Berg Estuary, November 2005 (Anchor Environmental Consultants, *unpub. data*).

3.2. Biotic Variables

Phytoplankton chlorophyll *a* concentration distribution differed considerably between the August and November 2005 sampling trips as a result of the difference in river flow entering the estuary (Figure 7). The average concentration was significantly higher in August ($t = -7.88$; $n_{(Nov)} = 38$; $n_{(Aug)} = 78$; $P < 0.001$), particularly in the middle and lower reaches of the estuary. In August there was no discernable pattern and the concentration was evenly distributed throughout the estuary. Chlorophyll *a* concentration ranged from 4.13 ± 0.25 µg·l^{-1}, 15 km from the mouth, to 6.09 ± 0.77 µg·l^{-1} at the mouth. There was a strong Pearson's correlation between chlorophyll *a* and ammonium ($r = 0.71$; $p < 0.01$; $n = 16$) and a negative correlation with TOxN ($r = -0.52$; $p < 0.05$; $n = 16$). In November there was a distinct increase in concentration with distance from the estuary mouth, ranging from 0.26 ± 0.04 µg·l^{-1} at the mouth to 6.63 ± 1.01 µg·l^{-1}, 43 km from the mouth. Total oxidised nitrogen and soluble reactive phosphorus (data provided by Anchor Environmental Consultants) increased with salinity, which was the inverse of the phytoplankton chlorophyll *a*. Vertically averaged phytoplankton chlorophyll *a*

measured in November was significantly correlated to light attenuation (r = 0.72; p < 0.05; n = 10) suggesting that phytoplankton cells either contributed to the turbidity, were closely associated to suspended solids or turbulence in the upper reaches of the estuary had resuspended fine sediments that were high in microalgal biomass.

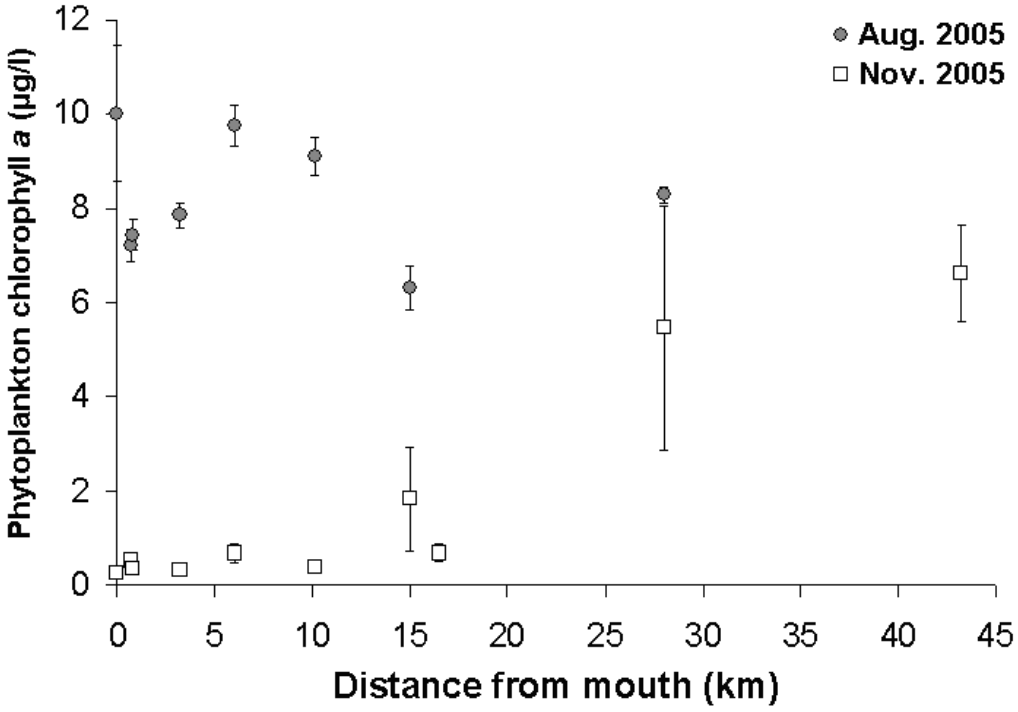

Figure 7 **Vertically averaged water column chlorophyll *a* measured along the longitudinal axis in the Berg Estuary, August and November 2005. Vertical bars represent standard error of the means.**

In August 2005 the phytoplankton was dominated by flagellates (80.9%) and diatoms (17.8%). The remaining three groups contributed less than 2 % of the phytoplankton cell density. The flagellates and diatoms had similar distribution patterns in the estuary and ranged from 5901 ± 1081 cells·ml^{-1} (28 km from the mouth) to 60527 ± 8934 cells·ml^{-1} (10.1 km from the mouth) and 3728 ± 674 cells·ml^{-1} (28 km from the mouth) to 16554 ± 4402 cells·ml^{-1} (10.1 km from the mouth) respectively (Figure 8). Using an all pairwise multiple comparison procedure (Tukey's Test) it was found that the density of flagellates at the two sites nearest to the head of the estuary (15 and 28 km) were significantly lower than at the next two sites closer to the mouth (6 and 10.1 km). This result indicates that the high density of flagellates did not enter the estuary, which would otherwise be evenly distributed throughout the upper reaches of the estuary. Instead, there was sufficient residence time for high cell densities to develop in the lower-middle reaches of the estuary (between 3.2 and 15 km from the mouth). A Tukey's Test on diatom density found no significant differences between the sampling sites. However, the distribution pattern is similar to that of the flagellates, which suggests that the high densities found between 3.2 and 15 km from the estuary mouth also developed within the lower-middle reaches of the estuary.

Of the five phytoplankton groups, only the flagellates (r = 0.97; p < 0.001; n = 39), diatoms (r = 0.79; p < 0.001; n = 39) and cyanophytes (r = 0.54; p < 0.001; n = 39) were significantly correlated to total cell

density in the estuary. This is largely due to the high cell density of these groups in the surface water 10.1 km from the mouth (Figure 8). Total phytoplankton density was significantly correlated to phytoplankton chlorophyll *a* in the estuary ($r = 0.34$; $p < 0.05$; n = 39), which showed a slight increase in the lower-middle reaches of the estuary and at the mouth of the estuary (Figure 9). Only the diatom group was significantly correlated to chlorophyll *a* ($r = 0.44$; $p < 0.01$; n = 39). It is possible that there was a high density of heterotrophic flagellates present resulting in the weak association between flagellate densities and chlorophyll *a*.

