ECOLOGICAL ROLE OF FREE-LIVING BACTERIA IN THE MICROBIAL FOOD WEB OF THE TEMPORARILY OPEN/CLOSED EAST KLEINEMONDE ESTUARY, SOUTH AFRICA

A thesis submitted in fulfilment of the requirements for the degree of

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by

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ABSTRACT

The main aim of this study was to assess the "top-down" and "bottom-up" control of bacterial production in the small temporarily open/closed East Kleinemonde Estuary, situated on the south-eastern coastline of southern Africa. Spatial and temporal patterns in bacterial abundance, biomass and production and the importance of abiotic and biotic factors were investigated over the period May 2006 to April 2007. The trophic interactions between bacteria, phytoplankton, nanoflagellates (< 20 μm), microzooplankton (< 200 μm) and mesozooplankton (< 2 000 μm) were investigated during winter and summer. Bacterial abundance, biomass and production ranged between 1.00×10^9 and 4.93×10^9 cells l^{-1}, 32.4 and 109 μg C l^{-1} and 0.01 and 1.99 μg C l^{-1} h^{-1}, respectively. With a few exceptions there were no spatial patterns in the values. Bacterial abundance, biomass and production, however, demonstrated a distinct temporal pattern with the lowest values consistently recorded during the winter months. Nanoflagellate and bacterial abundances were significantly correlated to one another (lower reaches: $r = 0.818$, $p < 0.001$; middle reaches: $r = 0.628$, $p < 0.001$; upper reaches: $r = 0.484$, $p < 0.05$) suggesting a strong predator-prey relationship. The frequency of visibly infected bacterial cells and the mean number of virus particles within each bacterial cell during this study demonstrated no temporal or spatial patterns and ranged from 0.5 to 6.1 % and 12.0 to 37.5 virus particles per bacterium, respectively. Viral infection and lysis was thus a constant source of bacterial mortality throughout the year. The estimated percentage of bacterial production removed by viral lysis ranged between 7.8 and 88.9 % of the total which suggests that viral lysis represented a very important source of bacterial mortality during this study.

The biological interactions between the selected components of the plankton community demonstrated that among the heterotrophic components of the plankton, the nanoflagellates were identified as the most important consumers of bacteria and small phytoplankton cells (< 20 μm). In the presence of microzooplankton the impact of the nanoflagellates on both the bacteria and phytoplankton was reduced, indicating that larger heterotrophs were preying upon the nanoflagellates. Mesozooplankton, however, appeared to exert the greatest impact

on nanoflagellates. In the cascading experiments, the data suggest that mesozooplankton consume nanoflagellates, which resulted in a decrease in the predation impact of these organisms on the bacteria. This result is consistent with predator-prey cascades. The presence of the larger heterotrophs therefore, mediates the interactions between the primary bacterivores, the nanoflagellates, and the bacteria within the temporarily open/closed East Kleinemonde Estuary.

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PREFACE

This study has been divided into two components:

1. Spatial and temporal patterns in bacterial abundance, production and viral lysis in the temporarily open/closed East Kleinemonde Estuary.
2. Trophic interactions between the plankton with particular interest in bacteria during winter and summer.

Work from the first study has been accepted for publication in the journal *Estuarine, Coastal and Shelf Science*:

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DECLARATION

The following thesis has not been submitted to a university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author.

CHAPTER 1
GENERAL INTRODUCTION

1.1. IMPORTANCE OF BACTERIA

Bacteria play a significant role in the total production and recycling of nutrients within a variety of aquatic ecosystems (Calbet and Landry, 1999). Bacteria have the potential to be significant biomass producers due to high growth efficiencies and rapid growth rates, which may exceed two divisions per day (Gasol, 1994; Fuhrman and Noble, 1995). Bacteria are an important source of carbon at the base of plankton food webs, however, bacterial production is only made available to higher consumers through trophic intermediates such as heterotrophic protists (Stoecker and Capuzzo, 1990; Froneman and Balarin, 1998; Calbet and Landry, 1999). Bacteria, therefore, account for a large fraction of the carbon flow through aquatic ecosystems and as a result play an important role in maintaining the trophic state of these systems (Pace and Cole, 1994). Bacteria can therefore be regarded as a key component of aquatic food webs (Sherr and Sherr, 1994). It has been demonstrated that bacteria and phytoplankton are the two largest marine ecosystem components (Button, 1994). Phytoplankton, however, tend to have a patchy distribution unlike bacteria, which have a more uniform distribution with spatial variations in activity (Button, 1994). Trophic interactions will therefore vary substantially both spatially and temporally, depending on the level of activity at the base of the food web. From an ecological perspective, it is essential to understand the trophic interactions between the various trophic levels in order to determine the food web structure and energy dynamics within estuaries (Calbet and Landry, 1999).

Bacterial dynamics within various ecosystems from marine to freshwater environments have been investigated worldwide. Bacterial abundance, biomass and production rates obtained from some of these studies have been summarised in Table 1.1. As bacteria are an important trophic link to higher consumers in the food web it is important to understand which factors influence their abundance and production within these systems.

Table 1.1. Summary of values obtained for bacterial abundance, biomass and production from various aquatic ecosystems (ND = no data).

Location		Abundance $\times 10^9$ cells l^{-1}	Biomass µg C l^{-1}	Productivity µg C l^{-1} h^{-1}	Reference
International:					
Marine					
Sargasso Sea	oceanic gyre	0.32	ND	0.01 - 0.05	Caron *et al.*, 2000
Georges Bank	coastal	1.15	ND	0.19 - 0.46	
Pacific Ocean	open ocean	0.03 - 0.08	ND	ND	Calbet and Landry, 1999
Antarctica	coastal	0.20 - 0.80	13 - 64	0.33 - 0.58	Leakey *et al.*, 1996
Adriatic Sea	coastal	ND	ND	0.01 - 8.20	Puddu *et al.*, 1998
New Zealand	offshore	0.72 - 1.01	ND	0.01 - 0.23	Smith and Hall, 1997
	inshore	0.52 - 0.85	ND	0.03 - 0.19	
	subantarctic	0.42 - 0.74	ND	0.05 - 0.14	
	STC	0.32 - 1.16	ND	0.02 - 0.69	
	subtropical	0.30 - 1.24	ND	0.04 - 0.31	
Saanich Inlet	coastal	1.40 - 2.50	ND	0.28 - 2.96	Fuhrman and Azam, 1980
McMurdo Sound	coastal	0.06 - 1.00	ND	0.00 - 0.12	
Scripps Pier	coastal	0.66 - 2.90	ND	0.03 - 2.21	
Santa Monica Pier	coastal	1.88 - 6.50	ND	2.96 - 6.16	Fuhrman and Noble, 1995
Estuarine					
Tampa Bay	bay	0.13 - 0.76	ND	ND	Cochran and Paul, 1998
Choptank River	estuary	ND	ND	0.20 - 1.40	Bouvier and del Giorgio, 2002
Delaware	estuary	1.00 - 8.00	ND	0.07 - 2.73	Coffin and Sharp, 1987
Scheldt	estuary	ND	38 - 98	0.30 - 11.4	Goosen *et al.*, 1995
Elbe	estuary	ND	31 - 60	0.10 - 2.50	
Hudson River	estuary	1.90 - 7.60	ND	2.25	Vaqué *et al.*, 1992
Old Woman Creek	stream	2.00	50	ND	Lavrentyev *et al.*, 2004
	wetland	5.80	100	ND	
	mouth	10.1	150	ND	
	lake	5.20	90	ND	
Urdaibai	estuary	0.50 - 6.70	ND	1.04 - 3.46	Revilla *et al.*, 2000

Table 1.1 *continued*:

Location		Abundance $\times 10^9$ cells l^{-1}	Biomass µg C l^{-1}	Productivity µg C l^{-1} h^{-1}	Reference
Freshwater					
Cataniapo and Cuao	clearwater	0.42 - 0.96	ND	0.05 - 0.37	Castillo *et al.*, 2004
Sipapo and Autana	blackwater	0.53 - 0.99	ND	0.03 - 0.32	
Lake Plußsee	lake	2.80 - 11.0	ND	0.17 - 1.25	Weinbauer and Höfle, 1998
Piburger See	lake	2.10 - 4.30	50 - 77	ND	Posch *et al.*, 2001
Lake Pavin	lake	ND	ND	0.74	Bettarel *et al.*, 2004
Lake Aydat	lake	ND	ND	2.06	
Kühwörte	blackwater	4.0	ND	1.18 - 4.14	Mathias *et al.*, 1995
Lake Pavin	lake	2.50 - 4.00	ND	0.40 - 0.92	Bettarel *et al.*, 2003
Alte Donau	oxbow lake	1.40 - 3.90	ND	0.72 - 1.24	Fischer and Velimirov, 2002
Řimov	reservoir	3.40 - 4.10	75 - 100	1.67 - 2.50	Šimek *et al.*, 2001
San Francisco Bay	delta	ND	40 - 120	0.17 - 0.96	Sobczak *et al.*, 2005
Local:					
Marine					
Cape Peninsula	upwelling	2.12	40	0.68	Painting *et al.*, 1989
Southern Benguela	shelf	0.21 - 2.68	ND	ND	Linley *et al.*, 1983
Southern Benguela	inshore	ND	9 - 42	ND	Probyn, 1985
	shelf	ND	31 - 79	ND	
	oceanic	ND	12 - 25	ND	
Estuarine					
Kariega	estuary	0.09 - 0.10	ND	ND	Froneman, 2002a
Kasouga	estuary	0.12 - 0.17	ND	ND	Froneman, 2006

1.2. CONTROL OF BACTERIAL PRODUCTION

It is now accepted that bacterial production rates are dependent on both abiotic and biotic factors (Button, 1994; Almeida *et al.*, 2001; Sherr and Sherr, 2002; Bettarel *et al.*, 2004). Abiotic factors (bottom-up control) include temperature, salinity, substrate availability and nutrient concentrations, particularly nitrogen and phosphorus concentrations (Button, 1994;

Goosen *et al.*, 1999; Almeida *et al.*, 2001; Castillo *et al.*, 2004; Ameryk *et al.*, 2005). The biotic factors (top-down control) that may control bacterial production are predation by protists and bacterial loss due to viral infection and lysis (Fuhrman and Noble, 1995; Weinbauer and Höfle, 1998; Sherr and Sherr, 2002; Bettarel *et al.*, 2004). The relative importance of the "bottom-up" and "top-down" control on bacterial production demonstrates a high degree of spatial and temporal variability. In some instances both control mechanisms have been found to operate simultaneously, affecting different components of the planktonic community (Berninger and Wickham, 2005).

1.3. BOTTOM-UP CONTROL OF BACTERIA

Bacterial production rates depend on several environmental factors including temperature, salinity, nitrogen and phosphorus concentrations and chlorophyll-*a* (chl-*a*) concentration (Goosen *et al.*, 1999; Almeida *et al.*, 2001; Castillo *et al.*, 2004; Ameryk *et al.*, 2005). Among these variables, temperature appears to have the greatest impact on bacterial production rates (Ameryk *et al.*, 2005). At temperatures greater than 14 °C, however, bacterial production appears to become more dependent on substrate and nutrient availability (Scavia and Laird, 1987; Ochs *et al.*, 1995; Ameryk *et al.*, 2005). As bacteria generally utilise only a small portion of the *in situ* dissolved organic matter (DOM) that is excreted from phytoplankton, the input of allochthonous organic matter must play an important role in determining bacterial production (Almeida *et al.*, 2001; Ameryk *et al.*, 2005).

Phytoplankton and bacterial growth in aquatic systems are usually limited by essential elements, particularly nitrogen and phosphorus (Perissinotto, 1995; Correll, 1998; Cloern, 2001). The relative importance of nitrogen and phosphorus as limiting elements differs between freshwater and marine systems due to the different patterns of nutrient cycling that predominate within each system (Cloern, 2001). It has, however, been shown that both nitrogen and phosphorus are important in estuaries in which phosphorus generally becomes limiting during spring while nitrogen becomes limiting during summer (Conley, 2000).

As phosphorus is an essential component of nucleic acids and many intermediary metabolites and nitrogen is an important component of nucleic acids and amino acids, they are both vital for metabolism for all life forms (Perissinotto, 1995; Correll, 1998). Nitrogen

occurs in various forms, which are made available either through *in situ* ammonia, urea and amino acids derived from the excretion of organisms (autochthonous input) or from nitrates that enter the ecosystem through upwelling events in the marine environment, freshwater runoff or nitrogen fixation (allochthonous input) (Perissinotto, 1995). In lake, reservoir and estuarine systems phosphorus is trapped relatively efficiently by biological assimilation and the deposition of sediments (Correll, 1998). In aquatic systems, phosphorus only occurs in the pentavalent form of which orthophosphate is the only form that autotrophs can assimilate (Correll, 1998). In both freshwater and marine environments heterotrophic bacteria account for a large portion of the total uptake of both phosphate and ammonia, which would therefore place bacteria and phytoplankton in competition for these growth limiting nutrients (Bratbak and Thingstad, 1985; Caron, 1994; Kirchman, 1994; Caron *et al.*, 2000). Previous studies have shown that bacteria may experience nutrient limitation, in particular nitrogen and phosphorus, in a variety of freshwater and marine ecosystems (Le *et al.*, 1994; Hoch and Kirchman, 1995; Felip *et al.*, 1996; Thingstad *et al.*, 1998). Estuarine systems, however, usually have high concentrations of dissolved organic carbon due to the inflow of freshwater from the terrestrial environment (Grange and Allanson, 1995; Adams *et al.*, 1999; Froneman, 2002b; Meire *et al.*, 2005). Nutrient limitation of bacterial production within estuaries is therefore unlikely to occur.

1.4. TOP-DOWN CONTROL OF BACTERIA

1.4.1. BACTERIVORY

Predation by phagotrophic protists (< 200 μm), which are typically dominated by heterotrophic flagellates, ciliates and dinoflagellates, can be a significant source of mortality for suspended bacteria in both freshwater and marine ecosystems (Sherr and Sherr, 2002; Bettarel *et al.*, 2004). Studies have shown that protistan grazing can remove between 26 and 108 % of the total bacterial production (Weinbauer and Höfle, 1998; Bettarel *et al.*, 2003; Bettarel *et al.*, 2004). In a study conducted in two contrasting lake ecosystems the amount of bacterial production removed by flagellates was higher (19 - 38 %) than that of ciliates (3 - 9 %) (Bettarel *et al.*, 2004). Both flagellates and ciliates preferentially ingest the larger bacterial cells (Gonzalez *et al.*, 1990). Although flagellates tend to have a greater response to bacterial cell size, they do not show any preferential grazing based on cell type or shape (Gonzalez *et al.*, 1990). Selective grazing by protozoa on larger bacterial cells, which are

generally the cells actively growing or dividing, will result in cropping the bacterial production rather than removing the standing stock (Gonzalez *et al.*, 1990; Sherr and Sherr, 1994). This may explain why most suspended bacterial assemblages in natural aquatic systems generally consist of small sized cells with low growth rates and only a few dividing cells (Gonzalez *et al.*, 1990; Sherr *et al.*, 1992).

Protistan grazing is an important source of regenerated nutrients due to the excretion of limiting nutrients, providing nutrients for further growth of their heterotrophic bacteria and phytoplankton prey (Caron, 1994; Kirchman, 1994; Sherr and Sherr, 2002). Protists therefore play a crucial role in nutrient cycling by consuming nitrogen and phosphorus-rich bacterial biomass (Caron, 1994). In addition, predation on bacteria will release phytoplankton from their competition with bacteria for the limiting nutrients (Bratbak and Thingstad, 1985). It has, however, been found that some protists can compete with herbivorous mesozooplankton and macrozooplankton for all size classes of phytoplankton (Sherr and Sherr, 2002). As protists can grow at the same rate or faster than their phytoplankton prey, protistan herbivory may be able to limit the development of phytoplankton blooms (Sherr and Sherr, 1994). Heterotrophic protists can also be an important food source for mesozooplankton (Stoecker and Capuzzo, 1990; Calbet and Landry, 1999). For example, it has been shown that protists may be a essential source of food for copepods and other zooplankton in regions of the ocean where most phytoplankton are less than 5 μm in size (Stoecker and Capuzzo, 1990; Perissinotto, 1995; Calbet and Landry, 1999). Phagotrophic protists, therefore, have an important ecosystem function, to transfer the production of bacteria and phytoplankton from the base of the food web to higher trophic levels (Anderson and Rivkin, 2001; Sherr and Sherr, 2002).

1.4.2. TROPHIC CASCADES

Previous studies have documented that predators play an important role in regulating abundance, biomass and community composition of the plankton (Pace *et al.*, 1998; Calbet and Landry, 1999; Sherr and Sherr, 2002). Through direct predation and trophic cascading, consumers can exert a strong control on the population dynamics of other organisms (Miller *et al.*, 1995). For example, in the oligotrophic North Pacific Ocean, copepods preying on microzooplankton enhanced the net growth of phytoplankton, nanoflagellates and bacteria

(Calbet and Landry, 1999). Dissolved organic material (DOM) is mainly utilised by bacteria and it is only through protistan bacterivory that this carbon is transferred to higher trophic consumers (Calbet and Landry, 1999; Sherr and Sherr, 2002). A theoretical model of a microbial food web is shown in Figure 1.1. This food web shows the potential pathways and trophic interactions in an aquatic system. The interactions between various components of the plankton food web are complex, reflecting feeding constraints of larger zooplankton, the availability of preferred food and zooplankton species composition (Fortier *et al.*, 1994; Hansen *et al.*, 1994; Calbet and Landry, 1999).

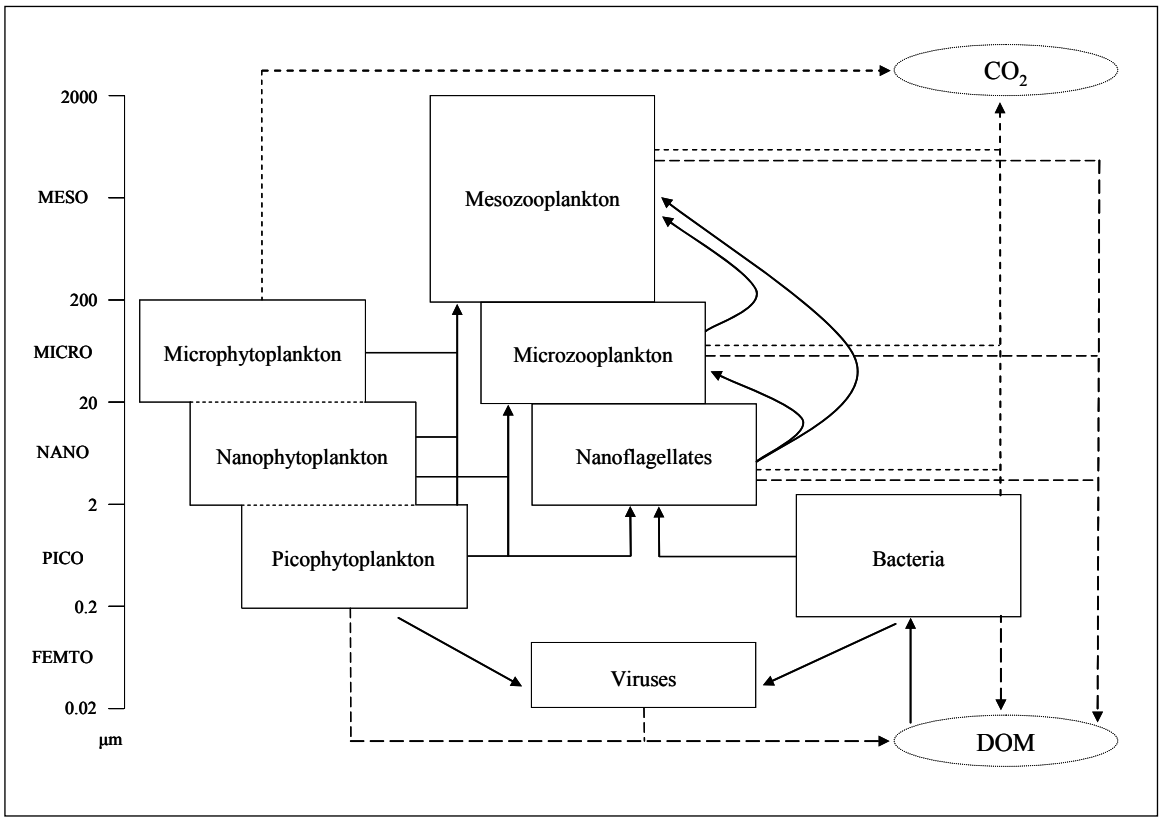

Figure 1.1. Conceptual model of a microbial food web in an aquatic system showing the size classification and possible trophic interactions (microbial loop and "classical" food chain). Solid lines indicate pathways of consumption of carbon while dotted lines indicate waste pathways (modified from Perissinotto, 1995 and Fuhrman, 1999).

