DIRECT AND INDIRECT EFFECTS OF ZOOPLANKTIVOROUS PREDATORS ON THE ESTUARINE PLANKTON COMMUNITY

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by

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PREFACE

This thesis is comprised of a general introduction (Chapter 1), a general methods chapter (Chapter 2), a series of data chapters (Chapters 3, 4, 5, 6 and 7) and a general synthesis and conclusion (Chapter 8). The data chapters are organised as scientific papers, some of which have been published, with the remaining chapters in press or under review (see below). The incorporation of the general methods section and a combined reference list at the end of the thesis has ensured limited repetition.

- Wasserman, R.J., Noyon, M., Avery, T.S. and Froneman, P.W. (2103). Trophic level stability-inducing effects of predaceous early juvenile fish in an estuarine mesocosm study. ***PLOS ONE*, 8**, e61019.

- Wasserman, R.J. and Froneman, P.W. (2013). Risk effects on copepods: preliminary experimental evidence for the suppression of clutch size by predatory early life-history fish. ***Journal of Plankton Research*, 35**, 421-426.

- Wasserman, R.J., Vink, T.M.F., Kramer, R. and Froneman, P.W. (*in press*). Hyper-benthic and pelagic predators regulate alternate key planktonic copepods in shallow temperate estuaries. ***Marine and Freshwater Research***. doi:10.1071/MF13233.

- Wasserman, R.J., Matcher, G.F. and Froneman, P.W. (submitted). Bacterial diversity in response to manipulation of estuarine plankton communities, using 16S rRNA Pyrosequencing analysis (*under review in* ***Microbial Ecology***).

- Wasserman, R.J., Kramer, R. Vink, T.M.F. and Froneman, P.W. (*in press*). Conspecific alarm cue sensitivity by the estuarine calanoid copepod, *Paracartia longipatella*. ***Austral Ecology***. doi: 10.1111/aec.12135.

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DECLARATION

I, Ryan John Wasserman, hereby declare that the work described in this thesis was carried out in the Department of Zoology and Entomology, Rhodes University under the supervision of Professor P.W. Froneman. The various components of the thesis comprise original work by the author and have not been submitted to any other university.

GENERAL ABSTRACT

Although predation has been identified as a potentially important driver in terrestrial and freshwater ecosystems, estuarine planktonic research has focused largely on the so-called "bottom-up" drivers of community assemblages. As such, this thesis focuses on the direct and indirect effects of zooplanktivorous predators on the planktonic community in an estuarine environment. By using a suite of *in situ* mesocosm experiments, a number of hypotheses, pertaining to the major research themes associated with predator-prey interactions, are tested. These themes included trophic cascading, risk effects associated with predation events and the importance of predator diversity in maintaining prey communities. The first experiment assessed the significance of apex predation pressure for the planktonic community through trophic cascades. Various treatments using *in situ* mesocosms were established in a closed oligotrophic estuary to highlight the importance of predation in stabilising estuarine plankton abundances. Through either the removal (filtration) or addition of certain planktonic groups, varied trophic scenarios were established. The experimental treatment containing apex zooplanktivores had consequences for multiple trophic levels, exerting a stabilising pressure throughout the food web (Chapter 3). Furthermore, pyrosequencing of filtered water samples revealed that when compared to the remaining treatments, the treatment containing stable apex predatory pressure experienced limited temporal deviation-from-initial in bacterial community structure (Chapter 4). These findings are consistent with trophic cascade theory whereby predators mediate interactions at multiple lower trophic levels with consequent repercussions for diversity. To assess the non-consumptive effects of predators on prey, two experiments were conducted. Firstly, using egg numbers per clutch as a measure of potential reproductive output, the non-lethal effects of predatory pressure on reproductive success in a key planktonic copepod was investigated. In this study, the average clutch size of fecund

female copepods was found to be consistently lower in the presence of predators when compared to females not exposed to predation threat (Chapter 5). The second study assessed the effects of conspecific chemical alarm cues associated with predation, on population dynamics of a copepod species. This study revealed that the copepods appear to detect the presence of chemical alarm cues associated with predation events, with repercussions for population demographics over time. Furthermore, it showed that in the absence of actual predation, copepod prey responses to alarm cues were adjusted over time, consistent with the threat sensitive predator avoidance hypothesis (Chapter 6). The final data chapter dealt with predator diversity and its implications for zooplankton community structure. By experimentally monitoring the effects of two alternate model predators on the metazoan community over time, dissimilarities in community level control emerged. Alternate key prey populations were regulated by the different model predators, highlighting the importance of predator and prey behaviour in mediating predator-prey interactions (Chapter 7). These results highlight the potential importance of predators in maintaining community dynamics in estuarine planktonic communities under certain conditions. This study represents some of the first work to address these various aspects of predator-prey dynamics within the context of planktonic estuarine ecology.

CHAPTER 1.
GENERAL INTRODUCTION

"If there is magic on the planet, it is contained in water."

- Loren Eiseley, The Immense Journey, 1957

Plate 1: A common sighting in the Eastern Cape: early life-history fish swimming in the shallows of an estuarine habitat (Photo: Ryan Wasserman).

Under natural conditions, community structure is a result of the evolution of species interacting with one another, within the physico-chemical constraints of their surroundings (Krebs 1994; Smetacek *et al.* 2004). Ecological investigations have long emphasised the importance of physico-chemical, or the so-called "bottom-up" processes, in the establishment of ecosystem organisation (Pace and Cole 1994; Ripple *et al*. 2010). This is largely because physical processes relate directly to primary productivity within ecosystems and can therefore be readily observed (Lindeman 1942; Odum 1957; Ivlev 1966; Sampson *et al.* 1993; Ripple *et al*. 2010). In planktonic ecology, despite the wide range of zoological predators that graze on phytoplankton, biologists generally consider these communities as being bottom-up controlled (Glibert 1997; Ripple *et al*. 2010). This is ascribed to the tight coupling between productivity and physical processes in the sea, more so than in terrestrial environments (Ripple *et al*. 2010). More recent work, however, has suggested that biological interactions are also important in maintaining ecosystem health. The role of predators and their so-called "top-down" contributions to structuring food webs and maintaining the ecological integrity of systems is now well recognised (Strong 1992; Glibert 1997; Schmitz *et al*. 2004; Borer *et al*. 2005; Estes *et al.* 2011; Schoener *et al.* 2011). The relative importance of the biological versus physico-chemical factors in controlling community structure, however, appears to be related to scale (Cornell and Lawton 1992; Pearson and Dawson 2003). It has been suggested that at the global, continental and regional scale, communities are largely determined by climatic and other abiotic variables, while at the local to micro scale, biotic interactions are paramount (Pearson and Dawson 2003).

Arguably the most important of all biotic interactions, often playing an essential organisational role in community and ecosystem ecology are those relationships between predators and their prey (Brooks and Dodson 1965; Lima 1998; Eklöv and VanKooten 2001;

Polis *et al*. 2000). These interactions form the basis of food-web ecology, with food webs comprising various taxonomic components, related through a variety of direct and indirect linkages (Heath *et al. in press*). Loss of biota can therefore, have implications for food-web structure and subsequent ecosystem functioning (Hooper *et al.* 2005; Diaz *et al*. 2006; Losey and Vaughan 2006). The relative importance of each taxon to a food web is, however, highly variable with many communities being dominated by the activities of a few species (Cox *et al.* 1991; Mills *et al.* 1993; Tilman and Downing 1994; Navarrete and Menge 1996). In many cases predators govern communities and these species are often more vulnerable to perturbation, an aspect of various intrinsic biological traits, including lower population densities and slower reproductive rates than their prey (Navarrete and Menge 1996; Purvis *et al.* 2000; Estes *et al*. 2011).

Predator-prey interactions

Studies on interactions between predators and their prey are widespread, with varied direct and indirect exchanges observed (Navarrete and Menge 1996; Sih *et al.* 1998; Creel and Christianson 2008; Ferarri *et al.* 2010; Letnic and Symon 2011). The following section outlines some of the more pertinent features of predator-prey interactions which have received attention across a range of ecosystem types. These features include the indirect effects of predators on multiple trophic levels, the effects of predation on prey diversity, and the relevance of predation-related risk effects on prey species.

An arena of sustained research interest is that of the notion of trophic cascading. Trophic cascades are broadly defined as the transmission of consumer impacts downwards through food webs (Paine 1980; Estes *et al*. 2011). Consequently, predators have been shown to affect not only the prey they directly consume, but also organisms further down the food web. This

concept was first introduced by Paine (1980), although its principles were used to manage ecosystems well before its formalisation into theory (Brooks and Dodson 1965; Shapiro and Wright 1974; Sommer and Sommer 2006). Trophic cascade investigations and their elucidation with regard to empirical and theoretical science have subsequently marked some of the most exciting breakthroughs in food-web ecology (Polis *et al*. 2000). This notion highlights the vitally important role predators play in community ecology despite their low relative abundance and biomass contributions to ecosystems (Estes *et al.* 2011). Indeed, it has been suggested that research and conservation priorities should be directed at the identification and exposition of the various processes associated with important predatory organisms (Cox *et al.* 1991; Navarrete and Menge 1996; Berlow *et al.* 1999; Ripple *et al.* 2010; Estes *et al*. 2011).

In addition to the effects of a predator on multiple trophic levels, the importance of maintaining predator diversity has received increasing attention in recent years (Sokol-Hessner and Schmitz 2002; Schmitz *et al.* 2004; O'Connor *et al.* 2008). Classically, ecologists and modellers often moderated the importance of predator diversity, assuming that predatory species have indistinguishable effects within their habitats (Fretwell 1987; Chalcraft and Resetarits 2003; Schmitz 2007). Assigning organisms at a similar trophic level to that of a single functional ecological unit may not represent ecological reality (Sih *et al.* 1998; Sokol-Hessner and Schmitz 2002; Schmitz *et al.* 2004; O'Connor *et al.* 2008). Behavioural traits such as diel foraging activity, mode of foraging, and microhabitat utilisation all affect the outcomes of predator-prey interactions (Fretwell 1987; Burks *et al.* 2002; Chalcraft and Resetarits 2003; Iglesias *et al.* 2007; Schmitz 2007; Muška *et al.* 2013). The partitioning of prey organisms among predatory species is therefore an important

consideration in predator-prey dynamics (Sih *et al.* 1998; O'Connor *et al.* 2008), with implications for biodiversity conservation (Chave *et al.* 2002; Amarasekare *et al.* 2004).

Prey population control, regardless of the mechanism, can be vital for community health at the system level (Estes *et al.* 2011). When assessing the impacts of the predator on a prey population, the latter are affected in a number of ways. The first and most obvious effect is that of direct predation, whereby predators kill and consume individuals within a population. More subtle effects, however, are those of the secondary mechanisms of population control, the so-called non-consumptive effects (Schmitz *et al.* 2008; Letnic and Symon 2011). This aspect of predator-prey interactions is largely centred on anti-predator behaviour and the associated energy costs induced by the presence of a predator (Fraser and Gilliam 1992; Peckarsky *et al.* 1993; Boonstra *et al.* 1998; Creel and Christianson 2008). Prey, in an attempt to avoid or reduce encounters with predators, modify their behaviour, often at the cost of optimal habitat, food or mate acquisition (Sih 1986; Ferarri *et al.* 2010). These effects, also referred to as risk effects, have been attributed with decreased survival, individual development and reproductive output of potential prey groups (Ruxton and Lima 1997; Pangle *et al.* 2007).

Risk effects on prey populations have been shown, at times, to be comparable to that of direct predation (Schmitz *et al.* 1997; Preisser *et al.* 2005; Creel and Christianson 2008). Risk effects are largely a result of predatory threat identification and avoidance by prey (Lima 1998; Brown and Chivers 2005). As visual reception is often lacking or under-developed in zooplanktonic organisms, recognition of predation threat is largely restricted to hydro-mechanical and chemical detection (Bronmark *et al.* 2000; Buskey and Hartline 2003). With regard to chemical detection, organisms have been shown to respond to two sources of chemical signals, those released by the predator (Kairomones), and those released by injured

conspecifics (alarm cues) (Bronmark *et al.* 2000). The recognition and subsequent evasion of predators is likely to be very costly for prey, reducing time and energy expenditure on other necessary activities (Brown 2003). The persistence of a prey organism therefore lies in its ability to balance energetically costly predator avoidance, and the required behavioural activities of foraging and kin interaction, associated with population success (Ferrari *et al*. 2007). One way in which these trade-offs can be optimised is for the prey to adjust their level of response to the level of predatory threat (Brown *et al*. 2006; Kusch *et. al* 2004). This aspect of predator-prey interactions is referred to as threat-sensitive predator avoidance, and is one of the least studied facets of predator-prey dynamics (Helfman 1989; Ferrari *et al*. 2008; Ferrari *et al*. 2010).

While considerable work has been conducted on the various aspects of predator-prey interactions, the relevance and importance of these issues have received limited attention in certain environments (Sommer 2008; Estes *et al.* 2011). One of the reasons for this is that biological interactions are often difficult to detect and quantify, as processes are frequently confounded by abiotic factors, the relative contributions of which are often impossible to determine (Weissenberger 1998). Biological interactions were, therefore, classically observed under laboratory conditions, allowing for the control and removal of confounds (Lafontaine and Leggett 1987; MacKenzie *et al*. 1990). More recently, however, experimental methods have emerged which maintain a greater degree of ecological reality (Sommer 2008).

Mesocosm studies

The increase in trophic-theory interest through the 1980s and 1990s, gave rise to a progression in the use of experimental tools in trophic ecology (Sommer 2008). This resulted in the development of the field of mesocosm science. Mesocosms can be broadly defined as

stable experimental ecosystem models generally expected to contain representative subsamples of the system being modelled (Lauth *et al*. 1996). Initially, Banse (1982) considered a mesocosm as an enclosure "larger than a bench-top container but smaller than, and isolated from, a subunit of the natural environment" (Lauth *et al.* 1996). In his classic paper "The mesocosm", Odum's definition was largely similar, although exclusively reserved for outdoor experimentation (Odum 1984).

The use of mesocosms in trophic and other ecosystem level studies has become increasingly important in recent years (Benton *et al.* 2007). Since the understanding of systems requires not only a working knowledge of the type, number and interaction of biota, but also of the nature of the abiotic factors interacting with the biota, studies at system levels are often very costly. Unfortunately, the difficulty in quantifying an economic value for the services provided by the natural environment often leads to a limitation or lack of prioritisation in funding or action (Losey and Vaughan 2006), ultimately making ecosystem-wide studies impossible. In this regard the mesocosm is invaluable, offering a cost-effective alternative while still enabling the rigorous testing of ecological theory with replicated experiments on populations or communities (Lawton 1995; Daehler and Strong 1996; Jessup *et al*. 2004; Benton *et al*. 2007). Benton *et al*. (2007) highlighted an additional advantage of the mesocosm. As opposed to mathematical and computational models, mesocosms provide the fundamental understanding of ecological processes. This allows for the development of theory based on biological and ecological interactions without the assumptions associated with mathematical and computational methods, while providing invaluable calibration information for such models (Benton *et al*. 2007).

Table 1.1: Selected seminal research articles employing mesocosms to observe ecological processes in the plankton. Outlined are the relative contributions of key abiotic-biotic interactions, and interactions between biological components; within freshwater, marine and estuarine environments. NL = nutrient level, A = acidification, C = competition, T = trophic, O = other.

	Response of plankton to abiotic variables			Biological interactions			
Environment	**NL**	**A**	**O**	**C**	**T**	**O**	**References**
Freshwater	*				*		Vanni (1987)
	*				*		Elser and Goldman (1991)
	*				*		Cottingham *et al.* (1997)
	*				*		Elser *et al.* (1998)
		*					Klug and Fischer (2000)
		*					Fischer *et al.* (2001)
	*				*		Pogozhev and Gerasimova (2001)
			*				Kreutzweiser *et al.* (2002)
	*				*		Williams *et al.* (2002)
					*		Jones and Sayer (2003)
	*				*		Cottingham *et al.* (2004)
				*			Dzialowski *et al.* (2004)
				*	*		Feuchtmayr *et al.* (2004)
	*				*		Moss *et al.* (2004)
	*				*		Romo *et al.* (2004)
	*				*		Vakkilainen *et al.* (2004)
					*		Chang and Hanazato (2005)
			*				Flies *et al.* (2005)
			*				Fischer *et al.* (2006)
	*						Forrest and Arnott (2006)
					*		Nagata *et al.* (2006)
					*		Castilho-Noll and Arcifa (2007)
					*		Boveri and Quiros (2007)
					*		Nogueira *et al.* (2008)

Table 1.1: (Continued)

	Response of plankton to abiotic variables			Biological interactions			
Environment	**NL**	**A**	**O**	**C**	**T**	**O**	**References**
Freshwater	*		*				Ogbebo and Ochs (2008)
					*		Rondel *et al.* (2008)
					*		von Rückert and Giani (2008)
Marine					*		Stoecker and Capuzzo (1990)
					*		Houde *et al.* (1994)
						*	McCormick and Kerrigan (1996)
	*						Suzuki *et al.* (1997)
	*					*	Svennson (1997)
	*						Duarte *et al*. (2000)
			*				Keller and Klein-MacPhee (2000)
					*		Nejstgaard *et al.* (2001)
					*		Granéli and Turner (2002)
	*				*		Stibor *et al.* (2004a)
	*				*		Sommer *et al*. (2005)
					*		Mowitt *et al.* (2006)
	*				*		Olsen *et al*. (2006)
	*				*		Sommer *et al.* (2007)
					*		Jeong *et al.* (2008)
		*					Andersson *et al.* (2009)
			*		*		Jónasdóttir *et al.* (2011)
					*		Zolner *et al.* (2009)
				*	*		Löder *et al.* (2011)
Estuarine	*						Oviatt *et al.* (1984)
	*						Nowicki and Oviatt (1990)
	*						Taylor *et al.* (1995)

Table 1.1: (Continued)

	Response of plankton to abiotic variables			Biological interactions			
Environment	**NL**	**A**	**O**	**C**	**T**	**O**	**References**
Estuarine			*				Sanford *et al*. (2001)
			*				Marino *et al.* (2002)
	*						Riedel and Sanders (2003)
	*				*		Troussellier *et al.* (2005)

Since their introduction to the biological sciences, much mesocosm-based work has been conducted on a range of topics and fields (Table 1.1). Such studies include investigations into ultraviolet light effects on crustacean movements (Fischer *et al.* 2006), toxicological effects on aquatic fauna (Kreutsweizer *et al*. 2002) and even the effects of sediment nutrients on seagrasses (Short 1987 and references therein). The vast majority of this work has, however, been conducted in freshwater limnetic environments, investigating various aspects of planktonic ecology including both the so-called "bottom-up" and "top-down" control of organisms (Brett and Goldman 1996; Cottingham *et al*. 1997; Boveri and Quiros 2007; Castilho-Noll and Arcifa 2007). Initially, freshwater ecologists dominated the field of trophic and mesocosm studies, with few comparable marine plankton studies having been conducted by the commencement of the 1990s (Sommer 2008 and references therein). There has subsequently, however, been much progress in the marine mesocosm field ranging from mesocosm design (Davis *et al*. 1996; Weissenberger 1998) to investigations on bottom-up effects on community structure (Duarte *et al.* 2000; Nowicki and Oviatt 1990; Diehl and Feissel 2001; Jeong *et al*. 2008) as well as classic trophic cascading studies on the plankton (Stibor *et al.* 2004a, Stibor *et al.* 2004b; Mowitt *et al.* 2006; Sommer 2009; Loder *et al*. 2011). Relatively few mesocosm studies have, however, been conducted in shallow estuarine habitats (Table 1.1).

Estuaries

In terms of ecosystem services, estuaries are of exceptionally high economic value, potentially producing \$ 22 832 ha^{-1} yr^{-1} (Costanza *et al.* 1997). Estuarine habitats naturally act as nutrient traps receiving enrichment from those elements of catchment origin (Nixon *et al.* 1986). As a consequence, these ecosystems support high levels of primary productivity (Gowan *et al.* 1992; Gobler *et al.* 2005; Painting *et al*. 2007), which in turn supports an

abundance of consumers (Houde and Rutherford 1993; Deyzel 2012). Of these consumers, zooplankton have been identified as a key link between the primary producers and organisms further up the food web (Calbet and Landry 1999; Calbet and Saiz 2005). Since these organisms attain extremely high levels of abundance in estuarine habitats compared to coastal and open-ocean waters, estuaries offer particularly important foraging areas for zooplanktivorous predators (Whitfield 1998; Mehner and Thiel 1999). Ichthyofauna constitutes a large portion of zooplanktivorous pressure in these systems, with utilisation of estuaries occurring at both the facultative and obligatory level across a range of life-history stages (Whitfield 1998; Mehner and Thiel 1999; Wasserman and Mostert *in press*). In this way, estuaries are of particular trophic interest, yet relatively little work has been conducted on trophic and other interesting biological interactions within these systems, particularly in the southern African systems.

It has been suggested that these habitats are now among the most disturbed aquatic environments on earth (Nixon *et al.* 1986; Costanza *et al.* 1997). This has been attributed mainly to anthropogenic disturbances, many of which are anticipated to intensify with the onset of predicted climate change (Christensen *et al.* 2007; Clark 2006; Mead *et al*. 2013).

Estuaries and estuarine ecology: the South African context

Approximately 259 estuaries intersect the ≈ 3100 km stretch of South African coastline (Whitfield 2000). Of these, the vast majority (72%) are temporarily open/closed estuaries (TOCEs), being blocked off from the sea for a duration of time (Whitfield 1998). A result of the characteristic state of the mouth of these systems is the vast variation in physico-chemical state, ranging from a typical horizontal salinity gradient to homogenous fresh or even

homogenous marine water dominated states, depending on the amount of freshwater entering the estuary (Whitfield and Bruton 1989).

South Africa is, for the most part, a semi-arid country with an uneven rainfall pattern distribution (Department of Water Affairs 1986). This, in combination with increased industrial development and an ever increasing human population, has resulted in the construction of numerous impoundments and water-transfer schemes in the catchment areas of estuaries (O'Keeffe and de Moor 1988; Coetzee *et al.* 1996; Schlacher and Wooldridge 1996). This ultimately affects downstream supply of freshwater to estuaries. Freshwater input is a major driver in estuarine structure and functioning, establishing salinity gradients along the length of estuaries and contributing to heterogeneity of abiotic and biotic components within the water column (Gibson *et al.* 1995; Whitfield and Bruton 1989; Allanson *et al.* 1999). As a consequence, a variety of habitats are typically found in estuaries (Whitfield 1983). In addition, freshwater input represents the primary source of macronutrients necessary for primary production and ultimately secondary productivity in estuaries (Wooldridge and Bailey 1982; Hilmer and Bate 1991; Schlacher and Wooldridge 1996). Reductions in freshwater input into estuaries change hydrodynamics within these systems, often resulting in reduced habitat heterogeneity, increased river siltation and prolonging of mouth closure events (Cyrus 1991; Adams and Bate 1994). These effects ultimately have repercussions for biodiversity and ecosystem functioning (Adams and Bate 1994; Mead *et al.* 2013).

Due to reductions in freshwater flow, South African estuarine research has prioritised investigations pertaining to the influence of freshwater flow on estuarine ecosystem functioning (Allanson and Read 1995; Schumann and Pearce 1997; Snow *et al.* 2000;

Wooldridge and Callahan 2000; Allanson 2001; Adams *et al.* 2002; Bate *et al.* 2004; Wooldridge 2007; Deyzel 2012; Adams *in press* and many more). This necessary work has provided insight on the importance of freshwater for estuarine health (Turpie *et al*. 2004; Bornman *et al*. 2002; Whitfield 2005 amongst others), with the findings of these studies heavily incorporated into water policy legislation and use for the region (Adams *in press* and references therein). Much of the additional ecological work on estuarine zooplankton studies in South Africa have focused on other bottom-up forces driving community dynamics (Jerling and Wooldridge 1991; Isla and Perissinotto 2004; Wooldridge and Deyzel 2012). As a result, only a handful of studies have assessed predator-prey dynamics within the context of South African estuarine zooplankton ecology (Wooldridge and Webb 1988; Jerling and Wooldridge 1995a, b; Whitfield and Harrison 1996; Perissinotto *et al*. 2000; Froneman 2000; Perissinotto *et al*. 2003).

Climate change models predict that climate-driven perturbations are likely to affect TOCEs (New *et al.* 2006; Mead *et al.* 2013). While biological responses to predicted climate-change related conditions are being considered (Mead *et al*. 2013), this is only being done at a coarse scale. Furthermore, the secondary repercussions of such changes will be relatively difficult to assess, given the paucity of baseline biological-interaction information. The lack of sufficient studies on key biological interactions in this regard permits limited prediction for perturbation repercussions on interactions among species (Stenseth and Mysterud 2002; Daufresne *et al.* 2009).

Present study

Due to the paucity of such information in estuarine habitats, the aim of the present thesis was to provide insight into the role of predation in structuring the estuarine plankton, and the

mechanisms by which predators affect prey organisms. The thesis is therefore centred on trophic interactions between various components of the plankton, whereby a number of these key aspects, embedded in classic ecological theory but within context of estuarine zooplankton, are addressed. Included in the thesis are the effects of direct predation on prey populations and the repercussions of these effects on lower trophic levels. In addition, light is shed on aspects of indirect effects of prey control by predators, a field completely understudied in marine and estuarine systems. Finally, the thesis highlights the potential importance of maintaining predator diversity for estuarine zooplanktonic communities, as has been revealed in certain terrestrial and freshwater ecosystems. More specifically, using early life-history fish as model apex planktonic predators, the following aspects are investigated:

- The role of zooplanktivores in stabilising the estuarine plankton community through trophic cascading.
- The role of apex predation in determining the diversity of the microbial community.
- The risk effects associated with direct predation and its repercussions for reproductive success in a dominant copepod species.
- Threat sensitivity by a key copepod species, and its implications for population dynamics over time.
- The effects of varied predatory species on the estuarine metazoan community.

One of the conclusions postulated by Sommer (2008) in his review on trophic studies in aquatic environments was that trophic cascades are widespread in the plankton and should not differ between marine and limnetic environments. The above-mentioned aspects, comprising the data chapters of the present study, are all considered important in well-studied freshwater ecosystems. As such, biological interactions and their contribution to community structure in estuaries needs to be investigated.

CHAPTER 2.
STUDY SITE AND *IN SITU* EXPERIMENTAL SET-UP

"When the well's dry, we know the worth of water."