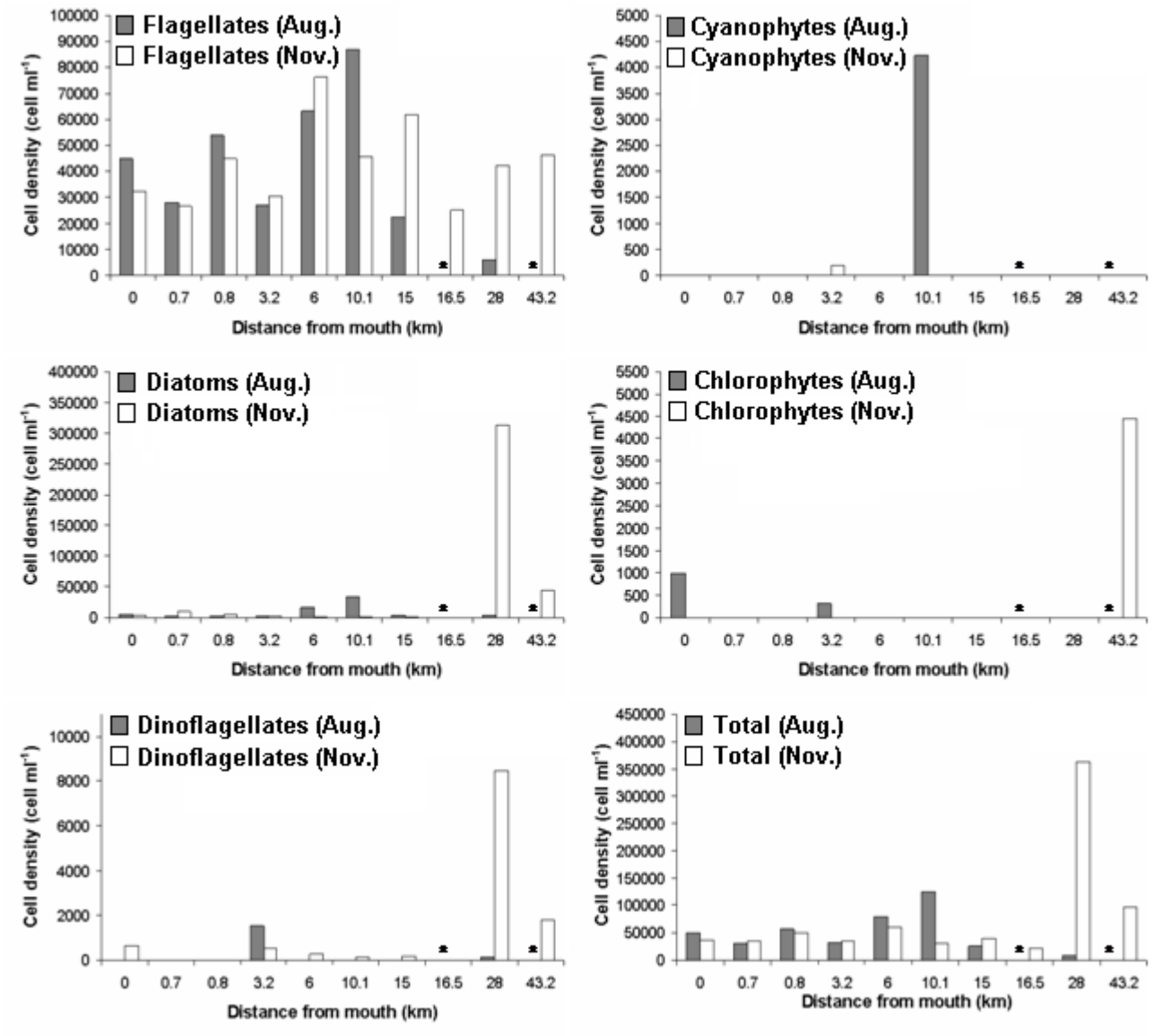

Figure 8 Surface phytoplankton cell density (cell·ml^{-1}) measured in August and November 2005 along the longitudinal axis of the Berg Estuary.

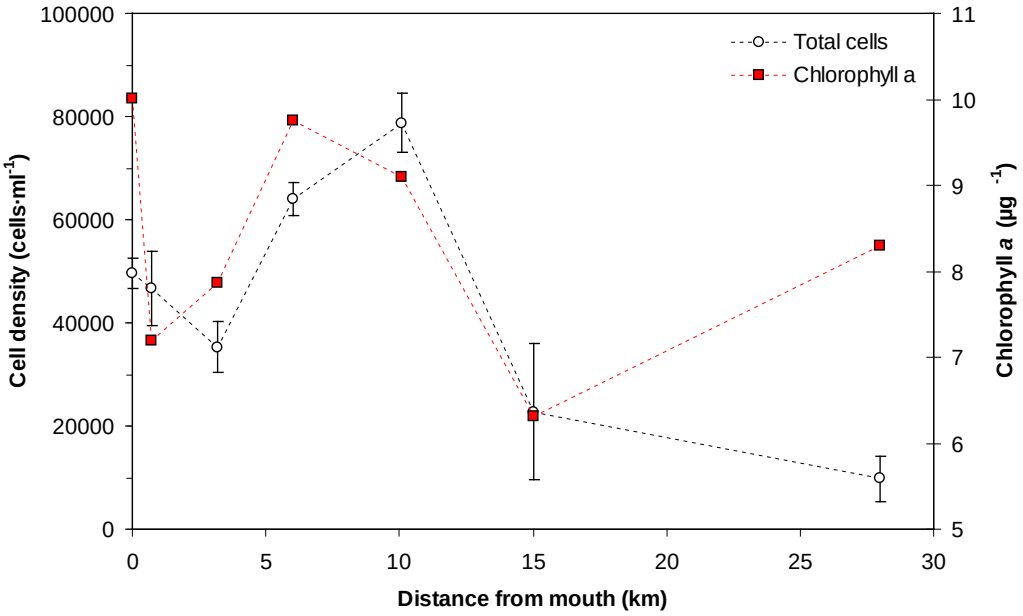

Figure 9 **Vertically averaged total phytoplankton cell density (cell·ml^{-1}) and chlorophyll *a* (µg·l^{-1}) measured in August 2005 along the longitudinal axis of the Berg Estuary. Vertical bars represent standard error of the means.**

In November 2005, phytoplankton at the water surface was dominated by flagellates (79.2%) and diatoms (19.5%). The flagellate:diatom ratio ranged from 3.0 to 175.0 in the lower to middle reaches of the estuary respectively. High densities (21366 to 58429 cells·ml^{-1}) of flagellates were present throughout the estuary (Figure 8) and were largely dominated by a 'small' flagellate (Figure 10C). In addition to the 'small' flagellates, a high density of 'large' flagellates (Figure 10A) was present in the estuary from 6.0 to 16.5 km from the mouth (3728 to 17714 cells·ml^{-1}), which contributed to the high flagellate: diatom ratio. A bloom of 'small' diatoms (Figure 10B) in the middle and upper reaches of the estuary (28 and 43.2 km), with densities in excess of 300 000 cells·ml^{-1} (Figure 8), resulted in the low flagellate: diatom ratio at these sites.