The phytoplankton size structure has been demonstrated to influence the trophic interactions within the plankton community (Calbet and Landry, 1999; Froneman, 2002a; Froneman and Bernard, 2004; Froneman, 2006). In regions where small cells (< 2 µm) dominate the phytoplankton size structure, microheterotrophs (< 200 µm) are the most

important grazers of the phytoplankton community (Froneman and Balarin, 1998; Froneman, 2006). Larger zooplankton such as mesozooplankton are unable to feed efficiently on these small phytoplankton cells due to feeding constraints (Fortier *et al.*, 1994; Hansen *et al.*, 1994). However, the primary production can be made available to larger zooplankton via trophic intermediates such as protists, in particular nanoflagellates (Stoecker and Capuzzo, 1990; Froneman and Balarin, 1998; Calbet and Landry, 1999). Even in mesotrophic systems, which are characterised by diatom blooms, phagotrophic protists have been shown to serve as an important trophic link between the bacterial and phytoplankton base of the food webs and higher trophic levels (Sherr and Sherr, 2002). Although bacteria are an important carbon source, bacterial production is only made available to higher consumers through trophic intermediates such as heterotrophic protists (Stoecker and Capuzzo, 1990; Froneman and Balarin, 1998; Calbet and Landry, 1999).

1.4.3. VIRAL IMPACTS

It was originally thought that the majority of the bacterial production in aquatic systems was passed onto higher trophic levels via bacterivory (Sherr and Sherr, 2002). It is, however, now accepted that a significant fraction of the bacterial population is lost through viral infection and lysis (Bratbak *et al.*, 1990; Fuhrman and Noble, 1995; Weinbauer and Höfle, 1998; Bettarel *et al.*, 2004). The density of viruses generally corresponds to other biological parameters, including bacterial abundance and total chl-*a* concentration, both spatially and temporally (Weinbauer *et al.*, 1993; Bratbak *et al.*, 1994; Wommack and Colwell, 2000; Fuhrman and Schwalbach, 2003). Viral counts, however, tend to be more correlated with bacterial counts than chl-*a*, suggesting that even though viral infection of eukaryotes does occur, the majority of viruses within natural aquatic ecosystems infect bacteria (Fuhrman, 1999; Wommack and Colwell, 2000). As viruses result in mortality of bacteria they, together with predation by protists, provide top-down control on the population (Weinbauer and Höfle, 1998).

Bacterial mortality resulting from viral lysis can be as high as that removed by protistan grazing and may account for about 10 - 50 % of the total bacterial mortality in surface waters (Suttle, 1994; Fuhrman and Noble, 1995; Weinbauer and Höfle, 1998; Fuhrman, 1999). In environments that are unfavourable to protists, such as low-oxygen waters, viral lysis may

however be higher, contributing between 50 - 100 % of the total bacterial mortality (Fuhrman and Noble, 1995; Fuhrman, 1999). It can thus be concluded that viruses often have a significant effect on bacterial mortality, which may at certain times even exceed that of grazing (Fuhrman, 1999). It is worth noting that protistan grazing may stimulate viral activity as viral infection was found to correlate with ciliate and nanoflagellate grazing (Šimek *et al.*, 2001). Since viruses are generally species-specific they will affect the species composition and diversity of a bacterial community unlike other population controlling factors (nutrient limitation and grazing), which often affect the community rather than a single species (Bratbak *et al.*, 1994; Fuhrman and Noble, 1995). Viruses may therefore affect the species composition of different trophic levels as well as the structure of the entire food web (Bratbak *et al.*, 1994).

Bacterial mortality due to viral infection and lysis within various aquatic ecosystems has been investigated worldwide. The frequency of visibly infected bacterial cells (FVIC) and the burst size (BS) obtained from some of these studies has been summarised in Table 1.2.

Table 1.2. Summary of values obtained for viral lysis (FVIC) and burst size (BS) from freshwater, coastal and open ocean environments.

	Location	FVIC (%)	BS	Reference
Open Ocean	Mediterranean Sea	1.4 - 1.7	26 - 31	Weinbauer *et al.*, 2003
	Baltic Sea	1.5 - 2.0	27 - 32	Weinbauer *et al.*, 2003
	Bering and Chukchi Seas	0.2 - 3.3	50	Steward *et al.*, 1996
Coastal Shelf	Northern Adriatic Sea	0.9 - 2.7	16 - 32	Weinbauer *et al.*, 1993
	Santa Monica	3.3 - 4.6	20	Fuhrman and Noble, 1995
	Gulf of Mexico	0.1 - 4.4	11 - 45	Weinbauer and Suttle, 1996
Freshwater	Lake Plußsee	0.7 - 2.5	19 - 35	Weinbauer and Höfle, 1998
	Alte Donau	2.8 - 9.0	22 - 42	Fischer and Velimirov, 2002
	Rimov Resevoir	2.0	8 - 47	Šimek *et al.*, 2001
	Lake Pavin	0.5 - 4.1	12 - 52	Bettarel *et al.*, 2004
	Lake Adyat	0.5 - 2.8	19 - 36	Bettarel *et al.*, 2004
	Danube River	1.0 - 4.0	17 - 36	Mathias *et al.*, 1995

If a significant fraction of the bacterial population is lost through viral infection and lysis, then that fraction of bacterial carbon is unlikely to be directly transferred to higher trophic levels (Fuhrman and Noble, 1995; Wommack and Colwell, 2000). Instead nutrients will be released from lysed cells and will therefore be available for the remaining bacterial community (Figure 1.2) (Bratbak *et al.*, 1990; Bratbak *et al.*, 1994; Fuhrman and Noble, 1995; Weinbauer and Höfle, 1998; Wommack and Colwell, 2000; Weinbauer *et al.*, 2002; Fuhrman and Schwalbach, 2003). Viral lysis therefore plays a significant role in the regeneration of nitrogen and phosphorus and is important for the carbon flow in the microbial food web (Bratbak *et al.*, 1994; Fuhrman and Noble, 1995; Fuhrman and Schwalbach, 2003).

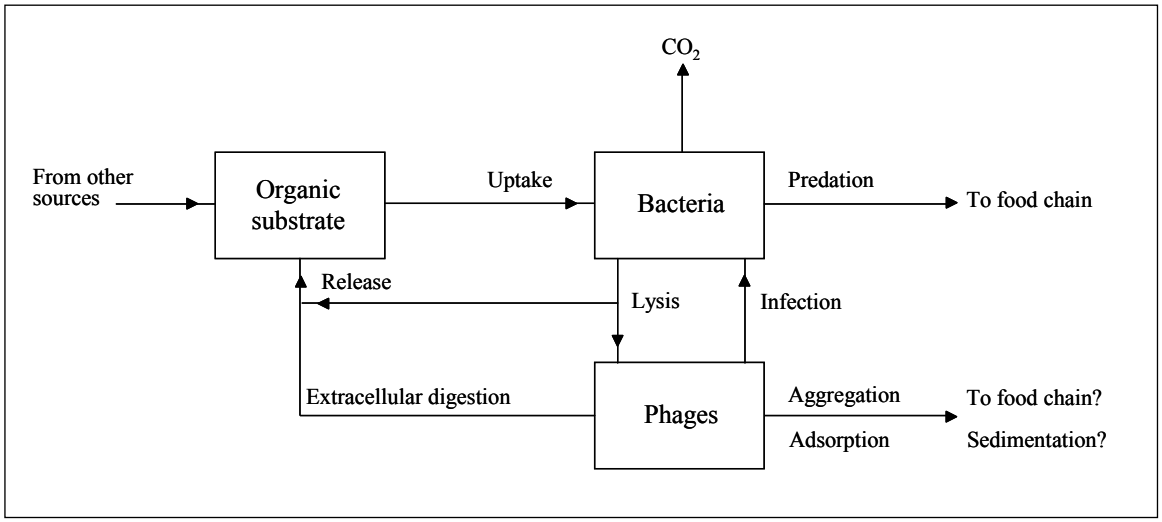

Figure 1.2. The potential role of viruses (bacteriophages) in the carbon flow within the microbial food web (after Bratbak *et al.*, 1990).

For lytic viruses, the rate of infection depends on the density and the activity of the host population and therefore could be important in controlling algal blooms where hosts are abundant (Bratbak *et al.*, 1994; Fuhrman, 1999; Fuhrman and Schwalbach, 2003). Viruses will therefore affect actively growing and dense populations of cells, ultimately having an important impact on the diversity in the community, as well as an extensive impact on the planktonic system as a whole (Fuhrman, 1999; Fuhrman and Schwalbach, 2003).

1.5. ESTUARIES

Estuaries are highly dynamic and rapidly changing systems that are characterised by sharp gradients in salinity, temperature, turbidity and nutrient concentrations (Lavrentyev *et al.*, 2004; Meire *et al.*, 2005). They occupy a transitional site between land and sea and as a result provide an interface between terrestrial and marine environments (Harrison *et al.*, 2000). As a consequence, estuaries are essential sites for bacterial degradation of terrestrial and riverine organic carbon and may sustain many important ecosystem functions including cycling and the movement of nutrients (Posey *et al.*, 1995; Meire *et al.*, 2005).

Initially an estuary was described as "a semi-enclosed body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage" (Pritchard, 1967). Day (1980) revised the definition of an estuary to "a partially enclosed body of water which is either permanently or periodically open to the sea, and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage". This revised definition was created to accommodate the smaller South African estuaries that, during the dry season and with naturally reduced freshwater inflow, close frequently, as well as systems that become hypersaline (Day, 1980). There are 250 estuaries recorded along the South African coastline, exhibiting a broad range of geomorphological and physico-chemical characteristics (Whitfield, 1992). Five estuary types have been determined based primarily on mouth characteristics and size, and to a lesser extent volume of freshwater and interaction with the marine environment (Whitfield, 1992). The five types are: permanently open estuaries, temporarily open/closed estuaries (also known as intermittently open/closed estuaries), estuarine lakes, estuarine bays and river mouths (Whitfield, 1992).

Of the 250 estuaries recorded along the South African coastline, approximately 70 % can be classified as temporarily open/closed systems (Whitfield, 1992). An important feature of temporarily open/closed estuaries is the presence of a sand bar at the mouth, which separates the estuary from the sea during low rainfall periods (Schumann *et al.*, 1999; Cowley and Whitfield, 2001; Froneman, 2002b). After periods of high rainfall and freshwater run-off, the volume of the estuary increases until the height of the sandbar is exceeded and the estuary opens to the sea (Schumann *et al.*, 1999; Cowley and Whitfield, 2001; Froneman, 2002b).

These breaching events result in a dramatic decrease in the water level of the estuary (Froneman, 2002b). A link between the marine environment and the estuary may also be re-established during spring-high tides or during severe storms (so called overtopping events) in which marine water flows over the sandbar, separating the estuary and the marine environment (Froneman, 2002b) (Figure 1.3). Due to the highly variable nature of rainfall along the south-east coastline of southern Africa mouth opening events of temporarily open/closed estuaries within this region occur sporadically throughout the year (Cowley and Whitfield, 2001; Froneman, 2002b).

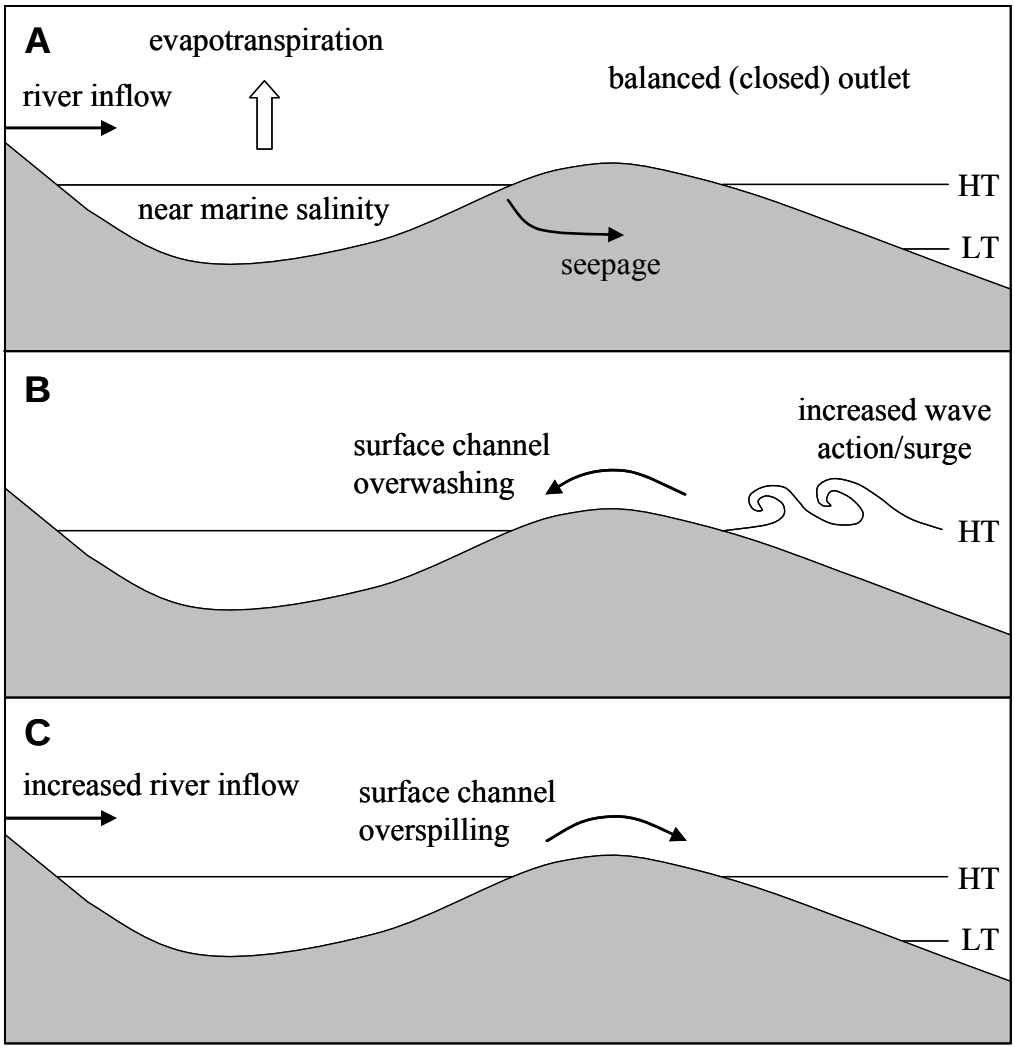

Figure 1.3. A diagram of a temporarily open/closed estuary under balanced (**A**), overtopping (**B**) and river inflow (**C**) conditions. During balanced conditions river inflow is balanced by losses through evapotranspiration and seepage (**A**). During high wave action overtopping introduces marine water into the system (**B**). During high inputs from overtopping (**B**) and river inflow (**C**) the system may breach (after Harrison *et al.*, 2000).

In temporarily open/closed estuaries, water temperature is affected by seasonal trends in atmospheric temperature as well as the temperature of marine and river water entering these systems (Snow and Taljaard, 2007). During mouth open state, sea conditions, such as upwelling events may influence the water temperature in these estuaries, particularly in the lower and middle reaches (Snow and Taljaard, 2007). Salinity is usually determined by the amount of freshwater entering the system and the mouth status of the estuary (Froneman, 2002b; Perissinotto *et al.*, 2002). Duration of stratified conditions in these systems depends on the amount of river inflow, the strength of wind-mixing forces and the depth of the estuary (Snow and Taljaard, 2007). A common feature of temporarily open/closed estuaries along the south east coast of southern Africa is the virtual absence of vertical and horizontal gradients in salinity and temperature (Froneman, 2002b; Perissinotto *et al.*, 2002). The absence of these gradients can be related to several factors, including limited freshwater inflow, shallow depth and wind induced mixing, which facilitates both horizontal and vertical mixing of the water column (Froneman, 2002b; Perissinotto *et al.*, 2002).

Previous studies on the trophodynamics of vertebrates and invertebrates in temporarily open/closed estuaries in southern Africa have shown that the plankton community composition and dynamics are strongly linked to mouth phase and amount of freshwater inflow (Wooldridge, 1999; Vorwerk *et al.*, 2001; Froneman, 2002b; Perissinotto *et al.*, 2002). Breaching events within these systems are typically associated with a decline in the total abundance and biomass of both vertebrates and invertebrates due to the outflow of estuarine rich waters into the marine environment and habitat loss resulting from exposure of submerged macrophytes (de Villiers *et al.*, 1999; Froneman, 2002b; Perissinotto *et al.*, 2002; Bernard and Froneman, 2005). These breaching events, however, provide an opportunity for marine breeding invertebrates and vertebrates to recruit into these systems (Vorwerk *et al.*, 2001; Bernard and Froneman, 2005). Overtopping events in these systems also represent important recruitment opportunities for marine breeding invertebrates and vertebrates (Kemp and Froneman, 2004). The inflow of freshwater into these systems is associated with elevated primary and secondary production rates, largely mediated by increased macronutrient availability (Adams *et al.*, 1999; Froneman, 2002b). The absence of horizontal gradients in temperature and salinity typically observed within temporarily open/closed estuaries contributes to the virtual absence of spatial patterns in the biology (fish and zooplankton) in these systems (Vorwerk *et al.*, 2001; Froneman, 2002b).

Estuaries are ecologically important areas of South Africa due in part to their contribution in terms of nutrient sinks and sources to the large marine ecosystems, particularly on the east coast of the subcontinent (Harrison *et al.*, 2000). The impacts of human pollution and alteration of aquatic ecosystem function is increasing and the effects of this will be predominantly evident at the microbial level as this is where the majority of production and nutrient cycling occurs (Perissinotto, 1995; Cloern, 2001; Paerl *et al.*, 2003; Meire *et al.*, 2005). Eutrophication, which is the over-enrichment of surface waters, has become a central issue in many aquatic ecosystems as it often results in excessive production by autotrophs, such as algae and cyanobacteria, resulting in high respiration rates which in turn leads to anoxia in poorly mixed waters (Correll, 1998; Cloern, 2001).

1.6. AIMS

Despite their importance in nutrient recycling and as a carbon source for selected components of plankton within aquatic systems, the role of bacteria in southern African estuarine ecosystems to date has largely been ignored. Only a single study has focussed on bacterial production within southern African estuaries (Tibbles *et al.*, 1992). This study focused on bacterial dynamics within the sediments and to a lesser extent the water column of a larger permanently open west coast estuary. To the author's knowledge, there have been no studies on bacterial dynamics within southern African temporarily open/closed estuaries. The main aims of this study are to:

1. Assess top down (biological) and bottom up (abiotic) control of bacterial abundance, biomass and production in a temporarily open/closed Eastern Cape estuary.

2. Investigate the biological interactions between selected components of the plankton community within the temporarily open/closed estuary, with special emphasis on bacteria.

The study was conducted in the small temporarily open/closed East Kleinemonde Estuary situated along the south-east coast of southern Africa.

CHAPTER 2
STUDY AREA

2.1. EAST KLEINEMONDE ESTUARY

2.1.1. POSITION

The East Kleinemonde Estuary (33º 32′ S; 27º 02′ E) is situated in the Eastern Cape of South Africa, approximately 15 km north east of the coastal town of Port Alfred (Figure 2.1) (Cowley and Whitfield, 2001). The small coastal town of Seafield surrounds the lower reaches and the coastal road (R72) between Port Elizabeth and East London crosses the system approximately 500 m from the mouth (Vorwerk, 2000).