- Benjamin Franklin, Poor Richard's Almanac

Plate 2: On site preparation of sampling gear, in the middle reach of the Kasouga Estuary (Photo: Ryan Wasserman).

Study area

Given the experimental nature of the study, the research was conducted on an estuary which had been well described with regards to its physico-chemical and biological characteristics. This mitigated much of the guesswork associated with expectations of experimental starting-points for the estuarine water chemistry and zooplankton communities. The investigation was therefore conducted in the Kasouga Estuary in the warm-temperate Eastern Cape of South Africa (Figure 2.1). The medium-sized Kasouga Estuary is located on the south-eastern coastline of South Africa, and enters the Indian Ocean at 33°39'17"S; 26°44'08"E (Whitfield 2000). The system is largely pristine, with relatively little agricultural development over its 39 km^2 catchment area (Froneman 2002a; Henninger *et al*. 2008). As such, the estuary is considered to be in good ecological condition (Whitfield and Baliwe 2013).

Like the vast majority of South African estuaries, the Kasouga Estuary is regarded as a temporarily open/closed system (TOCE) as it is separated from the marine environment by a sandbar at the mouth for varied periods of time (Figure 2.2). The Kasouga Estuary hydrological phase cycle is typical of that of TOCEs in the Eastern Cape region (Froneman 2002c). Long-shore drift in the nearshore marine environment contributes to the development of a sandbar across the mouth of the estuary, facilitating estuary mouth closure (Whitfield 1992; Froneman 2004b; Whitfield *et al.* 2008). The closed-phase can last for extended periods of time and is often exacerbated by low rainfall episodes and/or freshwater abstraction (Froneman 2002c). During mouth closure, supplemental seawater intermittently washes over the sandbar at the mouth into the estuary, elevating the water levels and salinity within the system. These over-wash events are limited to peak spring tides and large storm events, where seawater level or wave action is particularly high (Perissinotto *et al.* 2000; Walker *et al*. 2001; Froneman 2002b). When water within the estuary rises to a level higher

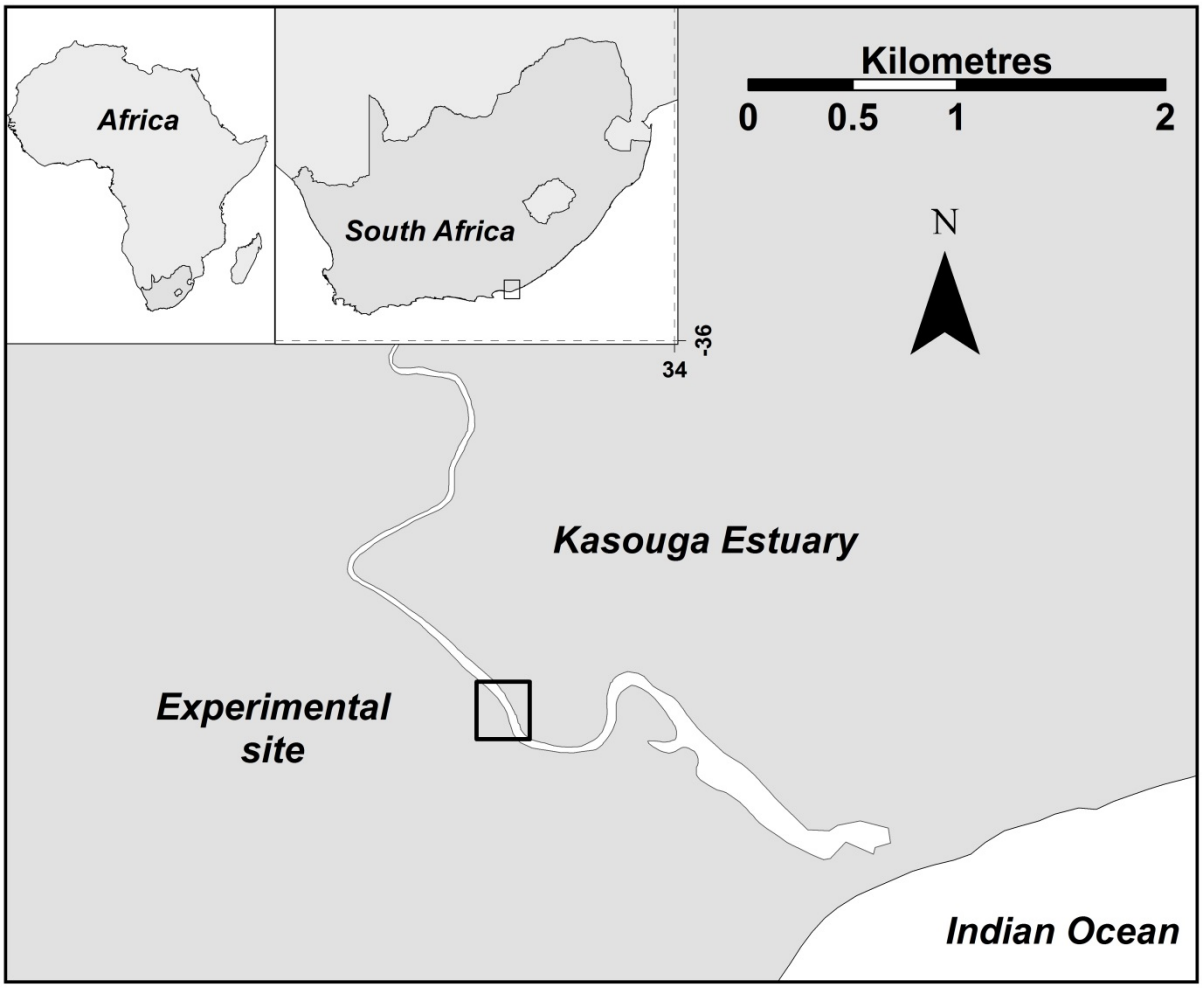

Figure 2.1: The geographic position of the warm temperate, temporarily open-closed Kasouga Estuary, South Africa, showing the location of experimental mesocosm deployments.

than that of the sandbar at the mouth the estuary breaches, contributing to a dramatic decrease in water levels within the system (Whitfield 1992; Perissinotto *et al.* 2000; Froneman 2002b). During these periods, river conditions dominate, but this is relatively short lived, as the sandbar at the mouth is soon re-developed, with subsequent seawater wash-over providing marine influence into these closed systems once more (Perissinotto *et al.* 2000; Froneman 2004c). During the closed phase, at the region near the mouth, the estuary is roughly 100-200 m in width, narrowing in the upper reaches to between 30-40 m (Henninger *et al*. 2008).

Figure 2.2: The lower reach of the Kasouga Estuary, South Africa, during a closed mouth phase, showing the sandbar across the estuary mouth.

The estuary is navigable for approximately 2.5 km during these periods and is around 1.8 m deep in the lower and middle reaches and 1.5 – 2 m in the main channel of the upper reaches (Froneman 2002c; Henninger *et al*. 2008). In the middle and upper reaches of the Kasouga Estuary, the dominant submerged macrophyte is *Ruppia maritima*, with the reed *Phragmites australis* growing along the estuary banks (Henninger 2009). Minimal vegetation, however, is found within the estuary in the lower sandy reaches of the system.

The biology of the Kasouga Estuary has been extensively studied (Whitfield and Baliwe 2013) including the ichthyofauna (Jubb 1979; Tweddle 2005; James and Harrison 2010) and selected components of the hyperbenthos (Henninger *et al*. 2008; Froneman and Henninger

2009) and avifauna (Beamish 2002). Most investigations have, however, focused on the planktonic components (Froneman 2002a; Froneman 2002b; Froneman 2002c; Froneman 2004a). Phytoplankton communities are most often dominated by picophytoplankton (< 2µm) and therefore support relatively low levels of zooplankton biomass when compared to the permanently open estuaries of the region (Jerling and Wooldridge 1991; Froneman 2002b; Froneman 2002c). This is largely ascribed to the low levels of macronutrient concentrations that characteristically occur in the system (Froneman 2002b). Zooplankton communities within the Kasouga Estuary are typically comprised of copepods at this time, with the calanoids *Pseudodiaptomus hessei* and *Paracartia longipatella* completely dominating this contribution (Froneman 2004c). Furthermore, contributions of larger zooplanktivorous components into the system, including mysids, amphipods and fish larvae, are supplemented by over-wash of seawater into the estuaries (Froneman 2004c). These organisms are associated with zooplanktivory (Wooldridge and Webb 1988; Jerling and Wooldridge 1995a; Whitfield 1998; Wasserman 2012), but their relative contribution to structuring the estuarine plankton in these systems, during closed phases, has received limited attention.

Given the nature of the present study, specific conditions were required to optimally observe planktonic organisms in response to biotic manipulations. One of the major requirements was that the system be deep enough for the establishment of mesocosm enclosures. Wave action, tidal fluctuation and strong coastal winds were all additional factors that required consideration for the successful establishment of experimental enclosures over the required time-period. As such, all experimental studies were conducted during the closed-mouth phase in the Kasouga Estuary, mitigating many of the logistical hindrances described above.

Experimental site and set-up

The thesis is comprised of a suite of independent experimental studies, each designed to address specific hypotheses. While the manipulations differed among the distinct investigations, the general logistics of the experimental procedures were similar across the studies. The present chapter therefore describes the construction and deployment of the experimental mesocosms employed in the thesis. The specific manipulations associated with each investigation are, however, discussed in the relevant data chapters (Chapters 3, 4, 5, 6 and 7). Site selection for the experiments was based on water depth and relative shelter from the strong coastal winds. All experimental investigations were therefore conducted in the middle reaches of the Kasouga Estuary (Figure 2.1).

Mesocosm enclosures (1.4 m deep) were constructed of translucent 200 µm thick, virgin polyethylene bags, secured to a square 80 cm × 80 cm frame (Figure 2.3). Each corner of the frame was attached to an airtight 5 L buoy, elevating the top end of the bag from the water's surface by ≈ 40 cm. The bottom end of the mesocosm bags were then secured, using a 50 cm long, 10 mm thick elasticated rope, to a concrete mooring anchored in the estuarine sediment. This was done to mitigate any wave action that the enclosure would potentially be exposed to. Enclosures were therefore open to the atmosphere at the top, yet isolated from surrounding estuarine waters. The top (open) end of each mesocosm was then covered by a plastic grid (4 cm x 4 cm mesh aperture) for protection from aerial predators. Each mesocosm was filled with 1000 L of estuarine water. This volume was selected based on the findings by Spivak *et al.* (2011) who showed that extrapolation of results from mesocosm planktonic studies were comparable to system level results when mesocosm volumes of 500 L or more were employed. This was largely ascribed to the reduction in relative surface area for algal

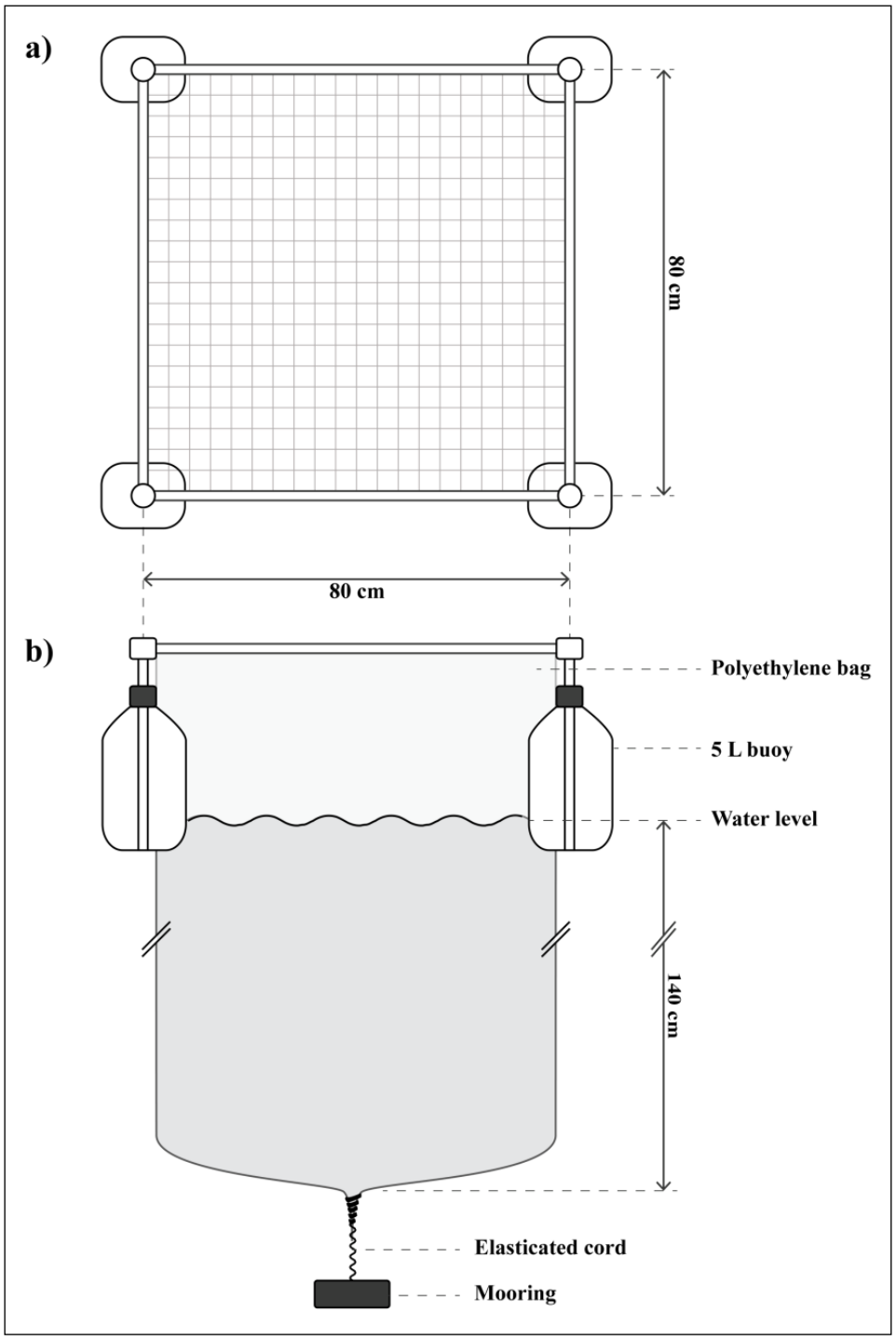

Figure 2.3: Illustration of mesocosm design, including a.) aerial, and b.) side views. Each 80 cm x 80 cm x 140 cm mesocosm contained 1000 L of gravity-fed estuarine water, the top-ends of which were open to the atmosphere. Each mesocosm was fitted with a 4 cm x 4 cm plastic grid, protecting the upper trophic levels from external, aerial predators.

settlement in enclosures greater than 500 L when compared to smaller mesocosms (Spivak *et al.* 2011). Employing mesocosms of 1000 L therefore more than adequately minimised any so-called “bottling effects” and their associated confounds.

Mesocosms were assembled and filled with water after sunset, maximising the incidence of representative taxa including those that demonstrate diel vertical migrations (Lampert 1989; Jerling and Wooldridge 1995b). Each mesocosm was filled with 1000 L of estuarine water collected from the experimental site adjacent to the mesocosms. This was done by collecting the estuarine water into 100 L containers, elevated on the bow of a 3 m long boat. Water was then gravity fed by polyethylene hose into each mesocosm. Mesocosms were deployed along the length of the channel in such a way that they all received the same amount of sunlight, between sunrise and sunset (Figure 2.4). To mitigate any further confounding factors associated with position or proximity concerns, treatment and replicate mesocosms were randomly dispersed along the length of their distribution.

Laboratory and data analyses

While all the data chapters were similar with regard to their experimental design, differences among hypotheses dictated the use of independent laboratory techniques and fundamental distinct data analyses for each chapter. All detailed information regarding enumeration and quantification of the various physico-chemical and biological components of the study are therefore dealt with in the materials and methods section of each of the data chapters. Furthermore, all statistical analyses and interpretation are also outlined in the relevant data chapters.

Figure 2.4: Treatment and replicate mesocosms positioned in random order, along the length of the channel, in the middle reach of the Kasouga Estuary, South Africa.

CHAPTER 3.
TROPHIC LEVEL STABILITY-INDUCING EFFECTS OF PREDACIOUS EARLY JUVENILE FISH IN AN ESTUARINE MESOCOSM STUDY

"If water is too clear, it will not contain fish."

- Chinese Proverb

Plate 3: Mesocosms deployed in the channel of the middle reaches of the Kasouga Estuary (Photo: Ryan Wasserman).

Abstract

Classically, estuarine planktonic research has focused largely on the physico-chemical drivers of community assemblages, leaving a paucity of information on important biological interactions. Within the context of trophic cascades, various treatments using *in situ* mesocosms were established in a closed estuary to highlight the importance of predation in stabilising estuarine plankton abundances. Through either the removal (filtration) or addition of certain planktonic groups, five different trophic systems were established. These treatments contained varied numbers of trophic levels and thus different "predators" at the top of the food chain. The abundances of zooplankton (copepod and polychaete), ciliate, micro-flagellate, nano-flagellate and bacteria were investigated in each treatment, over time. The reference treatment containing apex zooplanktivores (early juvenile mullet) and plankton at natural densities mimicked a natural, stable state of an estuary. Population variability (PV) and coefficient of variation (CV) of temporal abundances were calculated for each taxon and showed that apex predators in this experimental ecosystem, when compared to the other systems, induced stability. The presence of these predators therefore had consequences for multiple trophic levels, consistent with trophic cascade theory. PV and CV proved useful indices for comparing stability. Apex predators exerted a stabilising pressure through feeding on copepods and polychaetes, which cascaded through the ciliates, micro-flagellates, nano-flagellates and bacteria. When compared with treatments without apex predators, the role of predation in structuring planktonic populations in closed estuaries was highlighted.

Introduction

Trophic interactions play an essential organisational role in community and ecosystem ecology (Polis *et al.* 2000). Biological communities are comprised of numerous species interacting through complex relationships, yet coexisting in equilibrium (May 1976; Beddington *et al*. 1976). Of the many kinds of organisms comprising food webs, top predators are often the most vulnerable to extinction, an aspect of various intrinsic biological traits, including lower population densities and slower reproductive rates (Purvis *et al*. 2000). Therefore, the loss of a top predator could have consequences for a community (Carpenter *et al.* 1985; Estes *et al.* 2011). As such, predator contributions to community structure have received much attention and their importance has been highlighted in certain biological communities (Strong 1992; Polis 1994; Blaustein *et al*. 1996; Persson 1999; Fey *et al*. 2009). Predatory top-down control is observed through trophic cascades, across multiple lower trophic levels, underscoring the importance of top predators in food webs and underlying community structure (Strong 1992; Sommer 2008).

Empirically, trophic dynamics are generally under-studied and inadequately understood, largely because of the complex nature of community relationships that exist within them (Eveleigh *et al.* 2007). The classic trophic cascade theory originates in the "community" paradigm, whereby distinct trophic levels comprise linear food chains (Persson 1999). Community interactions have however been shown to be much more complex and are more aptly described as food webs, with a myriad of trophic relationships that are challenging to identify and disentangle (Persson 1999). Characterising food web interactions are complicated by predation at and on multiple trophic levels (Polis *et al.* 1989; Polis 1991; Diehl 1993), competition among species within a trophic level (Cáceres 1998; Hu and Tessier 1995), and intra-guild predation, where competing species also engage in predator-prey

interactions (Polis *et al*. 1989; Polis and Holt 1992). In this way, defining planktonic trophic levels is problematic and can perhaps best be described as those organisms within the size limits of a predator's foraging attainability. This attainability varies greatly, however, depending on the planktonic predator (Hansen *et al.* 1994) and as such, investigating indirect effects of predators across multiple trophic levels is challenging, especially when trying to assess cascade mechanisms. However, detecting cascades is possible without elucidating all mechanisms involved (Polis *et al*. 2000).

Within aquatic ecosystems, most demonstrated trophic cascades have been in limnetic environments (Carpenter *et al*. 1985) with comparably few marine and estuarine examples (Sommer 2008). Furthermore, trophic studies on plankton have either investigated zooplanktivore-zooplankton-phytoplankton interactions (Hansson 1992; Persson *et al*. 1992) or relationships at the microbial level between bacteria and bacterivorous protists (Fenchel 1982; Porter *et al*. 1985) with few studies having assessed interactions between these two arenas (Tranvik and Hansson 1997). When investigating planktonic trophic studies, *in situ* mesocosms are often employed (Blaustein *et al*. 1996; Castilho-Noll and Arcifa 2007; Davis *et al.* 1996).

The use of mesocosms in trophic studies has become increasingly important because they allow investigations of ecological theory without the assumptions and constraints of mathematical and computational methods (Benton *et al*. 2007). Mesocosms are easily manipulated, thus particularly useful for studying biological interactions across multiple trophic levels and testing specific predator-prey relationships under various environmental conditions (Blaustein *et al.* 1996; Davis *et al*. 1996; Zöllner *et al*. 2009). A further advantage is that they can be employed in the field, better simulating natural conditions and mitigating

laboratory artefacts (Davis *et al.* 1996). Mesocosms, then, can be broadly defined as stable experimental ecosystem models, generally expected to contain representative subsamples of the system being simulated (Lauth *et al*. 1996).

Predator-prey relationships are often size-related, with predators being larger than prey (Hansen *et al*. 1994). The experimental manipulations therefore involved either the removal of certain size-class planktonic predators using a simple filtration approach, or the addition of macroplanktonic predators, to induce a variety of artificial trophic scenarios. The planktonic trophic level interactions were investigated, highlighting the presence of trophic cascades in estuarine plankton. It was hypothesised that apex-planktonic-predators would stabilise the planktonic community through the maintenance of interactions that transcend multiple trophic levels. The impetus for this study originates from marine and estuarine food web studies that have mostly focused on effects of bottom-up services (Verity and Smetacek 1996), leaving top-down regulatory effects, crucial for the understanding of trophic dynamics, largely unknown.

Materials and methods

Experimental set-up

Fifteen 1000 L mesocosm enclosures (Figure 2.3) comprising five trophic treatments were established (Table 3.1). Based on a variation of the planktonic predator-prey size ratio theory (Hansen *et al*. 1994), three treatments (T1-T3) involved the exclusion of size-class plankton, and therefore trophic levels, via filtration of gravity fed water through mesh sizes 20, 80 and 500 µm, respectively. Treatment 4 (T4) used unfiltered water while Treatment 5 (T5) contained unfiltered estuarine water with the addition of zooplanktivorous “apex” predators. Early-juvenile freshwater mullet, *Myxus capensis* (Valenciennes, 1836) (31.3 ± 1.72 mm total

length) of the Mugilidae family, stocked at natural larval fish densities (two individuals per mesocosm), were employed as the apex predator (Strydom *et al.* 2003). Fish captured at the study site using a 25 m seine net with 1 mm mesh on the first evening of the study, were measured and stocked immediately. Triplicate mesocosms were used for each treatment and the entire study was conducted over a 19-day period spanning the new moon.

Table 3.1: Three replicate mesocosms were established for each of the five treatments (T). T1-T3 involved the filtration of gravity fed water through various mesh sized sieves, while T4 was filled with unfiltered estuarine water. For T5, unfiltered estuarine water was employed and stocked with early life-history fish as model apex planktonic predators.

Treatment	Manipulation
T1	Estuary water filtered through 20 µm mesh
T2	Estuary water filtered through 80 µm mesh
T3	Estuary water filtered through 500 µm mesh
T4	Unfiltered estuary water
T5	Unfiltered estuary water, with addition of two early life-history fish (natural densities)

Physico-chemical and biological sampling

Daily measurements of salinity, temperature (°C) and dissolved oxygen ($mg.L^{-1}$) were recorded from each mesocosm between 14:00 and 15:00 using an Aquaread Aquameter. Biological samples were collected from each mesocosm at the start and every third day (Day 0, 3, 6, 9, 12, 15 and 18) shortly after physical measurements and during daylight, with the exception of zooplankton samples, which were collected after sunset, between 19:30 and 20:30 to capture those organisms that demonstrate diel vertical migrations. Each mesocosm was stirred in a figure of eight pattern using an oar prior to sampling. Bacterial numbers were estimated by direct counting. Triplicate 1 mL water samples were collected from each mesocosm (see Chl-*a* collection below) and preserved with acidified Lugols' iodine following recommendations by Nishino (1986). Samples were gently vacuum filtered at <5

cm Hg through 0.1 µm polycarbonate black membranes, mounted onto glass slides (Nishino 1986), examined under an epifluorescent microscope at × 1000 and bacterial numbers estimated as a mean of triplicate samples (Turley 1993).

Size fractionated Chlorophyll-*a* concentrations (Chl-*a*) were determined from a 250 mL water sample collected, at a depth of ≈ 0.5 m from each mesocosm. Samples were serially vacuum filtered at <5 cm Hg through 20 µm, 2 µm and 0.7 µm filters and then placed in 8 mL of 90% acetone at -20°C for 24 hours. Chl-*a* concentrations were then determined using fluorometry following the method of Lorenzen (1966).

Water (250 mL) for micro- and nano-plankton analysis was collected and preserved using Lugol's iodine solution (Broglio *et al.* 2004). At a broad taxonomic level, blue-green algae, dinoflagellates, diatoms, nano-flagellates (<10 µm), micro-flagellates (>20 µm) and ciliates (>20 µm) were identified and enumerated using an Olympus CKX41 inverted microscope at × 400 magnification and using the Utermöhl settling technique (Reid 1983). Zooplankton was sampled vertically, after sunset using a WP-2 type net with 80 µm mesh size and a 155 mm hoop diameter filtering 26.43 L water for each sampling event. Where possible, zooplankton was identified to the lowest taxonomic level (Boltovskoy 1999) using a Wild M5A stereomicroscope. After Day 18, water from the mesocosms of T4 and T5 were filtered through a 1mm mesh sieve to collect and determine the abundance of zooplanktonic predators in these treatments.