Microalgal biomass was highest in the middle and upper reaches of the estuary where chlorophyll *a* concentrations were 5.47 ± 2.59 µg·l^{-1} (28 km) and 6.63 ± 1.01 µg·l^{-1} (43.2 km) (Figure 11). The estuary was shallow (< 1 m) 28 km from the mouth, which possibly reduced tidal flow and provided a more stable habitat in the upper reaches of the estuary. There was a strong association between phytoplankton chlorophyll *a* and the diatoms (r = 0.67; p < 0.05; n = 10), dinoflagellates (r = 0.70; p < 0.05; n = 10) and chlorophytes (r = 0.74; p < 0.05; n = 10). This indicates that the peak in chlorophyll *a* in the upper reaches of the estuary, referred to as the river-estuary interface (REI), is primarily the result of chlorophyll *a* pigments from these three phytoplankton groups. As was the case in August, the weak association between flagellates and chlorophyll *a* may be the result of a high proportion of heterotrophic cells within the flagellate group.

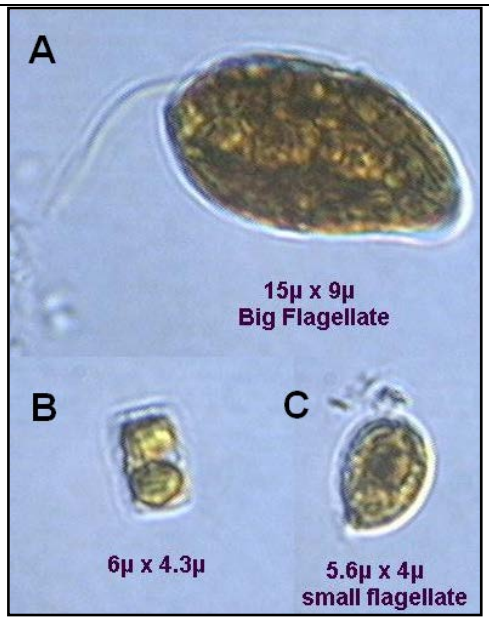

Figure 10 **Dominant phytoplankton cells collected in November 2005 (A = large flagellate, B = small diatom and C = small flagellate).**

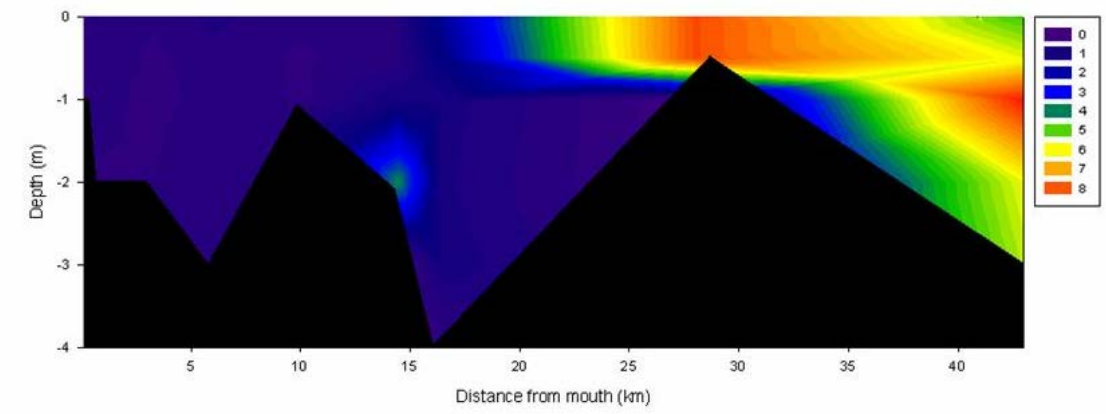

Figure 11 **Filled contour diagram of phytoplankton chlorophyll *a* (µg·l^{-1}) in the Berg Estuary, November 2005.**

Benthic chlorophyll *a* concentration measured in August ranged from 2.6 to 31.5 µg·g^{-1} in the intertidal zone and 2.1 to 12.6 µg·g^{-1} in the subtidal zone (Figure 12). Highest concentrations were measured in the soft sediments in the blind arm near to the mouth of the estuary. In November the concentration ranged from 1.5 to 3.9 µg·g^{-1} in the intertidal zone and 0.8 to 14.9 µg·g^{-1} in the sub-tidal zone. Intertidal chlorophyll *a* was lowest at the two sites nearest to the mouth of the estuary and subtidal chlorophyll *a* increased significantly with distance from the mouth (r = 0.84; p <0.01; n = 10).