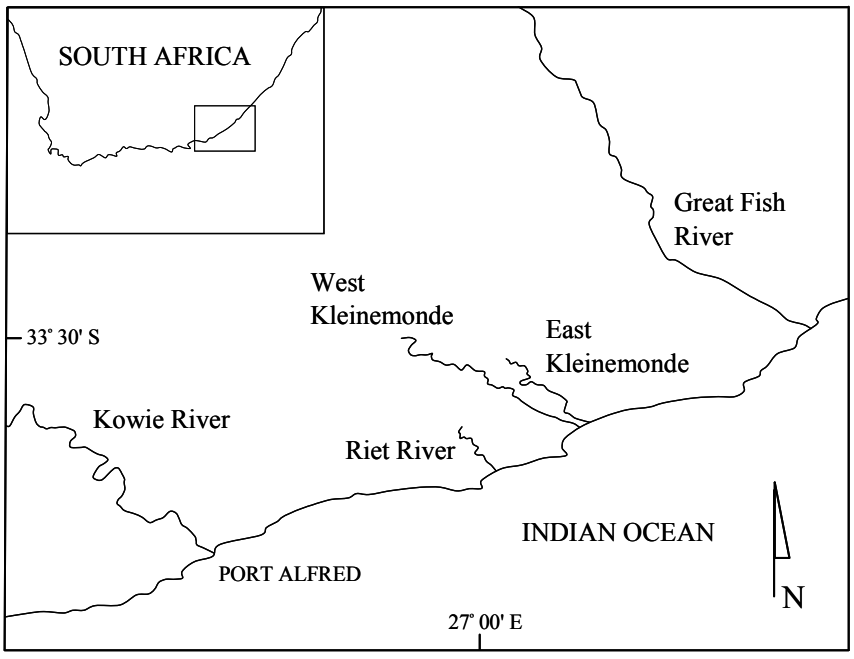

Figure 2.1. Map of the southeast coast of South Africa showing the position of the East Kleinemonde Estuary and adjacent estuaries.

2.1.2. COASTAL DYNAMICS

The East Kleinemonde Estuary is situated on the Eastern Cape coastline, within the warm-temperate biogeographic region (Whitfield, 2000). This region is dominated by the

Agulhas Current which is formed by the joining of the Mozambique Current and the East Madagascar Current (Lutjeharms, 2007). The Agulhas Current flows southwards along the shelf edge of southern Africa finally turning back on itself to form the "Agulhas Return Current" along the Subtropical Convergence (Figure 2.2) (Lutjeharms, 2007).

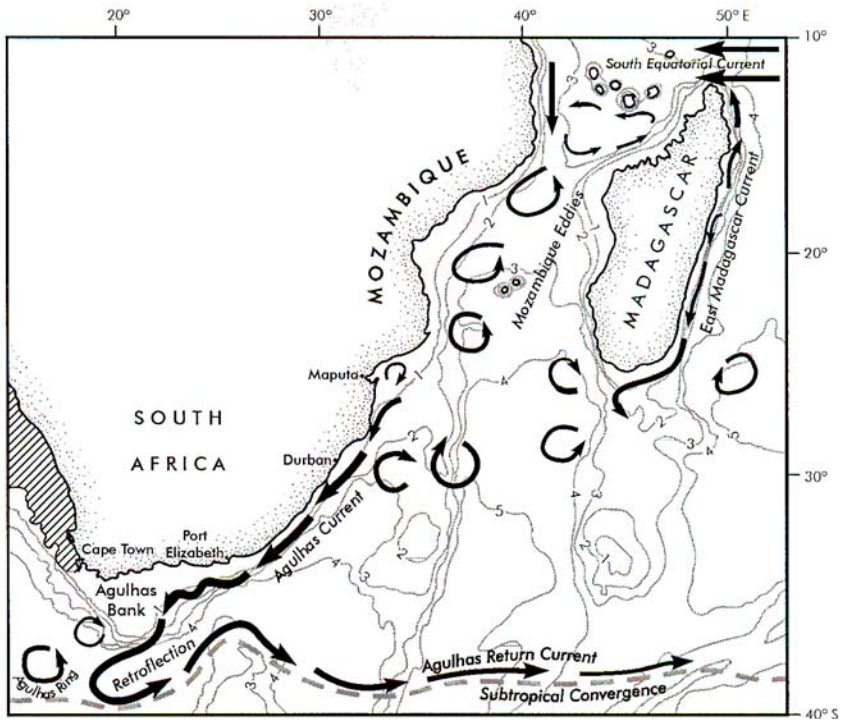

Figure 2.2. Map of the Agulhas Current showing its southward flow along the east coast of South Africa and the return current (after Lutjeharms, 2005).

Two regions of upwelling have been recorded within the Agulhas Current: the first off northern Kwazulu-Natal where the continental shelf widens and the second near Port Alfred, which is in close proximity to the study area (Lutjeharms *et al.*, 2000; Lutjeharms, 2007). At Port Alfred this persistent upwelling of colder water has been recorded 45 % of the time over a six year period (Lutjeharms *et al.*, 2000). The upwelling is likely to be due to the broadening of the continental shelf and the subsequent moving of the Agulhas Current further offshore (Lutjeharms *et al.*, 2000; Lutjeharms, 2007). It has also been found that smaller upwelling events occur within the coastal inshore region and are driven by wind and shear-edge eddies (Lutjeharms *et al.*, 2000; Lutjeharms, 2005). Upwelling events result in higher concentrations of nutrients in the surface waters which may result in higher levels of primary production (Lutjeharms *et al.*, 2000).

2.1.3. CLIMATE

The Southern and Eastern Cape coastal zone can be considered subtropical on the basis of the Koppen system of climate classification (Lubke, 1998). Temperatures along the coastline usually range between 9.5 and 26.0 °C (Stone *et al.*, 1998; Vorwerk, 2006). Fluctuations in air temperatures along the coastline are reduced due to the buffering effect of the sea (Stone *et al.*, 1998). Rainfall along the Southern and Eastern Cape coastal region is highly variable (Stone *et al.*, 1998). In the coastal region around Port Alfred, rainfall exhibits a bimodal pattern with an autumn-spring maximum (Stone *et al.*, 1998; Vorwerk, 2006). The mean monthly rainfall between 1996 and 2005 ranged from 20 to 85 mm (Vorwerk, 2006).

2.1.4. DESCRIPTION OF THE ESTUARY

The East Kleinemonde Estuary (Figure 2.3) is classified as a temporarily open/closed estuary (Whitfield, 1992; Cowley and Whitfield, 2001). It has a surface area of approximately 17.5 ha and a catchment size estimated at 46.3 km^2, which provides a mean annual run-off (MAR) of approximately 2×10^6 m^3 yr^{-1} (Badenhorst, 1988). The catchment area consists primarily of gentle sloped high-lying regions used for cattle farms and steep sloped stream and river valleys, which are covered by relatively undisturbed Valley Bushveld vegetation (Cowley and Whitfield, 2001).

The estuary is approximately 120 m at its widest section in the lower reaches and narrows to 25 m in the upper reaches (Cowley and Whitfield, 2001; Vorwerk *et al.*, 2001). The system is approximately 3 km long with a depth of between 1 and 2 m in the main channel, however, most of the estuary is shallow with a littoral zone less than one metre deep (Cowley and Whitfield, 2001; Vorwerk *et al.*, 2001). During periods of extended mouth closure, the water level of the estuary often exceeds that of the mean sea level due to the development of an extensive sandbar on the seaward side of the mouth (Cowley and Whitfield, 2001). Following a mouth opening event the estuary becomes very shallow with a maximum channel depth of approximately 1 m (Cowley and Whitfield, 2001).

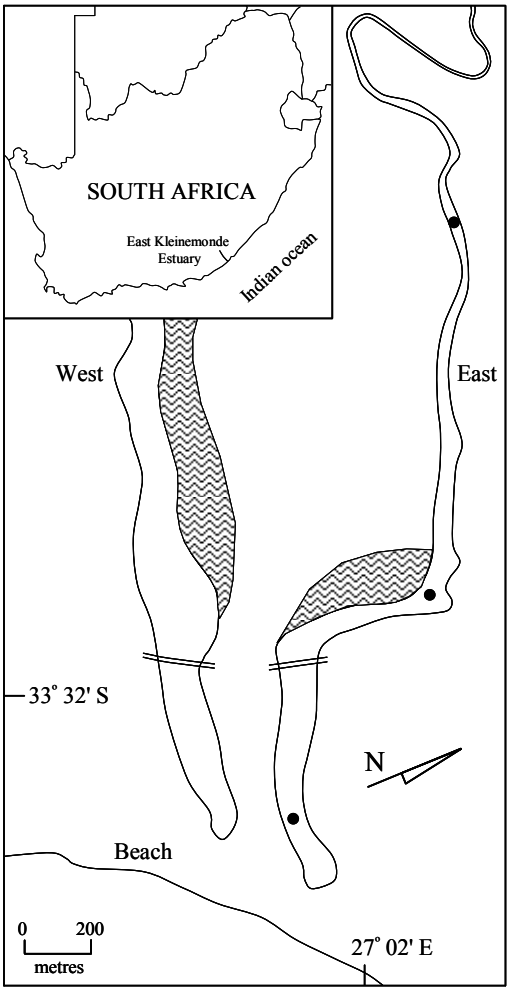

Figure 2.3. Position of the sampling sites located in the lower, middle and upper reaches of the temporarily open/closed East Kleinemonde Estuary and the adjacent West Kleinemonde Estuary (salt marsh areas indicated by shading; bridge of the coastal road indicated by double lines).

Previous studies have shown that the mouth of the estuary remains predominantly closed (≈ 72 % of the time) but opens sporadically (≈ 3 %) while overtopping events were evident approximately 25 % of the time (Bell *et al.*, 2001; Cowley and Whitfield, 2001). These mouth opening events were associated with periods of high rainfall (≥ 100 mm of precipitation) and were recorded during every month of the year except March and July (Cowley and Whitfield, 2001; Cowley *et al.*, 2001). Small overtopping events (less than 3 hours duration) were found to occur relatively frequently (≈ 24 %), while larger overtopping events (3 - 6 hours duration), primarily due to storm conditions and high seas, occurred less frequently (≈ 2 %) (Bell *et al.*, 2001; Cowley and Whitfield, 2001).

Previous studies have shown that the mean surface water temperature of the estuary ranged from 14.5 °C (winter) to 27.0 °C (summer), while the salinity ranged from 0 to 34 (practical salinity scale) depending on the amount of rainfall and the condition of the estuary mouth (Vorwerk, 2000; Cowley and Whitfield, 2001; Vorwerk *et al.*, 2001). During periods of extensive mouth closure, salinities were found to be relatively uniform throughout the estuary, with the exception of the upper reaches, where a decline in salinity regularly occurred due to riverine input (Cowley and Whitfield, 2001). During the open mouth phase, however, a steep salinity gradient frequently occurred between the mouth and head of the estuary as the seawater rarely extended beyond the lower reaches (Cowley and Whitfield, 2001).

The middle reaches of the estuary are characterised by an extensive salt marsh, which is comprised primarily of *Sarcocornia perennis*, *Sarcocornia decumbens, Sporobolus virginicus* and patches of *Juncus kraussii* (Adams, 1997). The salt marsh is largely inundated during times when water levels within the estuary are high, either as a result of freshwater inflow or marine overtopping. The littoral vegetation within the estuary is largely composed of *Ruppia cirrhosa* (Adams, 1997). Based on a survey of the fish community structure and water quality, the East Kleinemonde Estuary has been classified as being in a "good" ecological condition reflecting low levels of human impact on this system (Harrison *et al.*, 2000).

CHAPTER 3

SPATIAL AND TEMPORAL PATTERNS IN BACTERIAL ABUNDANCE, PRODUCTION AND VIRAL LYSIS

3.1. INTRODUCTION

Bacteria play a significant role in the total production and recycling of nutrients within aquatic ecosystems (Calbet and Landry, 1999). In addition they represent an important link between dissolved nutrients and higher consumers in the food web (Pace and Cole, 1994). Bacterial production in aquatic systems can either be passed onto higher trophic levels via bacterivory or it can be lost through viral infection and lysis (Fuhrman and Noble, 1995; Weinbauer and Höfle, 1998; Sherr and Sherr, 2002; Bettarel *et al.*, 2004). Bacterial production has been shown to be dependent on both abiotic (bottom-up control) and biotic (top-down control) factors. The bottom-up control of bacterial production is mediated by temperature, salinity, substrate availability and nutrient concentrations, in particular nitrogen and phosphorus concentrations (Goosen *et al.*, 1999; Almeida *et al.*, 2001; Castillo *et al.*, 2004; Ameryk *et al.*, 2005). Predation by protists and bacterial loss due to viral infection and lysis are generally considered as the most important biotic factors limiting bacterial production (Fuhrman and Noble, 1995; Weinbauer and Höfle, 1998; Sherr and Sherr, 2002; Bettarel *et al.*, 2004). The relative importance of bottom-up and top-down control on bacterial production may demonstrate a high degree of spatial and temporal variability.

Estuaries are rapidly changing systems that are characterised by sharp gradients in salinity, temperature, turbidity and nutrient concentrations (Lavrentyev *et al.*, 2004; Meire *et al.*, 2005). These highly dynamic systems are important sites for bacterial degradation of terrestrial and riverine organic carbon and may sustain many important ecosystem functions (Posey *et al.*, 1995; Meire *et al.*, 2005). Of the 250 estuaries recorded along the South African coastline, the vast majority (70 %) can be classified as temporarily open/closed systems (Whitfield, 1992). An important feature of temporarily open/closed estuaries is the presence of a sand bar at the mouth, which during low rainfall periods separates the estuary

from the sea (Schumann *et al.*, 1999; Cowley and Whitfield, 2001; Froneman, 2002b). Following periods of high rainfall and increased freshwater run-off, the volume of the estuary increases until the height of the sandbar is exceeded and the estuary opens to the sea (Schumann *et al.*, 1999; Cowley and Whitfield, 2001; Froneman, 2002b). These breaching events result in a dramatic decrease in the water level of the estuary due to the outflow of estuarine water into the marine environment (Froneman, 2002b).

Over the past decade, aspects of the biology of temporarily open/closed southern African estuaries have been studied in detail including the trophodynamics of both invertebrates (Wooldridge, 1999; Froneman, 2002b; Perissinotto *et al.*, 2002) and vertebrates (Cowley and Whitfield, 2001; Vorwerk *et al.*, 2001). Results of these studies indicate that the plankton community composition and feeding dynamics within these systems are strongly linked to mouth phase (open vs. closed) and magnitude of freshwater inflow (Wooldridge, 1999; Froneman, 2002b). Breaching events within these systems are typically associated with a decline in the total abundance and biomass of both invertebrates and vertebrates due to the outflow of estuarine rich waters into the marine environment (Froneman, 2002b; Perissinotto *et al.*, 2002). Conversely, the inflow of freshwater into these systems is associated with elevated primary and secondary production rates, largely mediated by increased macronutrient availability (Adams *et al.*, 1999; Froneman, 2002b). To date no studies have been conducted on the microbial systems within these estuaries. The main aim of this chapter is to investigate the spatial and temporal patterns in bacterial abundance, production and viral lysis in the temporarily open/closed East Kleinemonde Estuary.

3.2. MATERIALS AND METHODS

Sampling was conducted monthly from May 2006 to April 2007 in the lower, middle and upper reaches of the East Kleinemonde Estuary (see Figure 2.3). Exceptions occurred in June and August, in which upper reach samples could not be collected as breaching events had resulted in particularly low water levels in the upper reaches. In each reach, triplicate samples were collected from surface and bottom waters. Each month chl-*a* concentrations, bacterial abundances, biomass and nanoflagellate abundances were determined while temperature and salinity were measured *in situ*. Bacterial production and viral infection of bacteria were investigated seasonally. Samples were taken twice a season (winter, spring, summer and

autumn) from surface and bottom waters in the lower, middle and upper reaches of the estuary.

3.2.1. ENVIRONMENTAL VARIABLES

The temperature and salinity were measured *in situ* for the surface and bottom waters at each site. Temperature was measured using a YSI 550 DO probe, while salinity was measured using a hand-held refractometer and the Practical Salinity Scale.

3.2.2. TOTAL CHLOROPHYLL-*a* CONCENTRATION

The total chl-*a* concentration was determined from surface and bottom waters at each site from a 250 ml water sample that was gently filtered (vacuum < 5 cm Hg) through a GF/F filter (Whatman) and extracted in 8 ml of 90 % (v/v) acetone for 24 hours at -20 °C. After centrifugation at 5 000 rpm, the chl-*a* concentration was then determined using a Turner Designs 10AU Fluorometer according to the method of Holm-Hansen and Riemann (1978). Three replicates were prepared for each site and depth.

3.2.3. BACTERIAL ABUNDANCE AND BIOMASS

A 1 ml sample from surface and bottom waters at each site ($n = 3$) was preserved in 2.5 % glutaraldehyde (final concentration) and stained with acridine orange (100 µl ml^{-1}) for 5 minutes and then gently filtered (low vacuum < 5 cm Hg) through a 0.1 µm polycarbonate black membrane filter (Sterlitech), which was then mounted onto a slide. Bacterial numbers were then estimated using an Olympus BX60 epifluorescent microscope fitted with an exciter filter (BP 490), a blue excitation filter combination (Dichronic mirror 500 with built in barrier filter 0 - 515) and an additional filter (0 - 530) and operated at × 1 000 magnification. A minimum of 20 fields were counted for each sample and bacterial numbers were estimated according to the method of Turley (1993). Images were generated using a JEOL JEM 1210 transmission electron microscope (TEM) (× 40 000) from which length and width was determined using the imagery package AnalySIS. Cell volume (μm^3) was determined from the length (L) and width (W) of the bacterial cells as described by Heldal (1993):

$$\text{cell volume (V)} = (\pi/4) \times (W^2) \times (L - W/3)$$

Bacterial biomass (pg C) was calculated from the allometric relationship between cell volume and carbon content as described by Simon and Azam (1989) and modified by Norland (1993):

$$\text{bacterial cell carbon} = 0.12\ V^{0.7}$$

3.2.4. BACTERIAL PRODUCTION

Bacterial production rates were estimated from the rate of [^{3}H] thymidine incorporation (Bell, 1993). Three 1 % formaldehyde-killed controls and three replicates per site and depth were incubated at *in situ* temperatures in sterile 20 ml glass scintillation vials containing 20 nM [^{3}H] thymidine (specific activity, 85.0 Ci mmol^{-1}; Amersham). Following a 50 minute incubation period, the thymidine incorporation was terminated by the addition of formaldehyde to a 1 % final concentration. The 20 ml samples were then gently filtered (vacuum < 5 cm Hg) through 0.2 µm cellulose nitrate membrane filters (Whatman) and extracted with three 1 ml rinses with 5 % ice-cold trichloroacetic acid followed by five 1 ml rinses with 96 % ice-cold ethanol. The filters were then placed in scintillation vials and allowed to dry overnight. The filters were then dissolved with 1 ml of ethyl acetate. After 30 minutes 10 ml of scintillation cocktail was added to each vial and the radioactivity was determined using a Beckman 5801 liquid scintillation counter. The rate of thymidine incorporation was first converted to moles of thymidine per unit volume and per unit time, using the following equation described by Bell (1993):

$$\text{moles thymidine } l^{-1}\ h^{-1} = ((\text{dpm } 4.5 \times 10^{-13}) / (\text{SA t v})) \times 10^{-3}$$

where 4.5×10^{-13} = curies per dpm; SA = specific activity of [^{3}H] thymidine (curies per mmol); t = incubation time (h); v = filtered volume (L); 1×10^{-3} = mmol per mole.

The quantity of [^{3}H] thymidine incorporated into DNA was converted into bacterial production (µg C l^{-1} h^{-1}) using the conversion factor of 2×10^{18} cells produced per mole of thymidine incorporated and a carbon per cell value of 2×10^{-8} µg C cell^{-1} (Simon and Azam, 1989) in the following equation described by Bell (1993):

$$\text{bacterial production} = (\text{mole } l^{-1}\ h^{-1}) \times (2 \times 10^{18}) \times (2 \times 10^{-8})$$

3.2.5. PROTOZOOPLANKTON DENSITIES

For the determination of nanoflagellate densities, 50 ml samples from surface and bottom waters were fixed in glutaraldehyde (6.0 % V/V) and stained with proflavine (50 µl ml^{-1} for 2 minutes) and gently filtered (vacuum < 5 cm Hg) through a 0.2 µm pre-stained Irgalan Black Nucleopore filter (Sherr *et al.*, 1993). Permanent slides were then prepared according to the method of Booth (1993). The slides were then examined using a Zeiss Fluorescent microscope equipped with a 450 - 490 excitation filter, an FT 510 chromatic beam splitter and a long pass 528 barrier filter (Sherr *et al.*, 1993). Phototrophic plankton were distinguished from heterotrophic forms by the red auto-florescence of chl-*a* (Booth, 1993; Sherr *et al.*, 1993). Three replicates were prepared for each site and depth.