Statistical analyses

Temporal variation in population abundances is related to stability (MacArthur 1955; Holling 1973; Link and Nichols 1994), therefore temporal variability in taxa abundances was

employed as the measure of stability using coefficient of variability (CV) and proportional variability (PV; [Heath 2006]) as metrics. Definitions of community stability vary (Westman 1978; Connel and Sousa 1983; Pimm 1984; Cottingham and Schinder 2000), but most require some equilibrium point from which differences can be measured (Ives *et al*. 2003). For temporal variability, a proportional metric is typically used with the equilibrium point being the average abundance of the taxon under consideration. Proportional measures provide a degree of independence from the mean unless the underlying dynamics of the system are density dependent, which is typical in ecological systems (Gaston and McArdle 1994). Given that a mesocosm is essentially a closed population, CV has suitable properties for measuring temporal variability provided there are no (or few) zeros and variability is independent of the mean (McArdle *et al.* 1990; Gaston and McArdle 1994; Link and Nichols 1994). However, CV can be biased by zero counts, rare events and other 'non-normal' behaviour of population data requiring different indices that allow comparisons across taxa (Heath 2006). In contrast to CV, PV is calculated as an average difference in abundance among sampling events and reduces the effects of rare events by comparing all abundances relative to each other rather than to the mean (Heath 2006).

For each replicate mesocosm, CV was calculated as the standard deviation divided by mean for all days, while PV was calculated as the average proportional difference between all measured abundances at each day (Heath 2006). A PV value of 0 equals no change i.e. complete stability, and a value of 1 represents complete instability. More specifically, PV is calculated as: 1) C is derived as the number of all possible combinations of sampled abundances in a time series of length n (Equation 1); 2) proportional differences are then calculated as $D(z)$ (Equation 2), with z expressed as a pair of abundances (z_i and z_j) at any two

time steps; and 3) both *C* and *D*(*z*) are then used to calculate PV (Equation 3) as the average proportional variability of all time steps.

$$C = \frac{n(n-1)}{2} \quad (1)$$

$$D(z) = 1 - \frac{MIN(z_i, z_j)}{MAX(z_i, z_j)} \quad (2)$$

$$PV = \frac{\sum D(z)}{C} \quad (3)$$

For each replicate mesocosm, CV and PV values were calculated per taxon and for each treatment as overall CV or PV i.e. using data from the entire time series (all sampling days). Means (± standard deviations) of the resulting three overall CV and PV values for each treatment were used to compare CV and PV among treatments using ANOVA. Since T5 was assumed to be the most stable or "natural" environment, Dunnett's post-hoc test was used to determine treatment differences with T5 as the reference treatment. PV is a relatively new measure of variability; therefore CV was presented and compared to provide a reference point for historical studies.

Results

Physico-chemical variables

Temperature and salinity were largely similar across treatments and reflected those values recorded in the estuary (Figure 3.1). Initial mesocosm dissolved oxygen levels were, however, slightly higher than those within the nearby estuary, with the highest levels being recorded in T1. Nonetheless, the mean (overall) dissolved oxygen values were similar across treatments and remained slightly above those recorded in the estuary (Table 3.2).

Biological samples

The picophytoplankton size fraction (<2.0µm) dominated the total Chl-*a* concentration within each treatment, followed by the nanophytoplankton (2 -20 µm) size fraction (Table 3.3). Total Chl-*a* concentrations were broadly similar among treatments within each size fraction, but decreased in the 2 µm and 20 µm fractions from initial to overall, suggesting that resources in this fraction were being depleted over the study period.

Nano-flagellates (<10 µm) numerically dominated the plankton (99.8%), followed by the micro-flagellates (>20 µm) and ciliates (Table 3.4). In contrast, blue-green algae, diatom and dinoflagellate numerical contributions were minuscule. Similarly, of zooplankton sampled with the WP-2 type net, the calanoid copepod, *Pseudodiaptomus hessei* numerically dominated the adult copepod abundance (99.5%) while *Prionospio* sp. dominated polychaete numbers (99.9%) with the vast majority at an early life-history stage. No mortality of stocked young fish occurred during the study at all, and only the initially stocked young fish were collected at the end of the study. Furthermore, upon filtering mesocosm water at the end of the study, only four isopods (*Exosphaeroma hylecoetes*) were collected from T4 (two from replicate 1, one from replicate 3) and T5 (replicate 2). Therefore, taxonomic groups were broadly defined as: macrozooplanktonic predators (fish), copepods, copepodites and nauplii, polychaetes, ciliates, micro-flagellates, nano-flagellates, and bacteria. Responses to trophic manipulation were evident in all taxa when comparing abundance patterns among treatments and especially when T1 to T4 are compared with T5 (Figure 3.2). The initial lack of copepods in T1 contributed to microplankton (micro-flagellates and ciliates) occupying the top trophic level. This resulted in pronounced and quick increases in abundances of these taxa by Day 3 and 6 respectively, presumably through the released predation pressure by the removal of larger zooplankton.

Table 3.2: Physical and chemical parameters of mesocosm and estuary water samples. Values are mean ± standard deviation of 3 replicates. D.O. = dissolved oxygen; *single measurement taken on day of observation.

	Day 0			All Days (Overall)		
	Temp. (°C)	**Salinity**	**D.O. (mg/L)**	**Temp. (°C)**	**Salinity**	**D.O. (mg/L)**
Treatment 1	19.1 ± 0.2	21.7 ± 0.3	7.7 ± 1.2	19.2 ± 1.4	19.1 ± 1.9	6.3 ± 1.2
Treatment 2	19.2 ± 0.1	21.3 ± 0.3	6.7 ± 1.3	19.2 ± 1.4	19.5 ± 1.8	6.3 ± 1.1
Treatment 3	19.0 ± 0.1	21.3 ± 0.3	6.6 ± 0.8	19.2 ± 1.4	18.8 ± 1.9	6.2 ± 1.2
Treatment 4	19.1 ± 0.1	21.7 ± 0.3	6.2 ± 1.1	19.1 ± 1.4	19.7 ± 1.8	6.1 ± 0.9
Treatment 5	19.2 ± 0.1	21.3 ± 0.3	5.4 ± 1.1	19.2 ± 1.4	19.8 ± 1.9	6.0 ± 1.1
Estuary	19.0*	21.5*	5.2*	19.5 ± 1.3	21.1 ± 2.4	5.3 ± 1.7

Table 3.3: Fractionated Chlorophyll-*a* concentrations. Initial (Day 0) and overall (All Days) concentrations recorded as mean ± standard deviation of 3 replicates.

	Day 0 ($\mu g.L^{-1}$)			All Days (Overall) ($\mu g.L^{-1}$)		
	0.7 µm	**2 µm**	**20 µm**	**0.7 µm**	**2 µm**	**20 µm**
Treatment 1	0.06 ± 0.04	0.08 ± 0.01	0.02 ± 0.01	0.06 ± 0.03	0.03 ± 0.02	0.01 ± 0.01
Treatment 2	0.06 ± 0.01	0.07 ± 0.03	0.02 ± <0.01	0.06 ± 0.03	0.03 ± 0.02	0.01 ± < 0.01
Treatment 3	0.06 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0.05 ± 0.03	0.03 ± 0.02	0.01 ± 0.01
Treatment 4	0.06 ± 0.01	0.06 ± <0.01	0.02 ± 0.01	0.05 ± 0.03	0.03 ± 0.02	0.01 ± < 0.01
Treatment 5	0.07 ±0.06	0.06 ± 0.01	0.02 ± <0.01	0.08 ± 0.05	0.03 ± 0.02	0.01 ± < 0.01

Table 3.4: Mesocosm taxa abundances. Values represent overall abundance and the range (min - max) in values per taxon. Each of bacteria, diatom, dinoflagellate, blue-green algae, flagellate and ciliate abundances are presented in numbers per mL. All other taxa abundances presented as total numbers per sample (26.43 L).

		Treatment 1		**Treatment 2**		**Treatment 3**		**Treatment 4**		**Treatment 5**	
		Total	**Range**	**Total**	**Range**	**Total**	**Range**	**Total**	**Range**	**Total**	**Range**
Bacteria		4.8^{10}	5.7^{8} - 4.5^{9}	4.4^{10}	1.0^{9}- 5.3^{9}	4.3^{10}	9.1^{8}- 3.4^{9}	4.2^{10}	4.8^{8}- 3.7^{9}	3.0^{10}	9.8^{8} - 1.9^{9}
Diatoms	(< 10 µm)	135	0 - 29	160	0 - 43	168	0 - 46	145	0 - 39	35	0 - 7
Diatoms	(> 10 µm)	0	-	0	-	1	0 - 1	2	0 - 1	6	0 - 2
Dinoflagellates	(< 10 µm)	30	0 - 3	32	0 - 7	30	0 - 7	30	0 - 9	14	0 - 3
Blue-green algae		0	-	1	0 - 1	2	0 - 1	1	0 - 1	2	0 - 1
Nano-flagellates	(< 10 µm)	2.0^{5}	4.9^{3} - 2.1^{4}	1.8^{5}	4.9^{3} - 2.2^{4}	1.7^{5}	2.5^{3}- 1.8^{4}	2.0^{5}	3.9^{3}- 1.7^{4}	3.3^{5}	1.0^{3}- 2.2^{4}
Micro-flagellates	(> 20 µm)	1720	9 - 204	1264	29 - 124	1083	4 - 134	991	9 - 118	1438	27 - 120
Ciliates		208	0.7 - 36.2	194	1 - 26	132	0 - 22	208	0 - 28	190	3 - 21
Polychaetes											
	Prionospio sp.	235	0 - 78	12085	0 - 1873	16321	7 - 1892	13425	3 - 2285	1390	9 - 334
	Polychaete spp.	0	-	2	0 - 1	0	-	0	-	0	-
Nauplii		2376	0 - 664	1349	0 - 598	18934	8 - 3906	11257	4 - 2040	1757	4 - 237
Copepodites		539	0 - 137	286	0 - 43	5430	0 - 644	2873	0 - 312	520	0 - 49
Copepods											
	Pseudodiaptomus hessei	113	0 - 24	100	0 - 18	1673	0 - 238	1308	0 - 179	97.5	0 - 13
	Paracartia longipatella	0	-	2	0 - 2	10	0 - 3	4	0 - 2	1	0 - 1
	Euterpina acutifrons.	1	0 - 1	4	0 - 2	7	0 - 2	9	0 - 3	9	0 - 5
	Cyclopoid spp.	3	0 - 3	11	0 - 4	42	0 - 11	23	0 - 5	4	0 - 2
Amphipods											
	Grandidierella sp.	0	-	0	-	0	-	1	0 - 1	0	-
	Amphipod sp.	0	-	0	-	1	0 - 1	1	-	0	-
Isopod											
	Exosphaeroma hylecoetes	0	-	1	0 - 1	0	-	1	0 - 1	2	0 - 2

Concomitantly, increased grazing pressure by microzooplankton contributed to a decrease in nano-flagellate abundances and, consequently, an increase in bacterial cell counts.

An increase in polychaete numbers in T2 seemingly dampened the initial increase of micro-flagellate and (less so) ciliate abundances, although phytoplankton and bacterial trends were similar to T1, likely because polychaetes were feeding on both the microzooplankton and phytoplankton. The general decrease in micro-flagellate and ciliate numbers in the last two sampling days in T1 and T2 is attributed to the marginal increase of copepod, and copepodites and nauplii abundances. This decrease in micro-flagellates and ciliates probably resulted in a slight increase in nano-flagellate abundance, which likely directly caused a decrease in bacteria abundance.

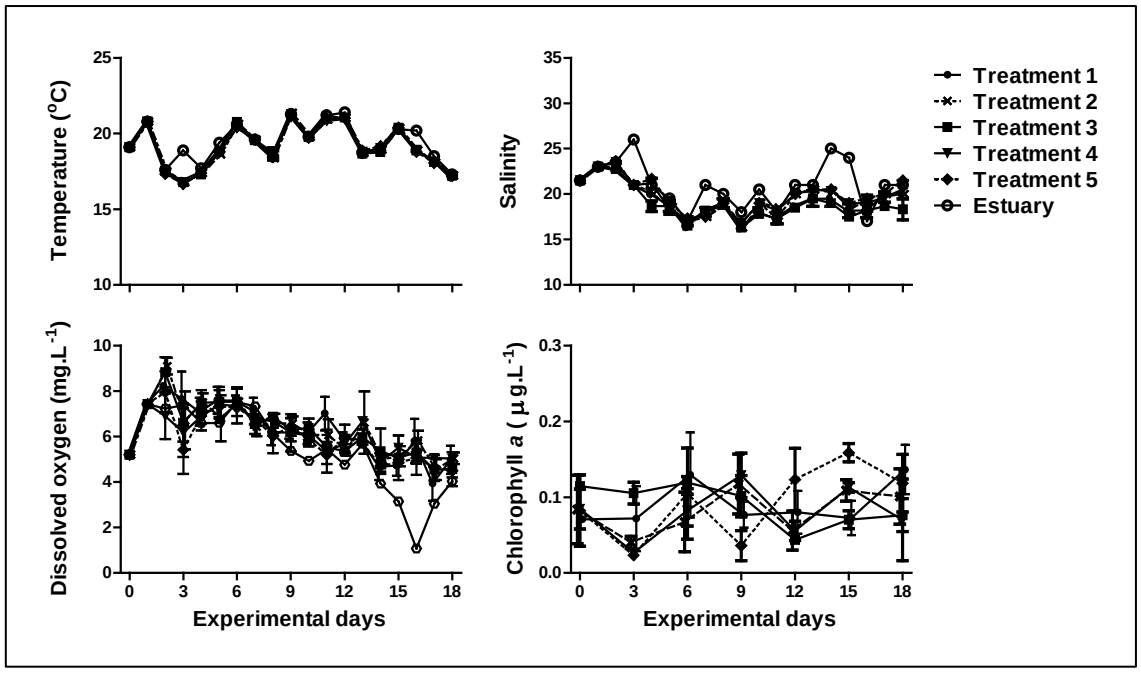

Figure 3.1: Physico-chemical measurements and Chlorophyll-*a* concentrations over time. Mean ± standard deviation of temperature, salinity, dissolved oxygen and total Chlorophyll-*a* concentrations over time, per experimental treatment and from the estuarine waters at the experimental site. Temperature, salinity and dissolved oxygen measurements recorded daily. Chlorophyll-*a* samples collected every third day.

In general, responses observed in T3 and T4 (*in situ*, but with no fish) were largely similar across taxa. The lack of apex-predation pressure within these two treatments resulted in a steady increase in copepods, copepodites and nauplii numbers over the duration of the study with a noticeable decrease in adult copepods only occurring on Day 18. While the abundance of micro-flagellates generally decreased in these two treatments, the ciliates showed an initial increase until about Day 6 (T3) and Day 5 (T4) before decreasing, potentially highlighting a predator-prey relationship between these two taxa. The polychaete abundance trends in these two treatments were similar to those of copepods, but at an order of magnitude higher, while nano-flagellate abundances showed an inverse trend dropping substantially from Day 0 to Day 12, then increasing from Day 12 to 18. The abundance and variability (standard deviation) of three trophic groups (copepod, copepodites and nauplii and polychaete) in T5 (containing the young zooplanktivorous fish) were low and, relative to other taxa, day-to-day variability was stable over the study period as shown in less variable trends. Again, there was a general decrease in micro-flagellate and ciliate abundances, but these trends were less pronounced than those of other treatments. Finally, nano-flagellate and bacteria abundances in T5 decreased initially, increased over the middle period, and then slowly decreased towards the end of the study. All taxa in T5 had relatively low replicate variability compared with other treatments except for phytoplankton. Nano-flagellate abundance initially decreased until Day 9, then increased and stabilised until Day 18. Bacterial abundances in T5 were lower and had relatively stable day-to-day variability in comparison with other treatments.

Statistical analyses

PV values corroborate differences in temporal stability shown in raw abundance values of Figure 3.2 (Figure 3.3). PV values in T5 were consistently the lowest and, thus, more stable over the study period across all treatments. That is, taxa in T5 were less variable overall than

taxa in all other treatments and support the observations shown in Figure 3.2 of less variable abundances. Statistically, 17 of 28 PV comparisons between T5 and the remaining treatments were significantly lower at $\alpha = 0.05$ and 9 others significantly lower at $\alpha = 0.1$ (Table 3.5). CV values confirm PV values and the increased stability (lower PV values) in T5. There was a reduction in significant comparisons with only 13 CV comparisons between T5 and other treatments being significantly lower at $\alpha = 0.05$ with a further 4 lower at $\alpha = 0.1$. In general, copepod, ciliate, micro-flagellate and nano-flagellate differences were upheld between the two metrics, but copepodites and nauplii showed a reversal in significance in treatments T5 vs. T1 and T5 vs. T2, and similar reversals in bacteria were more widespread. Some of the statistical differences found between CV and PV may be attributable to underlying properties of these metrics. However, only a few zeros were present in the entire dataset (4%) and zeros did not appear to affect overall abundance trends in those taxa with zero counts in any replicate or on any day. Yet, there was some variation between CV and PV trends. For example, three taxa, copepods, copepodites and nauplii, and ciliates, have CV values that do not follow as closely the pattern of PV values, whereas the other taxa show strikingly similar patterns in the two metrics (Figure 3.3). The greatest number of zeros was seen in the first three sampling days of copepods in T1 and T2, but the CV values in comparison with PV are quite similar for those two treatments, suggesting that zeros do not play a role in metric differences. The most important agreement between these metrics is firmly seen in T5, which had the lowest values across all taxa and most of these are significantly lower than other treatments within taxa.

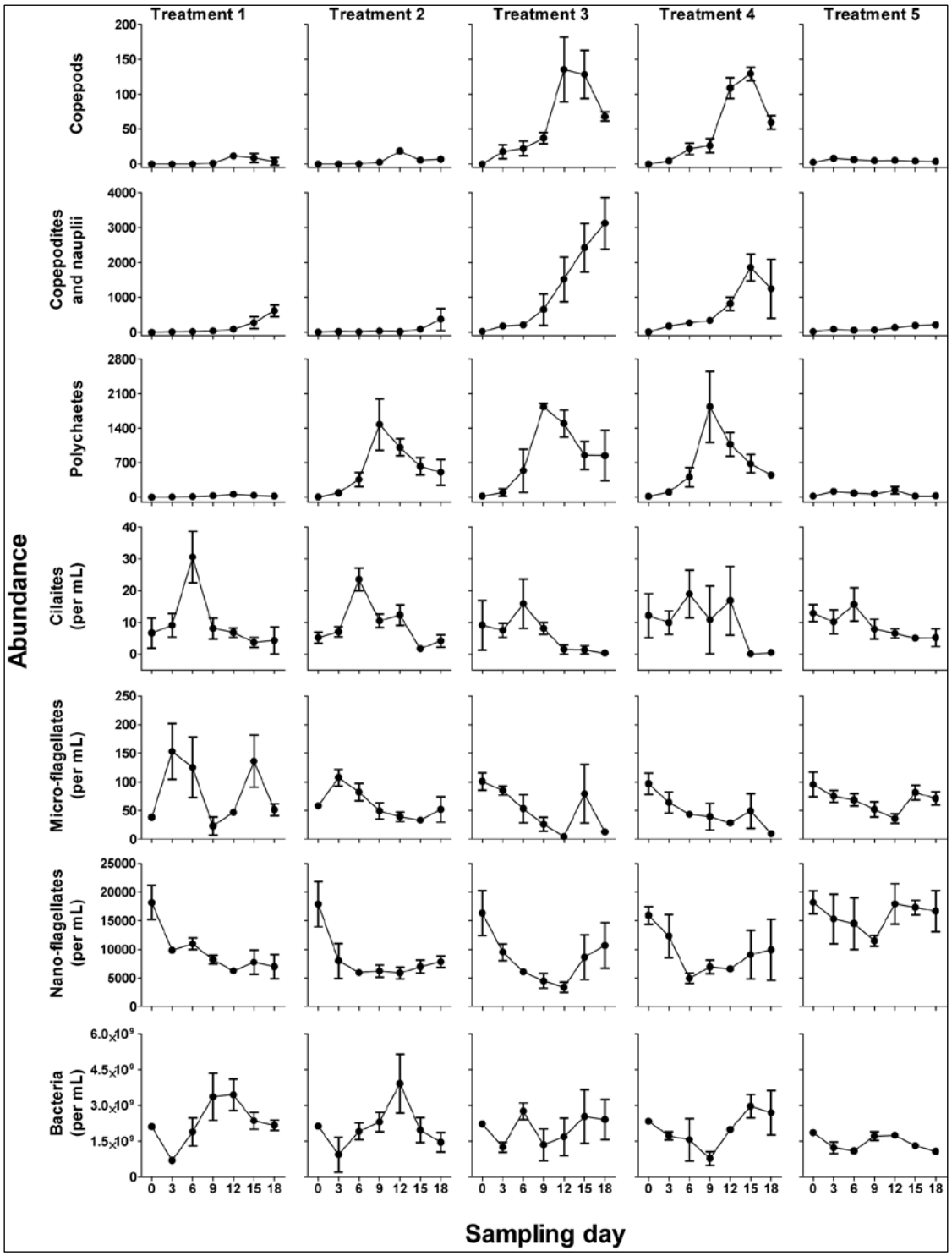

Figure 3.2: Taxon abundances over time. Bacteria, flagellate and ciliate values are expressed as numbers per mL, whereas polychaete, copepodite and nauplii and copepod values are expressed as numbers per sample (26.43 L). Mean ± standard deviation of abundances calculated from three replicates per treatment. Note different y-axes scales.

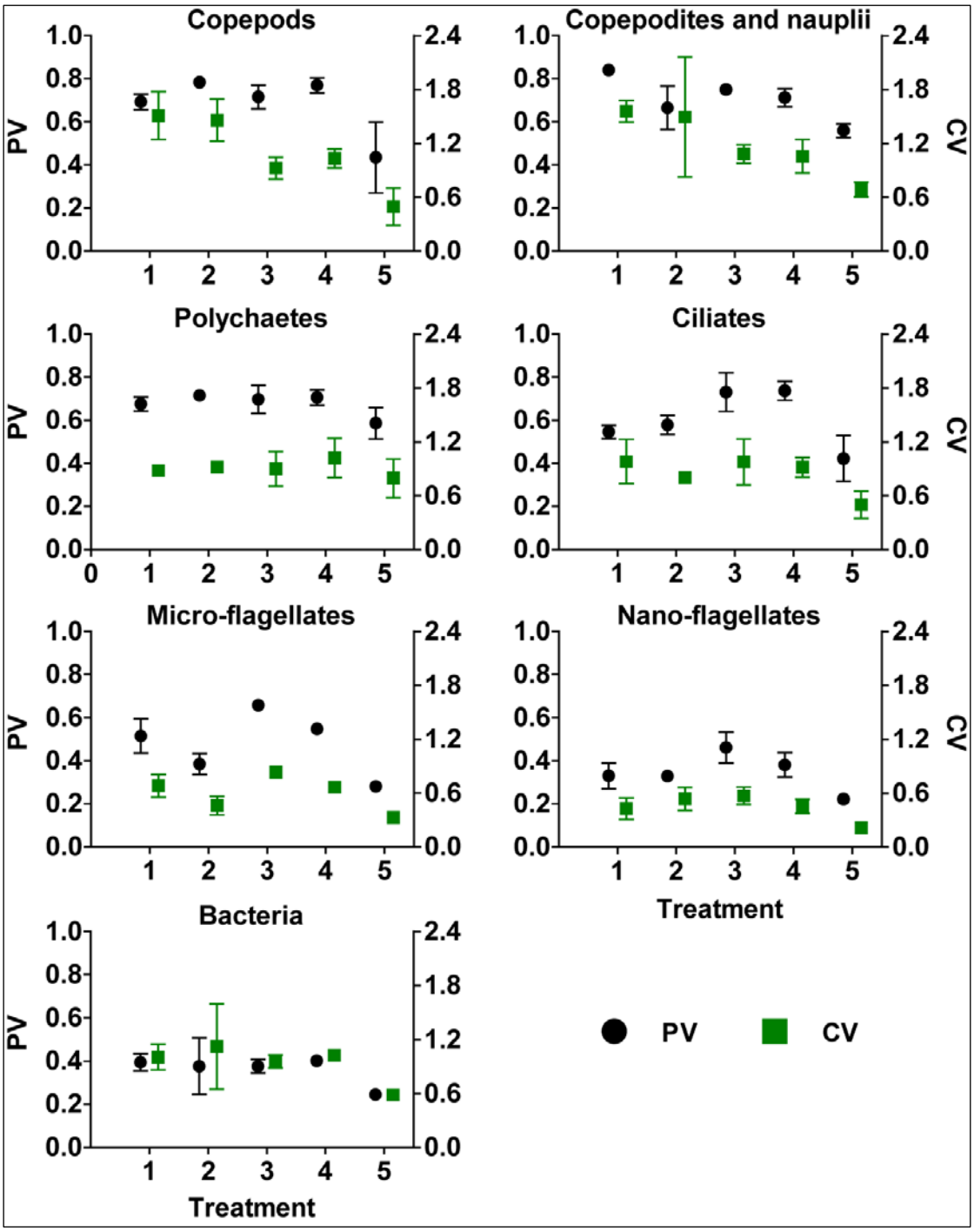

Figure 3.3: Comparison of the proportional variability (PV) and coefficient of variation (CV) for each taxon. Mean ± standard deviation of overall PV and CV calculated from three replicates per treatment. For PV (left y-axis), 0 = complete stability, 1 = complete instability; CV (right y-axis) has no upper bound.

Table 3.5: Dunnett's test results for comparisons between T5 (control) and treatments T1 to T4. P values for proportional variability (PV) and coefficient of variability (CV) are presented for comparisons among treatments. Values in red bold and black bold are $\alpha = 0.05$ and $\alpha = 0.01$ respectively.