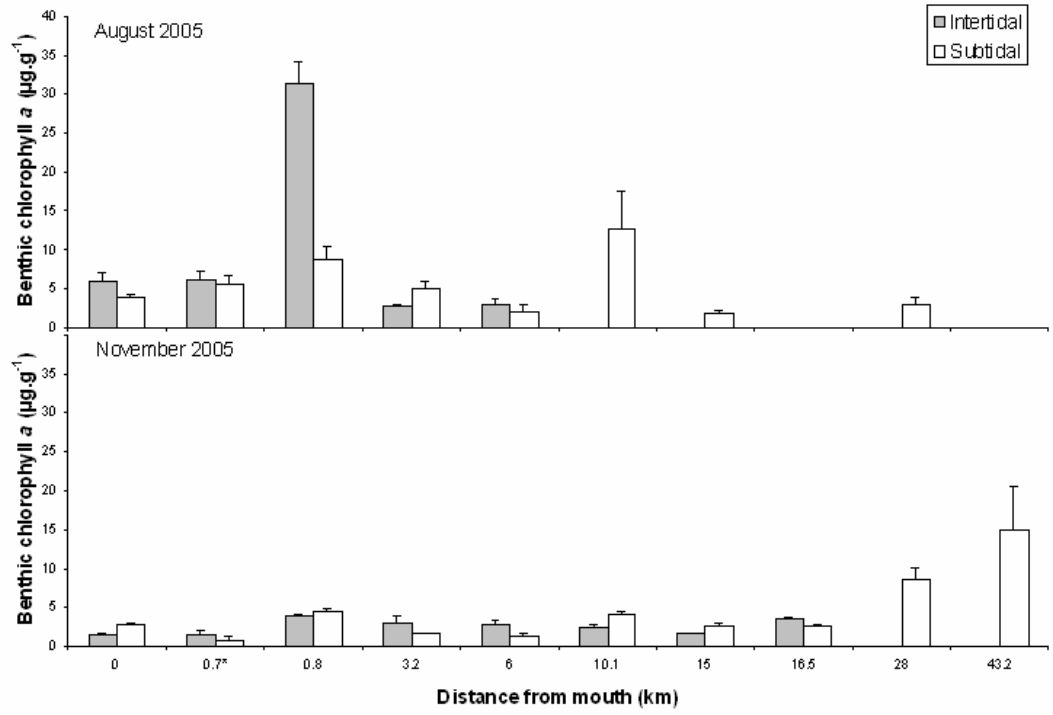

Figure 12 Intertidal and subtidal benthic chlorophyll *a* along the longitudinal axis of the Berg Estuary, August and November 2005.

There were 18 dominant benthic diatoms in the samples collected in August (Table 2) and 13 in November (Table 3). *Opephora minuta* was dominant at sites nearest to the mouth of the estuary in August and 10.1 km from the mouth in November. *Bacillaria paxillifer* var. *paxillifer* was present in the middle reaches of the estuary during both sampling sessions, not nearer than 6 km from the mouth. *Hippodonta* sp. was only dominant in the estuary in November at sites in the lower reaches of the estuary (from the mouth to 10.1 km). *Denticula sundaysensis* and *Planothidium delicatula* were examples of taxa dominant exclusively in sediment at sites nearest to the head of the estuary in November and August respectively.

Table 2 List of benthic diatom taxa collected in August 2005. The relative abundances of dominant species (dominance) and total number of species (# taxa) at each site are included.

Distance (km)	Zone	Taxon	dominance (%)	# taxa
0	Intertidal	*Opephora minuta* Cleve-Euler	45	23
		Catenula adhaerens (Mereschk.) Mereschk.	16	
		Odontella aurita (Lyngbye) Agardh	15	
	Subtidal	*Fallacia sp. 03* D.G. Mann	38	33
		Catenula adhaerens (Mereschk.) Mereschk.	19	
		Opephora minuta Cleve-Euler	17	
0.7	Intertidal	*Navicula gregaria* (Donkin)	80	28
	Subtidal	None		10
0.8	Intertidal	*Navicula salinicola* Hustedt	34	35
		Cocconeis sp. 02 Ehrenberg	13	
		Navicula gregaria (Donkin)	11	
	Subtidal	*Opephora minuta* Cleve-Euler	23	18

Distance (km)	Zone	Taxon	dominance (%)	# taxa
		Catenula adhaerens (Mereschk.) Mereschk.	18	
3.2	Intertidal	*Navicula gregaria* (Donkin)	70	32
		Nitzschia aff perindistincta Cholnoky	17	
		Amphora subacutiuscula Schoeman	10	
	Subtidal	None		11
6	Intertidal	*Amphora sp. 04*	28	12
		Navicula gregaria (Donkin)	24	
		Amphora cf. copulata (Kütz.) Schoe. & Arch.	16	
		Fallacia sp. 03 D.G. Mann	12	
	Subtidal	*Bacillaria paxillifer* var. *paxillifer* (O.F. Muller) Hendey	35	24
		Amphora ovalis var. affinis (Kützing) V Heurck	13	
10.1	Intertidal	*Rhopalodia brebissonii* Krammer	15	40
		Fallacia sp. 03 D.G. Mann	10	
15	Intertidal	*Navicula salinarum* Grunow	22	30
		Fallacia sp. 03 D.G. Mann	15	
		Tryblionella hungarica (Grunow) D.G. Mann	15	
28	Intertidal	*Hippodonta hungarica* (Grun.) Lange-Bertalot et al.	32	23
		Bacillaria paxillifer var. *paxillifer* (O.F. Muller) Hendey	22	
		Planothidium delicatula (Kütz) Round & Buktiyarova	14	

Table 3 List of benthic diatom taxa collected in November 2005. The relative abundances of dominant species (dominance) and total number of species (# taxa) at each site are included.

Distance (km)	Zone	Taxon	dominance (%)	# taxa
0	Intertidal	*Hippodonta sp.* Lange-Bertalot et al.	20	16
	Subtidal	*Amphora coffeaeformis* (Agardh) Kützing	60	14
0.7	Intertidal	None		
	Subtidal	None		
0.8	Intertidal	*Cocconeis scutellum var. scutellum* Ehrenberg	28	25
		Hippodonta sp. Lange-Bertalot et al.	22	
	Subtidal	*Amphora acutiscula* Kützing	85	29
3.2	Intertidal	*Hippodonta sp.* Lange-Bertalot et al.	80	25
	Subtidal	*Hippodonta sp.* Lange-Bertalot et al.	35	36
		Navicula sp. Bory	15	
		Navicula paeninsulae Cholnoky	10	
6	Intertidal	*Navicula sp.* Bory	60	28
		Nitzschia aremonica Archibald	20	
	Subtidal	*Hippodonta sp.* Lange-Bertalot et al.	33	20
		Bacillaria paxillifer var. *paxillifer* (O.F. Muller) Hendey	15	
10.1	Intertidal	*Opephora minuta* Cleve-Euler	85	19
	Subtidal	*Bacillaria paxillifer* var. *paxillifer* (O.F. Muller) Hendey	70	
		Hippodonta sp. Lange-Bertalot et al.	10	14
15	Intertidal	None		
	Subtidal	None		
16.5	Intertidal	*Nitzschia sigma* (Kützing) W.Smith	29	25
		Navicula salinicola Hustedt	23	
		Bacillaria paxillifer var. *paxillifer* (O.F. Muller) Hendey	20	
	Subtidal	*Navicula salinicola* Hustedt	90	19
28	Subtidal	*Fragilaria elliptica* Schumann	70	12
		Bacillaria paxillifer var. *paxillifer* (O.F. Muller) Hendey	23	
43.2	Subtidal	*Fragilaria elliptica* Schumann	75	25
		Denticula sundaysensis Archibald	15	