3.2.6. VIRAL LYSIS AND BURST SIZE

Bacteria from each site were analysed by ultracentrifuging 30 ml aliquots of glutaraldehyde-fixed samples using a Beckman Coulter Optima L-90K SW 28 Swing out rotor, run at 25 000 rpm (73 000 × g) for 25 minutes at 11 °C. Each pellet was transferred onto a 400-mesh electron microscope grid with carbon coated Formvar film and stained with phosphotungstic acid stain (PTA) for 30 seconds. The grids were examined at × 40 000 using a JEOL JEM 1210 TEM at 100 keV to identify virus infected and uninfected bacterial cells. Phages were identified by size and shape as described by Weinbauer *et al.* (1993) and a bacterium was considered infected if it contained five or more phages. For each grid 600 bacterial cells were examined from which the number of infected bacteria as well as the number of mature phages inside each host or burst size (BS) was recorded (Figure 3.1). To estimate virus induced bacterial mortality (VIBM) the frequency of infected cells (FIC) was first calculated from the frequency of visibly infected cells (FVIC) using the conversion factors determined by Proctor *et al.* (1993) with the model of Binder (1999) as follows:

$$FIC = 7.1\ FVIC - 22.5\ FVIC^2$$

FIC was then converted to VIBM according to the method of Binder (1999):

$$VIBM = (FIC + 0.6\ FIC^2) / (1 - 1.2\ FIC)$$

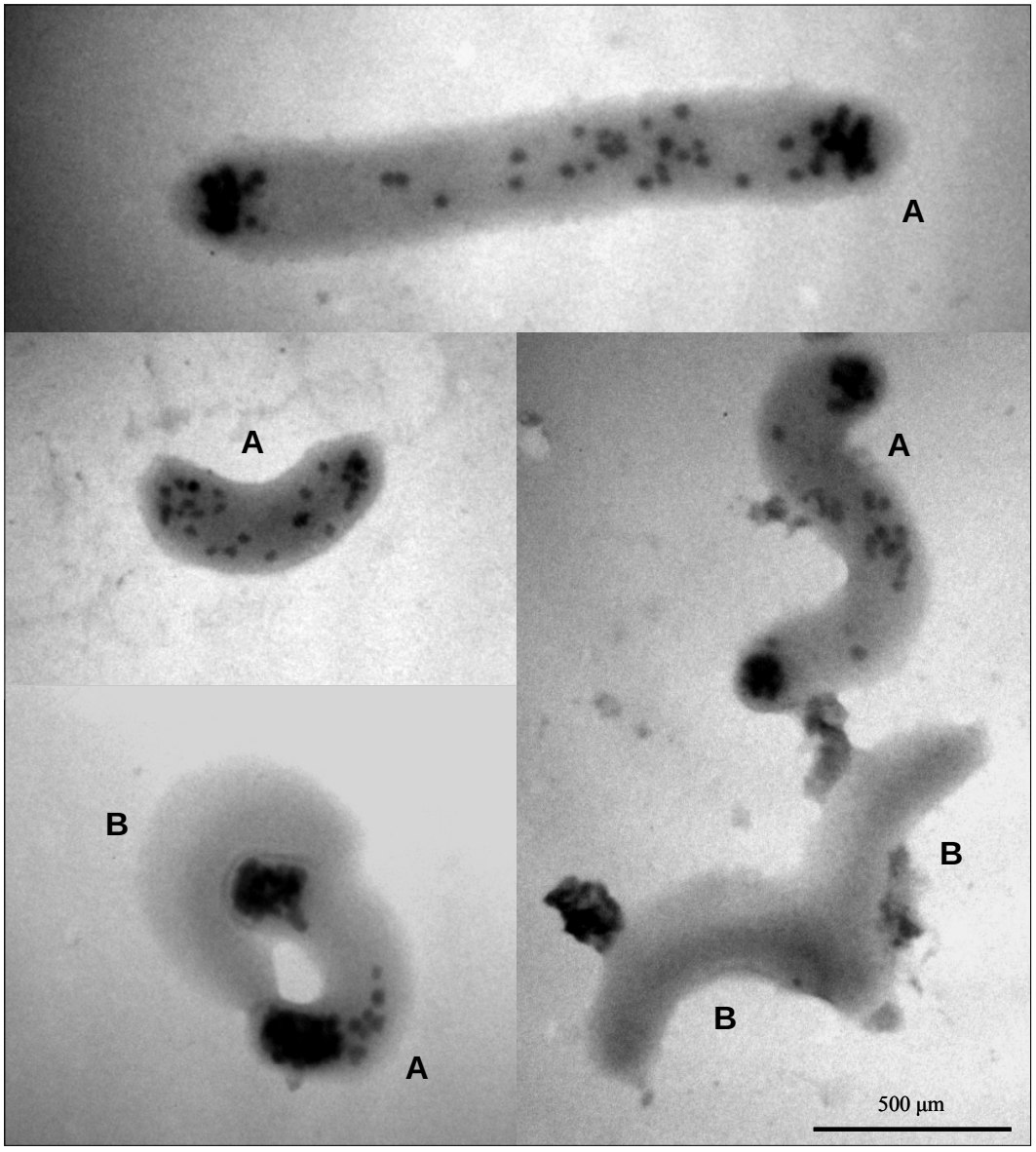

Figure 3.1. Transmission electron microscope images (× 40 000) showing bacterial cells that are infected with virus particles (**A**) and bacterial cells that are uninfected (**B**).

3.2.7. STATISTICAL ANALYSIS

To assess spatial and temporal patterns in total bacterial abundance, biomass, production and viral infection a one way or factorial ANOVA followed by a post hoc Scheffé test with a significance level of 5 % was undertaken. To assess predator-prey interactions between nanoflagellates and bacteria, a Pearson correlation analysis with a significance level of 5 % was undertaken. A correlation analysis with a significance level of 5 % was also undertaken between physical and biological variables. All analyses were conducted with the statistical package Statistica (version 7.0).

3.3. RESULTS

3.3.1. ENVIRONMENTAL VARIABLES

Water temperatures during the study showed strong seasonality with maximum values recorded during the summer (27.2 °C) and minimum values during winter (12.8 °C) (Figure 3.2). Intermediate values were recorded in autumn and spring.

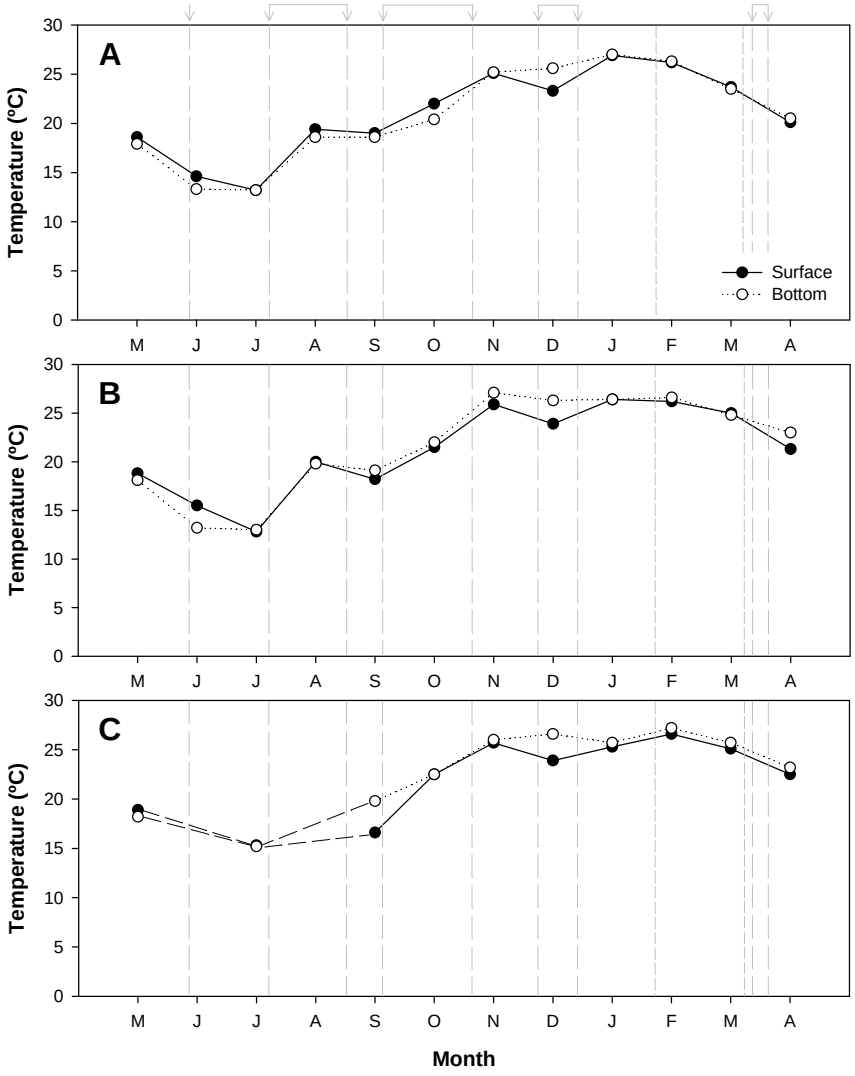

Figure 3.2. Temporal and vertical variation in temperature between the lower (**A**), middle (**B**) and upper (**C**) reaches. Arrows and long dashed lines indicate open phase due to breaching events while no arrows and short dashed lines indicate overtopping; letters represent the sampling months.

Salinity ranged from 1 to 33, however, it was highly variable and showed no effect of seasonality (Figure 3.3). There were however, distinct links between salinity and freshwater

inflow, with lowest salinities recorded immediately prior to breaching events and highest following overtopping events (Figure 3.3). Throughout the study salinity and temperature values for surface and bottom water were similar within each reach. An exception occurred in the middle reaches between August and December where stratification occurred within the water column resulting in the bottom water becoming an isolated water body.

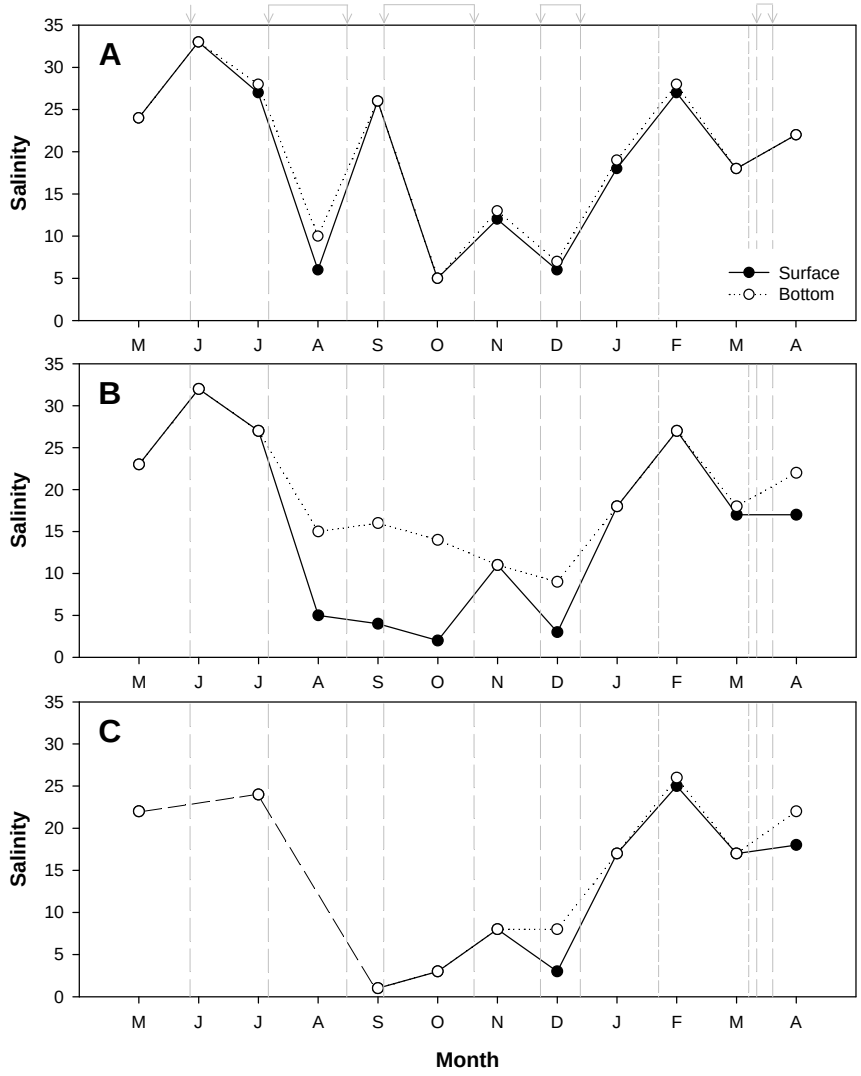

Figure 3.3. Temporal and vertical variation in salinity between the lower (**A**), middle (**B**) and upper (**C**) reaches. Arrows and long dashed lines indicate open phase due to breaching events while no arrows and short dashed lines indicate overtopping; letters represent the sampling months.

3.3.2. TOTAL CHLOROPHYLL-*a* CONCENTRATION

Total chl-*a* concentration during the study was highly variable, ranging between 0.12 and 6.19 mg chl-*a* m^{-3} and displayed no distinct seasonal patterns (Figure 3.4). Changes in total chl-*a* concentration were linked to freshwater inflow (increase) and mouth opening events (decrease). Total chl-*a* concentration during breaching events was significantly lower than during the closed phase ($F = 46.5$, $p < 0.001$). Post hoc Scheffé test conducted after ANOVA indicated that there was no significant difference in chl-*a* concentration between surface and bottom water within each reach throughout the study ($p > 0.05$). An exception was recorded in the middle reaches in September and the upper reaches in December when breaching events occurred ($F = 64.0$, $p < 0.01$; $F = 595$, $p < 0.001$, respectively).

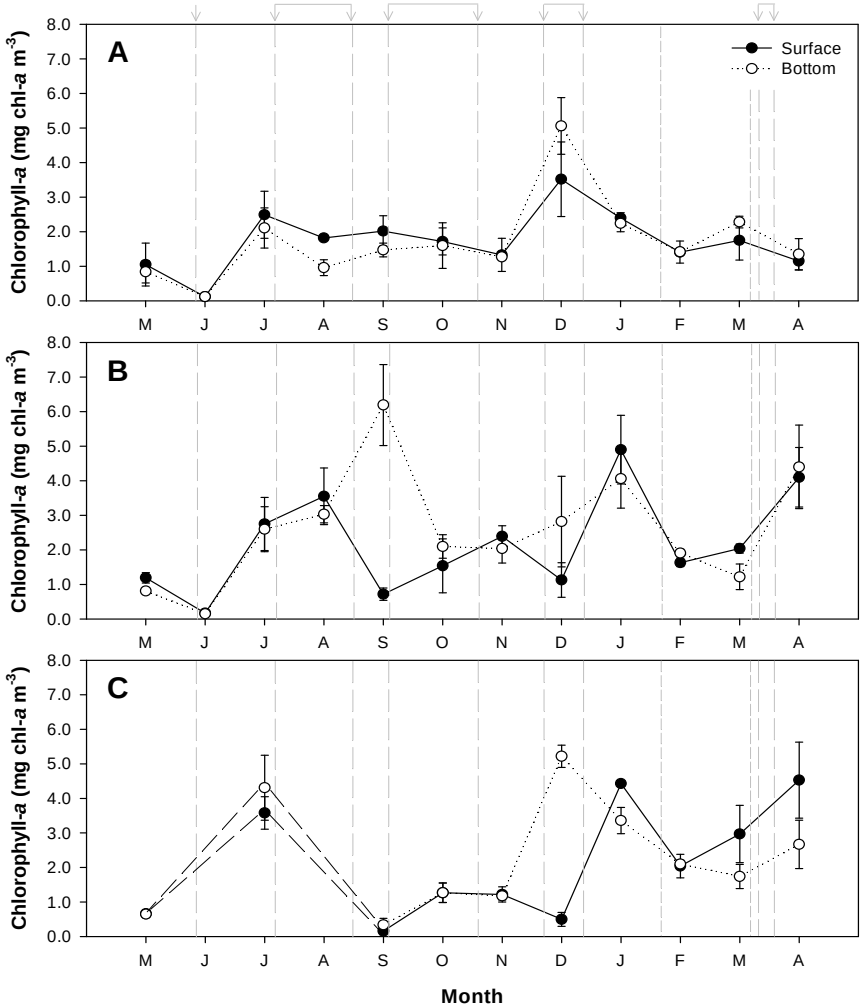

Figure 3.4. Temporal and vertical variation in total chlorophyll-*a* concentration between the lower (**A**), middle (**B**) and upper (**C**) reaches. Arrows and long dashed lines indicate open phase due to breaching events while no arrows and short dashed lines indicate overtopping. Error bars represent the standard deviation ($n = 3$), and letters represent the sampling months.

Total chl-*a* concentration in the lower reaches showed little temporal variation. The middle and upper reaches showed higher temporal variation, which was associated with freshwater inflow and breaching events. Pearson correlation analysis, however, indicated that there was no significant correlation between salinity and total chl-*a* concentration in all three reaches ($p > 0.05$ in all cases; Table 3.1). Total chl-*a* concentration showed a positive correlation with temperature, however, only in the lower reaches ($p < 0.01$; Table 3.1).

Table 3.1. Correlation coefficients for the described physical and biological variables in the lower, middle and upper reaches of the East Kleinemonde Estuary for the period May 2006 to April 2007.

	Temperature	Salinity	Chl-*a*	Nanoflagellates
Lower				
Chl-*a*	0.311 ⋆⋆	ns	-	ns
Bacterial abundance	0.732 ⋆⋆⋆	-0.508 ⋆⋆⋆	ns	0.818 ⋆⋆⋆
Bacterial biomass	0.862 ⋆⋆⋆	-0.809 ⋆⋆⋆	0.504 ⋆	ns
Bacterial production	0.522 ⋆	ns	ns	ns
Middle				
Chl-*a*	ns	ns	-	ns
Bacterial abundance	0.623 ⋆⋆⋆	-0.454 ⋆⋆⋆	ns	0.628 ⋆⋆⋆
Bacterial biomass	0.783 ⋆⋆⋆	-0.511 ⋆⋆⋆	ns	ns
Bacterial production	0.498 ⋆⋆⋆	ns	ns	ns
Upper				
Chl-*a*	ns	ns	-	ns
Bacterial abundance	ns	ns	ns	0.484 ⋆
Bacterial biomass	0.485 ⋆⋆	-0.399 ⋆⋆	ns	ns
Bacterial production	0.474 ⋆⋆	0.395 ⋆	ns	ns

ns: not significant; ⋆: significant at $p < 0.05$; ⋆⋆: significant at $p < 0.01$; ⋆⋆⋆: significant at $p < 0.001$

3.3.3. BACTERIAL ABUNDANCE AND BIOMASS

Bacterial abundance and biomass during the study ranged between 1.00×10^9 and 4.93×10^9 cells l^{-1} and between 32.4 and 109 µg C l^{-1}, respectively (Figure 3.5 and Table 3.2, respectively).