	Copepods	Copepodites and nauplii	Polychaetes	Ciliates	Micro-flagellates	Nano-flagellates	Bacteria
PV							
5 vs 1	**0.002**	**0.016**	**0.043**	**<0.001**	**<0.001**	**0.009**	**0.042**
5 vs 2	**0.006**	**0.004**	**0.061**	**0.001**	**<0.001**	**<0.001**	**0.090**
5 vs 3	**0.001**	**0.096**	**0.029**	**0.063**	**0.053**	**0.079**	**0.093**
5 vs 4	**0.010**	**<0.001**	0.145	0.158	**<0.001**	**0.073**	**0.052**
CV							
5 vs 1	**0.025**	0.461	0.316	**0.056**	**0.001**	**0.037**	0.115
5 vs 2	**0.073**	0.408	0.845	**0.029**	**<0.001**	**0.004**	0.203
5 vs 3	**<0.001**	**0.036**	0.774	0.191	0.189	**0.007**	**0.050**
5 vs 4	**<0.001**	**0.024**	0.917	**0.028**	**<0.001**	**0.066**	0.137

Discussion

The stability of copepod abundances in T5 with apex predator and its relative instability in treatments where copepods were not filtered out (T3 and T4), demonstrates the direct effect of stability exerted by the fish on copepods. It is no surprise that the calanoid copepod, *Pseudodiaptomus hessei*, dominated the mesozooplankton in our mesocosms as it often dominates zooplankton abundance and biomass in southern African estuaries (Wooldridge and Mellville-Smith 1979; Perissinotto *et al.* 2003). In the absence of fish in T3 and T4, copepod abundances increased which, in turn, negatively impacted the micro-flagellate and ciliate abundances, most likely due to increased grazing on these taxa by copepods. This grazing dynamic was consistent with predator-prey cascades (Tranvik and Hansson 1997; Granéli and Turner 2002). Trophic interactions of 'larger' plankton (copepods, micro-flagellates and ciliates) on smaller phytoplankton was less clear, however, and confounded in part by early copepod life stages (copepodites and nauplii) and the presence of polychaetes, which share overlapping prey size distributions with copepods (Pagano *et al*. 2003; Brucet *et al.* 2008; Martin *et al*. 1996). When copepods increased in abundance, ciliates and micro-flagellate numbers decreased, presumably a result of increased copepod grazing. While the micro-flagellates, ciliates, polychaetes and early life-history stage copepods could be capable of consuming the nano-flagellates (Hansen *et al*. 1994; Brucet *et al*. 2008; Martin *et al*. 1996), the latter would be largely unavailable for direct consumption by the adult calanoids as they are likely smaller than the prey size range of these copepods (Pagano *et al*. 2003).

Polychaete abundances were also stable in the presence of apex predators and ciliates showed a marked reduction in replicate variability, but were not more stable in T5 over T1. Predator-prey dynamics caused by size fractionation undoubtedly led to trophic pathway shifts and possibly prevented ciliates and polychaetes from operating within their predator-prey niche.

However, caution is required in interpreting these results, as removal of zooplankton every third day through sampling, without reposition of organisms could have affected overall numbers. While the trends in zooplankton data for most treatments seem to be largely unaffected by the sampling protocol, such removal may have exaggerated results for certain zooplanktonic components, such as the metazoans, especially where overall numbers were low. However, the sampling protocol was consistent, and vast differences in trends across treatments were evident, highlighting the effects of the treatments.

It is evident that removing biological size fractions was effective to limit taxa assemblages within treatments and clear trends in taxa dominance were noted. With the exception of T5, the taxon occupying the top trophic level initially increased in abundance as a result of predator release, and then decreased presumably as a result of density dependent factors or resource depletion. In some cases two taxa showed this pattern, e.g. copepods and polychaetes in T3 or ciliates and micro-flagellates in T1 (although not as pronounced for micro-flagellates), suggesting these taxa were not engaged in a predator-prey interaction at least over this period. In T1, ciliates and micro-flagellates initially occupied the top trophic level and increased dramatically over the first few days. In turn, they decreased nano-flagellate and bacterial abundances. When *Prionospio* polychaetes were included, as in T2, they appeared to dominate predation on micro-flagellates and potentially grazed on phytoplankton (Martin *et al.* 1996). Very little change was seen in ciliate abundance with polychaetes added suggesting that polychaetes did not feed on ciliates. Ciliates likely grazed on nano-flagellates and when ciliate abundances decreased in a consistent way (as in T3 and T5), nano-flagellate abundances increased. Where ciliates became variable, nano-flagellate abundances were also variable (see T4) suggesting that there was a strong coupling between ciliates and phytoplankton abundances. Intra-guild predation may also exist because ciliates

feed on micro-flagellates (Morin 1999). Competitive interactions between these two taxa may also be present because both have the ability to feed on nano-flagellates and bacteria (Hansen *et al*. 1994). Indiscriminate grazers will have effects on other taxa and some predator-prey relationships are more complex than simple one-to-one observations, but it was successfully demonstrated that successively higher trophic levels affect lower trophic levels in a cascading fashion (Polis 1994; Persson 1999). Proportional variability was shown as a powerful measure to tease out the stabilising role of predators on prey abundances throughout the ecological system highlighting some of these cascading effects.

Ecological investigations have long emphasised the importance of physical processes, so-called “bottom-up effects”, in structuring aquatic ecosystems (Ripple *et al*. 2010). In marine research the focus is often on the implications of various physico-chemical characteristics in structuring planktonic food webs (Verity and Smetacek 1996). Since the present study focused entirely on biological interactions, the study required physico-chemical homogeneity across treatments. Indeed, salinity and temperature measurements were consistently similar across treatments and comparable to that of the estuary. Furthermore, despite the initial differences in dissolved oxygen concentrations across treatments, likely resulting from increased aeration during the filtration procedure, the overall concentrations were similar. Particle aggregation and sinking, resulting in an export of materials from mesocosm water columns, were however not measured. While such information would be useful, potential differences in material export among treatments would likely be a result of the biological manipulations, rather than physico-chemical differences. As such, through physico-chemical homogeneity the significance of biological interactions in structuring ecosystems could be characterised, and the importance of predator-prey interactions highlighted.

A fundamental element of any manipulative trophic-interaction analysis is the adequate observation of predator numbers for the duration of the study (Salo *et al*. 2010). Since there is often a relative equilibrium of coexistence between predators and their prey in natural systems (May 1976; Beddington *et al*. 1976), it is necessary to establish a stable apex predatory pressure in experimental scenarios such as in the present study, where natural states were simulated (T5). The reproductive cycle of our apex predators precluded rapid reproduction; therefore, only the initial stocked young fish were present throughout the study and were healthy. Hence, verified stable apex predation pressure was qualified for T5, allowing for comparison with treatments whereby the top of the food web was less stable over time, ultimately highlighting the presence of trophic cascades.

In the oligotrophic warm-temperate Kasouga Estuary, the biological interactions between the taxa were not exclusively predator-prey in nature. The exposition of cascade mechanisms *per se* therefore proved elusive. However, the presence of trophic cascading was evident across treatments, where multiple lower trophic levels were affected, regardless of the varied dominant taxa at the top trophic level. The presence of the apex predator in this system provided a consistent pressure to stabilise copepod and polychaete numbers, and presumably through various cascade mechanisms, also stabilised ciliate, micro-flagellate, nano-flagellate and bacterial abundances. As such, it was shown that young fish can assume the role of apex planktonic predators, mediating interactions and stability at multiple lower trophic levels in oligohaline estuary environments.

CHAPTER 4.
EVIDENCE FOR THE ORGANISATION OF A BACTERIAL COMMUNITY BY ZOOPLANKTIVORES AT THE TOP OF AN ESTUARINE PLANKTONIC FOOD WEB

"Water is the best of all things."

- Pindar, Olympian Odes

Plate 4: Pristine vegetated margins of the middle reach of the Kasouga Estuary (Photo: Ryan Wasserman).

Abstract

The indirect effects of zooplanktivores on the microbial planktonic community remains poorly understood across aquatic environments. In the present study, the effect of apex predation on the bacterial community, through trophic cascading, was investigated using experimental *in situ* mesocosms in an estuarine habitat. Estuarine water was used to fill treatment mesocosms, while certain planktonic components were either removed or added, establishing different trophic scenarios. Only one treatment contained stable apex predation pressure, simulating a stable trophic food web. Water samples were then collected over time from each of the treatments. The bacterial community associated with these samples was assessed through pyrosequencing of the variable regions 4 and 5 of the bacterial 16S rRNA gene. In order to attain species level information (0.03 operational taxonomic unit distance) for each of the samples, the Mothur platform was utilised. Using the bacterial species level data, a community analysis procedure was employed to assess the variability in bacterial communities over time. Bacterial communities were the most similar among treatments at the commencement of the experiment, with all communities deviating over time. Compared to the remaining treatments, limited temporal deviation-from-initial in bacterial community structure was found within the stable apex-predatory pressure treatment. These findings are consistent with trophic cascade theory, whereby predators mediate interactions at multiple lower trophic levels with consequent repercussions for diversity.

Introduction

Eukaryotic phytoplankton and prokaryotic bacteria have been demonstrated to be the two largest contributors to primary production in marine ecosystems (Button 1994). Moreover, these organisms often represent an important carbon source for the heterotrophic components within the microbial loop (Smith 2007). As a consequence, these ubiquitous, abundant and diverse organisms have been shown to be key regulators of various biogeochemical processes, including the carbon and nitrogen cycles in aquatic environments (Kirchman 1994). Despite their importance within aquatic ecosystems, the various factors that determine the bacterial community composition within aquatic ecosystems have received little attention (Dolan 2005; Teira *et al*. 2008), especially those relating to biological processes.

Trophic interactions have been demonstrated to play an essential organisational role in community and ecosystem ecology (Polis *et al*. 2000). Substantial research has been conducted on this field of ecology which has revealed that communities and associated food webs are often controlled by predators through trophic cascades (Leibold *et al.* 1997, Persson, 1999, Estes *et al.* 2011). Cascade investigations and their elucidation with regard to empirical and theoretical science have in recent times marked some of the most exciting breakthroughs in trophic ecology (Polis *et al.* 2000). In addition to their contribution in controlling prey numbers, predators may through selective feeding also affect the diversity at lower trophic levels, potentially increasing species diversity by removing/decreasing superior competitors (Paine 1966; Worthen 1989; Addicott 1974; Spiller and Schoener 1998). Studies on the role of predators in determining the species composition of prey numbers in aquatic environments have, however, traditionally only focused on components of the classical food web; namely those interactions between zooplanktivores, zooplankton and phytoplankton (Porter 1996).

Estuaries are of particular ecological interest. Among the most productive of all ecosystems (Gobler *et al*. 2005), estuaries support high primary (Nixon *et al.* 1996; Gowan *et al.* 1992) and secondary consumer biomass (Houde and Rutherford 1993). While the classic food web has received much attention in these systems (Hansson 1992; Froneman 2002a), there is relatively little information available on microbial dynamics of estuaries and indeed, marine ecosystems as a whole. In southern African estuaries, as in many aquatic environments, zooplanktivorous predation is, at least partly, performed by early life-history fish (Whitfield 1998; Wasserman 2012). Zooplanktivorous fish have long since been shown to influence invertebrate prey communities (Brooks and Dodson 1965; Hall *et al.* 1976). Furthermore, zooplanktivores have been demonstrated to alter phytoplankton communities indirectly, through interaction with the zooplankton (Hansson 1992; Persson *et al.* 1992). However, the indirect effects of zooplanktivores on the microbial planktonic community composition and diversity remains poorly understood (Travnik and Hansson 1997).

In Chapter 3, the presence of an apex zooplanktivorous predator contributed to the control of zooplankton numbers, with a subsequent increase in stability of phytoplankton and bacterial abundances. That component of the study, however, focused only on absolute number of organisms at the broad taxonomic level, with no information pertaining to the species composition within these broad groups. In this chapter, the impact of the apex predators on prokaryotic diversity is emphasised, by using ribosomal RNA gene sequencing. Through pyrosequencing of select, reliable hypervariable regions of the 16S rRNA gene and taxonomic analysis, classification of sampled microbes is possible (Wang *et al.* 2007; Liu *et al.* 2007; Wang and Qian 2009). Furthermore, given the high volume of sequences produced with pyrosequencing, rare organisms are more readily represented, allowing for an accurate analysis of the microbial community, irrespective of the potential dominance of certain

populations (Wang *et al*. 2007; Liu *et al.* 2007; Wang and Qian 2009). Using selected water samples collected from the various treatments established in Chapter 3, the bacterial communities exposed to the varied trophic manipulations are compared over time. It was postulated that the presence of the stable apex-predatory pressure, which was shown to stabilise the entire food web (Chapter 3), would have repercussions for the bacterial community at the base of the planktonic food web.

Materials and methods

Sample collection and genomic DNA extraction

Water samples were collected for the determination of the aquatic bacterial community associated with selected treatments. Samples were collected from a single representative mesocosm for treatments 1, 2, 4 and 5 (see Table 3.1 for manipulation details). Water samples (100 ml) collected from 0.5 m depth from each mesocosm, were gravimetrically strained through 1 mm mesh to remove large debris and then gently filtered through a 0.22 µm filter. With the aid of the PowerWater DNA Isolation Kit (MioBio Laboratories), purified genomic DNA was acquired from the 0.22 µm filters and used in the PCR amplification process. These samples were collected from each treatment, on selected days of the 19-day experiment (Table 4.1).

PCR template preparation and multiplex pyrosequencing

The variable regions 4 and 5 of the bacterial 16S rRNA gene were amplified, using the E517F (5'-CAGCAGCCGCGGTAA-3') and E969-984 (5'-GTAAGGTTCYTCGCGT-3') primer pair (Wang and Qian, 2009). For PCR amplification of the 16S rRNA gene fragments, a 25 µl PCR mix comprising 0.1-1 µl of the extracted genomic DNA, 1X PCR buffer (containing $MgCl_2$), 300 µM dNTPs, 0.3 µM of each template specific primer and 0.5 units

KAPAHiFi Hotstart DNA Polymerase (KAPA Biosystems) were subjected to initial enzyme activation and DNA denaturation at 95°C for 5 minutes. This was followed by cycling parameters of 98°C (45 seconds), 45°C (30 seconds), 72°C (45 seconds) for five cycles, 98°C (45 seconds), 50°C (30 seconds) and 72°C (45 seconds) for 10 cycles and a final extension at 72°C for 5 minutes. Using the Zymo Gel DNA Recovery kit (Zymo Research), the ensuing 468 nt PCR products were gel purified. Using FLX fusion primers comprised of sequencer specific nucleotides, multiplex identifier tag and template-specific nucleotides, a second PCR amplification was then performed on approximately 2 ng of the 468 nt PCR product. Each sample was amplified with a primer set containing a unique multiplex tag, the amplification of which consisted of 2 ng template, 0.4 μM forward and reverse primers, 300 μM dNTPs, 1X PCR Buffer, 0.5 units KAPAHiFi Hotstart DNA Polymerase (KAPA Biosystems), in a final volume of 25 μℓ. Initial enzyme activation and DNA denaturation was done at 95°C for 5 minutes, followed by cycling parameters of 98°C (20 seconds), 52°C (45 seconds), 72°C (1 minute) for five cycles, and 98°C (20 seconds), 65°C (45 seconds) and 72°C (1 minute) for 15 cycles. A final 72°C, 5-minute extension was then done. The resultant 538 nt PCR products were gel purified as before with the relevant amplicons being pooled in equal amounts, subjected to emulsion PCR, and then sequenced using the GS Junior Titanium Sequencer (454 Life Sciences, Roche).

Computational methods

Sequence reads were quality filtered and cured of primer/tag sequences using the standard software provided by 454 Life Sciences. The dataset was then further curated using Mothur in which all reads containing an ambiguous nucleotide, a homopolymeric run greater than 7, or a read length of less than 200 nt were removed from the dataset (Schloss *et al.* 2009). The Ribosomal Database Project (RDP) Classifier, which assigns 16S rRNA sequences to

phylogenetic taxonomic groups down to the genus level using a Naïve Bayesian rRNA classifier algorithm (Cole *et al.* 2009; Wang *et al.* 2007) was used to classify and describe the curated sequences at a broad taxonomic level. In order to determine the operational taxonomic units (OTUs) for each of the sample sites, the Mothur platform was utilised (Schloss *et al.* 2009). Chimera were removed from the dataset using UChime (Edgar *et al.* 2011) and the reads were assigned taxonomic rankings using SILVA 16S rRNA database (Quast *et al.* 2013). Chloroplast 16S rRNA sequences were removed from the database and the remaining reads were aligned against the SILVA 16S rRNA database. Aligned sequences were then filtered to ensure that the sequences all overlap the same alignment space. A distance matrix was then generated and used to determine OTUs at a distance value of 0.03 and the resultant reads clustered using the average neighbor algorithm (Mothur).

Data analysis

As PCR amplification negates the ability to express the absolute numbers for each taxonomic unit present in the samples (Matcher *et al.* 2011), raw data was converted to percentage data to determine the relative contributions of each taxa to the overall numbers. Percentage (RDP classifier) data at the phylum level was used for descriptive statistics. At the species level (0.03 OTU distance), the percentage data was pre-treated using an arcsine transformation before being imported into PRIMER v6 statistical software package (Clarke and Warwick 1994) for resemblance and community analysis, whereby samples are analysed according to their levels of similarity and dissimilarity.

In PRIMER v6, each sample was assigned both an experimental treatment (T1, T2, T4 or T5), and sampling day (0, 9, 12 or 18) label. These two separate labels were then used as factors for analyses, prior to any statistical investigation, as recommended by Clarke and

Warwick (1994). Using non metric MDS (Multiple Dimension Scaling) analyses, a 2-dimensional scatter plot was constructed. In an exploratory fashion, clusters at a given statistical similarity level were then overlaid on the 2-dimensional MDS plot to determine and visually represent the relative sample similarity (Clarke and Warwick 1994). Similarly, trajectories for samples within each treatment were overlaid on the 2-dimensional MDS plot for visual representation of differences between treatments, based on the spatial spread of the samples in the plot. An MVDISP (Index of Multivariate Dispersion) test was then employed to statistically measure the relative rank dissimilarity between these samples. Within group similarity was also determined for samples within each treatment factor using SIMPER analysis according to the method described in Clarke and Warwick (1994).

Results

Subsequent to sequence quality control, primer trimming and size exclusion, the present study produced 101 066 16S rRNA sequence reads, which were classified to the level of genus using the RDP 16S rRNA database. Of these sequenced reads, 6 869 did not show significant homology to any sequence in the current RDP database and were grouped together as unclassified bacteria. The remaining reads were successfully assigned to 17 phyla and 362 genera. The number of sequences assigned to taxa did, however, vary between treatments and sampling days due to variations in the sizes of the sequence datasets generated for each of the samples (Table 4.1). At the commencement of the experiment, the relative numerical contributions of each of the phyla were similar, with the Bacteroidetes, Proteobacteria and the Cyanobacteria/Chloroplast group (hereafter referred to as the blue-green group) being the most numerically dominant across all the experimental treatments (Figure 4.1).

Table 4.1: Number of unique sequences, representing species (0.03% dissimilar), as well as species richness (D) and evenness (‘H) from each sequenced water sample collected from experimental mesocosm enclosures. Samples were collected from four treatments (T1-T4) over four temporal sampling events (Day 0-18). Samples absent for Day 12 in Treatments 2 and 3.

	T1	**T2**	**T3**	**T4**
Day 0				
Unique sequence no.	358	326	385	644
D	42.2	38.72	45.47	68.86
H’	4.05	3.96	4.15	3.85
Day 9				
Unique sequence no.	239	116	301	336
D	26.9	15.23	34.33	37.04
H’	3.45	3.47	2.64	3.529
Day 12				
Unique sequence no.	268	-	-	429
D	30.97	-	-	46.16
H’	3.71	-	-	3.80
Day 18				
Unique sequence no.	481	414	419	342
D	53.14	46.55	46.39	38.83
H’	4.18	3.69	3.93	4.00

By Day 9, however, the relative abundance of Bacteroidetes increased in T1, T2 and T5, at the cost of the blue-green group, but returned to near initial contributions by the end of the experiment. In contrast, in T4 on Day 9, the blue-green group and unclassified bacteria seemed to out-compete the Bacteroidetes, but returned to a state more reflective of the other treatments by Day 18.

Within the blue-green group, however, contributions of unicellular algae, Cyanobacteria and unclassified blue-green organisms, varied considerably between treatments and over time (Figure 4.2). Almost completely dominating upon experimental commencement, the unicellular algae contributions were drastically reduced by Day 9 in all the treatments, while the unclassified-chloroplast-holders increased. These trends, like those of the Bacteriodetes and Proteobacteria, returned to near initial contributions, but for an elevated percentage of Cyanobacteria at Day 18 in all of the treatments (Figure 4.2).

Many of the less dominant bacterial phyla contributions were relatively similar across the experimental treatments at the onset of the study. A few groups, however, generally increased in contribution over time across the treatments, including the Chlamydiae and Planctomycetes (Figure 4.1), while others were absent from samples collected after Day 0, such as the Nitrospira and Tenericutes (Figure 4.1).

Operational taxonomic units (OTUs) of the bacterial components were determined for each sample at a distance value of 0.03 which is the generally accepted distance at the level of species. The results of the OTUs analysed in PRIMER v6 reflected those results at the phyla level. Upon commencement of the study, the mesocosms contained similar communities

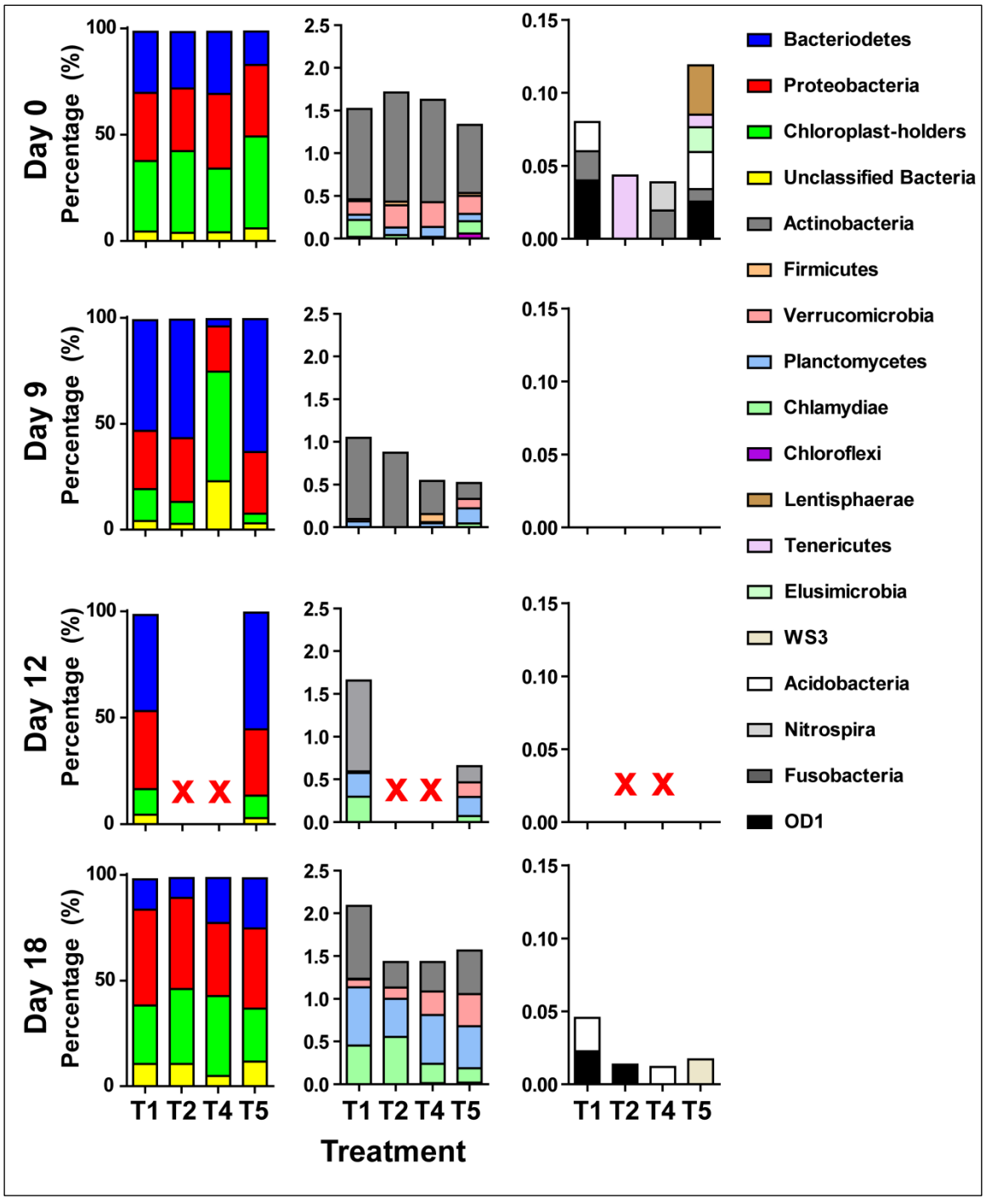

Figure 4.1: Percentage contribution of each phyla to the microbial community, sampled from four experimental treatments (T1-T4) over time (Day 0-18). Cumulative values within in treatment for each sampling day equal 100%. Samples absent for Day 12 in Treatments 2 and 3.

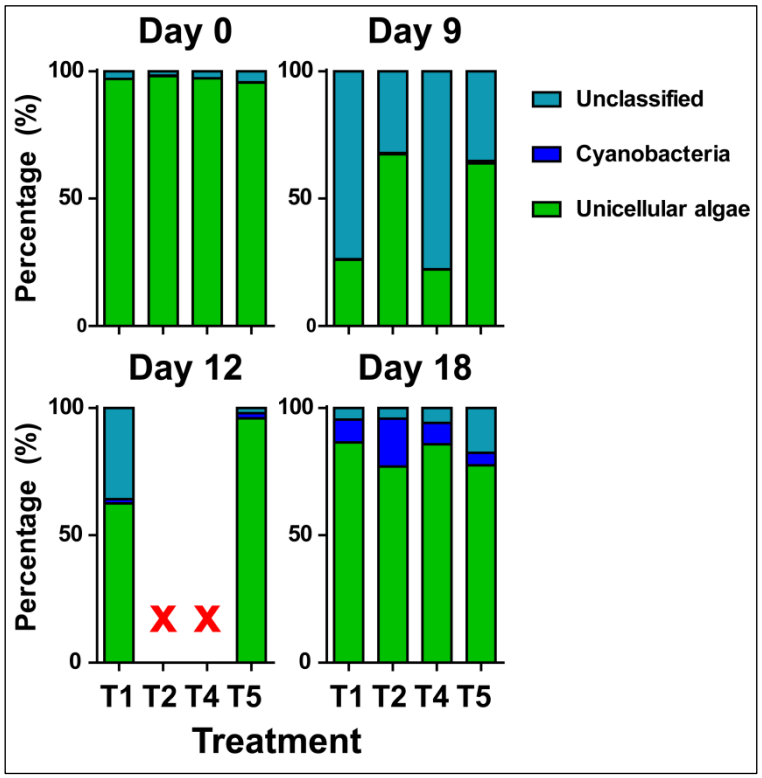

Figure 4.2: Percentage contribution of the green (unicellular algae), blue-green (cyanobacteria) and unclassified-chloroplast holders to chloroplast-holding organisms, sampled from four experimental treatments over time (Day 0-18). Samples absent for Day 12 in Treatments 2 and 4.

among all four experimental treatments. At the start of the study, all the samples in the different treatments grouped out, separate from those of the other sampling days (Figure 4.3). At a similarity level of ≈ 70%, all the Day 0 samples were clustered into a single grouping (Figure 4.3) while those of the remaining T5 samples clustered into a separate grouping. The outstanding samples were split between three additional groups, identified with the MDS analysis. The most distant group comprised a single T4 sample on Day 9, while the Day 18 samples of T1, T2 and T4 also clustered out. The last group comprised Day 9 samples from T1 and T2, as well as that of T1 from Day 12.