3.3. CSIR Study (1989-1990)

During the flood tide on 18 September 1989 there was a full salinity gradient from near marine (33.1 ‰) at the mouth to near fresh (0.7 ‰) at the surface 9.8 km from the mouth of the estuary (Figure 14). Water in the estuary during the following ebb tide (19 September 1989) was river-dominated ranging in salinity from 32.8 ‰ at the mouth to 0.6 ‰ just 3.1 km from the mouth. Phytoplankton chlorophyll *a* was low throughout the estuary, ranging from 0.7 to 2.9 $\mu g \cdot l^{-1}$ during the two sampling dates (average ± standard deviation; 1.8 ± 0.5 $\mu g \cdot l^{-1}$) (Figure 13). It is important to note that samples were collected from the surface and bottom of the water column, probably missing the peak in biomass that generally occurs at a depth of between 0.5 and 1.0 m. Biomass was fairly even throughout the estuary showing no association with salinity.

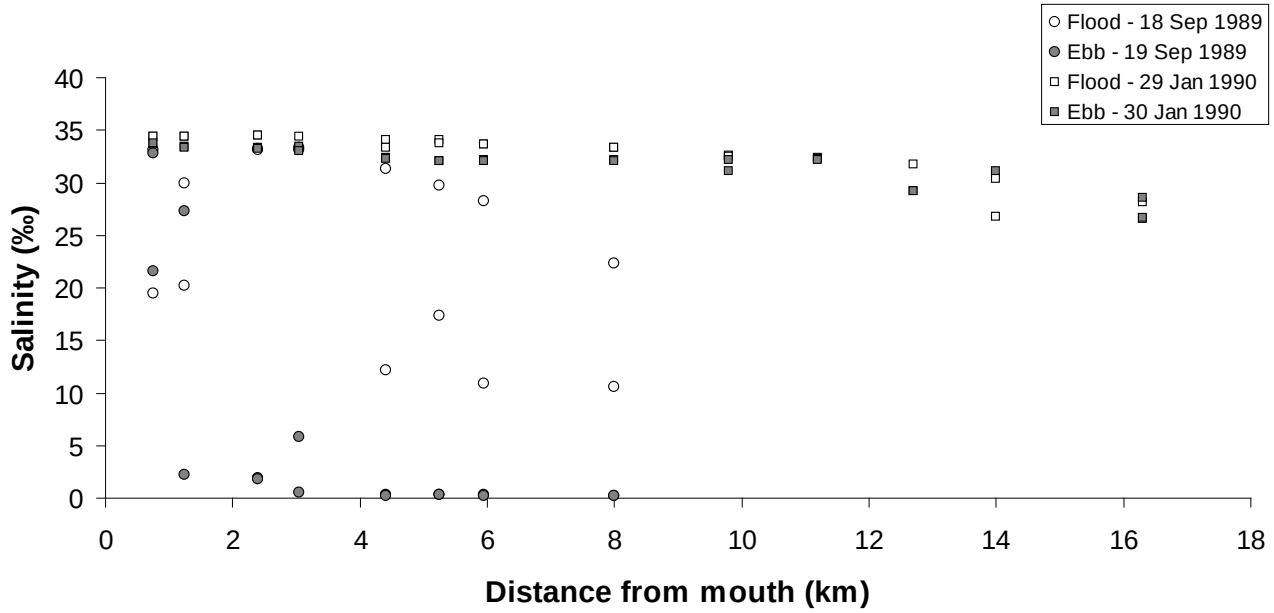

Figure 13 Surface and bottom salinity measured along the longitudinal axis of the Great Berg Estuary during flood and ebb tides in September 1989 and January 1990.

On 29 January 1990 the estuary was marine-dominated during both the flood and the following ebb tide (30 January 1990); salinity ranged from near marine at the mouth (34.4 and 33.7 ‰ respectively) to 26.6 ‰ at the surface 16.3 km from the mouth. Phytoplankton chlorophyll *a* was extremely low on these two days, ranging from 0.1 $\mu g \cdot l^{-1}$ to 0.4 $\mu g \cdot l^{-1}$ throughout the estuary. The average concentration (0.2 ± 0.1 $\mu g \cdot l^{-1}$) is more typical of oligotrophic systems.

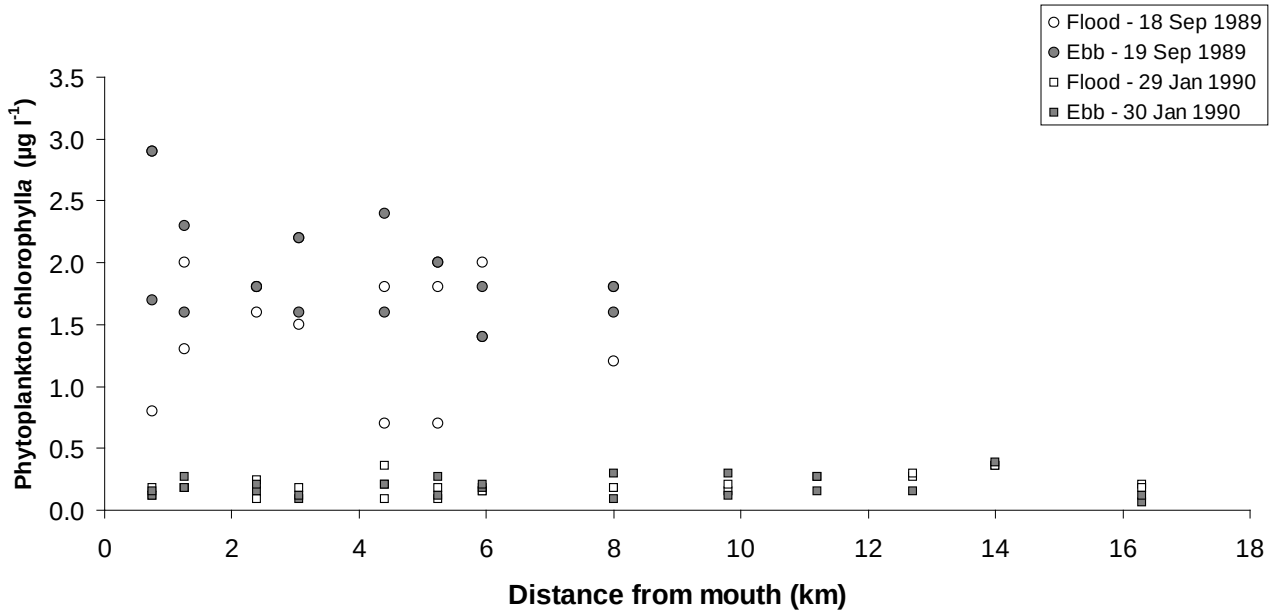

Figure 14 **Surface and bottom phytoplankton chlorophyll *a* measured along the longitudinal axis of the Great Berg Estuary during flood and ebb tides in September 1989 and January 1990.**