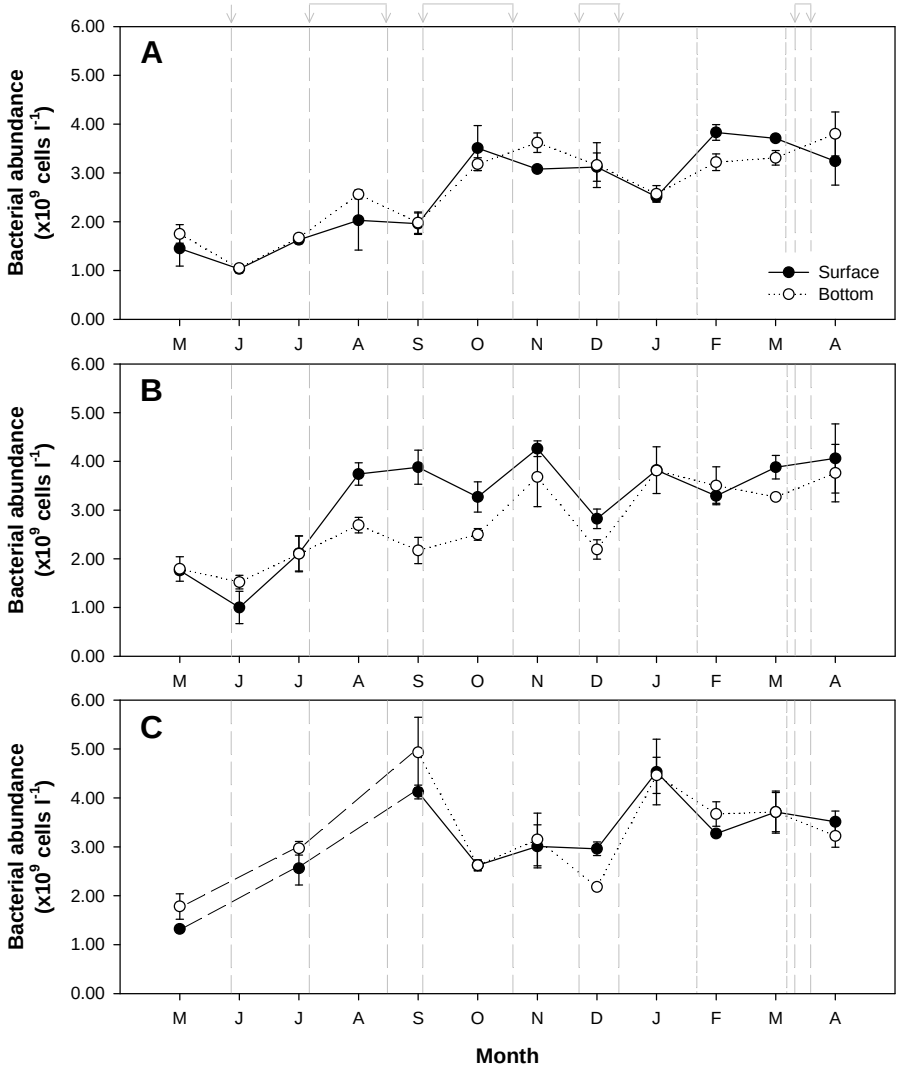

Figure 3.5. Temporal and vertical variation in bacterial abundance between the lower (**A**), middle (**B**) and upper (**C**) reaches. Arrows and long dashed lines indicate open phase due to breaching events while no arrows and short dashed lines indicate overtopping. Error bars represent the standard deviation ($n = 3$), and letters represent the sampling months.

Bacterial abundance and biomass exhibited a distinct temporal pattern with highest values recorded in autumn and summer (respectively) and the lowest in winter (Table 3.2). Indeed, the total bacterial abundance and biomass in winter was significantly lower than in spring, summer and autumn ($F = 50.9$, $p < 0.001$; $F = 82.5$, $p < 0.001$, respectively). There

was no significant difference in bacterial abundance between spring, summer and autumn ($p > 0.05$ in all cases). There was, however, a significant difference in bacterial biomass between spring and summer ($F = 13.0$, $p < 0.001$) while there was no significant difference between spring and autumn ($p > 0.05$). Throughout the study there was no significant difference in abundance and biomass between surface and bottom water ($p > 0.05$) except in the middle reaches in August and September when stratification of the water column occurred ($F = 42.6$, $p < 0.01$; $F = 45.1$, $p < 0.01$, respectively). In the lower and middle reaches bacterial abundance showed a significant positive correlation with temperature and nanoflagellates ($p < 0.001$ in all cases; Table 3.1) and a significant negative correlation with salinity ($p < 0.001$ in all cases; Table 3.1). In the upper reaches, however, bacterial abundance only showed a positive correlation with nanoflagellate abundance ($p < 0.05$; Table 3.1). Bacterial biomass showed a significant positive correlation with temperature and a significant negative correlation with salinity in the lower, middle and upper reaches ($p < 0.001$; $p < 0.001$; $p < 0.01$, respectively; Table 3.1). In the lower reaches bacterial biomass also showed a positive correlation with total chl-*a* concentration ($p < 0.05$; Table 3.1). During the study the ratio of phytoplankton (chl-*a*) carbon to bacterial carbon ranged from 1.0 to 2.8 (Table 3.2).

Table 3.2. Seasonal variation in bacterial abundance, biomass and the ratio of phytoplankton to bacterial carbon. Values shown are mean ± standard deviation for the combined surface and bottom waters of the lower, middle and upper reaches of the estuary for the period May 2006 to April 2007 ($n = 36$ per season).

	Abundance ($\times 10^9$ cells l^{-1})	Biomass (μg C l^{-1})	Chl-*a* C: Bacterial C
Winter	1.92 ± 0.73	32.4 ± 7.55	2.8: 1
Spring	3.20 ± 0.85	84.6 ± 26.12	1: 1
Summer	3.25 ± 0.70	109 ± 28.40	1.5: 1
Autumn	3.60 ± 0.44	95.8 ± 11.63	1.2: 1

3.3.4. BACTERIAL PRODUCTION

Bacterial production was highly variable during the study and ranged from 0.01 to 1.99 μg C l^{-1} h^{-1} (Figure 3.6). A distinct temporal pattern in the bacterial production rate was evident with values recorded in autumn being significantly higher than values recorded in

winter ($F = 62.1$, $p < 0.001$). There was no significant difference in bacterial production between winter and spring ($p > 0.05$). Values obtained in both winter and spring, however, were significantly different from summer and autumn (winter: $F = 36.2$, $p < 0.001$; $F = 62.1$, $p < 0.001$; spring: $F = 37.1$, $p < 0.001$; $F = 74.7$, $p < 0.001$, respectively), which were significantly different from one another ($F = 31.6$, $p < 0.001$). Bacterial production was highly variable spatially with significant differences occurring between each reach in December, January and March ($F = 68.7$, $p < 0.001$; $F = 74.9$, $p < 0.001$; $F = 19.3$, $p < 0.001$, respectively). There was no significant vertical difference between surface and bottom water ($p > 0.05$) except in the middle reaches in September ($F = 12.2$, $p < 0.05$). Bacterial production showed a positive correlation with temperature in the lower, middle and upper reaches ($p < 0.05$; $p < 0.001$; $p < 0.01$, respectively; Table 3.1) as well as a positive correlation with salinity in the upper reaches ($p < 0.05$; Table 3.1).

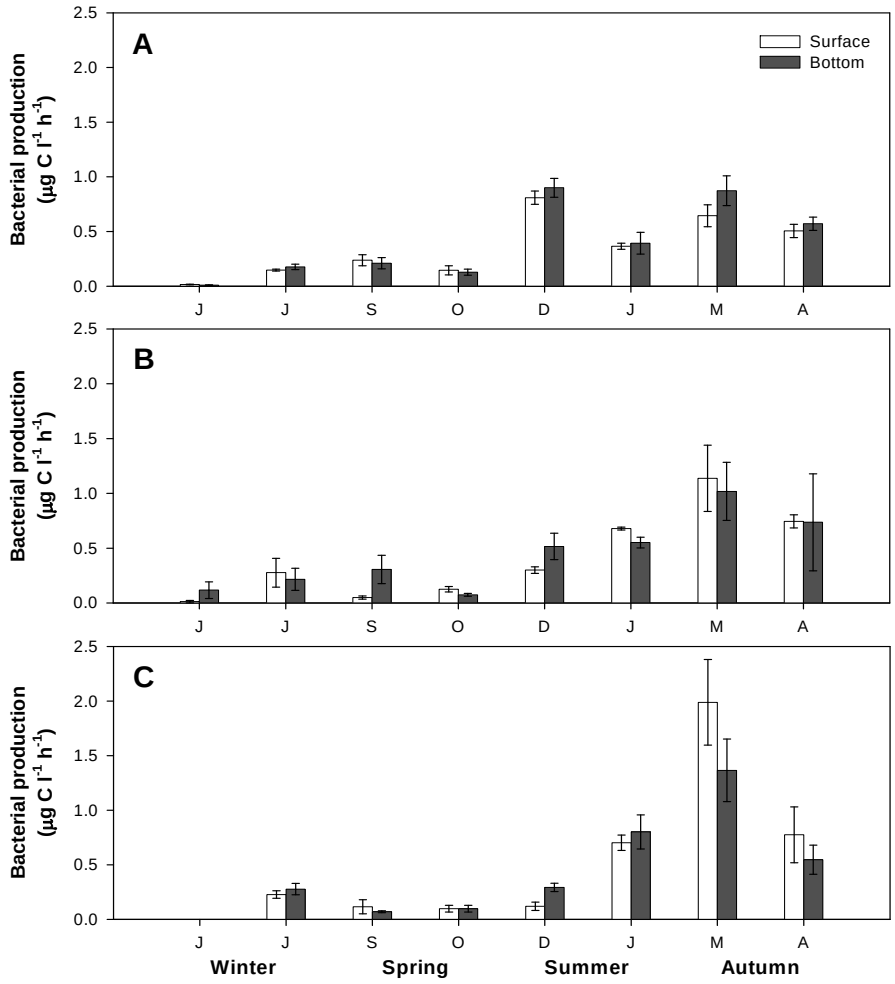

Figure 3.6. Temporal and vertical variation in bacterial production between the lower (**A**), middle (**B**) and upper (**C**) reaches for winter, spring, summer and autumn. Error bars represent the standard deviation ($n = 3$), and letters represent the sampling months.

3.3.5. PROTOZOOPLANKTON DENSITIES

As there were no significant spatial patterns in nanoflagellate abundances between surface and bottom waters ($p > 0.05$), values were pooled. Nanoflagellate abundances during the study ranged from 0.18×10^4 to 5.64×10^4 ind. l^{-1} (Figure 3.7). Total nanoflagellate abundances showed a significant positive correlation with total bacterial abundances in the lower and middle reaches ($p < 0.001$; $p < 0.001$, respectively; Table 3.1) and a weak positive correlation in the upper reaches ($p < 0.05$; Table 3.1).

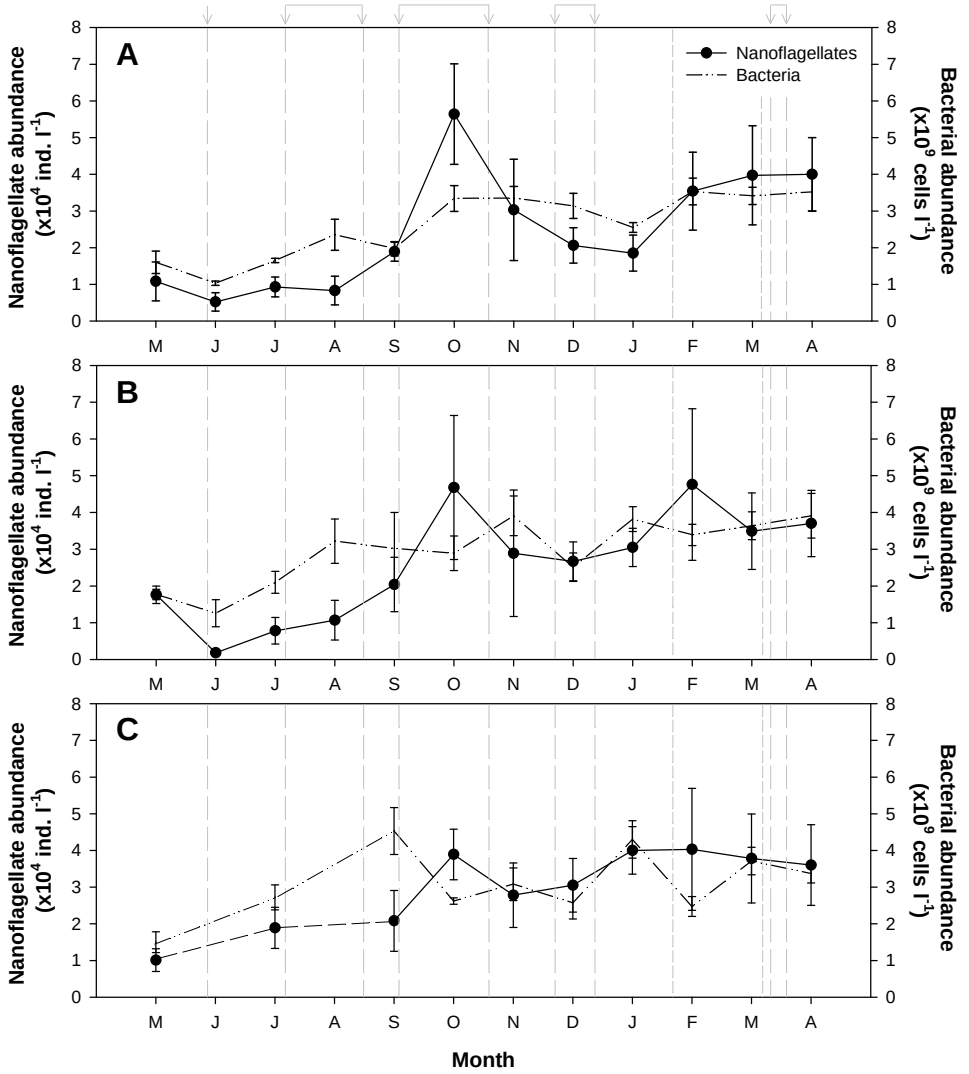

Figure 3.7. Temporal variation in nanoflagellate abundances (mean values for surface and bottom water) between the lower (**A**), middle (**B**) and upper (**C**) reaches. Dashed lines show fluctuations in total bacterial abundance to highlight predator-prey relationship. Arrows and long dashed lines indicate open phase due to breaching events while no arrows and short dashed lines indicate overtopping. Error bars represent the standard deviation (n = 6), and letters represent the sampling months.

3.3.6. VIRAL LYSIS AND BURST SIZE

The mean frequency of visibly infected cells (FVIC) during the study ranged from 0.5 to 6.1 % (Figure 3.8). There were no significant spatial (between reaches or between surface and bottom waters) or temporal (between seasons) patterns in FVIC during the study ($p > 0.05$ in all cases). FVIC showed a significant positive correlation with bacterial production in the middle and upper reaches ($p < 0.001$; $p < 0.001$, respectively; Table 3.3) and a weak positive correlation in the lower reaches ($p < 0.05$; Table 3.3).

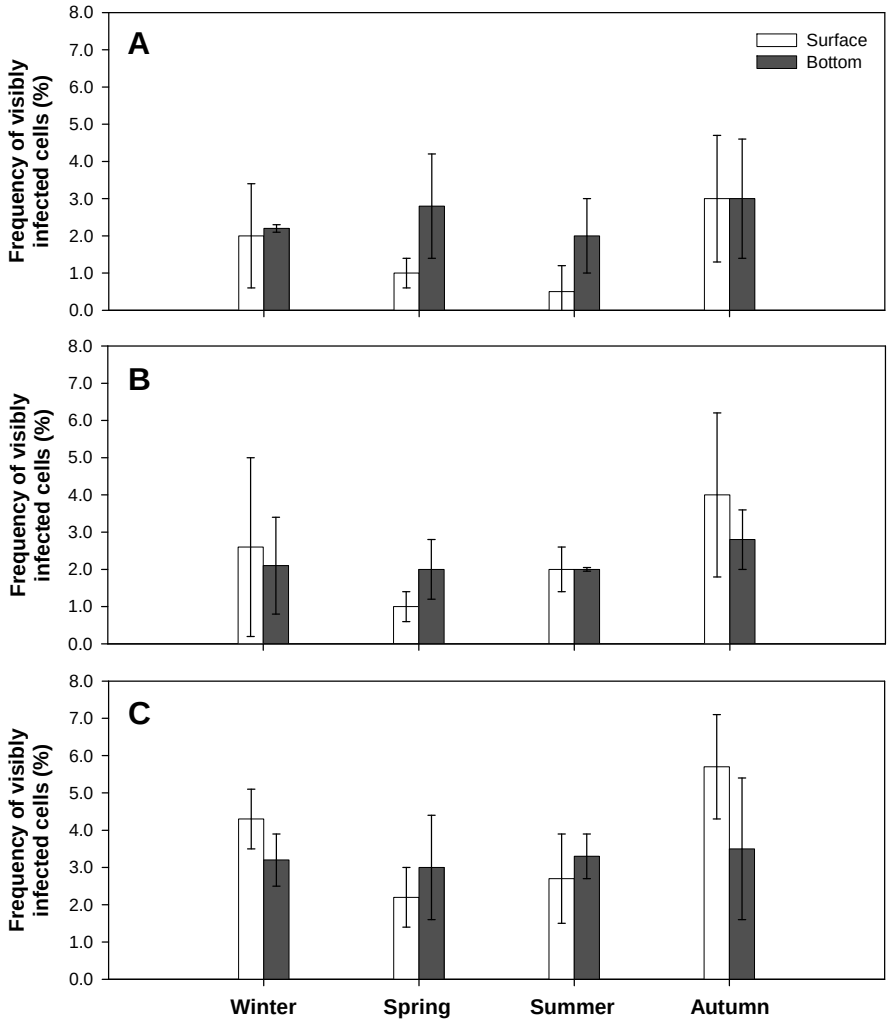

Figure 3.8. Temporal and vertical variation in the frequency of visibly infected bacterial cells (FVIC) between the lower (**A**), middle (**B**) and upper (**C**) reaches. Error bars represent the standard deviation ($n = 3$).

The estimated bacterial production removed by viral lysis (VIBM) for each season during the study ranged between 17.6 and 37.2 % of the total (Figure 3.9) and exhibited no significant difference vertically, horizontally and between seasons ($p > 0.05$ in all cases).

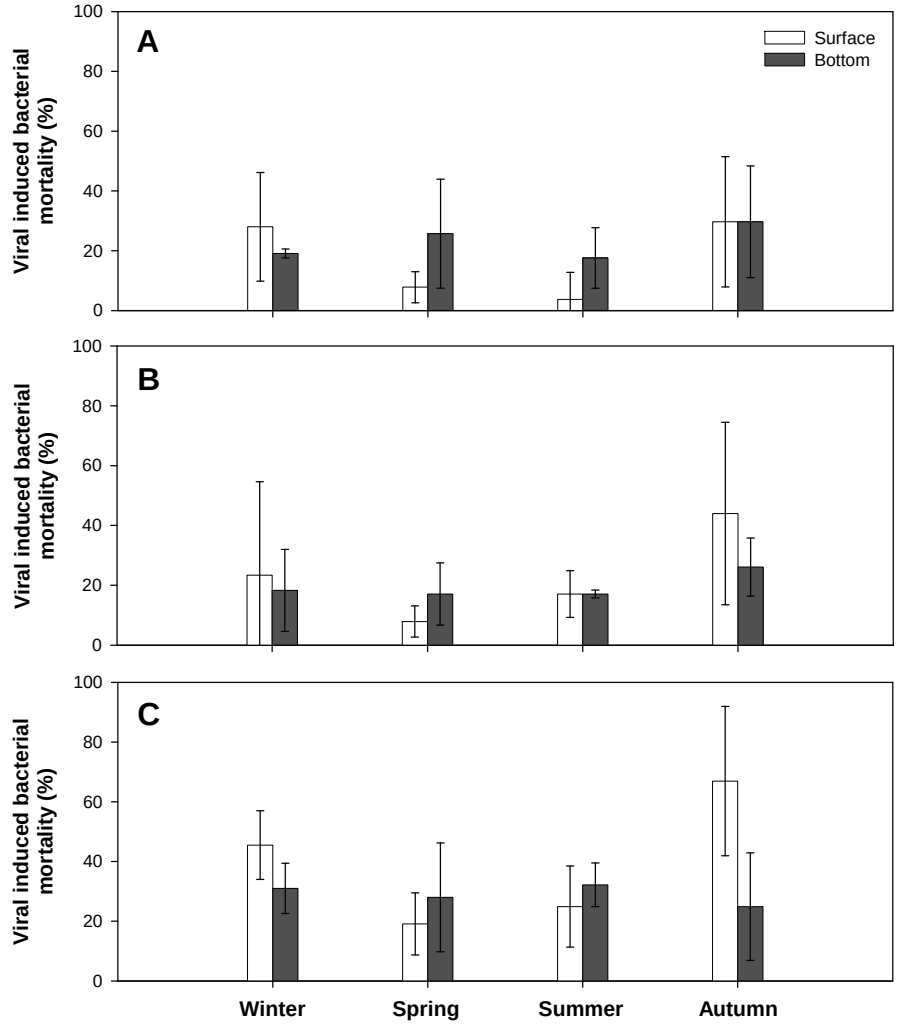

Figure 3.9. Temporal and vertical variation of virus induced bacterial mortality (VIBM) between the lower (**A**), middle (**B**) and upper (**C**) reaches. Error bars represent the standard deviation ($n = 3$).