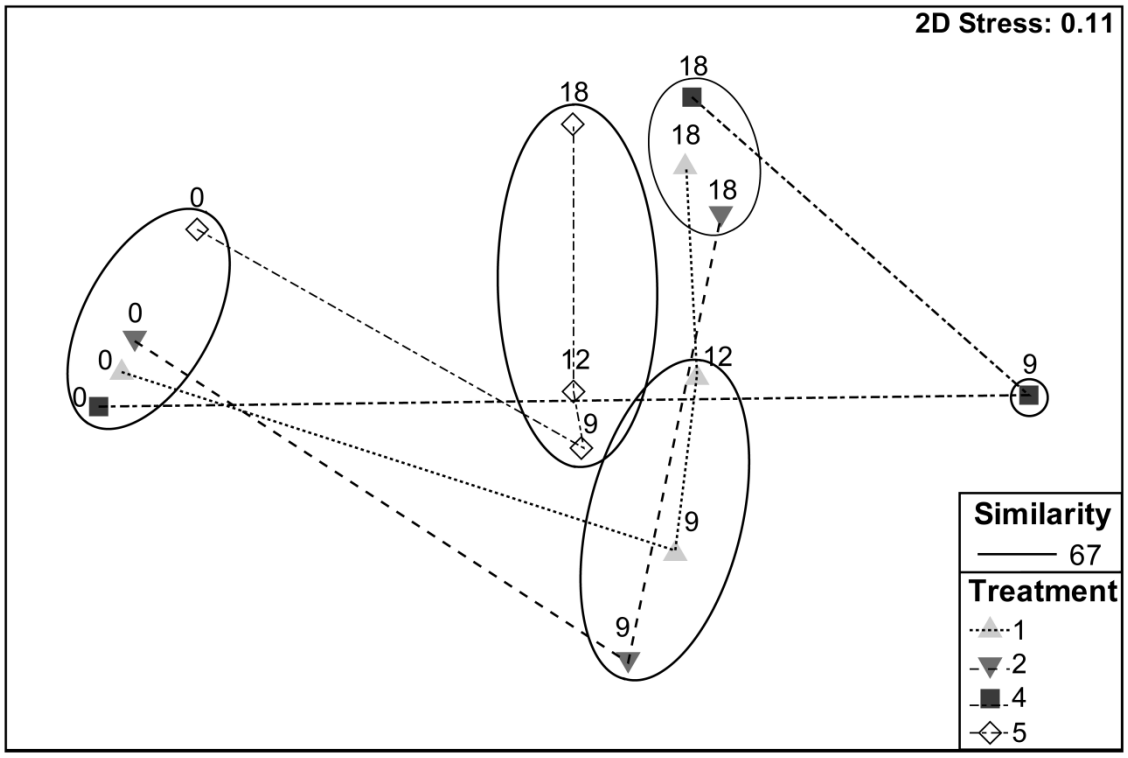

Figure 4.3: Two dimensional MDS (Multiple Dimension Scaling) plot showing relative similarity between each sample. Each sample is coded, with the digit representing those of the sampling days (0, 9, 12 and 18). Samples are clustered at a 67% similarity level (encompassed within a solid-line ring). Trajectory sequences represented by hashed-lines, for each treatment, and are chronological.

Visual trends, observed in the trajectories produced and overlaid on the same 2D MDS plot show that the microbial community in Treatment 5, based on distance between samples, was the least dissimilar over time (Figure 4.3). Treatment 4 was revealed to be the most temporally dissimilar. Analysis of the sampled microbial community for dissimilarity in PRIMER, using MVDISP, showed that for the selected factor of sampling day, Day 0 samples were the least dispersed (Table 4.2), as these samples were the most similar of all sampling days. The treatment factor MVDISP, however, revealed that the predator treatment (T5) was the least dispersed of all the treatments over time. These results were corroborated by the SIMPER analysis whereby the microbial communities in the four treatments on Day 0 were the most similar (56.45%) while T4 samples were the most similar (44.01%) over time.

Table 4.2: MVDISP (Index of Multivariate Dispersion) and SIMPER test results, respectively showing the relative dispersion and similarity of samples within each factor. The lower the MVDISP value, the less dispersed the samples within the factor. The greater the SIMPER percentage, the more similar the samples within the factor. Both sampling day and experimental treatment were selected as factors for analysis.

Sampling day	MVDISP	SIMPER (%)	Treatment	MVDISP	SIMPER (%)
Day 0	0.65	56.45	T1	0.93	39.38
Day 9	1.37	43.92	T2	1.30	32.60
Day 12	1.30	45.76	T3	1.51	31.24
Day 18	0.93	50.07	T4	0.67	44.01

Discussion

The important role that predators play in structuring ecosystems has been emphasised in numerous studies (Paine 1966; Addicott 1974; Spiller and Schoener 1998). While this notion makes reference to direct interactions between the predator and its prey community, it also highlights another important biological interaction, that of competition (Paine 1966). The removal of a predator not only affects the prey community but also that of lower trophic levels as shifts in resource partitioning often ensue, potentially altering much of the food-web structure and energy flow (Persson 1999; Estes *et al.* 2011). It was shown that the predatory pressure of the zooplanktivorous fish contributed to temporal stability of metazoan zooplankton, microzooplankton, phytoplankton and bacterial numbers (Figure 3.3). In the treatments that did not have the stable apex predatory pressure, instability across multiple trophic levels ensued. Here, the role of predation in structuring the prokaryotic community composition during this mesocosm experiment was investigated.

Compared to other components of the plankton, bacteria are thought to be more uniformly distributed in upper surface waters of aquatic systems (Button 1994). As such, the bacterial communities within the different treatments were expected to be broadly similar at the onset of the mesocosm experiment. Indeed, at a broad taxonomic resolution, the numerical analyses

(MDS, SIMPER and MVDISP) grouped the treatments on Day 0 into a single grouping with the Bacteriodetes, Proteobacteria and the blue-green group dominating the reads (Figure 4.3). Bacteriodetes and Proteobacteria often dominate bacterial contributions within marine/estuarine environments, being primarily associated with marine animals, phytoplankton and organic aggregates (DeLong *et al.* 1993; Cottrell and Kirchman 2000; Grossart *et al*. 2005). These organisms play an important role in aquatic environments contributing to the degradation of complex dissolved and particulate organic matter, the cycling of carbon and potentially, the uptake and mineralisation of dissolved inorganic matter (Cottrell and Kirchman 2000; Kirchman 2002). Similarly the Actinobacteria and Verrucomicrobia are also implicated in nutrient cycling of high molecular weight organic matter. The predominance of these microbes is not surprising given the importance of the detrital food web within estuarine ecosystems. Indeed, the prevalence of the Bacteriodetes and Proteobacteria in estuarine systems has recently been demonstrated in a study conducted in the nearby permanently open, Kariega Estuary (Matcher *et al.* 2011).

In the present study, the largest temporal changes in the microbial community composition were a result of fluctuations in the Bacteriodetes, Protobacteria and blue-green group (Figure 4.1). Within the blue-green group, the observed changes concerned the decrease in unicellular algae with a concomitant increase in cyanobacteria and unclassified chloroplast-holders (Figure 4.2). In each case, however, the fluctuations in group contributions of the different phyla were reduced in the presence of the predator. It is a worth noting in the absence of the predator (Treatment 4), the total zooplankton abundances (proto-zooplankton and metazoans) attained the highest levels in Treatment 3 (Figure 3.2). The decrease in unicellular algae in Treatment 4 during the study is probably the result of grazing by the zooplankton (Chapter 3). Such a decrease likely resulted in competitive release for other organisms such as the

cyanobacteria. Interestingly, of the treatments under investigation, Chapter 3 showed that this treatment (unfiltered estuarine water) was among the most unstable over time with regard to components of the zooplankton and phytoplankton total counts (Figure 3.3). This instability seems to have been reflected in the present study, with the Treatment 4 bacterial community being the most dispersed of all treatments over time. Although microbial samples from the predator treatment also deviated over time, these samples were clustered together, as the most diverse, most similar and least spatially dispersed samples over time (Figure 4.3). This suggests that the model apex-predators do indeed have far-reaching repercussions on the plankton community, ultimately affecting the microbial community diversity. This highlights the importance of "top-down" effects in controlling the microbial community structure within the estuary.

Despite the variability in the composition of the microbial community within the different treatments during the first 12 days of the experiment, by the end of the mesocosm experiment the microbial community within the different treatments were broadly similar (Figure 4.1). This was likely the result of an unidentified "bottom-up" process, such as that of increased substrate availability, offered by the walls of the mesocosms. Regardless of the identity or mechanism of the "bottom-up" processes that may have been in play, the presence of the predators did have an effect on the community, mediating and minimising the extent of the change over time. As such, consistent with our hypothesis, the microbial community was demonstrated to be affected by the presence of an apex-predator as samples from the predator treatment were more similar and less dispersed over time than those of the other treatments (Figure 4.3). Our findings are consistent with trophic cascade theory, whereby predators mediate interactions at lower trophic levels (Leibold 1996; Lynch and Shapiro 1981), with the subsequent repercussions for diversity.

CHAPTER 5.
RISK EFFECTS ON COPEPODS: EXPERIMENTAL EVIDENCE FOR THE SUPPRESSION OF CLUTCH SIZE BY PREDATORY EARLY-LIFE HISTORY FISH

"All nature wears one universal grin."

- Henry Fielding, Tom Thumb the Great, 1730

Plate 5: Female, egg-sac bearing *Pseudodiaptomus hessei* (Photo: Ryan Wasserman).

Abstract

Using egg numbers per clutch as a measure of potential reproductive output, the risk effects of zooplanktivorous early life-history fish on the calanoid copepod, *Pseudodiaptomus hessei*, were investigated in an *in situ* estuarine mesocosm experiment. Two treatments were established, both containing similar numbers of *P. hessei*. One treatment was stocked with zooplanktivores, while the other contained no added predators. Zooplankton samples were collected from each of the mesocosms at discrete time intervals over the course of 18 days, along with additional physico-chemical and biological information. Clutch size (number of eggs) was determined for all sampled egg-carrying *P. hessei* over the duration of the experiment. The average clutch size of fecund *P. hessei* females was consistently lower in the presence of predators, despite the elevated food resources (total Chlorophyll-*a* concentrations) available in this treatment. The present study highlights an alternative mechanism by which fish predators maintain copepod prey populations, consistent with risk-effects theory.

Introduction

The importance of predators in structuring biological communities through the control of prey abundance is now well recognised (e.g. May 1976; Estes *et al.* 2011). The impact of predators on prey numbers is a result of both direct predation and the indirect energy costs associated with anti-predator responses (Fraser and Gilliam 1992; Peckarsky *et al.* 1993; Boonstra *et al.* 1998; Creel and Christianson 2008), which include decreased survival, individual development and reproductive output of potential prey (Pangle *et al.* 2007; Ruxton and Lima 1997). The indirect predation impacts on prey, also known as risk effects or non-lethal effects, have been shown at times to be comparable to those of direct predation (Schmitz *et al.* 1997; Preisser *et al.* 2005; Creel and Christianson 2008).

The calanoid copepod, *Pseudodiaptomus hessei* is frequently a principal contributor to zooplankton communities in southern African estuaries, often dominating abundance and biomass in these systems (Wooldridge and Melville-Smith 1979; Isla and Perissinotto 2004). While the bottom-up effects on the energetics and secondary production of this species have been investigated (Isla and Perissinotto 2004; Jerling and Wooldridge 1991), the role of biological interactions, particularly predation, in structuring their populations is less well studied. The role of zooplanktivorous predators in southern African temporarily open/closed estuaries are, at least partially, filled by the early life-history fish (Whitfield 1998; Wasserman 2012). Here, a preliminary assessment of the risk effects of a zooplanktivorous early-life history fish on *P. hessei* is presented, using number of eggs per clutch as a measure of reproductive output.

Materials and methods

Experimental set-up

For this component of the study two experimental mesocosms treatments, of three replicates each were established. Estuarine water was collected into 100 L containers and gravity fed from the bow of a 3 m long boat by polyethylene hose into each mesocosm (Figure 2.3). For the first treatment, mesocosms were left free of any stocked predators (Treatment 1). For Treatment 2, however, mesocosms were stocked with natural densities (two individuals each) of a zooplanktivorous early life-history fish, the freshwater mullet, *Myxus capensis* (Valenciennes, 1836), Family Mugilidae (31.3 ± 1.72 mm total length).

Physico-chemical and biological sampling

Salinity, temperature (°C) and dissolved oxygen (mg/l) were recorded from each mesocosm and from the estuary, every third day, between 14:00 and 15:00 using an Aquaread Aquameter. Water samples were collected from each mesocosm and filtered through 0.22 µm filter syringes for dissolved nutrient examination. Using the method described by Bate and Heelas (1975), total oxidised nitrogen (TOxN) was determined via the reduced copper cadmium method while soluble reactive phosphorus (SRP) and ammonium (NH_4) were determined manually using standard colorimetric methods (Parsons *et al*. 1984). A 250 mL water sample was collected from each mesocosm to determine total Chlorophyll-*a* (Chl-*a*) concentrations. Each Chl-*a* water sample was filtered through a 0.7 µM filter, placed in 8 mL of 90% acetone at -20°C for 24 hours, after which concentrations were determined using fluorometry (Lorenzen 1966).

Zooplankton samples were collected every third day from each mesocosm after sunset via vertical tows using a 80 µm mesh size and 155 mm diameter WP-2 type net (filtering 26.43 L

water) with filtering proportions according to Sameoto *et al.* (2000). Zooplankton were identified and quantified at a broad taxonomic level (with the exception of *P. hessei*), using a Heerenburg dissecting microscope operated at × 400 magnification. From these samples, male, female and egg-sac bearing female *P. hessei* were identified and quantified, as were the egg numbers per clutch (egg-sac) for each relevant female.

Statistical analyses

Using STATISTICA (version 10, StatSoft Inc. 2011) and a significance level of $P < 0.05$, t-tests were employed to determine statistical differences between treatments for physico-chemical variables, total Chl-*a* concentrations and *P. hessei* abundances.

Results and Discussion

No significant differences ($P > 0.05$, df = 40) were found between treatments for temperature, salinity or dissolved oxygen. Furthermore, for the duration of the experiment, the estuarine water within each mesocosm was largely ambient, with trends reflecting those physico-chemical measurements recorded in the estuary (Figure 5.1). Similarly, no significant differences were found for any of the measured dissolved nutrient concentrations (SRP, TOxN or NH_4) between the two treatments ($P > 0.05$, df = 12). Ammonium concentrations were more variable, however, in the predator treatment (Treatment 2) (Figure 5.2). Total Chl-*a* concentrations in the two treatments were similar on experimental days 0 and 3, but thereafter, the highest concentrations were generally recorded in the treatment containing the fish, presumably a result of lower grazing pressure in this treatment. A notable exception was recorded on Day 9 of the study when the highest total Chl-*a* concentrations were recorded in Treatment 1 (Figure 5.1). However no statistical difference between overall Chl-*a* concentrations were found ($P = 0.42$, df = 40).

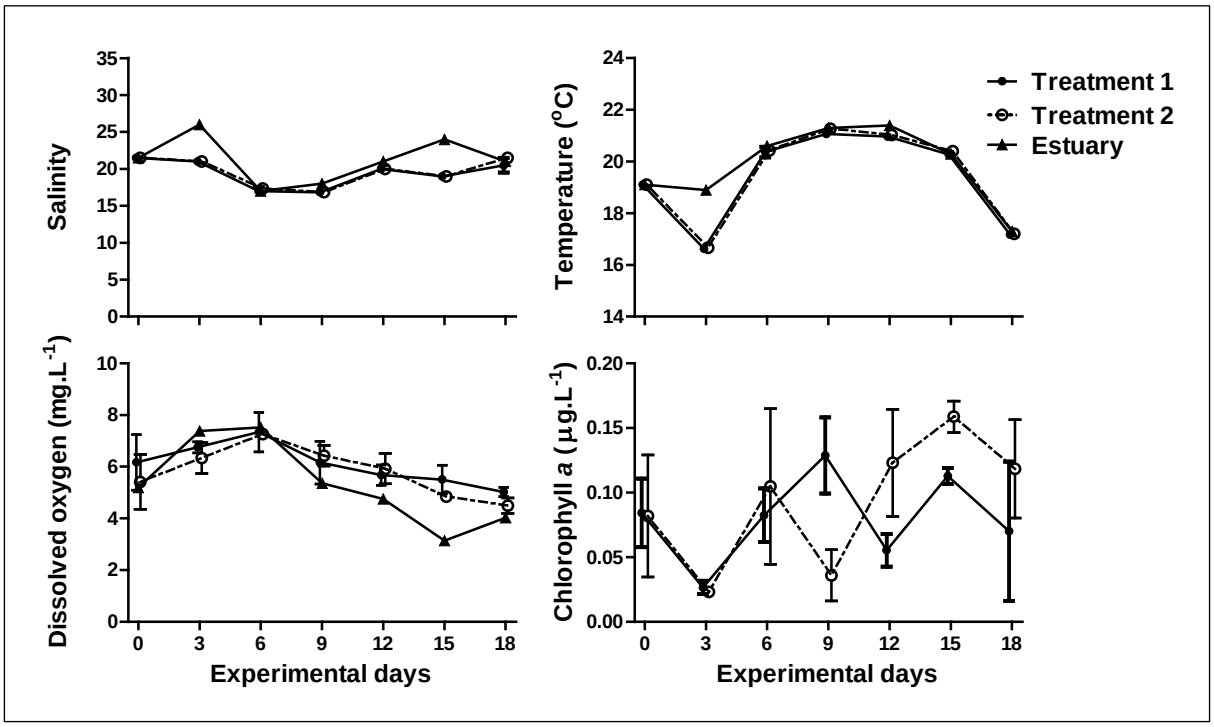

Figure 5.1: Mean ± standard deviation of physico-chemical measurements over time, from each *in situ* mesocosm treatment and from the estuarine waters at the experimental site.

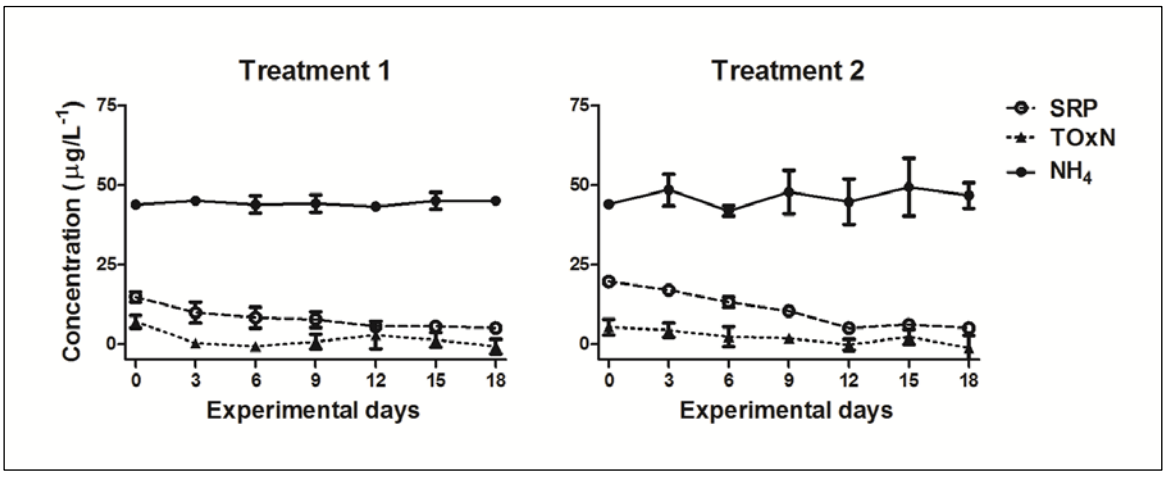

Figure 5.2: Mean ± standard deviation of dissolved nutrient concentrations from each treatment. Values derived from three replicate mesocosms per treatment. SRP = soluble reactive phosphorus, TOxN = total oxidised nitrogen, NH_4 = ammonium.

Furthermore, with the exception of sampling Day 9 (P = 0.01, df = 4) where concentrations were higher in Treatment 1, and Day 15 (P = 0.004, df = 4) with higher concentrations found in the predator treatment, no significant differences between Chl-*a* concentrations were found per sampling day (P > 0.05, df = 4 in each case). Of the zooplankton samples, overall polychaete, copepod and early life-history copepod numbers were much lower in the treatment containing the zooplanktivorous fish (Table 5.1). Crustaceans were dominated by the calanoid copepod, *Pseudodiaptomus hessei*, comprising 96.2% of the total adult crustacean catch and 96.6% of total copepod catch. Adult *P. hessei* numbers were also vastly different between treatments (P = 0.0001, df = 40), with much lower numbers collected from mesocosms containing the predators (Figure 5.3).

Table 5.1: Mean and standard deviation (SD) of zooplankton catches (number of individuals) per sampling day from Treatment 1 containing no stocked predators, and Treatment 2 containing stocked zooplanktivorous fish. Values derived from three replicate mesocosms per treatment.

	Treatment 1		Treatment 2	
	Mean	SD	Mean	SD
Polychaeta	651.4	44.1	67.3	9.9
Nauplii	536.0	122.2	83.6	8.1
Copepodites	136.8	45.4	24.8	7.7
Copepods (adults)	50.0	0.9	4.8	1.6
Other	0.1	0.2	0.1	0.2

Total Chl-*a* concentrations were atypically low in the mesocosms, reflecting the oligotrophic state of the estuary at this time. This was likely the result of a large flooding event, which flushed and drained the estuary just weeks before the study (author's pers. obs.). The experiment was only conducted once the estuary mouth closed, following this flooding event. This also possibly explains the dominance of *Pseudodiaptomus hessei* in the study as this species is often the first metazoan to pioneer estuarine habitats following stochastic flooding

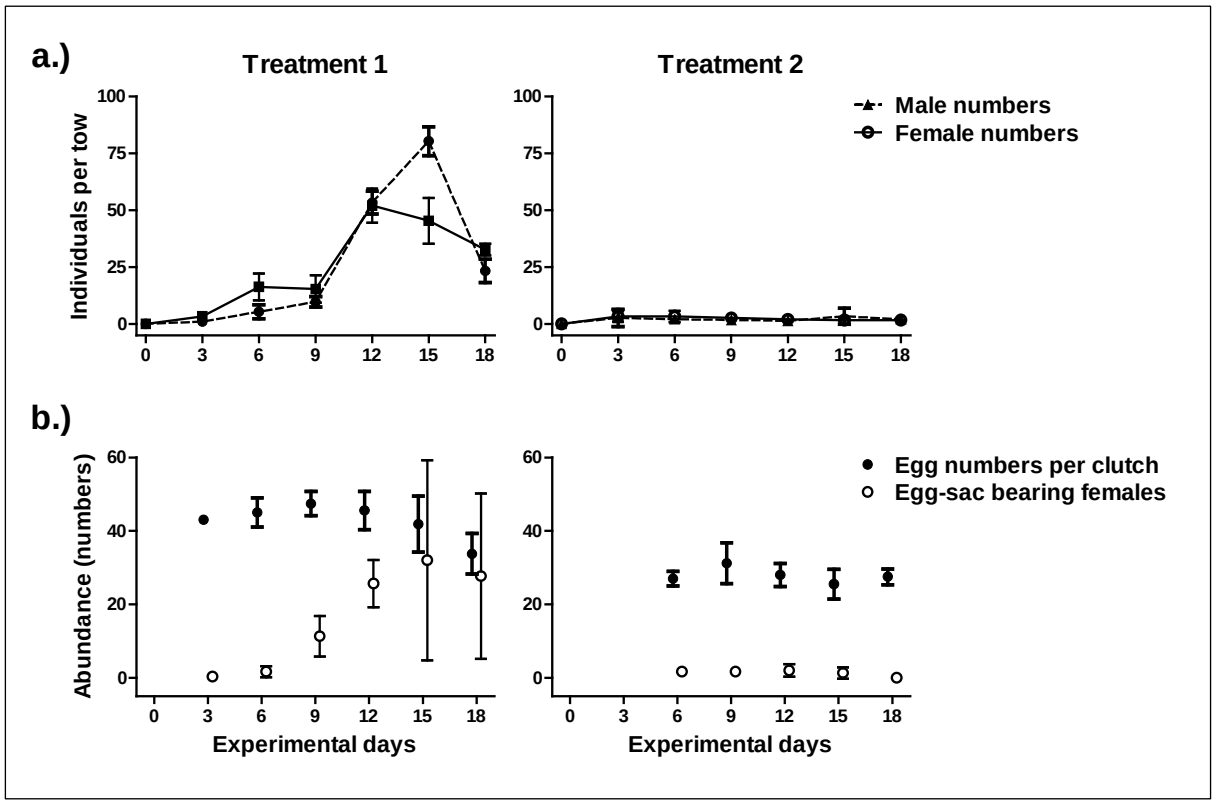

Figure 5.3: Mean ± standard deviation of a.) male (hatched line) and female (solid line) numbers as well as b.) egg-sac bearing female numbers (open circle), and egg numbers per clutch (closed circle) for each treatment over time. Values derived from three replicate mesocosms per treatment.

events (Wooldridge and Melville-Smith 1979). The abundances of *P. hessei* in the different treatments were similar at the commencement of the experiment (Figure 5.3a). Temporal patterns in *P. hessei* abundances in Treatment 1 (predator-free), however, increased considerably between days 3 and 15 only to decrease on Day 18. Conversely, in the treatments containing the fish (Treatment 2), copepod abundances were maintained at near-initial abundances for the duration of the investigation. Overall male to female ratios were similar between treatments (Treatment 1 = 1: 1.52, Treatment 2 = 1: 1.69), with more females recorded than males.

Egg-sac bearing females were recorded from Day 3 in Treatment 1 and from Day 6, in Treatment 2 (Figure 5.3b). Upon initial detection, both treatments contained similar numbers of egg-sac bearing females, but those in Treatment 1 generally increased in abundance over the course of the study, while those of Treatment 2 remained relatively consistent. The average number of eggs per female in Treatment 1 was generally higher than that of Treatment 2, but decreased after Day 12, likely the result of a resource limitation that occurred earlier in the experiment in Treatment 1. Like *P. hessei* numbers, the average number of eggs per female in Treatment 2 remained relatively and consistently low.