4. DISCUSSION

Based on historical data (1975 to 1996), Taljaard (2004) described the Berg River Estuary as having a strongly seasonal hydrological regime. During winter, strong freshwater flushing generally limited saline intrusion during the flood tide up to the R27 Bridge (Figure 1). During summer the river inflow to the estuary was low and the system became marine-dominated. Characteristically estuarine water (salinities between 30 and 5 ppt) occurred between Kliphoek (site 7 in figure 1) and 40 km from the mouth of the estuary.

The Berg Estuary was well mixed in August and November with distinct changes in environmental variables between the sampling sessions. Temperature followed local climatic conditions with highest temperatures recorded in November (~20°C) and lowest temperatures in August (~15°C). The water temperature in the lower reaches of the estuary was lower, by up to 6°C, than in the upper and middle reaches of the estuary because of seawater intrusion.

An important morphological feature in the Berg Estuary is the depth of the channel 28 km from the mouth. The estuary broadens in this area but is very shallow (0.5 m at low tide), which causes a slight bottle-neck in flow. In November the salinity was low at this site (<2 ‰) and increased to 10 ‰ at the next site downstream (16.5 km). In addition, the attenuation of light through the water column was highest (>3 m^{-1}) at this site and further upstream.

Nutrient input into the estuary is moderate to high as a result of agricultural inputs. Taljaard (2004) stated that nitrogen in the estuary is mainly in the form of nitrate (NO_3), as opposed to nitrite (NO_2), which is typical of a well-oxygenated system. Turpie & Clark (2005) estimated that freshwater contains 60 to 90 µM total inorganic nitrogen in winter as opposed to 20 to 40 µM in summer. Under low flow conditions, nitrogen concentration is highest in the lower reaches of the estuary, decreasing upstream and in winter the concentration is highest in the upper reaches. Results of this study have

confirmed this finding in August with a high concentration of TOxN (>60 µM) at the head and decreasing to less than 40 µM at the mouth of the estuary. Soluble reactive phosphorus was generally low in the estuary (<1.2 µM) and the molar DIN:P ratio (i.e. $[NO_3 + NO_2 + NH_4]$:$[PO_4]$) was above 40 throughout the estuary and was >500 in the blind arm near to the mouth. This could be indicative of P-limited microalgal growth. However, at high river flows the residence time of water in the estuary and turbulent currents may be more important limiting factors than nutrient supply.

Phytoplankton chlorophyll *a* measured in the Berg Estuary during this study and the CSIR 1989-1990 studies were low, generally less than 8 $\mu g.l^{-1}$, which compares well to other Western Cape estuaries (Table 4). However, sampling was limited to two sessions, during high and low flow periods and does not include the transition phases between the two flows. The transition phases would be a period of medium flow and would be most likely to occur during spring and autumn, seasons when phytoplankton blooms are most likely to occur.

An estuarine freshwater requirement (EFR) study of the Olifants Estuary, a permanently open estuary approximately 125 km north of the Berg, was sampled in March and August 2004. Results showed that the system functions as a permanently open estuary and has a well developed, but not eutrophic, REI region (Bate 2006). The pattern of water-column chlorophyll *a* was similar to the Berg Estuary, decreasing from the head of the estuary to the mouth. Maximum chlorophyll *a* concentrations were low, generally less than 10 $\mu g \cdot l^{-1}$, and were similar in March and August. This pattern of microalgal biomass appeared to be a function of macro-nutrient concentration and grazing pressure (Bate 2006). In March 2004, flagellates were the most dominant phytoplankton group and, together with dinoflagellates, peaked in the middle reaches of the estuary. Diatoms increased in density with decreasing salinity, reaching a maximum 20.5 km from the mouth. In August, the slightly higher salinity in the estuary indicated a slightly lower river water input. Flagellates were still the dominant group in the estuary but the density was much lower and, together with the diatoms, were evenly spread throughout the estuary.

Table 4 Range of phytoplankton chlorophyll *a* concentrations published for South African estuaries (modified from Adams & Bate 1999). Western Cape estuaries are marked with an asterisk.

	Chlorophyll a ($\mu g.l^{-1}$)		
Estuary	**Min**	**Max**	**Reference**
Olifants*	1.7	10.3	Bate 2006
Palmiet*	2	8	Branch & Day 1984
Bot*	0	6	Bally et al. 1985
Gamtoos	1.6	115.2	Snow 2000
Sundays	12	23	Hilmer & Bate 1991
Sundays	> 100 (bloom)		Hilmer & Bate 1990
Kariega	1	8	Allanson & Read 1995
Great Fish	0	52	Allanson & Read 1995
Great Fish	> 100 (bloom)		Lucas 1986
Keiskamma	0	19	Allanson & Read 1995
Nahoon	1	6	Campbell et al. 1991
Gqunube	5	15	Campbell et al. 1991
Kwelera	0	10	Campbell et al. 1991
St Lucia	0	16	Fielding et al. 1991

Phytoplankton dominance in permanently open estuaries is a function of inorganic nutrient concentration in the river water and favourable water retention times (days to weeks). A study of the Gamtoos Estuary (Snow et al. 2000) found that a flow of 1 $m^3 \cdot s^{-1}$ provided the ideal residence time, resulting in the highest vertically averaged chlorophyll *a* concentration (115 $\mu g \cdot l^{-1}$), or REI, in the upper reaches of the estuary. As river flow increased, the position of the REI moved further downstream and the chlorophyll a concentration decreased to 10 $\mu g \cdot l^{-1}$ at a flow of 30.5 $m^3 \cdot s^{-1}$. In this study, phytoplankton chlorophyll *a* in the Berg was highest in the lower reaches of the estuary in August. The highest vertically averaged chlorophyll *a* (10 $\mu g \cdot l^{-1}$) was located at the mouth of the estuary. In November the highest phytoplankton chlorophyll *a* was in the upper reaches of the estuary. There was a distinct decrease in concentration in the middle and lower reaches of the estuary below the shallow 28 km site. The highest vertically averaged chlorophyll *a* concentration (7 $\mu g \cdot l^{-1}$) was at the head of the estuary (43.2 km).