The mean number of virus particles observed in each infected bacterial cell which is know as the burst size (BS) ranged from 12.0 to 37.5 (Figure 3.10). Again there were no significant spatial or temporal patterns in burst size during the study ($p > 0.05$ in all cases). Burst size showed a significant positive correlation with bacterial abundance in the lower reaches ($p < 0.01$; Table 3.3) and a weak positive correlation in the middle reaches ($p < 0.05$; Table 3.3). In the upper reaches BS showed a significant positive correlation with bacterial production ($p < 0.01$; Table 3.3).

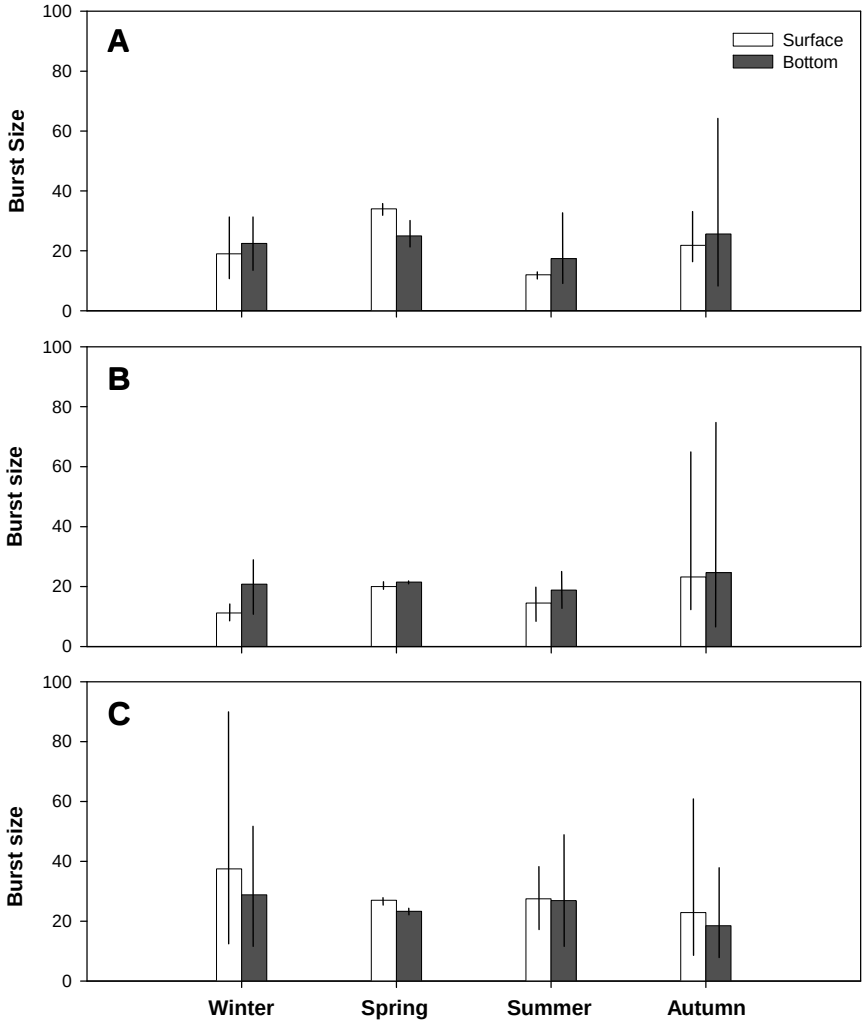

Figure 3.10. Temporal and vertical variation in burst size (virus particles per bacterium) between the lower (**A**), middle (**B**) and upper (**C**) reaches. Vertical bars indicate the range of virus particles observed.

Table 3.3. Correlation coefficients for bacterial and virus variables in the lower, middle and upper reaches of the East Kleinemonde Estuary for the period May 2006 to April 2007.

	Bacterial Abundance	Biomass	Production
Lower			
FVIC	ns	ns	0.488 ⋆
BS	0.504 ⋆⋆	ns	ns
Middle			
FVIC	ns	ns	0.734 ⋆⋆⋆
BS	0.390 ⋆	ns	0.522 ⋆⋆
Upper			
FVIC	ns	ns	0.718 ⋆⋆⋆
BS	ns	ns	-0.576 ⋆⋆

ns: not significant; ⋆: significant at $p < 0.05$; ⋆⋆: significant at $p < 0.01$; ⋆⋆⋆: significant at $p < 0.001$

3.4. DISCUSSION

The virtual absence of vertical and horizontal gradients in salinity and temperature within the East Kleinemonde Estuary during this study is a common feature of temporarily open/closed estuaries along the south east coast of southern Africa (Froneman, 2002b; Perissinotto *et al.*, 2002). The absence of these gradients can be related to several factors including limited freshwater inflow, shallow depth and wind induced mixing, which facilitates horizontal and vertical mixing of the water column (Froneman, 2002b; Perissinotto *et al.*, 2002). The importance of horizontal gradients in salinity and temperature in determining the distribution of estuarine organisms in southern African estuaries is now well documented (see for example Wooldridge, 1999; Froneman, 2002b; Froneman, 2004). The lack of any horizontal and vertical patterns in selected components of the biology during this study is therefore, likely the result of the absence of gradients in the physico-chemical variables. In agreement with previous studies conducted in temporarily open/closed estuaries within the same region, mouth opening events were associated with a decline in the total chl-*a* concentrations within the estuary (Froneman, 2002b; Perissinotto *et al.*, 2002). The observed pattern can be linked to the outflow of biomass rich estuarine water into the

marine environment. Surprisingly, no such trend was observed for bacterial abundances, biomass and production during this investigation. The absence of any distinct pattern in bacterial values could be a result of similar bacterial densities reported for both coastal and estuarine environments. Bacterial abundances reported for estuaries in the northern hemisphere range from 1.9×10^9 to 8.0×10^9 cells l^{-1} (Coffin and Sharp, 1987; Vaqué *et al.*, 1992; Cochran and Paul, 1998; Lavrentyev *et al.*, 2004), which are in the range reported for coastal areas (1.8×10^9 to 6.0×10^9 cells l^{-1}) (Rosso and Azam, 1987; Jiang and Paul, 1994; Fuhrman and Noble, 1995).

Studies conducted in a variety of estuarine systems in both the northern and southern hemisphere have shown that the inflow of freshwater into estuaries promotes the growth of phytoplankton and increases primary production due to the increase in macronutrient availability (Mallin and Paerl, 1994; Adams *et al.*, 1999; Froneman, 2002b). Pearson correlation analysis indicates that during this study the total chl-*a* concentration was not significantly correlated to salinity (Table 3.1). The observed pattern can likely be attributed to breaching events following rainfall in the catchment, which were associated with a decline in the total chl-*a* concentrations due to the outflow of biomass rich estuarine waters into the marine environment. Finally, it is worth noting that the total chl-*a* concentration values obtained during this study are within the range reported for studies conducted in estuarine systems within the same geographic region (Perissinotto *et al.*, 2000; Perissinotto *et al.*, 2002; Froneman, 2004).

Bacterial abundance, biomass and production values exhibited a weak seasonal pattern with the highest values recorded in autumn and summer and the lowest in winter (Figures 3.5 and 3.6; Table 3.2). The abundance, biomass and production values obtained during this study are within the lower range reported for studies conducted in permanently open systems in the northern hemisphere (Coffin and Sharp, 1987; Vaqué *et al.*, 1992; Goosen *et al.*, 1995; Cochran and Paul, 1998; Lavrentyev *et al.*, 2004) (see Table 1.1). For example, bacterial abundance and production values recorded in the Delaware Estuary ranged from 1.00×10^9 to 8.00×10^9 cells l^{-1} and 0.07 to 2.73 µg C l^{-1} h^{-1}, respectively (Coffin and Sharp, 1987). Similarly bacterial biomass and production values in the Elbe Estuary ranged from 31 to 60 µg C l^{-1} and 0.10 to 2.50 µg C l^{-1} h^{-1} (Goosen *et al.*, 1995). The significantly lower values obtained during the colder winter months suggests that temperature may at times limit

bacterial growth. This result is in agreement with studies conducted in temperate lakes and coastal regions which have demonstrated the importance of reduced temperatures in controlling bacterial populations (Scavia and Laird, 1987; Ochs *et al.*, 1995; Ameryk *et al.*, 2005). On the other hand, at elevated temperatures (higher than 14 ºC), bacterial growth appears largely to be dependant on abiotic factors such as substrate, nutrient availability and biotic interactions with other microorganisms such as bacterivores.

Previous studies have demonstrated that the bacterial carbon biomass may exceed that of phytoplankton by 2 to 4 times in low production areas of the world's oceans (Cho and Azam, 1990; Smith and Hall, 1997). To estimate the amount of phytoplankton carbon biomass available during this study, total chl-*a* concentrations were converted to carbon equivalents assuming a chl-*a*: carbon ratio of 50 (chl-*a* carbon = chl-*a* × 50) (Redalje, 1983). Throughout this study the ratio of phytoplankton carbon to bacterial carbon ranged from 1.0 to 2.8 (Table 3.2). These results suggest that bacterial carbon does indeed represent a potentially important carbon source for the heterotrophic components of the plankton food web, particularly nanoheterotrophs (Sherr and Sherr, 2002). Indeed, it is worth noting that during this study nanoflagellate abundances were significantly correlated to bacterial abundances (Table 3.1). This importance of bacteria as a carbon source is not unexpected as temporarily open/closed estuaries are characterised by low chl-*a* concentrations due to reduced freshwater inflow (Perissinotto *et al.*, 2000; Froneman, 2002b; Perissinotto *et al.*, 2002; Froneman, 2004). The highly variable ratio, however, suggests that the significance of bacteria as a carbon source demonstrates a high degree of temporal variability within the estuary.

A key feature of this study was the extreme variability in bacterial abundance, biomass and production values during each season. Numerous studies have shown that bacterial production can be regulated by resource availability such as organic carbon and nutrients (bottom-up control) or by predators (top-down control) (Button, 1994; Pace and Cole, 1994; Calbet and Landry, 1999; Sherr and Sherr, 2002; Froneman, 2006). In some instances both control mechanisms have been found to operate simultaneously, which may affect different components of the planktonic community (Berninger and Wickham, 2005). During this study phytoplankton and bacterial dynamics fluctuated independently of one another suggesting that each community was being affected by a different control mechanism. Bacterial

abundance in the lower and middle reaches showed a negative correlation with salinity indicating that freshwater inflow may contribute to bacterial growth. Nanoflagellate and bacterial abundances, however, had a stronger correlation indicating that top-down control is likely to be a more important control mechanism in this system.

It is well established that predation by phagotrophic protists, which are typically dominated by flagellates, ciliates and dinoflagellates (< 200 μm), can be a significant source of mortality for suspended bacteria in both freshwater and marine ecosystems (Sherr and Sherr, 2002; Bettarel *et al.*, 2004). It is now also accepted that a significant fraction of the bacterial population is lost through viral infection and lysis (Fuhrman and Noble, 1995; Weinbauer and Höfle, 1998; Bettarel *et al.*, 2004). The role of viral lysis and flagellate grazing in bacterial mortality may change spatially and temporally. The importance of viral lysis has been shown to increase in situations where flagellate grazing is reduced. For example, top-down control of larger microzooplankton on flagellates will reduce the grazing impact of these organisms (Gasol, 1994). In anoxic waters grazing rates are also low since there are only a few anaerobic protozoan species, this results in viral lysis dominating as the major source of bacterial mortality (Weinbauer and Höfle, 1998; Bettarel *et al.*, 2004). It has also been shown that occasionally at high bacterial abundances the majority of bacterial mortality can shift from grazing to viral lysis (Weinbauer and Peduzzi, 1995).

The percentage of bacteria infected with virus particles as well as the burst size during the present study showed no significant spatial or temporal variability during this study (post hoc Scheffé test ANOVA $p > 0.05$ in all cases). Viral infection and subsequent lysis were therefore a constant source of bacterial mortality throughout the year. The nanoflagellate and bacterial abundances, however, followed a similar pattern both spatially and temporally. The positive correlation observed in the lower and middle reaches between these two components indicates that a predator-prey interaction is likely to be occurring. Since viral lysis was a constant source of bacterial mortality, increases in the bacterial population are likely to have been controlled by flagellate bacterivory. The estimated percentage of bacterial production removed by viral lysis during this study ranged between 7.8 and 88.9 % (mean = 30.3 %), which suggests that viral lysis represents a very important source of bacterial mortality within the East Kleinemonde Estuary. It is worth noting that the number of infected bacterial cells recorded during this study is within the range reported for studies conducted in the

northern hemisphere freshwater lake and coastal environments (Weinbauer and Suttle, 1996; Weinbauer and Höfle, 1998; Fischer and Velimirov, 2002; Bettarel *et al.*, 2004) (see Table 1.2). For example, FVIC and BS values recorded in Lake Plußsee ranged from 0.7 to 2.5 % and 19 to 35 virus particles per bacterium, respectively (Weinbauer and Höfle, 1998). In the coastal shelf region in the Gulf of Mexico FVIC and BS ranged from 0.1 to 4.4 % and 11 to 45 virus particles per bacterium, respectively (Weinbauer and Suttle, 1996).

It is important to note, however, that there are several potential sources of error in the estimates of bacterial production and viral lysis during this study. Firstly, a widely used conversion factor of 2.0×10^{18} cells mol^{-1} was employed to estimate bacterial production. This factor was generated from the median of 97 marine studies (Bell, 1993) and may not be applicable to this study, which was conducted in an estuary. Secondly, for viral lysis it has been suggested that the use of ultracentrifugation at high speeds could lead to rupturing of infected bacterial cells and therefore result in lower estimates of FVIC (Weinbauer and Höfle, 1998; Weinbauer *et al.*, 2002). Finally, it has been shown that estimates of FVIC and BS obtained using the TEM approach are lower than those obtained using the virus dilution approach (Weinbauer *et al.*, 2002). Using whole-cell examination rather than thin sections has also been found to produce lower estimates of FVIC (Weinbauer *et al.*, 1993; Fuhrman and Noble, 1995; Weinbauer and Höfle, 1998; Weinbauer *et al.*, 2002). As a result values obtained for FVIC and BS in this study have the potential to be underestimated and should be considered conservative estimates.

In conclusion, results of this study indicate that there were limited spatial (horizontal and vertical) differences in the biology of the East Kleinemonde Estuary reflecting the hydrodynamics of the system. Bacterial abundance, biomass and production showed some evidence of a seasonal pattern, however, the only real difference occurred between winter and the rest of the seasons. At low temperatures bacterial production appears to be controlled by temperature. During this study viral infection was found to be a constant and extremely important source of bacterial mortality. The significant positive correlation between nanoflagellate and bacterial abundances during the present study suggests strong top-down control of bacteria within the estuary. The result of this study highlights the complex interactions at the base of the food web and suggests the need for further investigation of the microbial dynamics within temporarily open/closed estuaries.

CHAPTER 4
TROPHIC INTERACTIONS BETWEEN THE PLANKTON: EVIDENCE FROM TROPHIC CASCADING EXPERIMENTS

4.1. INTRODUCTION

The interactions between different trophic levels can, via trophic cascading, influence the structure of the plankton community (Pace *et al.*, 1998; Calbet and Landry, 1999; Froneman, 2002a; Froneman and Bernard, 2004). For example, previous studies have demonstrated that predators play an important role in regulating abundance, biomass and the community composition of the plankton (Pace *et al.*, 1998). Through direct predation and trophic cascading, consumers can therefore control the population dynamics of other organisms (Miller *et al.*, 1995). This coupling between various components of the food web via trophic cascading was demonstrated in the oligotrophic North Pacific Ocean where the predation of copepods on microzooplankton was shown to enhance the net growth of phytoplankton, nanoflagellates and bacteria (Calbet and Landry, 1999). From an ecological perspective, it is essential to understand the trophic interactions between the various trophic levels in order to determine the food web structure and energy dynamics within estuaries (Calbet and Landry 1999).

In oligotrophic regions, the phytoplankton community is typically dominated by picophytoplankton (< 2 μm), which are largely unavailable to direct consumption by metazoans, particularly copepods, due to feeding constraints (Calbet and Landry, 1999). These primary producers, however, are linked to higher trophic levels via cascading impacts of mesozooplankton grazing on intermediate consumers (protozoans) i.e. carnivory (Calbet and Landry, 1999). Trophic intermediates such as protozoans therefore provide an important carbon source to the larger zooplankton in regions where small cells dominate the phytoplankton community (Stoecker and Capuzzo, 1990; Froneman and Balarin, 1998). The predation impact of mesozooplankton on the microheterotrophs will coincide with a decrease in their feeding impact on the bacteria and phytoplankton thereby reducing the top-down

control on the bacterial and phytoplankton production (Froneman, 2006). The presence of higher trophic consumers, such as mesozooplankton, can also have a small indirect positive effect on bacteria due to the excretion of nitrogenous waste products such as ammonia, which promote the growth of bacteria (Calbet and Landry, 1999). Even in mesotrophic systems, which are characterised by diatom blooms, phagotrophic protists have been shown to serve as an important trophic link between the base of the food web and higher trophic levels (Sherr and Sherr, 2002). In regions that are dominated by nanophytoplankton (2 - 20 μm), however, mesozooplankton are able to feed directly on the phytoplankton resulting in a decrease in the level of carnivory and therefore trophic cascading (Froneman, 2006).

Results of studies conducted during this investigation demonstrated a strong significant correlation between bacterial and nanoflagellate abundances (Figure 3.7; Table 3.1), suggesting a strong top-down control of bacteria within the East Kleinemonde Estuary (see Chapter 3). The interactions between various components of the plankton food web are, however, complex reflecting the availability of preferred food, zooplankton species composition and size structure of the phytoplankton community (Fortier *et al.*, 1994; Calbet and Landry, 1999; Froneman, 2006). The main aim of this chapter is to investigate the biological interactions between selected components of the plankton community within the temporarily open/closed East Kleinemonde Estuary during winter and summer.

4.2. MATERIALS AND METHODS

Trophic interactions between the bacteria, phytoplankton, nanoflagellates (2 - 20 μm), microzooplankton (20 - 200 μm), and mesozooplankton (200 - 2 000 μm) was investigated in the middle reaches of the East Kleinemonde Estuary (see Figure 2.3). Experiments were only carried out in the surface water in the middle reaches as the previous study demonstrated that there were no significant spatial patterns in the biology of the system (Chapter 3). Bacterivory, direct-feeding interactions and cascading effects of zooplankton grazing were investigated in winter (July 2006) and summer (January 2007) to enable seasonal comparisons. Experiments were conducted during the closed phase to negate possible breaching effects on the biology of the system. Environmental and biological variables were determined in Chapter 3.

4.2.1. *IN SITU* CHLOROPHYLL-*a* CONCENTRATION

Size fractionated chl-*a* concentration was determined from 250 ml water samples which were serially filtered (low vacuum < 5 cm Hg) through 20.0 µm, 2.0 µm and GF/F filters to determine micro-, nano- and picophytoplankton concentrations. Size fractionated chl-*a* concentrations were determined fluorometrically as described in Chapter 3.2.2. Three replicates were prepared for each of the two seasons.