The impact of predators on the prey populations reflect direct effects and indirect risk effects (Creel and Christianson 2008; Estes *et al.* 2011). The direct effects of the early-life history fish on the abundances of the calanoid copepod, *P. hessei*, in Treatment 2 are clearly evident as abundances of male, female and egg-sac bearing females were maintained at near initial stock densities for the duration of the study (Figure 5.3). In contrast, in the absence of the fish, abundances of the copepod initially increased until Day 15 after which time they declined, presumably the result of a density dependent factor. In addition to decreased abundances of *P. hessei*, the presence of the early-life history fish seemed to contribute to a decline in clutch size (Figure 5.3). Physico-chemical characteristics were similar between the two treatments, implying that the observed effects were not driven by any measured bottom-up process. Ammonium concentrations, however, did fluctuate more in the predator treatment (Figure 5.2), but did not accumulate or deviate significantly from that of the predator-free mesocosms. Total Chlorophyll-*a* concentrations were generally lower in Treatment 1, likely a consequence of elevated grazing pressure due to the rapidly increasing number of zooplankton. Despite the reduced food availability, the reproductive output of *P. hessei* was generally higher in Treatment 1, suggesting that resource availability can be discounted as

contributing to the observed differences in reproductive output between the two treatments. However, within the predator-free treatment (Treatment 1), resource limitation presumably occurred after Day 12, when *P. hessei* abundances increased substantially, as clutch sizes were reduced.

While the clutch sizes of fecund female *P. hessei* sampled from the predator treatment were consistently lower than those sampled in the predator-free environment, these results need to be interpreted with caution owing to the low number of fecund females recorded in the predator treatment. Reduced clutch sizes in response to the presence of predators have, however, been reported for other species of calanoid copepods (Svensson 1997). What is less clear is the mechanism of predator recognition eliciting such responses. The identification and response to predators by prey is facilitated through varied sensory modes, including visual and tactile detection, and chemoreception (Boothby and Roberts 1995; Amo *et al.* 2004; Gonzalo *et al.* 2012).

Previous studies indicate that predator avoidance behaviour impacts on both the energetics and reproductive output of prey species (Fraser and Gilliam 1992; Boonstra *et al.* 1998). In addition, the act of mating is often associated with increased predation risk as copulating pairs are larger and more visible to predators than individual adults (Ward 1986; Maier *et al.* 2000). As such, it is proposed that the early life-history fish that utilise southern African estuaries as nursery areas contribute to the control of *P. hessei* numbers through both direct and indirect mechanisms of predatory control.

CHAPTER 6.
THREAT SENSITIVE PREDATOR AVOIDANCE BY AN ESTUARINE CALANOID COPEPOD: EVIDENCE OF POPULATION LEVEL EFFECTS OF CONSPECIFIC ALARM CUES

"Water is the driver of nature."

- Leonardo da Vinci

Plate 6: *In situ* study site (Photo: Ryan Wasserman).

Abstract

The present study aimed to assess the effects of conspecific chemical alarm cues associated with predation on population dynamics of the estuarine copepod *Paracartia longipatella* over a 12-day period. Using experimental *in situ* mesocosms, *P. longipatella* adult, copepodite and nauplii numbers were compared among three treatments; those inoculated with conspecific alarm cues, those containing direct predation pressure (zooplanktivorous fish), and those accommodating no predation threat. Population trends were similar between the direct predation and alarm cue treatments for the first six days of the experiment, decreasing in abundance. During the latter half of the study, however, *P. longipatella* abundances in the alarm cue treatment increased, while those in the presence of direct predation continued to decrease. In the treatment absent of any predation threat, *P. longipatella* abundances increased consistently over time for the duration of the study. It is suggested that the observed trends in the alarm cue treatment are a result of the prey organisms' ability to adjust their sensitivity to background levels of the perceived predation threat, which is consistent with the threat sensitive predator avoidance hypothesis.

Introduction

Prey organisms have been shown to identify predation threat in aquatic environments in a number of ways, including those of visual (Helfman 1989; Buskey and Hartline 2003), hydro-mechanical (Buskey *et al.* 2002) and chemical detection (Zhao and Chivers 2005; Kesavaraju *et al.* 2007; Ferrari *et al.* 2008). Predation risk identification is important for the successful evasion of predators by prey (Lima 1998; Brown and Chivers 2005), but threat-induced avoidance strategies can be energetically costly, altering behaviour (Mirza *et al.* 2006), affecting growth (Teplitsky *et al.* 2003) or even fecundity (Fraser and Gilliam 1992; Svensson 1997) of potential prey organisms. The persistence of each prey entity therefore lies in its ability to balance energetically costly predator avoidance and the required behavioural activities of foraging and kin interaction associated with ecological success (Ferrari *et al.* 2007). One way in which this can be achieved is through threat sensitive predator avoidance (Ferrari *et al.* 2008). The threat sensitive predator avoidance hypothesis predicts that prey response to predation threat is flexible (Helfman 1989). In this way, prey organisms can adjust their response to the intensity of a predatory threat thereby minimising unnecessary energy expenditure and optimising advantageous behaviour.

The purpose of the investigation was to assess the effects of a specific chemical cue on an estuarine copepod species within the context of the threat sensitive predator avoidance hypothesis. It has been shown across a variety of prey taxa that prey responding to predator cues elevate the intensity of their response to increased levels of the perceived threat (Ferrari *et al.* 2005; Zhao and Chivers 2005; Kesavaraju *et al.* 2007). Most of these studies have, however, been conducted over relatively short periods of time and have investigated the responses of individuals to varied concentrations of a given chemical cue (degree of threat). In the present study, however, responses to predation-event cues were investigated at the

population level. As such, copepods exposed to chemical cues were observed over a time period (13 days) long enough to detect changes at the population level (Chapter 3).

The focus of the present study is on the conspecific alarm cues and their effects on the population of calanoid copepods *Paracartia longipatella* (Acartiidae) (Connell and Grindley, 1974). Rather than employing a variety of cue concentrations, *P. longipatella* were exposed to a single concentration of alarm cue over an extended time period. Population numbers were compared over time, among a treatment inoculated with conspecific alarm cues, a treatment containing direct predation pressure, and one with a complete absence of any predation threat. It was postulated that, initially, exposure of the estuarine calanoid copepod to conspecific alarm cues would have similar repercussions at the population level to direct predation. Furthermore, it was postulated that given sufficient time, the response of the copepods to the alarm cues alone would diminish, as proposed by the threat sensitive predator avoidance hypothesis.

Materials and methods

Experimental set-up

This component of the study was conducted for 13 days, in austral summer. Water was collected from the estuary using 10 L buckets and transferred into each mesocosm (Figure 2.3) through a 1 mm sieve to ensure exclusion of large predatory zooplankters such as mysids and early life-history stage fish (Hansen *et al.* 1994). Three experimental treatments, of three replicates each were established. The first treatment employed estuarine water with no inoculation of alarm cues, or of zooplanktivorous predators to control zooplankton numbers. Treatment 2 was inoculated with alarm cue water (see below). Treatment 3 was stocked with zooplanktivorous young fish, *Monodactylus falciformis* (Lacèpede, 1800) (50 ± 0.6 mm TL;

4.1 ± 0.1 mm GW; 2.0 ± 0.3 g), at natural early life-history fish densities (2 per mesocosm) (Strydom *et al.* 2003). The fish were captured on site using a 15 m long, 1 mm mesh seine net on the first evening of the study (Day 0).

Conspecific alarm cue solution

A preliminary survey of the zooplankton community in the Kasouga Estuary revealed that *Paracartia longipatella* almost completely dominated the surface waters numerically (see Chapter 7). Conspecific alarm cues were therefore prepared from natural zooplankton captured one evening prior to experimental set-up. Zooplankton was collected from the estuary channel, after sunset using a boat-based plankton tow. A single WP-2 plankton net (570 mm mouth diameter, 0.2 mm mesh aperture) was towed for 5 minutes at a speed of 1-2 knots. The sample was fraction filtered through a 1 mm mesh sieve to remove larger unwanted organisms and then through 0.5 mm mesh to retain adult and copepodite stages of *P. longipatella*. The retained catch was immediately transported in estuarine water to the field station. Once in the field station the zooplankton sample used for alarm cue establishment was split using a Folsom plankton splitter. A 1/16 subsample of the zooplankton was preserved for identification and mass determination. The remaining zooplankton in the sample was used to establish the conspecific alarm cue solution.

Zooplankton for conspecific alarm cues were vacuum filtered (< 5cm Hg) onto a 20 µm filter. Using a ceramic pestle and mortar the sample was then emulsified (Wisenden and Millard 2001) and transferred to 75 L of estuarine water, collected from the site of experimental set-up (pre-strained through 20 µm mesh sieves). The estuarine water containing the emulsified zooplankton was homogenised, strained through GF/C (Whatman, glass microfiber) filters and frozen in 2 L aliquots at -20°C (Hylander *et al.* 2012). To rule out the confounding

factors associated with the supplemented water itself, a further 150 L of pre-strained (GF/C filtered) estuarine water, collected from the same site at the same time, but containing no added conspecific cues, was also frozen in 2 L aliquots at -20°C to be used in the non-chemical cue treatments (Treatments 1 and 3). Inoculation of mesocosms was done on a daily basis, with 2 L aliquots of non-chemical cue (Treatments 1 and 3), and chemical cue water (Treatment 2) added to each respective mesocosm.

Physico-chemical and biological sampling

From each mesocosm, daily records of temperature (°C), salinity and dissolved oxygen (D.O.) ($mg.L^{-1}$) were measured, while water samples for the determination of total Chlorophyll-*a* (Chl-*a*) concentrations were collected on Days 0, 6 and 12. For zooplankton collection, the entire water column of each mesocosm was sampled, through a vertical haul of a WP-2 type net with 80 µm mesh size and a 155 mm diameter on Days 0, 6 and 12, after sunset. Once in the laboratory, Chl-*a* concentrations were determined using spectrophotometry, following the method of Lorenzen (1966). Zooplankton samples were processed in their entirety, whereby organisms were identified to the lowest taxonomic level and life-history stage (Jerling and Wooldridge 1989; Boltovskoy 1999), and enumerated using a Wild M5A stereomicroscope operated at X 120 magnification.

Statistical analyses

Using the STATISTICA (version 10, StatSoft Inc. 2011) software package, one-way analysis of variance (ANOVA) tests were employed to assess differences between treatments for Day 0 measurements of temperature, salinity and D.O. Kruskal-Wallis tests (STATISTICA) were, however, employed to assess differences between treatments for starting (Day 0) values of Chl-*a*, and abundances of *P. longipatella*, as these data were non-parametric. For analysis of

these biological components over time, time was considered a covariate of abundance and therefore, an analysis of covariance (ANCOVA) test was selected to assess differences in overall abundances between treatments (Hamilton 1994). As the variance of the data was not homogeneous, differences were tested via a permutation procedure (pANCOVA). This was conducted using the ImPerm library package in R (v 3.0.1) (Wheeler 2010a; R Development Core Team 2013) via rank transformation of the data, overcoming variance heterogeneity (Kabacoff 2011). This analysis allowed for differences to be assessed at the treatment, time, and the treatment$^{\times}$time level.

Results

Using the 1/16 subsample of zooplankton for extrapolation, overall emulsified zooplankton concentration in the cue water, prior to GF/C filtering, was calculated as 264.8 mg/L. Adult and copepodite *Paracartia longipatella* completely dominated the sample, contributing ≈ 97.9% of the zooplankton biomass. As the mesocosm zooplankton samples were also dominated by the various life-history stages of the same copepods (Table 6.1), the chemical alarm cues extracted from the emulsified zooplankton represented conspecific cues (Wisenden and Millard 2001). Excluding the large numbers of unidentified calanoid copepod nauplii sampled from the mesocosms, *P. longipatella* completely dominated the overall mesocosm zooplankton catches, contributing 92.9%, 90.6% and 96.1% to the total zooplankton counts in treatments 1, 2 and 3, respectively. It was therefore presumed that similar proportions of the copepod nauplii belonged to this highly dominant copepod species (Table 6.1).

Estuarine water sampled from the mesocosms revealed no significant differences in initial

Table 6.1: Zooplankton abundances for each treatment, sampled from mesocosms during the study. Total represents the total number of organisms collected in the study. Range refers to the minimum and maximum numbers from each treatment. Life-history stage information presented where available.

	Treatment 1		Treatment 2		Treatment 3	
	Total	**Range**	**Total**	**Range**	**Total**	**Range**
Copepoda						
Paracartia longipatella	11911	556 - 2081	8082	605 - 1547	2611	0 - 798
P. longipatella copepodite	17953	783 - 3939	17858	462 - 5498	6795	23 - 1691
Nauplii	82732	624 - 25883	52051	231 - 22524	24849	61 - 8316
Pseudodiaptomus hessei	503	4 - 129	545	4 - 121	34	0 - 11
P. hessei copepodite	1506	4 - 549	1986	8 - 549	193	4 - 72
Euterpina acutifrons	57	0 - 15	45	0 - 15	49	0 - 19
E. acutifrons copepodite	15	0 - 11	19	0 - 8	0	-
Harpacticoid sp. 1	26	0 - 11	11	0 - 8	30	0 - 11
Harpacticoid sp. 2	0	-	0	-	4	0 - 4
Harpacticoid copepodite	11	0 - 8	23	0 - 8	11	0 - 11
Copepod sp. 1	4	0 - 4			0	-
Copepod sp. 2	0	-	4	0 - 4	0	-
Decapoda						
Hymenosoma orbiculare zoea	11	0 - 4	4	0 - 4	4	0 - 4
Amphipoda						
Amphipod sp.	4	0 - 4	0	-	0	-
Isopoda						
Exosphaeroma hylecoetes	4	0 - 4	8	0 - 4	8	0 - 4
Cirripedia						
Cyprid larvae	102	0 - 49	11	0 - 11	11	0 - 11
Balanoid nauplii	3	3 - 4	4	0 - 4	4	0 - 4
Polychaeta						
Prionospio sexoculata.	38	0 - 15	34	0 - 15	34	0 - 15
Diptera						
Chironomid sp.	4	0 - 4	0	-	0	-

temperature (df = 2, F = 1.5, $P > 0.05$), salinity (df = 2, F = 1.5, $P > 0.05$) or D.O. (df = 2, F = 0, $P > 0.05$) levels. Furthermore, while varying over time, they were similar among the treatments for the duration of the study (Figure 6.1). This was to be expected, as all mesocosms were filled with estuarine water at the same time, from the same site.

Similarly, initial differences in total Chl-*a* concentrations were not significant (df =2, H = 3.8, $P > 0.05$), as were initial stocked abundances of *P. longipatella* adult (df =2, H = 2.9, $P > 0.05$), copepodite (df =2, H = 6.0, $P > 0.05$) and calanoid copepod nauplii (df =2, H = 5.8, $P > 0.05$). Observed variations in population numbers of these organisms among treatments, over time, were therefore in response to the experimental manipulations rather than to any confounding bottom-up processes.

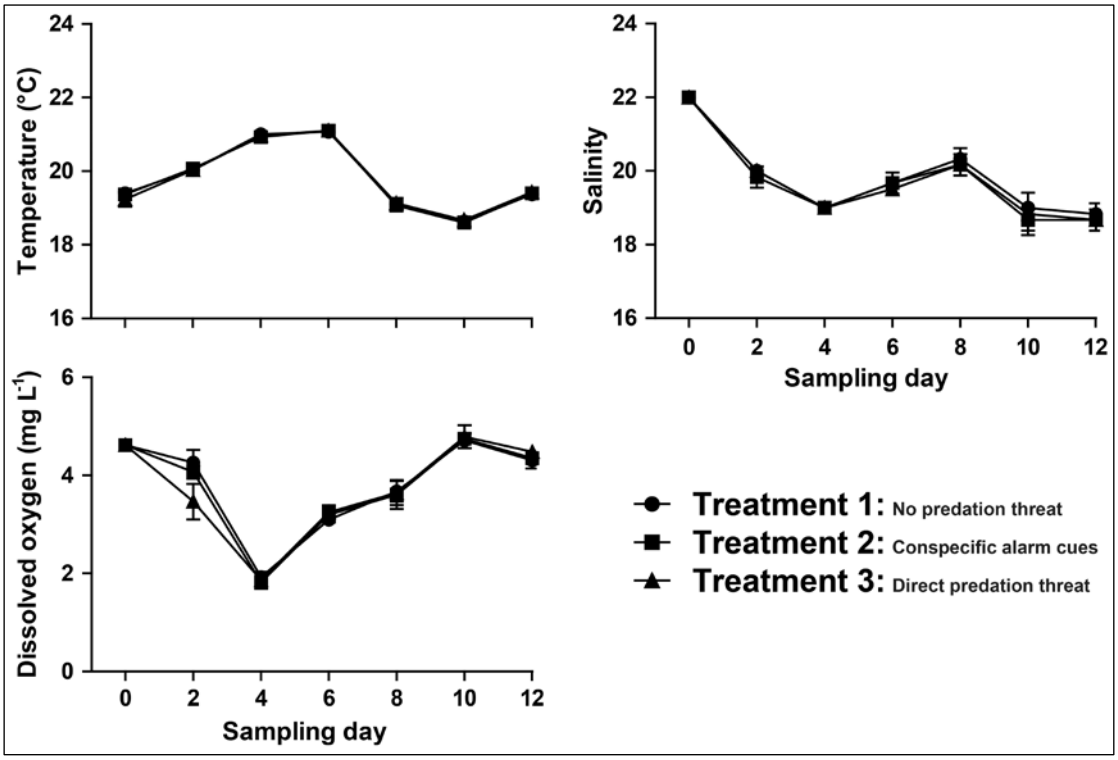

Figure 6.1: Temperature, salinity and dissolved oxygen measurements, recorded from each of the experimental mesocosms. Mean ± standard deviation of concentration values calculated from three replicates per treatment.

Variability among treatments in total Chl-*a* concentrations were not significant over the course of the study ($P > 0.05$) (Table 6.2). Differences among treatments were, however, observed in the key zooplankton components over the duration of the experiment (Figure 6.2). *Paracartia longipatella* adult numbers varied significantly over the duration of the study, differing among treatments, over time, and between treatment×time interactions (Table 6.2). In the predator-free treatment (Treatment 1), *P. longipatella* adult numbers increased between Day 0 and Day 6, while remaining relatively consistent between days 6 and 12 (Figure 6.2). *Paracartia longipatella* copepodites and copepod nauplii numbers also differed significantly over the course of the experiment (Table 6.2). However, the copepodites showed a consistent increase between days 0 and 12, while nauplii numbers increased by an order of magnitude between Day 0 and Day 6, only to decrease again by Day 12.

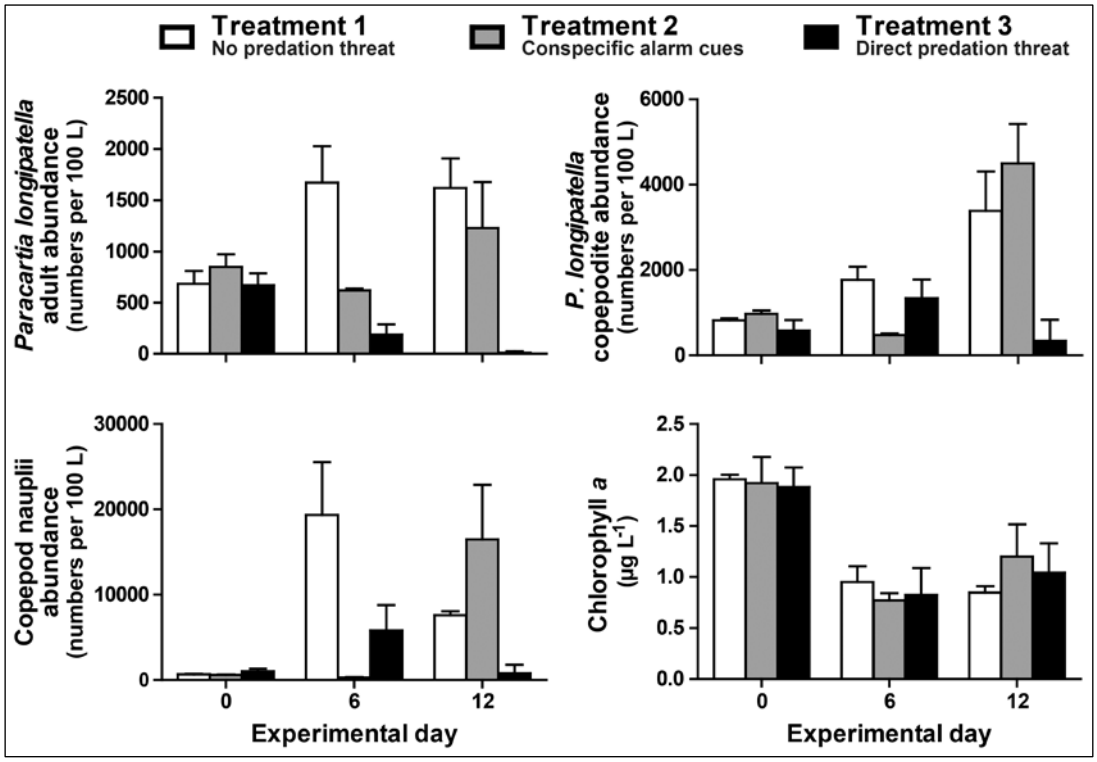

Figure 6.2: *Paracartia longipatella* adult and copepodite abundances as well as abundances of calanoid copepod nauplii sampled from each of the experimental treatments on sampling days 0, 6 and 12. Mean ± standard deviation values calculated from abundance data of three replicates per treatment.

Table 6.2: Analysis of covariance with permutations (pANCOVA) results for Chlorophyll-*a* (Chl-*a*) concentrations as well as adult and copepodite *Paracartia longipatella* and copepod nauplii abundances. RMS = Root Mean Square, *P* - perm = probability of significance (Wheeler 2010).

	Treatment		**Time**		**Treatment×Time**	
	RMS	***P* - perm**	**RMS**	***P* - perm**	**RMS**	***P* - perm**
Chl-*a*	0.009	-	1.214	-	0.116	-
Paracartia longipatella						
Adult	6713957	***	318926	*	2834895	***
Copepodite	9432424	***	27204818	***	7172836	**
Nauplii	205654926	**	336836110	***	61914388	-

*** = *P* < 0.05, ** = *P* < 0.01, *** = *P* <0.001**

Discussion

Adult *P. longipatella* numbers did not increase between Day 0 and Day 6 in the alarm cue treatment (Treatment 2), but increased considerably in the predator free treatment (Figure 6.2). These trends were observed in the *P. longipatella* copepodite and copepod nauplii numbers of treatments 1 and 2. It is highly likely that the observations in Treatment 2 were a response to the presence of conspecific alarm cues. Indeed, the initial demographic trends in Treatment 2 were more comparable to the predator treatment (Treatment 3) than to the predator-free treatment (Treatment 1). However, these similarities did not persist past Day 6. Between days 6 and 12, *P. longipatella* adult, copepodite and copepod nauplii numbers increased in the alarm cue treatment (Treatment 2), while these same groups decreased in the presence of direct predation (Treatment 3).

Differences in population trends between the predator-free (Treatment 1) and predator treatments (Treatment 3) support the expectations of typical predator-prey interaction outcomes whereby prey organisms increase in number in the absence of predators, but are maintained in the presence of predatory pressure (Beddington *et al.* 1976; Fey *et al.* 2009). In the mesocosms inoculated with conspecific alarm cues (Treatment 2), however, *P. longipatella* population numbers decreased for the first 6 days, despite the lack of any direct predation. In the latter half of the experiment, *P. longipatella* and copepod nauplii numbers increased in this same treatment. A possible explanation is that these prey organisms were able to adjust their responses to the threat, thereby minimising the associated indirect adverse effects. This would be consistent with the threat sensitive predator avoidance hypothesis postulated by Helfman (1989), as these prey organisms presumably altered responses to background levels of the perceived threat, becoming less sensitive to the inoculated conspecific alarm cues.

There is extensive evidence that indirect negative effects of predators on prey organisms can, at times, even be comparable to those effects of direct predation (Schmitz *et al.* 1997; Nelson *et al.* 2004; Pangle *et al.* 2007). While the mechanisms driving the observed trends in the *P. longipatella* abundances during the present investigation are unclear, studies have shown that predator-avoidance activities can have repercussions for the energetics and growth of prey species (Fraser and Gilliam 1992; Teplitsky *et al.* 2003; Mirza *et al.* 2006). Furthermore, these costs can even affect the reproductive output of organisms (Fraser and Gilliam 1992), as has been reported for similar species of calanoid copepods (Svensson 1997; results from Chapter 5). Another possible indirect mechanism of population control, mediated by the threat of predation, is that of the stimulation of resting-egg production. Studies have shown that the chemical cues associated with predation can result in increased production of resting eggs over non-resting eggs by female zooplankters (Ferrari *et al.* 2010). While this is well documented for daphniids (Ślusarczyk 1999), it has also been shown for certain cyclopoid and calanoid copepod species in freshwater habitats (Maier 1989; Hairston and Dillon 1990). Acartiid copepod species are known to produce resting and non-resting eggs (Belmonte and Puce 1994; Hairston *et al.* 1995), with *P. longipatella* being no exception (Deyzel 2012). The production of resting eggs by *P. longipatella* in response to the chemical cues may account for the reduction in hatched nauplii numbers on Day 6, in the conspecific alarm cue treatment in the present study. This is, however, speculation, as no studies, to the best of our knowledge, have investigated the effects of predation on the production of resting eggs in these, or any other marine copepod group.

As the actual chemical concentration of the alarm cue was not known, the alarm cue accumulation could not be measured in the study. Indeed, this is a problem that plagues marine chemical ecology studies as chemical cues are notoriously difficult to identify and

measure (Zimmer and Butman 2000). The response of *P. longipatella* to the perceived presence of predation threat does, however, suggest a degree of sensitivity to conspecific alarm cues. This sensitivity seems to be flexible, consistent with the threat sensitive predator avoidance hypothesis. While the effects of chemical cues associated with predation have been extensively studied on freshwater zooplankton (Maier 1989; Hairston and Dillon 1990; Ślusarczyk 1999; Ferrari *et al.* 2010), limited information is available on the marine and estuarine equivalents. Copepods in these habitats are indeed sensitive to chemical communication as these signals play a vital role in mate finding (Bagøien and Kiørboe 2005). As such, further studies exploring sensitivity to chemical cues from predators (kairomones), and chemical alarm cues from injured prey by marine and estuarine metazoans need to be conducted.