The Swan River Estuary, Western Australia, is similar to the Berg Estuary and could provide an invaluable glimpse into the effects of reduced river input on the phytoplankton community composition in the Berg. The Swan Estuary is in a Mediterranean climate, is micro-tidal and there are moderate to high nutrient loads from urban and rural catchments (Chan et al. 2002). The estuary is split into two distinct regions; the lower estuary (the mouth at Fremantle to the narrows near Perth) and the upper estuary (the narrows to the tidal limit). Salinity penetration is generally restricted at the narrows with the result that the upper estuary is much fresher than the lower estuary (Figure 15). The upper estuary has a series of deep pockets and these are reported to have higher concentrations of NH_4 and PO_4 (Rosser and Thompson 2001). A recognised pattern of bloom succession has been

established within the upper reaches (Rosser and Thompson; Chan et al. 2002) (Figure 16). Chlorophyte-dominated blooms occurred in early spring as river flow decreased. In mid summer the salinity in the upper estuary reached ~30 ‰ and estuarine diatoms dominated giving way to dinophyte- and cryptophyte-dominated blooms through summer and autumn periods. As flow increased and salinity decreased in the upper estuary, freshwater diatoms became dominant.
A reduction in flow from dam construction and increased nutrient loads from anthropogenic inputs caused an increased occurrence and biomass of cyanophyte blooms in the inner Neva Estuary, Gulf of Finland (Nikulina 2003).

Flagellates were dominant throughout the Berg Estuary in August and November, during high and reduced river inputs respectively. In August, the estuary was near-fresh throughout (<3 ‰) and a distinct phytoplankton community was present 10.1 km from the mouth, which consisted of flagellates (77%), diatoms (21%), cyanobacteria (1%) and chlorophytes (1%). In November a distinct community occurred 28 km from the mouth and consisted of flagellates (12%), a diatom-dominated bloom (86%) and dinoflagellates (2%).

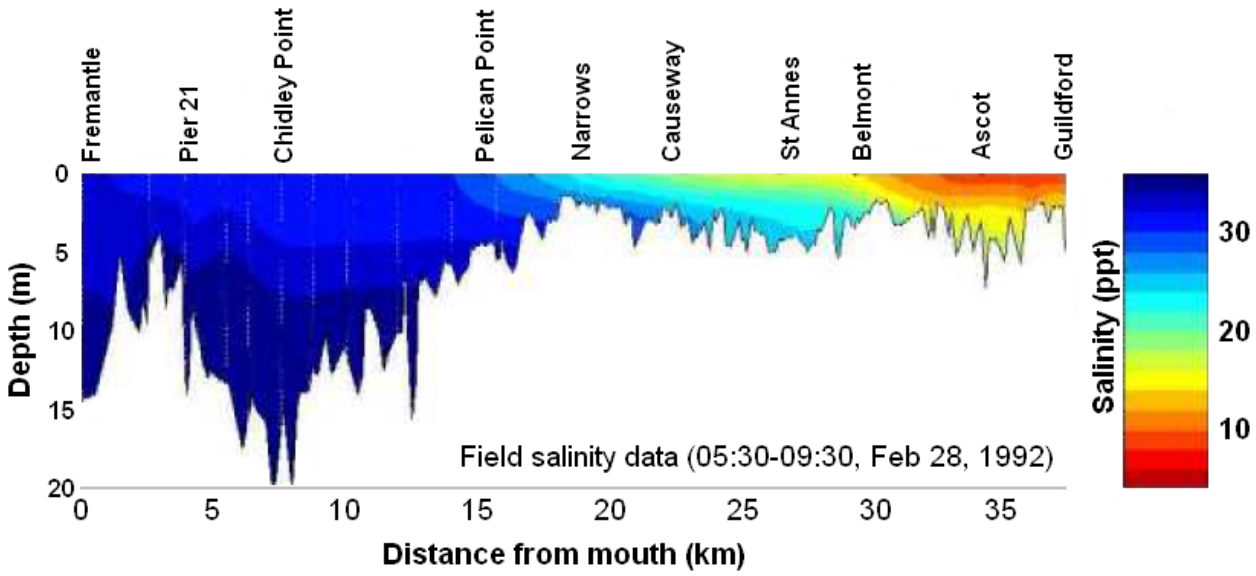

Figure 15 Filled contour plot of salinity in the Swan River Estuary, Western Australia (Chan *et al*. 2002).