4.2.2. BACTERIVORY BY HETEROTROPHS

Surface water was collected (2 L samples) and pre-screened through 2 µm (control), 20 µm (< 20 µm nanoflagellate treatment) and 200 µm (< 200µm microzooplankton treatment) filters. The fourth treatment was set up with unfiltered water to show natural conditions (> 200 µm mesozooplankton treatment). Each treatment resulted in a different trophic interaction: only bacteria (control), nanoflagellates and bacteria (< 20 µm), microzooplankton, nanoflagellates and bacteria (< 200 µm) and mesozooplankton, microzooplankton, nanoflagellates and bacteria (> 200 µm). For each treatment three replicates were prepared. The treatments were incubated for 24 hours in a constant environment (CE) room set at the specific temperature recorded at the estuary (18 °C during winter and 27 °C during summer) with a 12:12 light / dark cycle. For each treatment bacterial abundances were determined before and after the incubation using epifluorescent microscopy as described in Chapter 3.2.3. It was assumed that during the incubations the bacteria demonstrated exponential growth rates. The growth rates of the bacteria were estimated employing the following equation:

$$r = \ln (Nt / No) / \Delta t$$

where Nt = final values; No = initial values; Δt = time in days (Froneman, 2002a).

4.2.3. DIRECT FEEDING INTERACTIONS

To determine direct feeding relationships 2 L surface water samples were pre-screened through 10 µm (control), 20 µm (< 20 µm nanoflagellate treatment) and 200 µm (< 200 µm microzooplankton treatment) filters. The fourth treatment was set up with unfiltered water to simulate natural conditions (> 200 µm mesozooplankton treatment). Each treatment resulted

in a different trophic interaction: only phytoplankton (control), nanoflagellates and phytoplankton (< 20 μm), microzooplankton, nanoflagellates and phytoplankton (< 200 μm) and mesozooplankton, microzooplankton, nanoflagellates and phytoplankton (> 200 μm). For each treatment three replicates were prepared. The treatments were incubated for 24 hours in a CE room set at the specific temperature recorded at the estuary with a 12:12 light / dark phase. At the beginning and end of the incubation, aliquots of water were taken from each bottle for the determination of total chl-*a* concentration. Total chl-*a* concentrations were determined fluorometrically as described in Chapter 3.2.2. Net growth rates of the chl-*a* for each treatment were estimated from initial and final concentrations assuming an exponential change during the incubations.

4.2.4. CASCADING EFFECTS OF ZOOPLANKTON GRAZING

To assess the cascading effects of mesozooplankton on the lower levels of the food web, the net growth response of bacteria, total chl-*a*, nanoflagellates and microzooplankton to increasing concentrations of mesozooplankton was investigated according to the method of Calbet and Landry (1999). Surface water was pre-screened through a 200 μm filter to remove mesozooplankton. Mesozooplankton were collected from the sub-surface waters (depth 1 m) using a modified WP-2 net (mesh size 60 μm; nominal mouth area 0.2 m^2) fitted with a 1 L cod end. The zooplankton collected were carefully transferred into a 1 L bottle containing estuarine water. Aliquots of mesozooplankton were added to three replicate bottles per treatment, in addition three control bottles were prepared without mesozooplankton. The bottles were then incubated for a period of 24 hours in a CE room set at the specific temperature recorded at the estuary with a 12:12 light / dark cycle. At the beginning and end of each incubation, aliquots of water were removed for the determination of total chl-*a* concentration, bacterial counts, nanoflagellate and microzooplankton densities. Total chl-*a* concentration and bacterial abundance was determined as described in Chapter 3.2.2 and 3.2.3, respectively. Nanoflagellate and microzooplankton densities were determined using epifluorescent microscopy as described in Chapter 3.2.5. Net growth rates of total chl-*a*, bacteria, nanoflagellates and microzooplankton were estimated from initial and final concentrations assuming an exponential change during the incubations. At the end of the incubation period each treatment (1 L sample) was filtered through a pre-weighed GF 52

glass fibre filter and the dry weight of the added mesozooplankton was determined after oven drying for 24 hours at 60 °C.

4.2.5. STATISTICAL ANALYSIS

To assess bacterivory and direct feeding interactions a one way ANOVA followed by a post hoc Scheffé test with a significance level of 5 % was undertaken. For the cascading effects of zooplankton grazing, a regression analysis was performed. All analyses were conducted with the statistical package Statistica (version 7.0).

4.3. RESULTS

A summary of the environmental and biological variables recorded during the experiments is shown in Table 4.1. Water temperature in winter and summer was 12.8 and 26.4 °C, respectively, and salinity was 27 and 18, respectively. Bacterial, nanoflagellate and microzooplankton abundances were lower in winter (2.11×10^9 cells l^{-1}, 0.78×10^4 ind. l^{-1} and 1.03×10^3 ind. l^{-1}, respectively) than in summer (3.82×10^9 cells l^{-1}, 3.05×10^4 ind. l^{-1} and 1.64×10^3 ind. l^{-1}, respectively).

Table 4.1. Environmental and biological variables in the temporarily open/closed East Kleinemonde Estuary during winter and summer (mean ± standard deviation).

	Temperature (°C)	Salinity	Bacterial abundance ($\times 10^9$ cells l^{-1})	Nanoflagellate abundance ($\times 10^4$ ind. l^{-1})	Microzooplankton abundance ($\times 10^3$ ind. l^{-1})
Winter	12.8	27	2.11 ± 0.36	0.78 ± 0.07	1.03 ± 0.19
Summer	26.4	18	3.82 ± 0.48	3.05 ± 0.52	1.64 ± 0.73

4.3.1. *IN SITU* CHLOROPHYLL-*a* CONCENTRATION

Total chl-*a* concentration was higher in winter (1.53 mg chl-*a* m^{-3}) than in summer (0.39 mg chl-*a* m^{-3}) (Figure 4.1). Results of size fractionated total chl-*a* analyses indicated that in winter, the total chl-*a* concentration was dominated by the larger microphytoplankton (> 20 μm) (54 ± 5 % of the total pigment). The nanophytoplankton fraction (2 - 20 μm) was identified as the second largest contributor (26 ± 3 %) closely followed by the picophytoplankton (< 2 μm) (20 ± 3 %). In summer the reverse pattern was observed as the

total chl-*a* concentration was dominated by the picophytoplankton (54 ± 27 % of the total pigment). The nanophytoplankton fraction was identified as the second largest contributor (36 ± 20 %) while the microphytoplankton only contributed 11 % (± 5 %) of the total pigment.

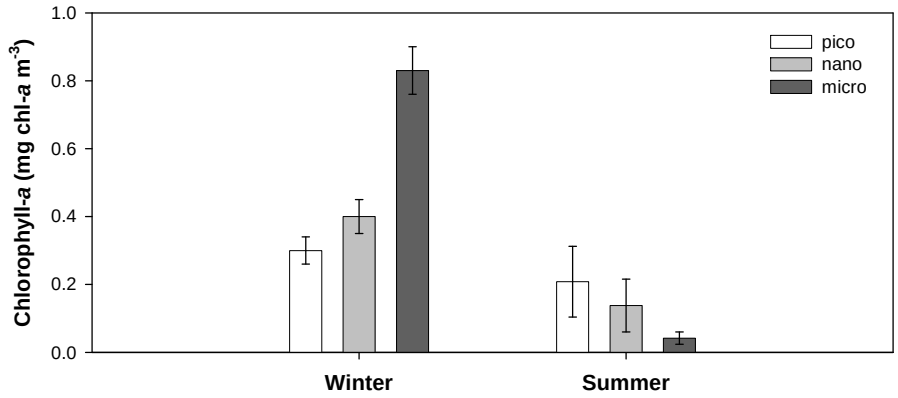

Figure 4.1. Size fractionated chlorophyll-*a* concentrations for trophic cascading experiments conducted during winter and summer. Error bars represent the standard deviation (*n* = 3).

4.3.2. BACTERIVORY BY HETEROTROPHS

In the absence of grazers (control), there was a positive mean net growth rate of the bacteria estimated at 0.26 ± 0.06 day^{-1} in winter and 0.60 ± 0.15 day^{-1} in summer (Figure 4.2 A and B, respectively). There was a significant difference in the net growth rates of the bacteria in the control between winter and summer ($F = 14.7$, $p < 0.05$). In the nanoflagellate treatment (< 20 µm), the mean net growth rate of the bacteria was estimated a -0.28 ± 0.08 day^{-1} in winter and 0.17 ± 0.07 day^{-1} in summer (Figure 4.2 A and B, respectively). In the microzooplankton treatment (< 200 µm), the mean net growth rate of the bacteria in winter and summer was estimated at 0.12 ± 0.08 day^{-1} and 0.32 ± 0.03 day^{-1}, respectively (Figure 4.2 A and B, respectively). Finally in the presence of all grazers (> 200 µm mesozooplankton treatment), the mean net growth rate of the bacteria was estimated at 0.32 ± 0.15 day^{-1} during winter and 0.42 ± 0.22 day^{-1} in summer (Figure 4.2 A and B, respectively). During winter, the estimated net growth rate of the bacteria in the nanoflagellate treatment was significantly lower than the control, micro- and mesozooplankton treatments ($F = 108$, $p < 0.001$; $F = 40.5$, $p < 0.01$; $F = 38.9$, $p < 0.01$, respectively). There was no significant difference in the estimated growth rates of the bacteria in the micro- and mesozooplankton treatments ($p > 0.05$). In summer, the net growth rate of the bacteria in the nanoflagellate treatment was again significantly lower than the

control ($F = 20.3$, $p < 0.05$). The net growth rate of the bacteria in the control and the nanoflagellate treatment were not significantly different from the micro- and mesozooplankton treatments ($p > 0.05$).

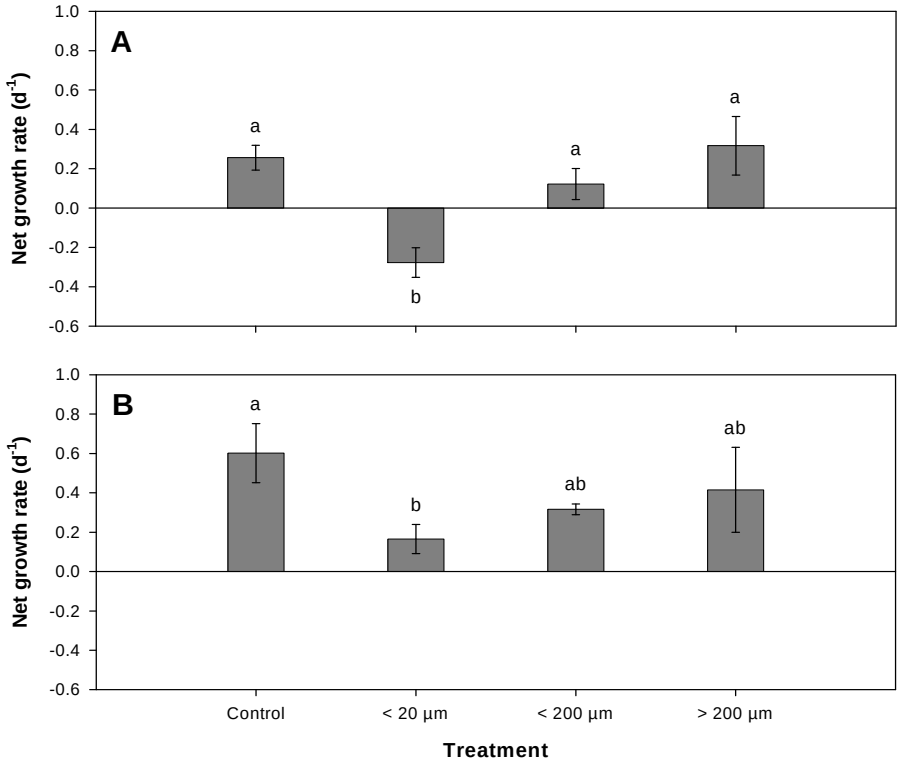

Figure 4.2. Impacts of nano-, micro- and mesozooplankton grazers on the net growth rate of the bacteria during winter (**A**) and summer (**B**). Error bars represent the standard deviation ($n = 3$). Letters denote significant difference between treatments (post hoc Scheffé test).

4.3.3. DIRECT FEEDING INTERACTIONS

In the absence of grazers (control), the net growth rate of the phytoplankton was estimated at 0.11 ± 0.03 day^{-1} in winter and 0.11 ± 0.02 day^{-1} in summer (Figure 4.3 A and B, respectively). There was no significant difference in the net growth rates of the phytoplankton between the winter and summer studies ($p > 0.05$). In the nanoflagellate treatment (< 20 µm), the mean net growth rate of the phytoplankton was variable between summer and winter. In winter there was a net increase in the growth rate of the phytoplankton from the control (0.14 ± 0.04 day^{-1}) while in summer there was a net decrease (0.02 ± 0.01 day^{-1}) over the duration of the incubation (Figure 4.3 A and B, respectively). In the microzooplankton treatment (< 200 µm) the mean net growth rate of the phytoplankton was estimated at 0.23 ± 0.02 day^{-1} in winter and 0.07 ± 0.02 day^{-1} in summer (Figure 4.3 A

and B, respectively). Finally in the presence of all grazers (> 200 µm mesozooplankton treatment) the mean net growth rate of the phytoplankton was estimated at 0.30 ± 0.04 day^{-1} in winter and 0.11 ± 0.04 day^{-1} in summer (Figure 4.3 A and B, respectively). During winter, the net growth rate of the phytoplankton in the control was significantly different from the micro- and mesozooplankton treatments ($F = 43.8$, $p < 0.01$; $F = 42.9$, $p < 0.05$, respectively). There was no significant difference in the estimated growth rate of the phytoplankton between the micro- and mesozooplankton treatments ($p > 0.05$). The net growth rate of the phytoplankton in the nanoflagellate treatment was significantly different from the mesozooplankton treatment ($F = 22.3$, $p < 0.05$). There was no significant difference in the estimated growth rate of the phytoplankton between the nanoflagellate and microzooplankton treatments ($p > 0.05$). During summer, the net growth rate of the phytoplankton was significantly lower in the nanoflagellate treatment than the control and mesozooplankton treatment ($F = 69.8$, $p < 0.01$; $F = 67.7$, $p < 0.05$, respectively). The control was not significantly different from the micro- and mesozooplankton treatments ($p > 0.05$).

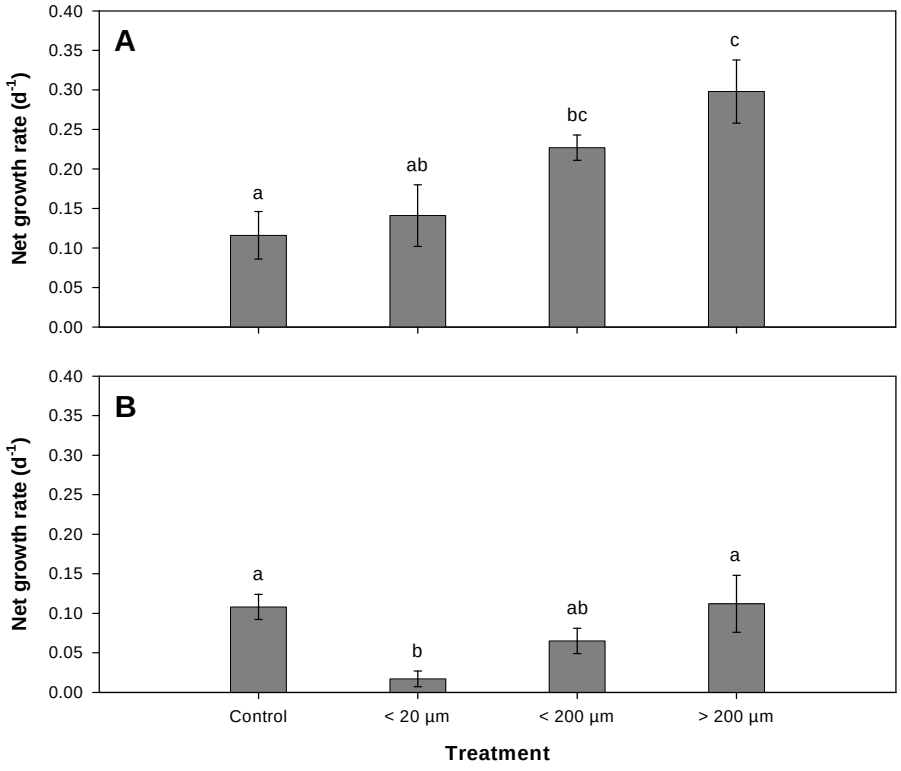

Figure 4.3. Impacts of nano-, micro- and mesozooplankton grazers on the net growth rate of the total chlorophyll-*a* during winter (**A**) and summer (**B**). Error bars represent the standard deviation ($n = 3$). Letters denote significant difference between treatments (post hoc Scheffé test).

4.3.4. CASCADING EFFECTS OF ZOOPLANKTON GRAZING

Zooplankton community during both winter and summer was numerically dominated by copepods of the genera *Pseudodiaptomus* (*P. hessei*), *Acartia* (*A. longipatella*), *Oithona* (*O. nana*) and *Halicyclops* species. Collectively these copepods accounted for > 90 % of all zooplankton counted during the two seasons (Froneman, unpublished data). The results of the trophic cascading experiments and regression coefficients are shown in Figure 4.4 and Table 4.2. Biomass of mesozooplankton within the bottles was within the range reported for the estuary (Vorwerk, 2006). The projected growth rates of the total phytoplankton, bacteria, nanoflagellates and microzooplankton in the absence of mesozooplankton (intercept) during both winter and summer were positive (Table 4.2). The addition of mesozooplankton resulted in an increase in the net growth rate of the bacteria and a decrease in the net growth rates of the nanoflagellates during both seasons (Figure 4.4 A and B, respectively). In contrast, the net growth rate of the total phytoplankton showed no apparent change during the winter study (Figure 4.4 A). In the summer study, however, there was an increase in the net growth rate of the total phytoplankton (Figure 4.4 B). There was no apparent change in the net growth of the microzooplankton in the winter study, however, in the summer study there was a decrease in the net growth rate of the microzooplankton (Figure 4.4 A and B, respectively).

Table 4.2. Regression parameter and correlation coefficients (r^2) of the experiments conducted to assess trophic cascades during winter and summer ($n = 6$).

Parameter	Intercept	slope	r^2	p value
Winter				
Total chl-*a*	0.30	-0.03	0.02	ns
Bacteria	0.37	0.86	0.97	< 0.01
Nanoflagellates	0.27	-0.54	0.98	< 0.01
Microzooplankton	0.22	0.003	0.0001	ns
Summer				
Total chl-*a*	0.12	0.21	0.87	< 0.01
Bacteria	0.44	0.49	0.95	< 0.01
Nanoflagellates	0.21	-0.37	0.94	< 0.01
Microzooplankton	0.14	-0.23	0.67	< 0.05

ns = not significant

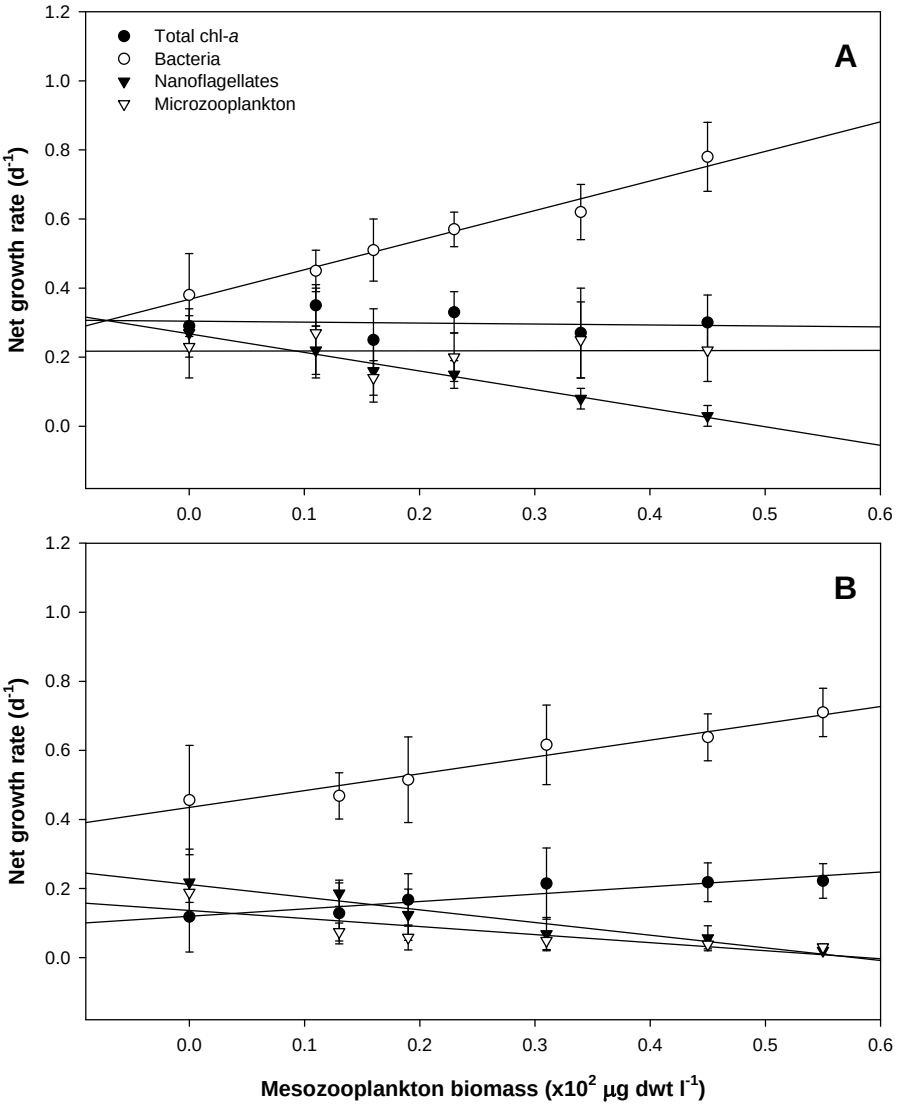

Figure 4.4. Impacts of mesozooplankton grazing on the net growth rates of the total chlorophyll-*a*, bacteria, nanoflagellates and microzooplankton during winter (**A**) and summer (**B**). Error bars represent the standard deviation (n = 3).