CHAPTER 7.
HYPER-BENTHIC AND PELAGIC PREDATORS REGULATE ALTERNATE KEY PLANKTONIC COPEPODS IN SHALLOW TEMPERATE ESTUARIES

"Water is H_2O, hydrogen two parts, oxygen one, but there is also a third thing, that makes it water and nobody knows what that is."

- D.H. Lawrence, Pansies, 1929

Plate 7: Juvenile Cape Moonfish (*Monodactylus falciformis*) and River Goby (*Glossogobius callidus*); alternate model zooplanktivorous predators (Photo: Ryan Wasserman).

Abstract

Although predation has been identified as an important community driver, the role of predator diversity in structuring estuarine zooplankton has not been assessed. As such the effects of two different zooplanktivorous fish species on the estuarine zooplankton community were investigated during a 12-day mesocosm study. Three experimental treatments were established whereby natural zooplankton communities were subject to either 1) no predatory pressure, 2) predation by a pelagic predator (*Monodactylus falciformis*) or 3) predation by a hyper-benthic predator (*Glossogobius callidus*). The pelagic *M. falciformis* largely fed on the numerically dominant mid-water copepod species, *Paracartia longipatella*. In contrast, the hyper-benthic fish had a greater predatory impact on the less numerically dominant copepod, *Pseudodiaptomus hessei,* which demonstrates strong diel vertical migration. Variations in prey population regulation are ascribed to the distinct behavioural differences of the predators, and mediated by the differences in behaviour of the copepod species.

Introduction

Predator contribution to structuring biological communities has received much attention (Polis 1994; Persson 1999; Estes *et al.* 2011). As almost all communities are comprised of a variety of predators, prey organisms are usually at risk from multiple predator species (Sih *et al.* 1998; O'Connor *et al.* 2008). Predatory species have distinguishable effects on lower trophic levels as they have evolved to occupy specific trophic niches within these habitats (Fretwell 1987; Chalcraft and Resetarits 2003; Schmitz 2007). Given the behavioural variability among species, potential prey organisms at a similar trophic level do not experience the same degree of predation risk when faced with a potential predator (Sih *et al.* 1998; Sokol-Hessner and Schmitz 2002; Schmitz *et al.* 2004; O'Connor *et al.* 2008). Similarly, predators of different identities pose varied levels of threat to specific prey organisms (Sih *et al.* 1998; Schmitz 2007). While much work has been conducted on these and other aspects of predator-prey interactions, the relevance and importance of these issues have received limited attention in certain environments (Sommer 2008; Estes *et al.* 2011).

In limnetic environments, diel invertebrate migrations appear to be an important mechanism for predator avoidance (Burks *et al.* 2002; Iglesias *et al.* 2007; Muška *et al.* 2013). In estuaries, diel vertical migration (DVM) has been identified as a significant behavioural feature for components of metazoan community (Hart and Allanson 1976; Fancett and Kimmerer 1985; Jerling and Wooldridge 1995b; Kouassi *et al.* 2001). Unlike those studies in freshwater habitats, the efficiency of this behavioural strategy in avoiding predation within shallow estuarine environments remains largely unknown.

Estuaries are important nursery and feeding areas for many fish species (Warlen and Burke 1990; Whitfield 1998; Wasserman and Strydom 2011). Among the most productive of all

ecosystems, these environments are of particular trophic interest, supporting high primary and secondary biomass (Houde and Rutherford 1993; Gobler *et al.* 2005). In estuaries, as in most marine environments, the mesozooplankton community is numerically dominated by copepods (Wooldridge and Melville-Smith 1979; Humes 1994; Isla and Perissinotto 2004; Montoya-Maya and Strydom 2009). These metazoans are trophically important, feeding on a wide range of micro- and meso-planktonic organisms and seston (Banse 1995; Buskey *et al.* 2011). Furthermore, they often comprise an important food source for many young and small fish species (Whitfield 1998; Wasserman 2012). As a consequence, mesozooplankton can be regarded as a key component of planktonic food webs. In shallow southern African estuaries, various metazoan species, including copepods, contribute considerably to both pelagic and hyper-benthic environments depending on the depth, site and time of day (Jerling and Wooldridge 1995b; Heyns and Froneman 2010). As such there is strong coupling between the pelagic and hyper-benthic zones (Perissinotto *et al.* 2003).

Zooplanktivorous fish have been shown to regulate invertebrate prey numbers in many aquatic environments (Brooks and Dodson 1965; Mehner and Thiel 1999). Indeed, zooplanktivorous predation in southern African estuaries is at least partly performed by young and small sized fishes (Whitfield 1998; Mehner and Thiel 1999). Using *in situ* mesocosms, the effects of two different zooplanktivorous fish species on the mesozooplankton community in a southern African estuary were investigated. *Monodactylus falciformis* (Lacèpede, 1800), Monodactylidae, feeds mainly mid water, while *Glossogobius callidus* (Smith, 1937), Gobiidae, forages primarily in the hyper-benthic zone (Whitfield 1998). Both species, overlapping in distribution, are abundant in the estuaries of the region (Whitfield 1998; Wasserman and Strydom 2011) and are therefore assumed to be major contributors to predatory pressure in their specific habitats. The experimental study was

conducted over 13 days, a time period sufficiently long enough to detect population level responses of the dominant metazoans to manipulations. Understanding the varied effects of predator species on lower trophic levels has implications for biodiversity conservation (Chave *et al.* 2002; Amarasekare *et al.* 2004), as activities that reduce or remove predators can have cascading effects on other organisms within a system (Persson 1999; Estes *et al.* 2011).

Materials and methods

Experimental set-up

As our study aimed to assess zooplankton community responses to different fish predators *in situ*, mesocosms (Figure 2.3) were filled with natural estuarine water collected on site (Figure 2.1). Operating from 2 m long inflatable boats, water was collected using 10 L buckets and immediately poured into each mesocosm through a 1 mm sieve to successfully exclude all large predatory zooplankters such as mysids and early life-history stage fish. Three treatments were established. The first treatment (T1) used estuarine water with no inoculation of an apex predator to control zooplankton numbers. Treatments 2 (T2) and 3 (T3) were, however, supplemented with the alternate predatory young fish stocked at natural pelagic early life-history fish densities (Strydom *et al.* 2003). Using a 15 m long, 1 mm mesh seine net, fish were captured on site and stocked, on the first evening of the study (Day 0). Two individual fish were used for each mesocosm. Fish of similar mass were selected, with total length (TL) and mouth gape width (GW) also determined for each individual (Wasserman 2012). In Treatment 2, the pelagic predator *Monodactylus falciformis* (50 ± 0.6 mm TL; 4.1 ± 0.1 mm GW; 2.0 ± 0.3 g) was added, while *Glossogobius callidus* (60.2 ± 0.9 mm TL; 4.5 ± 0.3 mm GW; 1.9 ± 0.1 g), the hyper-benthic predator was employed in Treatment 3.

Field sampling and laboratory analyses

Daily measurements of salinity, temperature (°C), and dissolved oxygen (D.O.) ($mg.L^{-1}$) were recorded in each mesocosm between 12:00 and 13:00 using an Aquaread Aquameter. For comparative purposes, estuarine water temperatures, salinities and D.O. levels were measured in parallel to those in the mesocosms. Water samples for the determination of dissolved nutrient (µM) and total Chlorophyll-*a* (Chl-*a*) concentrations were collected from a depth of 0.5 m, every third day (0, 3, 6, 9 and 12). Similarly, zooplankton samples were collected every third evening, after sunset, between 19:30 and 20:30 to capture those organisms that demonstrate diel vertical migrations. The entire water column was sampled for zooplankton in each mesocosm, through a vertical haul of a WP-2 type net with 80 µm mesh size and a 155 mm hoop diameter.

Water samples for the determination of dissolved nutrient concentrations were filtered through 0.22 µm filter syringes into polyethylene containers and frozen till analysis (within 2 weeks of collection). Using the method described by Bate and Heelas (1975), total oxidised nitrogen (TOxN) was determined via the reduced copper cadmium method while soluble reactive phosphate (SRP) and ammonium (NH_4) were determined manually using standard colorimetric methods (Parsons *et al.* 1984). Chlorophyll-*a* concentrations were determined from a 250 mL water sample collected from each mesocosm. Using vacuum filtration (<5 cm Hg), samples were filtered through 0.7 µm filters, and then placed in 8 mL of 90% acetone at -20°C in the dark for 24 hours. Chl-*a* concentrations were then determined using fluorometry following the method of Lorenzen (1966). In the laboratory, the entire zooplankton sample was processed, whereby organisms were identified to the lowest taxonomic level and life-history stage, and quantified using a Wild M5A stereomicroscope operated at × 12 magnification (Jerling and Wooldridge 1989; Boltovskoy 1999).

Data analysis

Using one-way analysis of variance (ANOVA) tests, differences between treatments for starting (Day 0) measurements of the physico-chemical variables, Chl-*a* and nutrient concentrations, and abundances of the dominant zooplankton group were assessed. For analysis of variables over time, however, specific tests were employed to cater for any autocorrelation associated with the lack of independence in the datasets (Nemec 1996). A repeated measures ANOVA and Tukey post-hoc analysis was therefore used for each of the physico-chemical variables (with time as the repeated measure), as this data did not violate any parametric assumptions. Each of the biological datasets, however, required the use of a repeated measures PERMANOVA, as variances in these data were not homogeneous (Anderson 2001; Kabacoff 2011; Holzmueller *et al.* 2012). Using the PERMANOVA procedure, data is assessed on a sample of possible permutations (Wheeler 2010b; Mos *et al.* 2011), whereby any variance heterogeneity is overcome (Kabacoff 2011). Time was also employed as the repeated measure using this analysis, with a Fisher LSD method used for pairwise multiple comparison procedure (post-hoc) investigation. All ANOVA tests were conducted in STATISTICA (version 10, StatSoft Inc. 2011, www.statsoft.com), while the PERMANOVAS were employed using the statistical software package R (v 3.0.1) (R Development Core Team 2013) and the "ImPerm" library (Wheeler 2010a).

Results

Temperature ($F = 10.8$, $P > 0.05$), salinity ($F = 1.0$, $P > 0.05$) and D.O. levels ($F = 10.8$, $P > 0.05$) demonstrated no significant differences among the various treatments at the commencement of the study (Table 7.1). Similarly, no effects were observed between interactions among the factors of treatment and time, for any of these physical measures (Table 7.1). These physical parameters therefore did not differ significantly between

treatments over the course of the study. Temperatures and salinities were relatively stable over the duration of the study, ranging from 18.3 to 21.2°C and 18 to 22 respectively (Figure 7.1). Dissolved oxygen levels, however, were more variable over time, dropping initially between Day 0 and Day 4, but increasing over the remainder of the experiment to near initial levels. Initial differences in SRP (F = 0.05, P > 0.05), NH_4 (F = 1.8, P > 0.05) and TOxN concentrations (F = 1.8, P > 0.05) were not significant on Day 0. However, unlike the physical measurements, a significant interaction was observed between treatment and time factors within each of these variables, highlighting the difference among treatments over time. Soluble reactive phosphates differed significantly between T1 and T2 on Day 6, while NH_4 levels differed significantly on Day 9, with T2 being higher than both T1 and T3. Total oxidised nitrogen differed significantly only at the end of the experiment, with T1 being greater than T2 and T3 (Table 7.2).

Initial Chl-*a* concentrations, ranging from 1.5 to 2.2 $\mu g.L^{-1}$, were similar across treatments (F = 2.3, P > 0.05). Significant interactions among treatment and time factors were, however, present, highlighting differences in the treatments over the duration of the study. (Mean Sq = 0.08, P < 0.01) (Table 7.1). Chl-*a* concentrations in all three treatments decreased initially, with T3 being significantly lower than both T1 and T2 on Day 3 (Table 7.2). Between Day 3 and Day 9, Chl-*a* concentrations increased steadily in Treatment 3, while decreasing steadily in Treatment 1. Treatment 2 demonstrated a significant increase in Chl-*a* concentration on Day 9 compared to T1 and T3 and remained at an elevated level until Day 12 (Figure 7.1) (Table 7.2).

Table 7.1: One way ANOVA results for Day 0 values, as well as repeated measure ANOVA and PERMANOVA results for time-series data. TOxN = total oxidised nitrogen, SRP = soluble reactive phosphate, NH_4 = ammonium, Chl-*a* = Chlorophyll-*a*, Overall abundance = total zooplankton numbers, Nauplii = copepod nauplii, F = F-stat (ANOVA), *P* = significance level (ANOVA), Mean Sq = Mean of Squares (PERMANOVA), *P* - perm = significance level (PERMANOVA).

	Day 0		**Temporal dataset**					
			Treatment		**Time**		**Treatment×Time**	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Temperature (°C)	10.8	-	20.0	**	859	***	2	-
Salinity	1.00	-	0.7	-	70.1	***	0.7	-
Dissolved oxygen ($mg.L^{-1}$)	10.8	-	7.0	-	251.0	***	2.2	-
TOxN (µM)	1.89	-	7.4	*	12.6	***	5.2	***
SRP (µM)	0.1	-	7.6	*	18.8	***	2.8	*
NH_4 (µM)	1.8	-	12.0	**	1.2	-	3.0	*
			Mean Sq	***P* -perm**	**Mean Sq**	***P* -perm**	**Mean Sq**	***P* -perm**
Chl-*a* ($\mu g.L^{-1}$)	2.3	-	0.5	**	6.887	***	1.1	**
Overall abundance	0.1	-	305779836	***	370009027	***	38057253	-
***P. longipatella* adult**	0.5	-	4236594	***	208680	**	393001	***
***P. hessei* adult**	3.6	-	8346.3	***	2104.2	***	1378.9	***
***P. longipatella* copepodite**	2.0	-	10593458	***	9510094	***	2184501	*
***P. hessei* copepodite**	1.1	-	96223	***	76795	***	22467	**
Nauplii	1.2	-	146947953	***	290450255	***	28266055	-

*** = *P*/ *P*-perm < 0.05, ** = *P*/ *P*-perm < 0.01, *** = *P*/ *P*-perm < 0.001**

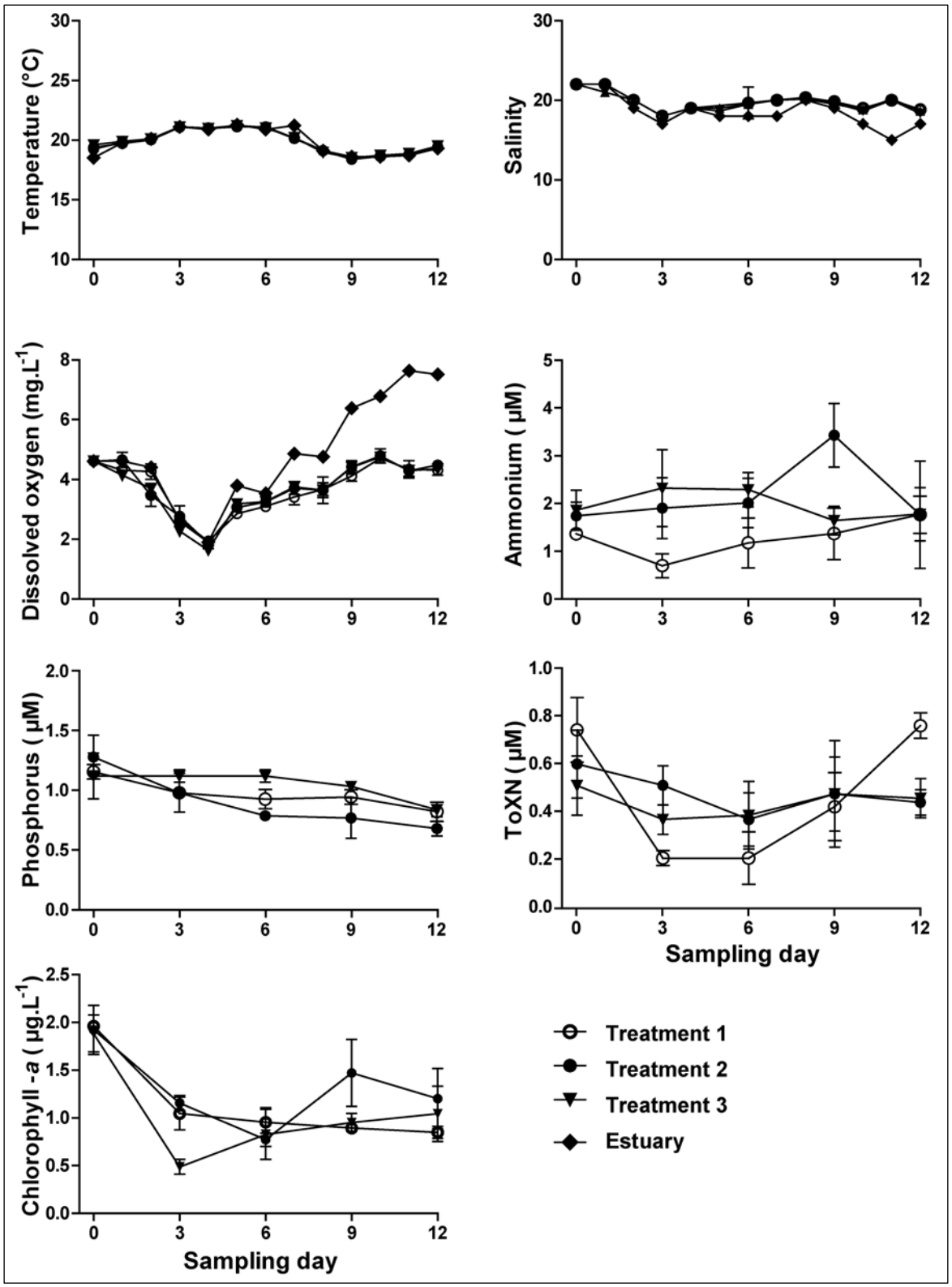

Figure 7.1: Physical recordings as well as nutrient and Chlorophyll-*a* concentrations measured from each of the experimental treatments. Mean ± standard deviation of concentration expressed for each variable. Measures calculated from three replicates per treatment.

No significant differences (F = 0.14, P = > 0.05) were found among treatments of Day 0 abundances for total zooplankton numbers. Furthermore, over the duration of the study overall zooplankton abundances were similar when assessing treatment×time interactions (Mean Sq = 38057253, P > 0.05), highlighting a lack of difference in total abundances between the treatments over time (Figure 7.2). Zooplankton samples yielded low taxonomic diversity, being dominated by the various life-history stages of copepods (Table 7.3). The various life-history stages of copepods dominated overall zooplankton abundances, contributing 99.8% to total numbers. Copepod nauplii contributed 64.5%, while large numbers of *Paracartia longipatella* copepodites (23.13%) and fewer *Pseudodiaptomus hessei* copepodites (1.7%) were recorded. The dominant adult copepods were *P. longipatella*, contributing 9.9%, followed by *P. hessei* (0.4%) of the total zooplankton counts. *Paracartia longipatella* and *P. hessei* contributed 94.9% and 3.9% toward overall adult copepod abundances, respectively. Numerous other invertebrates were found to occur in the mesocosms, but at very low numbers, with a combined numerical contribution of only 0.2% (Table 7.3).

When assessing abundances of each of the dominant contributors separately, differences in trends emerge across treatments. Of each of the dominant groups sampled from the mesocosms (Table 7.1), no significant differences (P > 0.05) were found between treatments for initial number of organisms (see Table 7.2 for relevant F statistics). Over the duration of the study, however, *P. longipatella* (Mean Sq = 393001, P < 0.001) and *P. hessei* adult numbers (Mean Sq = 1379, P < 0.001) differed significantly at the treatment×time level. *Paracartia longipatella* generally increased in abundance over time in the predator-free treatment but decreased in the presence of the mid-water predator *M. falciformis* (T2).

Table 7.2: Post-hoc results for repeated measures ANOVA (Tukey) and PERMANOVA (Fisher LSD) analyses. Each significant difference between treatment×time interactions presented within parenthesis. TOxN = total oxidised nitrogen, SRP = soluble reactive phosphate, NH_4 = ammonium, Chl-*a* = Chlorophyll-*a*, *P. longipatella* = *Paracartia longipatella*, *P. hessei* = *Pseudodiaptomus hessei*, ">" = greater than.

	Day 3	**Day 6**	**Day 9**	**Day 12**
			Treatment	
Tukey (ANOVA)				
TOxN (µM)	-	-	-	(1 > 2) (1 > 3)
SRP (µM)	-	(2 > 3)	-	-
NH_4 (µM)	-	-	(1 < 2) (2 > 3)	-
Fisher (PERMANOVA)				
Chl-*a* ($\mu g.L^{-1}$)	(1 > 3) (2 > 3)	-	(1 < 2) (2 > 3)	(1 < 2)
P. longipatella adult	(2 < 3)	(1 > 2) (2 < 3)	(1 > 2) (1 > 3) (2 < 3)	(1 > 2) (1 > 3) (2 < 3)
P. hessei adult	-	(1 > 3) (2 > 3)	(1 > 3) (2 > 3)	(1 > 2) (1 > 3) (2 > 3)
P. longipatella copepodite	-	(2 < 3)	(2 < 3)	(1 > 2) (2 < 3)
P. hessei copepodite	-	(1 > 3) (2 > 3)	(2 > 3)	(1 > 3) (2 > 3)

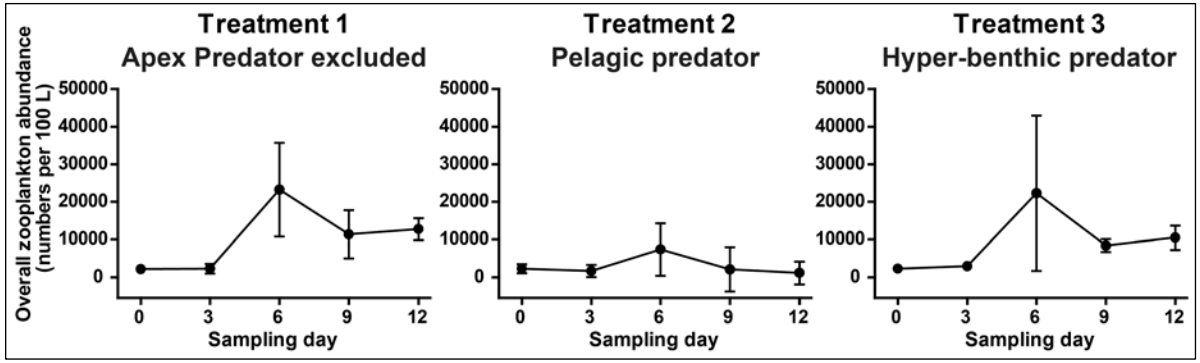

Figure 7.2: Total zooplankton abundances sampled from each of the experimental treatments over the 12-day period. Mean and standard deviation expressed per sampling day, as calculated from three replicates per treatment.

Table 7.3: Zooplankton overall abundances and range values sampled during the study. Life-history stage information presented where available. Total represents the total number of organisms collected in the study. Range refers to the minimum and maximum numbers sampled from during the study.

	Treatment 1		Treatment 2		Treatment 3	
	Total	**Range**	**Total**	**Range**	**Total**	**Range**
Copepoda						
Paracartia longipatella	4792	147 - 550	1041	0 - 211	3386	116 - 335
P. longipatella copepodite	7026	58 - 1041	2580	6 - 612	8458	78 - 1493
Pseudodiaptomus hessei	185	1 - 34	105	0 - 20	18	0 - 3
P. hessei copepodite	536	1 - 45	581	1 - 102	69	0 - 19
Euterpina acutifrons	18	0 - 4	12	0 - 3	2	0 - 1
E. acutifrons copepodite	4	0 - 3	1	0 - 1	2	0 - 1
Harpacticoid sp. 1	12	0 - 3	12	0 - 3	2	0 - 1
Harpacticoid sp. 2	0	-	2	0 - 1	2	0 - 2
Harpacticoid copepodite	5	0 - 2	4	0 - 3	7	0 - 3
Copepod sp. 1	0	-	1	0 - 1	1	0 - 1
Copepod sp. 2	2	0 - 1	0	-	0	-
Copepod sp. 3	1	0 - 1	0	-	0	-
Nauplii	28592	165 - 6841	7832	16 - 2198	24255	175 - 7718
Mysida						
Mesopodopsis wooldridgei	1	0 - 1	0	-	0	-
Decapoda						
Hymenosoma orbiculare zoea	4	0 - 1	2	0 - 1	0	-
Unidentified zoea	1	0 - 1	0	-	0	-
Amphipoda						
Amphipod sp.	1	0 - 1	0	-	0	0 - 1
Isopoda						
Exosphaeroma hylecoetes	3	0 - 1	3	0 - 1		0
Cirripedia						
Cyprid larvae	33	0 - 13	3	0 - 3	13	0 - 5
Balanoid nauplii	3	3 - 1	6	0 - 3	3	0 - 1

Table 7.3: (Continued)

	Treatment 1		Treatment 2		Treatment 3	
	Total	**Range**	**Total**	**Range**	**Total**	**Range**
Polychaeta						
Prionospio sexoculata.	14	0 - 4	11	0 - 4	8	0 - 2
Polychaete sp.	1	0 - 1	0	-	0	-
Diptera						
Chironomid sp.	1	0 - 1	1	0 - 1	1	0 - 1

As a result, all three treatments were significantly different from one another by Day 6, and remained so until the end of the experiment (Table 7.2). Similarly, adult *P. hessei* numbers were significantly different between treatments from Day 6 to Day 12 in the mesocosms (Table 7.2). These differences were, however, driven by alternate trends. Adult *P. hessei* numbers also increased over time in the absence of predation pressure (T1), but as opposed to adult *P. longipatella* abundances, did not decrease over the duration of the study in the pelagic predator treatment (T2). Instead, adult *P. hessei* numbers increased between Day 0 and Day 6 in T2, while decreasing over the reminder of the experiment. In contrast, adult *P. hessei* abundances were maintained at very low numbers in the hyper-benthic treatment (T3).