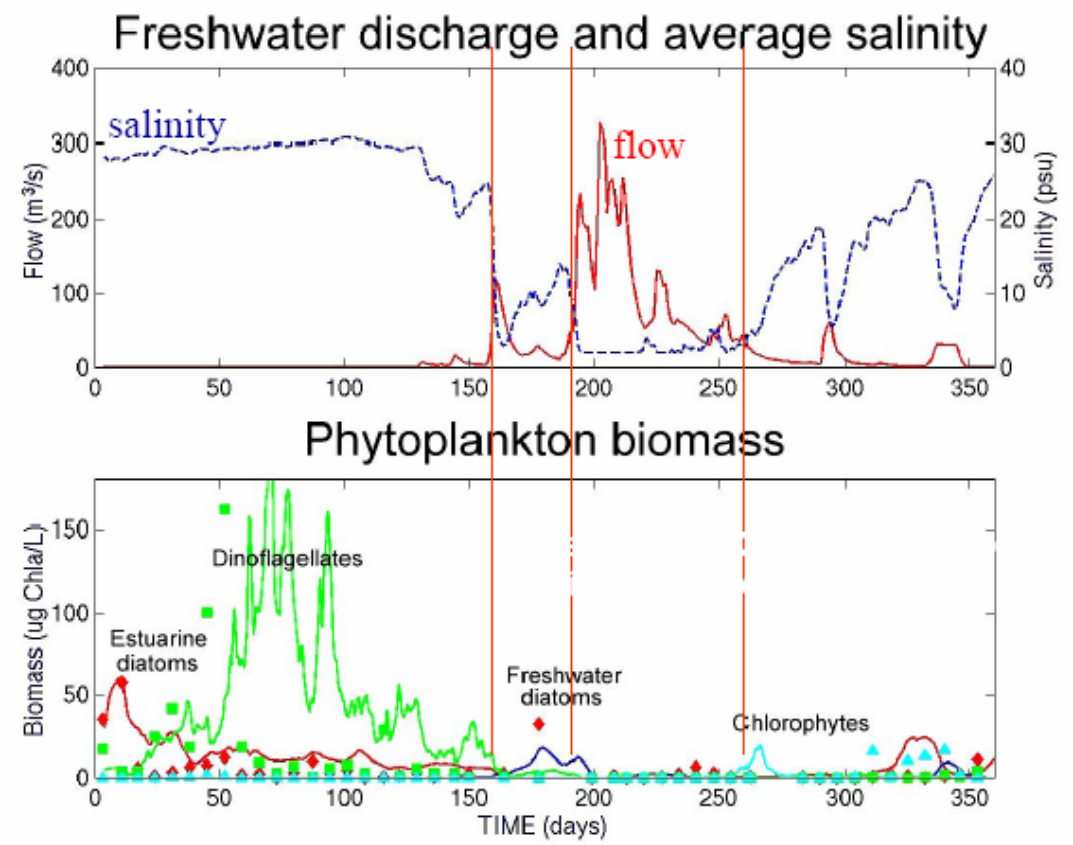

Figure 16 Phytoplankton group biomass in the upper reaches of the Swan River Estuary relative to freshwater discharge and salinity, 1995 (Chan et al. 2002).

Recent studies of the Keurbooms, Gamtoos, Swartkops, Sundays, Mngazana and Mngazi Estuaries found an intertidal chlorophyll *a* range of between 0.5 ± 0.3 µg·g^{-1} and 86.6 ± 7.3 µg·g^{-1} (Snow 2007). Benthic microalgal biomass followed a general distribution pattern based on the distributions of organic matter and fine sediment (<125 µm). Sites closest to the mouth and head of the estuaries were generally dominated by coarse sediment, were low in organic matter (<3% ash-free dry weight) and supported a low microalgal biomass. The process of flocculation, a process well described by Day (1981), leads to the settlement of organic matter, fine sediment particles, nutrients such as phosphorus and microalgal cells as floccules in the REI zone. This results in sediment in the middle reaches of the estuaries becoming high in organic matter, dominated by fine sediment and supporting a high microalgal biomass. However, results from the Berg Estuary did not follow this generalised pattern (Figure 12) and could be the result of the turbulent tidal currents. The highest biomass was measured in the blind arm during high flow in August, probably because this site was protected from the turbulent currents in the main channel (river water flow and tidal exchange). In November, biomass was highest in the upper reaches of the estuary and decreased towards the mouth. It is possible that suspended microalgal cells were settling out of the water column but it is more likely that benthic microalgal biomass increased as tidal currents, and the associated resuspension of microalgal cells, decreased with distance from the mouth.

4.1. Responses to Reduced Flow

Salinity profiles measured during this study compared well to profiles reported by Schumann (2004). In August 2005, salinity was 2.9 ‰ at the mouth and the 1 isohaline was close to site 4 (Figure 1). This is similar to the August 2003 spring low salinity profile (Schumann 2004), where flow was estimated to be 126.9 $m^3 \cdot s^{-1}$. The salinity profile during the spring low in November 2005 corresponded well with the profile measured during the spring low in November 2003. Unfortunately, base flows had not been investigated at the time of submitting the 2004 annual report 2004 and had not been updated in the 2005 annual report. However, based on flows reported for May 2003, flow was less than 0.2 $m^3.s^{-1}$.

Data supplied on the Berg Estuary showed that its volume is 12 million m^3. The actual annual average flow for the period 15 Jan 1995 to 1 November 2003 was 17.9 $m^3 \cdot s^{-1}$. The maximum was 892.6 $m^3 \cdot s^{-1}$ while the minimum was 0.8 $m^3 \cdot s^{-1}$. Applying the 42-Day "Optimum Rule", the flow rate that would give the maximum biomass ± 10% is 3.3 $m^3 \cdot s^{-1}$. The actual flow data show that the optimum flow rate is exceeded 84% of the time and only within the optimum range for 6% of the time. For 10% of the time the flow rate is below the optimum.

The implication of this is that during periods of high river inflows, the estuary is freshwater dominated and the "REI" would be out to sea. This must be interpreted with caution, however. Studies of freshwater requirements are usually undertaken when there is a necessity to decrease the flow in rivers in order to send it elsewhere. The data provided in this report might easily be interpreted to imply that because the average flow rate is far above the optimum for primary productivity, that by decreasing the flow, the estuary might increase its productivity and therefore improve. Assumptions of this nature are incorrect because there would be a shift from the natural condition and the holistic ecology will be altered in a manner that cannot be predicted without a complete understanding of how it is functioning. For example, if the flow rate were to decrease, there will probably be an increase in the TDS of the water flowing out to sea. In an area already prone to "Red Tides", this could have negative impacts.

Phytoplankton biomass; a reduction in flow during winter is not likely to lead to a change in the phytoplankton biomass peak but the peak is expected to move slightly upstream of the mouth. During summer, a reduction in flow could lead to hypoxic conditions in deeper areas of the estuary, causing the release of PO_4 and NH_4 from the sediment. This would favour the increased occurrence of phytoplankton blooms as experienced in the Swan-Canning Estuary.

Phytoplankton community structure; an estuarine diatom-dominated bloom occurred during the study so a reduced flow during summer is expected to increase the occurrence of these blooms. Chlorophytes were present in the river water so it is expected that they could bloom in spring as river flow begins to decrease. There has been no evidence of dinoflagellate or cyanophytes blooms but it is likely that this could occur during stable conditions in late summer, particularly in response to a pulse of nutrients from an unseasonable rainfall event.

Benthic biomass; Benthic chlorophyll *a* will increase in the estuary by up to 2 $mg \cdot m^{-2}$ in response to a reduction in river flow in winter due to decreased turbulent flow. Reduced flow in summer is likely to increase subtidal chlorophyll *a* in the upper reaches of the estuary but turbulent tide-driven currents will continue to limit benthic microalgal biomass in the lower reaches of the estuary.

Benthic community structure; there are insufficient data to predict the exact change in taxa in the estuary but the increased penetration of high salinity water and the more stable conditions of the water column is likely to support more marine species in the lower reaches and species that are better adapted to fine sediment and increased organic content in the upper reaches of the estuary.

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