4.4. DISCUSSION

In the absence of grazers, the estimated net growth rate of the bacteria was significantly lower in winter than in summer (F = 14.7, $p < 0.05$). The observed pattern is consistent with bacterial production studies estimated by thymidine incorporation experiments presented in Chapter 3. The reduced growth rates of bacteria in winter are also in agreement with studies conducted in temperate lakes and coastal regions, which have demonstrated the importance of reduced temperatures (< 14 ºC) in controlling bacterial populations (Scavia and Laird, 1987; Ochs *et al.*, 1995; Ameryk *et al.*, 2005). Changes in the net growth rate of the bacteria,

however, showed a similar trend during winter and summer suggesting that top-down control is an important controlling mechanism during both winter and summer.

Previous studies conducted in a variety of southern African estuaries have demonstrated that phytoplankton growth rates and community structure are strongly linked to freshwater inflow (Adams *et al.*, 1999; Froneman, 2002b; Froneman, 2006). The winter study was conducted shortly after rainfall had occurred within the catchment area (see Figure 3.3). Studies conducted in a variety of estuarine systems have shown that the inflow of freshwater into estuaries promotes the growth of phytoplankton as freshwater inflow represents the primary source of macronutrients necessary to sustain the growth of larger phytoplankton cells (Mallin and Paerl, 1994; Adams *et al.*, 1999; Froneman, 2002b; Froneman 2006). The predominance of larger microphytoplankton (> 20 μm) during the winter study was therefore likely to be related to the increased macronutrient availability due to freshwater inflow (Figure 4.1). On the other hand, the summer study was conducted during a prolonged period of dryness (see Figure 3.3). Macronutrient concentrations were therefore likely to have been depleted resulting in small cells (< 20 μm) dominating the phytoplankton community.

The net growth rate of the bacteria in both winter and summer was significantly lower in the nanoflagellate treatment than in the controls ($F = 108$, $p < 0.001$; $F = 20.3$, $p < 0.05$, respectively; Figure 4.2). This result is consistent with those obtained by Sherr and Sherr (2002) who proposed that within aquatic systems the primary bacterivores are typically small heterotrophic flagellates (cells < 5.0 μm in size). The addition of microzooplankton coincided with an increase in the net growth rate of the bacteria. Microzooplankton have been shown to feed on nanoflagellates greater than 5.0 μm in size (Calbet and Landry, 1999). As a result, it is likely that components of the larger microzooplankton would have preyed upon the nanoflagellates therefore reducing the impact of these organisms on the bacteria. In the mesozooplankton treatment, the estimated growth rate of the bacteria was not significantly different from the control. Mesozooplankton have been shown to typically feed on particles within the nano- (2 - 20 μm) size range (Fortier *et al.*, 1994; Calbet and Landry, 1999). The absence of any decrease in the net growth of the bacteria in the mesozooplankton treatments can therefore be related to larger grazers feeding on the nanoflagellates. The presence of higher trophic consumers such as mesozooplankton can also have small indirect positive effects on bacterial growth rates due to the excretion of nitrogenous waste products

such as ammonia, which can be utilized by the bacteria (Calbet and Landry, 1999). For example, a study conducted in the oligotrophic North Pacific Ocean showed that the organic substrates released by mesozooplankton promoted the net growth of phototrophic *Prochlorococcus* species and bacteria (Calbet and Landry, 1999). The absence of any significant difference in the estimated growth rates of the bacteria in the control and the mesozooplankton treatments ($p > 0.05$), however, suggests that mesozooplankton waste products did not promote the growth of bacteria during the incubations. Substrate availability therefore did not appear to limit bacterial growth during this study.

Changes in the net growth rate of the total phytoplankton in the different treatments, unlike bacteria, differed between the summer and winter studies. These observed differences are likely the result of the size structure of the phytoplankton community. Analysis of the literature indicates that heterotrophic components (< 2 000 µm) of the plankton preferentially feed on particles within the nano- size fraction (Fortier *et al.*, 1994; Hansen *et al.*, 1994; Calbet and Landry, 1999). The absence of any significant decrease in the growth rates of the phytoplankton in the various treatments during winter when microphytoplankton dominate total chl-*a* concentration, is therefore not surprising. Indeed, results of this study indicate that larger heterotrophic components may in fact promote the growth of the microphytoplankton, possibly mediated by the release of waste products, which would stimulate the growth of these phytoplankton cells (Figure 4.3 A). During summer, however, there was a significant decrease in the net growth rate of the phytoplankton when nanoflagellates were added which gradually increased as higher trophic levels were added. In summer, the phytoplankton community was dominated by small cells (< 20 µm) (Figure 4.1). A number of studies have demonstrated that nanoflagellates and microzooplankton are the most important consumers of phytoplankton production particularly in regions were small phytoplankton cells dominate total chl-*a* concentration (Froneman and Balarin, 1998; Sherr and Sherr, 2002; Froneman, 2004). The decrease in the net growth rate of the phytoplankton in summer when nanoflagellates and microzooplankton were added is as a consequence, not surprising. In areas where picophytoplankton are dominant, copepods are unable to feed directly due to feeding constraints and therefore alternative sources such as nanoflagellates and microzooplankton are consumed i.e. carnivory is prevalent (Stoecker and Capuzzo, 1990; Froneman and Bernard, 2004). This introduced predation on protozooplankton will result in a reduced grazing impact upon the phytoplankton, resulting in a net increase in the growth rate.

The increase in the mesozooplankton biomass during the cascading experiments in both winter and summer coincided with a net increase in the growth rate of the bacteria together with a net decrease in the growth rate of the nanoflagellates (Figure 4.4). In the summer study there was a net increase in growth rate of the total phytoplankton while in the winter study there was no apparent change. The phytoplankton community in summer was dominated by small picophytoplankton (< 2 μm), which are generally considered too small for direct utilisation by the mesozooplankton. Similarly, the phytoplankton community during winter (dominated by microphytoplankton) was too large to be efficiently grazed by the mesozooplankton (Fortier *et al.*, 1994; Calbet and Landry, 1999). It has been shown that in situations were phytoplankton are unavailable to direct consumption, carnivory by metazoans is most prevalent (Stoecker and Capuzzo, 1990). The increase in the net growth rate of the bacteria, and to a lesser extent the total phytoplankton (Figure 4.4), is therefore likely to be due to the predation impact of mesozooplankton on the nanoflagellates and microzooplankton. This predation impact is evident by the decrease in the net growth rate of the nanoflagellates and microzooplankton with an increase in the mesozooplankton biomass. The predation by the mesozooplankton would reduce the grazing impact of the nanoflagellates and microzooplankton on the bacteria and phytoplankton. It is important to note that the results of the trophic cascading experiments suggest that, even though 36 % of the available phytoplankton in winter were within the nano- size range, the mesozooplankton readily consumed nanoflagellates. It has been suggested that nanoflagellates maybe a particularly rich source of proteins, amino acids and essential lipids especially when compared to phytoplankton (Stoecker and Capuzzo, 1990). The zooplankton may therefore be preferentially feeding on the nanoflagellates to gain energy benefits.

In conclusion, results of this study indicate that phytoplankton community size composition results in different trophic interactions amongst the plankton. When small phytoplankton cells (< 20 μm) dominated the phytoplankton community, nanoflagellates were the most important consumers of both bacteria and these small phytoplankton cells. Complex food webs also resulted mostly due to the feeding constraints of the larger mesozooplankton. Mesozooplankton were found to readily consume nanoflagellates even when the phytoplankton community were within the size range suitable for direct utilisation. This indicates that nanoflagellates provide an important alternative energy source for higher trophic levels. Most importantly, nanoflagellates were identified as the most important

consumers of bacteria and that predation by larger components of the plankton on nanoflagellates reduced the impact of these organisms on the bacteria. The presence of the larger heterotrophs therefore mediates interactions between these primary bacterivores and the bacteria. The result of this investigation highlights the need for more complex studies to investigate top-down control of bacterial populations within aquatic ecosystems.

CHAPTER 5
GENERAL DISCUSSION

Studies conducted on the aspects of the biology of temporarily open/closed southern African estuaries indicated that the plankton community composition and feeding dynamics within these systems are strongly linked to mouth phase and magnitude of freshwater inflow (Wooldridge, 1999; Froneman, 2002b; Perissinotto *et al.*, 2002). Results of the present study indicate that there were limited horizontal and vertical patterns in the biology (total chl-*a* concentration, bacterial and nanoflagellate abundances) within the East Kleinemonde Estuary (Chapter 3). The lack of any spatial patterns is likely the result of the absence of gradients in the physico-chemical variables, which reflects the hydrodynamics of the system. The virtual absence of vertical and horizontal gradients in salinity and temperature observed within the East Kleinemonde Estuary during this study is a common feature of temporarily open/closed estuaries along the south east coast of southern Africa (Froneman, 2002b; Perissinotto *et al.*, 2002). This can be related to several factors including limited freshwater inflow, shallow depth and wind induced mixing, which facilitates horizontal and vertical mixing of the water column (Froneman, 2002b; Perissinotto *et al.*, 2002).

Mouth opening events were associated with a decline in the total chl-*a* concentrations within the estuary, which can be linked to the outflow of biomass rich estuarine waters into the marine environment (Chapter 3). This is in agreement with previous studies conducted in temporarily open/closed estuaries within the same region (Froneman, 2002b; Perissinotto *et al.*, 2002). This pattern, however, was not observed for bacterial abundances, biomass and production (Chapter 3). The absence of any distinct pattern in bacterial values could be a result of similar bacterial densities in both coastal and estuarine environments (Coffin and Sharp, 1987; Rosso and Azam, 1987; Vaqué *et al.*, 1992; Jiang and Paul, 1994; Fuhrman and Noble, 1995; Cochran and Paul, 1998; Lavrentyev *et al.*, 2004).

Bacterial production is dependent on both abiotic (temperature, salinity, substrate and nutrient availability) and biotic (bacterivory and viral lysis) factors (Button, 1994; Fuhrman

and Noble, 1995; Weinbauer and Höfle, 1998; Goosen *et al.*, 1999; Sherr and Sherr, 2002; Bettarel *et al.*, 2004; Ameryk *et al.*, 2005). Bacterial abundance, biomass and production showed some evidence of a weak seasonal pattern, however, the only significant difference was observed between winter and the rest of the year (Chapter 3). This pattern was also observed in Chapter 4 where the estimated net growth rate of the bacteria, in the absence of grazers, was significantly lower in winter than in summer. The significantly lower values and reduced growth rates of bacteria in winter are in agreement with studies conducted in temperate lakes and coastal regions, which have demonstrated the importance of reduced temperatures (< 14 ºC) in controlling bacterial populations (Scavia and Laird, 1987; Ochs *et al.*, 1995; Ameryk *et al.*, 2005). It is important to note, however, that in the absence of nutrient data, it is possible that some of the variability observed in bacterial production rates during this study may be a result of substrate availability.

A key feature of this study was the extreme variability in bacterial abundance, biomass and production values during each season. It is now well established that predation by protists and viral lysis can be significant sources of mortality for suspended bacteria in both freshwater and marine ecosystems (Fuhrman and Noble, 1995; Weinbauer and Höfle, 1998; Sherr and Sherr, 2002; Bettarel *et al.*, 2004). During this study the percentage of bacteria infected with virus particles and the number of virus particles present (burst size) showed no significant spatial or temporal variability (Chapter 3). Viral infection and lysis was therefore a constant source of bacterial mortality throughout the year. It is important to note, however, that a substantial portion of the bacterial production was lost to viral lysis (30.3 %), suggesting that viral lysis represents a very important source of bacterial mortality within the East Kleinemonde Estuary. The nanoflagellate and bacterial abundances followed a similar pattern both spatially and temporally (Chapter 3). The positive correlation in the lower and middle reaches between these two components indicates the presence of a predator-prey relationship. Since viral lysis was a constant source of bacterial mortality, increases in the bacterial population are likely to have been controlled by flagellate bacterivory rather than viral lysis.

Bacteria represent an important carbon source for the heterotrophic components of the plankton food web within a variety of aquatic systems (Stoecker and Capuzzo, 1990; Calbet and Landry, 1999; Sherr and Sherr, 2002). Bacteria have been shown to be potentially

important biomass producers due to their high growth efficiencies and rapid growth rates (Gasol, 1994; Fuhrman and Noble, 1995). During this study the ratio of phytoplankton carbon to bacterial carbon ranged from 1.0 to 2.8 (Chapter 3). In addition, nanoflagellate and bacterial abundances showed a strong correlation indicating that top-down control is likely to be important in this system (Chapter 3 and 4). These results suggest that bacterial carbon does indeed represent a potentially important carbon source for the heterotrophic components of the plankton food web. The importance of bacteria as a carbon source within the estuary is not unexpected as temporarily open/closed estuaries are generally characterised by low chl-*a* concentrations due to reduced freshwater inflow (Perissinotto *et al.*, 2000; Froneman, 2002b; Perissinotto *et al.*, 2002; Froneman, 2004).

Previous studies have documented that predators play an important role in regulating abundance, biomass and community composition of the plankton (Pace *et al.*, 1998; Calbet and Landry, 1999; Sherr and Sherr, 2002). Through direct predation and trophic cascading, consumers can exert a strong control on population dynamics of other organisms (Miller *et al.,* 1995). The interactions between various components of the plankton food web, however, are complex reflecting the availability of preferred food, zooplankton species composition and size structure of the phytoplankton community (Fortier *et al.*, 1994; Hansen *et al.*, 1994; Calbet and Landry, 1999). Different interactions were observed between the winter and summer studies, which are likely the result of the both size structure of the phytoplankton community and feeding constraints of the larger zooplankton (Chapter 4). The phytoplankton community in the winter study was dominated by larger microphytoplankton (> 20 μm) while small phytoplankton cells (< 20 μm) dominated the community in the summer study. A number of studies have demonstrated that nanoflagellates and microzooplankton are the most important consumers of phytoplankton production particularly in regions were small phytoplankton cells dominate total chl-*a* concentration (Froneman and Balarin, 1998; Sherr and Sherr, 2002; Froneman, 2004). The decrease in the net growth rate of the phytoplankton in summer when nanoflagellates and microzooplankton were added is as a consequence, not surprising. In winter the phytoplankton community was dominated by large phytoplankton cells that were largely unavailable to direct utilisation by the higher trophic levels. It has been shown that in situations were phytoplankton are unavailable to direct consumption carnivory by metazoans is most prevalent (Stoecker and Capuzzo, 1990).

Dissolved organic material (DOM) is mainly utilised by bacteria and it is only through the feeding activity of protists that this carbon is transferred to higher trophic consumers (Calbet and Landry, 1999; Sherr and Sherr, 2002). As a result, bacteria can account for a large fraction of the carbon flow through aquatic ecosystems (Pace and Cole, 1994). The most important consumers of bacteria in the East Kleinemonde Estuary during winter and summer were the nanoflagellates (2 - 20 μm) (Chapter 4). This result is consistent with previous studies that have shown that the primary bacterivores in aquatic systems are typically small heterotrophic flagellates (Calbet and Landry, 1999; Sherr and Sherr, 2002; Bettarel *et al.*, 2004; Froneman, 2006). In areas where picophytoplankton are dominant, mesozooplankton such as copepods are unable to feed directly on phytoplankton due to feeding constraints and therefore alternative food sources such as nanoflagellates and microzooplankton are consumed i.e. carnivory (Stoecker and Capuzzo, 1990; Calbet and Landry, 1999; Froneman and Bernard, 2004). The predation on nanoflagellates by larger components of the plankton, such as microzooplankton and mesozooplankton, will reduce the impact of the nanoflagellates on the small phytoplankton and bacteria, as observed during this study (Chapter 4).

During the winter study even though the phytoplankton were within the nano- size range, the mesozooplankton were found to readily consume nanoflagellates (Chapter 4). It has been suggested that nanoflagellates maybe a particularly rich source of proteins, amino acids and essential lipids especially when compared to phytoplankton (Stoecker and Capuzzo, 1990). The mesozooplankton may therefore be preferentially feeding on the nanoflagellates to gain energy benefits. Bacteria are therefore an important source of carbon for higher trophic levels, however, this bacterial production is only made available to higher consumers through trophic intermediates such as heterotrophic protists, as shown in other studies (Stoecker and Capuzzo, 1990; Froneman and Balarin, 1998; Calbet and Landry, 1999).

5.1. FINAL CONCLUSIONS

In conclusion, results of this study indicate that bacteria are an important source of carbon and account for a large portion of the carbon flow through the plankton food web in the East Kleinemonde Estuary. In agreement with previous studies conducted in temporarily open/closed estuaries within the same geographic region there were limited

spatial differences in the biology reflecting the dynamic nature of this system. At low temperatures bacterial abundance, biomass and production appeared largely to be controlled by temperature. A key feature of this study, however, was the extreme variability in the bacterial abundance and production within each season. Viral infection and subsequent lysis was found to be a significant source of bacterial mortality during this study, accounting for between 7.8 and 88.9 % of the total bacterial production removed. Predation by nanoflagellates on bacteria was also shown to represent an important source of bacterial mortality. Results of the viral studies and trophic cascading experiments suggest a strong top-down control of bacteria within the East Kleinemonde Estuary. The loss of bacterial production due to viral lysis, however, was a constant source of mortality suggesting that protistan grazing is likely to account for the observed fluctuations in the bacterial densities. Nanoflagellates were also readily consumed by higher trophic levels, which resulted in a reduced impact of these organisms on the bacteria. This study therefore demonstrates that bacteria can be an important carbon source for higher trophic levels, and that bacterial production is made available to these higher consumers through trophic intermediates such as heterotrophic protists.

5.2. FUTURE RESEARCH

The results of this study demonstrate the importance of bacteria as a carbon source for higher trophic consumers in the plankton food web within the temporarily open/closed East Kleinemonde Estuary. This study also highlights the complex interactions at the base of the food web. As a result, this study indicates the need for further investigation in microbial dynamics within temporarily open/closed estuaries. The following are required in order to expand our knowledge of what controls this important ecosystem component:

1. More detailed investigations into top-down control of bacterial populations such as grazing rates of heterotrophic protists.
2. Investigations into the role of substrate availability in controlling bacterial production.
3. Investigations into the extent of nutrient recycling within the microbial loop and nutrient regeneration through bacterivory.

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