Of the copepodites, *P. longipatella* and *P. hessei* differed significantly at the treatment$^{\times}$time level (Mean Sq = 2184501, $P < 0.05$ and Mean Sq = 22467, $P < 0.01$ respectively), highlighting their differences between treatments over the course of the study. *Paracartia longipatella* copepodites showed similar trends to their adult counterparts in the absence of an apex predator (T1), generally increasing in the absence of the predation pressure. The same was not found in the presence of the pelagic predator (T2), where they remained relatively consistent over the 12 experimental days. In the presence of the hyper-benthic predators, however (T3), *P. longipatella* copepodites generally increased over time. Significant differences between T1 and T2 *P. longipatella* copepodite abundances were only observed by Day 12, however, while those of T3 were significantly greater than T2 from Day 6 onwards (Table 7.2). *Pseudodiaptomus hessei* copepodites increased initially between Day 0 and Day 6, in T1 and T2, only to decrease again by Day 9 in T1, while remaining at an elevated level in T2 until Day 12. Similar to those of the adult *P. hessei* abundances in T3, *P. hessei* copepodite abundances remained significantly lower for the duration of the experiment in this treatment (Table 7.2).

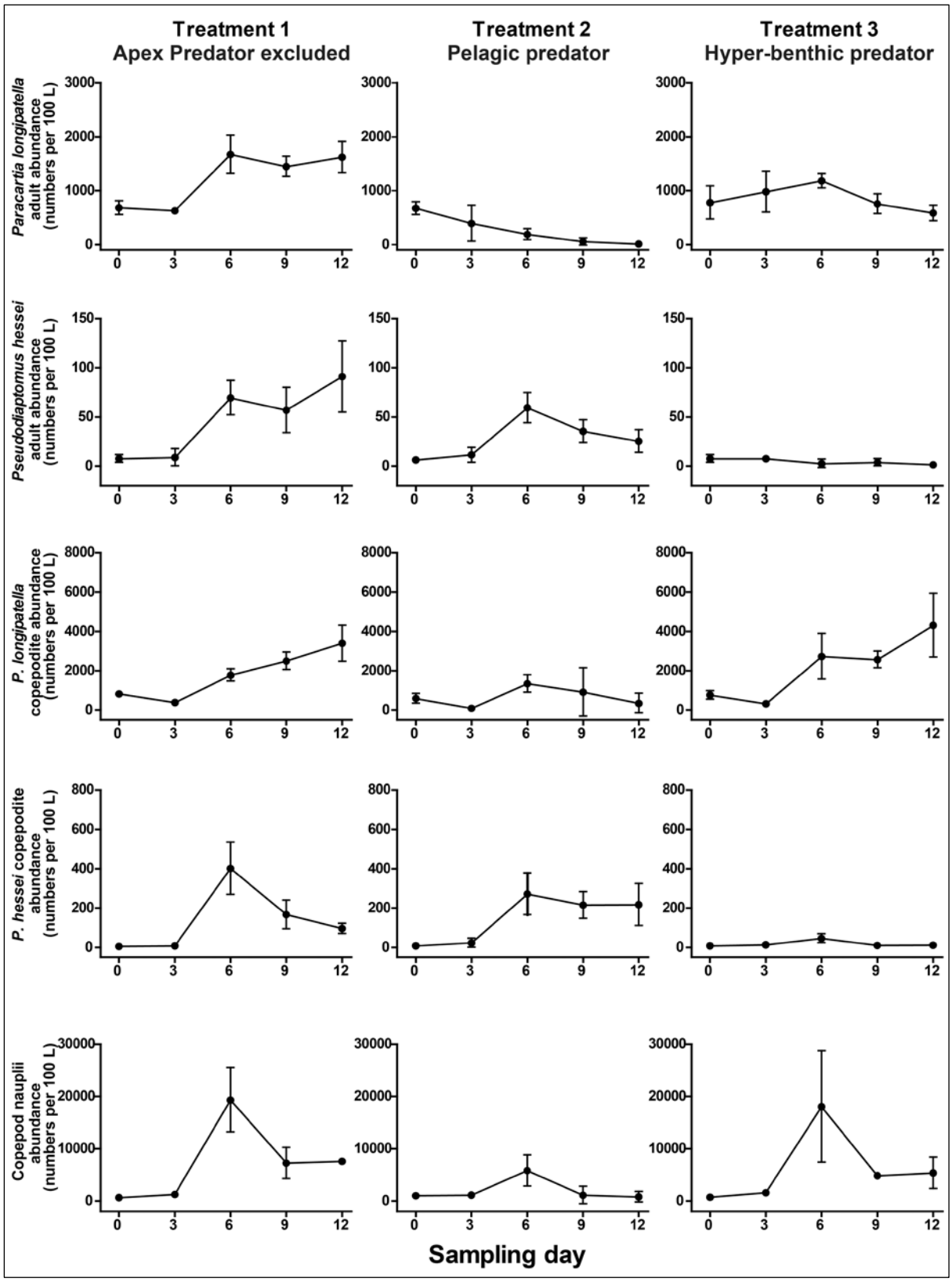

Figure 7.3: Dominant copepod taxa and life-history stage abundances sampled from each of the experimental treatments over time, contributing 99.8% of total catch. Mean and standard deviation expressed per sampling day, as calculated from three replicates per treatment.

Nauplii numbers were similar when assessing treatment×time interactions (Mean Sq = 28266055, $P > 0.05$), highlighting the lack of difference in total abundances between treatments over time. These copepod nauplii showed similar trends across treatments 1, 2 and 3, whereby numbers increased between Day 3 and Day 6, only to decrease again by Day 9. The increase between Day 6 and Day 9 was, however, less pronounced in the presence of the pelagic predator (T2) when compared to the other treatments.

Discussion

The absence of any significant differences in all the physical variables among the treatments during this investigation ($P > 0.05$) suggests that the observed change in the biology over time reflects the top-down effects. Dissolved nutrient concentrations were also similar on Day 0, but in contrast to the physical parameters, demonstrated changes over time. While the exact processes accounting for these differences remain unclear, they are likely driven by differences in trophic activities between the treatments. This has been well observed in other studies and as such, metazoans are considered key in the cycling and transfer of organic matter within aquatic ecosystems (Møller 2005; Saba *et al.* 2011). In this way, biological contributions to changes in water chemistry ultimately have repercussions for other biological organisms, including the zooplankton (Møller 2007). These results highlight the importance of biological interactions in the structuring of communities, an aspect often overlooked in marine planktonic studies, as bottom-up processes are usually investigated (Sommer 2008).

The low taxonomic diversity of the zooplankton recorded during the mesocosm study is consistent with previous studies conducted in TOCEs along the southern African coastline during the closed phase (Kibirige and Perissinotto 2003; Froneman 2004a). This can be ascribed to the virtual absence of marine species within the zooplankton community due to

the presence of a sandbar at the mouth, which separates the estuary from the sea for prolonged periods (Froneman 2002c). The numerical dominance of the zooplankton community by the various life-history stages of *Paracartia longipatella* and *Pseudodiaptomus hessei* in the mesocosms during the study is not surprising, given their abundance in estuaries of the region (Wooldridge and Melville-Smith 1979; Jerling and Wooldridge 1995b; Montoya-Maya and Strydom 2009).

Prey organisms typically increase in number in the absence of predators until some resource limitation is established (Beddington *et al.* 1976; Fey *et al.* 2009). This was indeed the case in the present study, whereby all major zooplankton components increased significantly over time in the absence of predation pressure (T1). Total zooplankton numbers in the predator-free treatment, largely driven by nauplii abundances, seemed to be controlled by resource limitation, as numbers increased between Day 0 and Day 6 only to decrease between Day 6 and Day 9. The trends in the predator treatments, however, were more complicated, with prey components responding to the predator species in different ways. The most numerically important adult copepod, *P. longipatella* was more affected by the pelagic than by the hyper-benthic predator, while for the less dominant adult *P. hessei* the inverse was true. The pelagic predator was also able to control *P. longipatella* copepodite numbers more efficiently than was the hyper-benthic gobiid. In contrast, *P. hessei* copepodite numbers were consistently lower in the presence of the hyper-benthic predator, while generally increasing over time when exposed to pelagic predatory pressure. While *Pseudodiaptomus* is considered a demersal copepod genus (Walter 1987), it still comprises an important part of the pelagic zooplankton community in shallow estuarine environments as it contributes considerably to the planktonic metazoan numbers (Froneman 2000; Pagano *et al.* 2003; Perissinotto *et al.* 2003). These copepods utilise estuarine surface waters under low light conditions, while

migrating to the hyper-benthic zone of the estuary during daylight hours (Jerling and Wooldridge 1995b; Kouassi *et al.* 2001). By contrast, *P. longipatella* activities are less regulated by light and are found within the water column throughout the diel period (Jerling and Wooldridge 1995b). Diel vertical migrations in zooplankton are thought to have evolved as a predator avoidance strategy (Fancett and Kimmerer 1985; Lampert 1989). Adults are typically found at depth during the day-time and in the surface waters at night-time when the majority of visual predators are presumably inactive. The observed increase in the total abundances of *P. hessei* in the treatment containing the pelagic predator highlights the effectiveness of this strategy in avoiding predation, as *M. falciformis* has been shown to be a diurnal feeder (Vumazonke *et al.* 2008). By contrast abundances of the mid-water copepod, *P. longipatella,* decreased in this treatment over the duration of the experiment. A different pattern emerges in the treatment containing the hyper-benthic predator, *G. callidus* which feeds both diurnally and nocturnally (Vumazonke *et al.* 2008). Here the copepodite abundances of the mid-water copepod, *P. longipatella* increased while *P. hessei* numbers remained at levels akin to those at the onset of the mesocosm experiment.

The behavioural differences of predators and the prey are an integral aspect of predator-prey dynamics. As much of the warm temperate South African estuarine habitat is relatively shallow (Whitfield 1998; Froneman 2002c; Wasserman *et al.* 2010; Wasserman and Strydom 2011), it is highly likely that the pelagic *Monodactylus falciformis* will on occasion forage in bottom waters. Similarly, *Glossogobius callidus* will likely emerge from the bottom waters to feed in the water column. The majority of their foraging is, however, likely to occur in the zones for which they are specialised. Such behavioural traits are important in trophic ecology as the degree of predation efficiency is often a function of the behaviour of both predator and prey (Warfe and Barmuta 2004).

While the experiment was only conducted for a short period of time, observations over the 13 days were sufficient to elucidate population level responses, at multiple life-history stages, for each dominant copepod species (see Chapter 3). Although mortality of zooplankton was likely associated with their stocking into the mesocosms, these factors can be considered consistent across the experimental treatments. The observed variation in response between these two key copepod species to the different zooplanktivorous fish highlights the significance of foraging activities of the predator and the behavioural activities of their prey in determining predator-prey outcomes. Overall, however, it appears that *M. falciformis* is a more efficient predator of all the life-history stages of copepods, as is evident from the decrease in the total numbers of zooplankton abundances within the treatment. Whether the reduction in copepod nauplii numbers in the pelagic predator treatment was a result of direct predation, or an indirect control measure linked to the lower numbers of reproducing adults, is unclear. It does, however, seem that the decrease in total zooplankton abundances within the pelagic predator treatment was coupled with an increase in the total Chl-*a* concentration. This result is consistent with the expectations of predator prey-cascades (Persson 1999; Estes *et al.* 2011) and highlights the importance of top-down control of phytoplankton production within the estuary.

Caution needs to be exercised when extrapolating these experimental results to the system level, as the mesocosms in the present study did not include representative benthic habitats which may have affected the outcome of certain interactions. However, the depth of our mesocosms was representative of vast portions of the Kasouga Estuary (Henninger *et al.* 2008), and prey population species were therefore able to demonstrate their natural DVM behaviour. In addition, both predatory model species (Strydom *et al.* 2003) and key prey copepod species are found in high numbers in the estuaries in the region (Jerling and

Wooldridge 1995b; Froneman 2000) and were stocked at natural densities. The observed differences in prey number trends over time, in response to the different predators, therefore highlights the role of behaviour of both predator and prey in determining the outcome of specific predator-prey interactions.

Niche-partitioning is a vital mechanism facilitating the stable coexistence of species competing for similar resources (Gilbert *et al.* 2008). Such partitioning is also present among predators and this consideration is important in the understanding of ecology at the community level, with implications for biodiversity conservation (Chave *et al.* 2002; Amarasekare *et al.* 2004). Investigations such as the present study, elucidating varied prey community responses to different predators, are useful in stressing the variability in trophic interactions. Such studies are scant in the estuarine and marine literature, when compared to freshwater research (Sommer 2008). The combined effects of both model predatory species simultaneously were not, however, assessed and compared. This work still needs to be conducted, as predation impacts by multiple predators do not necessarily combine additively (Griffen 2006). It is highly likely that inter-specific interference competition between predators will provide an additional dimension in predator-prey interaction variability (Dewitt *et al.* 1998; Sih *et al.* 1998) with subsequent effects on the other trophic levels.

CHAPTER 8.
CONCLUSION AND SYNTHESIS

"Nature is painting for us, day after day, pictures of infinite beauty."

- John Ruskin

Plate 8: Shallow sandy mouth region of the Kasouga Estuary, during a closed mouth period (Photo: Ryan Wasserman).

Introduction

In addition to the tight coupling between primary productivity and various physico-chemical processes, aquatic ecosystems are also strongly influenced by biological interactions, in particular, those exchanges between predators and their prey (Ivlev 1966; Sampson *et al.* 1993; Smetacek *et al.* 2004; Estes *et al.* 2011). Although these processes are well described in freshwater and marine systems, they have received limited attention in estuaries. The present study focused on predator-prey interactions in the estuarine planktonic community of an oligohaline, well mixed temporarily open/closed (TOCE) southern African estuary during the closed phase. The experimental procedures allowed for the adequate examination of key *in situ* biological interactions, while mitigating the confounding effects of environmental heterogeneity (Lauth *et al*. 1996; Spivak *et al.* 2011).

The thesis highlights the potential role of predatory zooplanktivores in structuring the estuarine plankton, emphasizing fundamental processes associated with predation events. The experimental components comprising the study were largely centered on the predation effects on selected prey populations within the planktonic community. In agreement with several previous studies (Wooldridge and Melville-Smith 1979; Montoya-Maya and Strydom 2009), copepods were the major component of the metazoan community and were therefore employed as prey organisms positioned at the highest trophic level. As such, the experiments were conducted over a period of time long enough to observe population level responses of these organisms, as the remaining organisms lower down the food web had faster life-history turn-over rates (Purvis *et al.* 2000). The experimental investigations addressed key issues associated with predation that have been well described and studied in numerous terrestrial and freshwater ecosystems but are largely lacking in estuarine habitats (Ferarri *et al.* 2007; Sommer 2008; Estes *et al*. 2011). The complementary data chapters through highlighting

important interactions therefore elucidate the role predators play in structuring estuarine planktonic communities under certain conditions. The present chapter synthesizes the discrete data sections and highlights the various relevant limitations. In conclusion, additional questions that emerged from the studies are raised and discussed.

Trophic cascading

The first experiment was centered on the notion of trophic cascading whereby predators affect not only their prey organisms but also organisms further down the food web (Paine 1980). Trophic cascade investigations are widespread, the results of which are often incorporated in ecosystem management (Paine 1966; Estes *et al*. 2011; see Table 1.1). Trophic cascade studies on planktonic communities are, however, often limited to either the so-called "classic food web", comprising zooplanktivore-zooplankton-phytoplankton interactions (Hansson 1992; Persson *et al*. 1992), or those interactions among microbial organisms in the so-called "microbial loop" (Fenchel 1982; Porter *et al*. 1985; Allan 2008; Allan and Froneman 2008). In addition to representing one of the first studies to examine trophic cascades in the estuarine plankton, the present study is one of the few experimental investigations which have considered the interactions between the classic food web and the microbial (Tranvik and Hansson 1997). The results of the thesis therefore highlight the potentially broad ecological impact of zooplanktivorous fish in estuaries. Since much of the zooplanktivorous predatory pressure is ascribed to early-life history fish in estuarine habitats (Whitfield 1998; Mehner and Thiel 1999), activities and perturbations that result in reductions or alteration in the abundances and biomass of these predators can have far reaching ecological implications within estuaries. Such findings are intuitive, as trophic cascade theory originates in the "community" paradigm whereby predators and their prey coexist in relative equilibrium (May 1976; Beddington *et al*. 1976). However, the empirical

assessments of such tropho-dynamics are often difficult to determine and have not been elucidated in all environments (Eveleigh *et al*. 2007). The present study therefore echoes the predictions by Sommer (2008), who postulated that planktonic trophic studies should not differ across aquatic environments. Indeed, estuarine planktonic food webs seem to respond to trophic cascades in a similar way to those of marine and freshwater systems (Carpenter *et al.* 1985; Stibor *et al.* 2004; Castilho-Noll and Arcifa 2007).

Although the precise pathway facilitating the trophic cascade in the present study could not be determined, a conceptual model of the food-web interactions between the main trophic groups was constructed (Figure 8.1). The trophic interactions between organisms in the model are based on those of similar organisms studied in other environments (see Chapter 3 for relevant interactions and references). Despite the lack of precise mechanistic information, the sum result of these complex interactions was manifested as a trophic cascade. Not only did the early life-history fish indirectly affect lower trophic levels, these repercussions were evident even at the bacterial base of the food web, a so-called “full cascade” (Sommer 2008).

The second component of the study highlighted the consequences of trophic level stability (at the broad taxonomic level) on species level diversity within the trophic guild. When comparing the PV and CV values calculated from enumerated bacteria using the direct counting method (Chapter 3) with the OTU data obtained from the pyrosequenced water samples (Chapter 4), it becomes evident that temporal stability in total bacterial counts seemed to affect bacterial diversity. It was therefore concluded that apex predatory pressure not only stabilises trophic levels, but that the relative stability of a given trophic level has implications for diversity within that guild.

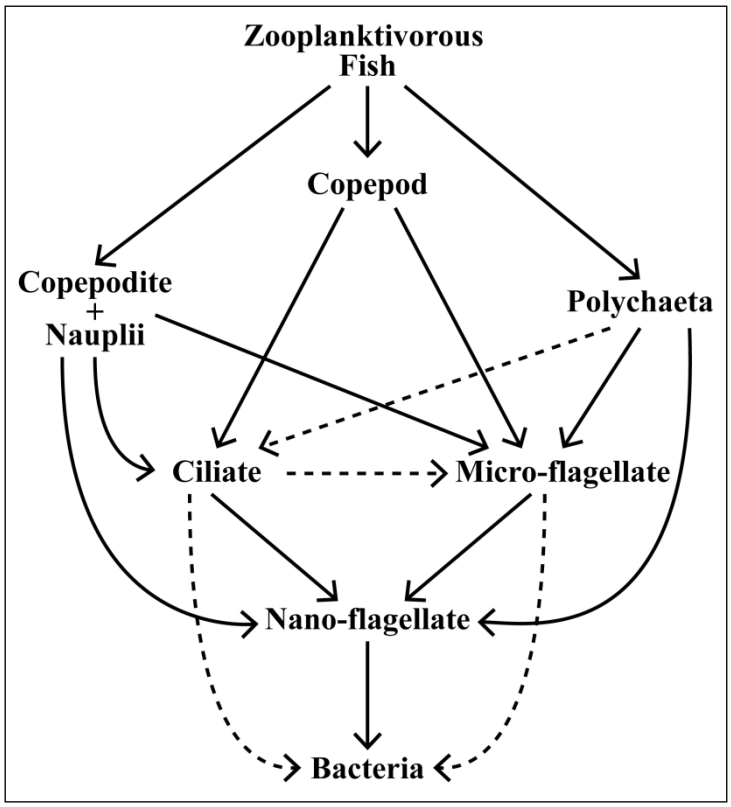

Figure 8.1: Conceptual model of trophic interactions between the various components of the experimental food web in the stable apex-predator treatment (Treatment 5, Chapter 3). Arrows depict direction of predatory pressure. Solid lines represent primary pressure and hatched lines represent secondary predation pressure.

These results are not unexpected as it has been suggested that within ecosystems the level of oscillation in community biomass or total abundance over time can be linked to species diversity with limited oscillation favoring the maintenance of diversity (Connell and Sousa 1983; Doak *et al.* 1998). Indeed, in the present study the treatment that produced the most deviation in bacterial diversity from the initial community was the same treatment that demonstrated the lowest stability (i.e. greatest oscillation) in overall bacterial abundances over time (see Figures 3.3 and 4.3). Such observations are often the result of competitive outcomes between species within a guild whereby in the absence of predatory pressure, competitively superior individuals dominate the community (Paine 1966). In this way

predators mediate competitive interactions by minimising the monopolization of resources by select species (Paine 1966).

While the effects of "top-down" pressure were evident for the bacterial community, potential sources of uncertainty must be raised. Firstly, there may have been bottom-up processes confounding the bacterial community dynamics within the mesocosms, therefore minimising the observed effects of the biological manipulations. Foremost amongst these was the possibility that the walls of the experimental enclosures (mesocosms) provided a substrate for bacterial settlement (Almeida *et al* 2001). The walls of the mesocosms offered unnaturally high settlement surface areas, potentially facilitating the dominance of certain substrate-associated species (Almeida *et al* 2001; Spivak *et al.* 2011), which may account for the overall similarity in bacterial community by the end of the 19-day experiment (Figure 4.3). The second and more serious limitation was that of the lack of replication. For this chapter, samples were collected from a single replicate for each treatment. Prohibitive budgetary constraints contributed to the lack of replication as pyrosequencing is an expensive analysis. The findings of the study, however, provide the necessary justification for a more thorough and robust assessment of the effects of zooplanktivores on bacterial diversity in the near future.

Non-consumptive effects

Once it had been established that apex-predation did indeed have implications for the entire food web, further studies on the specific non-consumptive impacts of predator-prey interactions were conducted. Non-consumptive predation effects refer to any effect, unrelated to direct capture and consumption, predators have on prey individuals or populations (Blaustein 1997). Non-consumptive predation effects on prey organisms have been observed

in many faunal groups across a range of environments (Schmitz *et al.* 1997; Preisser *et al.* 2005; Creel and Christianson 2008), but have never (to the best of the author's knowledge) been observed in estuarine metazoans.

Reduced fecundity is a well observed non-consumptive effect of predation risk on potential prey organisms (Svensson 1997; Lima 1998). The third experiment (Chapter 5) was therefore designed to assess the effects of predatory fish on the fecundity of an estuarine copepod species. The calanoid copepod *Pseudodiaptomus hessei* dominated estuarine metazoan counts during this component of the study, and as such was most represented in the mesocosms. As this copepod is an egg-sac carrier, the number of eggs per female could easily be quantified (Svensson 1997). The reduced clutch sizes observed in females exposed to direct predation was presumably in response to the risks related to predation pressure (Welton *et al*. 2003). Indeed, the energetic demands associated with detection and avoidance of predators have been shown to be considerable (Pangle *et al.* 2007; Ruxton and Lima 1997). Furthermore, these activities allow for less foraging time (Godin and Smith 1988; Welton *et al*. 2003). These principles seem to transcend taxonomic and environmental divides, having been highlighted in numerous vertebrate and invertebrate species across a range of habitats (Ward 1986; Peckarsky *et al.* 1993; Maier *et al.* 2000; Preisser *et al.* 2005; Pangle *et al.* 2007; Heath *et al. in press*). The selective pressures associated with non-consumptive and consumptive predatory effects on organisms make predation one of the most important and pervasive of the biological interactions (Kats and Dill 1998; Brown 2003).

For there to be indirect effects of predators on prey the identification of predation threat is pertinent, as this ensures prey responses which ultimately result in non-lethal effect (Gonzalo *et al.* 2012). The next experiment therefore delved into predation threat recognition, an area

of ecology that has received increased interest in recent years (Ślusarczyk *et al.* 2005; Ferrari *et al.* 2010; Santangelo *et al*. 2010; Gonzalo *et al*. 2012). Chemoreception is one of several ways in which predatory threat recognition by prey is facilitated (Boothby and Roberts 1995; Amo *et al.* 2004; Gonzalo *et al.* 2012). This is an important sensory mode in many invertebrates, including the copepods, as vision is often under-developed in comparison to the higher taxa (Bagøien and Kiørboe 2005). Ideally the copepod *P. hessei* should have been the focus of this study, as this model species was employed in the previous study investigating risk effects on clutch size. Unfortunately, when this particular field experiment was conducted, very few *P. hessei* were present in the estuary, with the metazoan community being almost entirely numerically dominated by another calanoid copepod, *Paracartia longipatella*. This necessitated the use of the latter species to investigate potential responses of estuarine copepods to conspecific alarm cues. Unfortunately, *P. longipatella* are not egg-bearing copepods, and release their eggs into the water column as they are produced (Deyzel 2012). As the number of eggs per clutch could not simply be counted as with *P. hessei*, abundances of the various *P. longipatella* life-history stages over time were enumerated. This approach gave an indication of population response to conspecific alarm cues at the demographic level, ultimately highlighting threat sensitivity in *P. longipatella*. The present study represents one of the first of its kind, addressing predation threat related chemo-reception in estuarine metazoans. This suggests that copepods in these habitats can detect conspecific cues associated with predation risk, as has been observed in daphniid (Ślusarczyk 1999) and copepod species in freshwater habitats (Maier 1989; Hairston and Dillon 1990). Chemoreception therefore needs to be considered as a predator avoidance mechanism in estuarine environments. This field of ecology is, however, still in its infancy in marine and estuarine environments, with much future work required (Hay 2009).

Predator diversity

The conduits for flow of organic matter in food webs are largely determined through partitioning of prey species by predators (Hansen *et al.* 1994). This partitioning is governed by a number of features invariably the result of an "evolutionary arms race" between predators and their natural prey (Dawkins and Krebs 1979; Brodie and Brodie 1999). It is thus incorrect to assume that organisms at a similar trophic level comprise a single functional ecological unit within ecosystems (Krupa and Sih 1998; Sih *et al.* 1998; Sokol-Hessner and Schmitz 2002; Schmitz *et al.* 2004; O'Connor *et al.* 2008).

Within this context, the last data chapter of the thesis assessed the impact of different prey species on the plankton community. The results of this investigation indicated that outcomes that ensued were ascribed to the distinct behavioural differences in foraging features of the predatory fish species. This was further mediated by the differences in behaviour of alternate copepod prey species, whereby the species that practise strong diel vertical migrations (DVM) were more susceptible to benthic foraging while those copepod species that did not practise DVM were more heavily preyed upon by the pelagic predator (Figure 8.2). These findings are consistent with similar studies conducted in other aquatic environments, whereby behavioural aspects of both the predator and prey determine predator-prey outcomes (Burks *et al.* 2002; Iglesias *et al.* 2007). Behavioural differences between species are, however, often overlooked when modelling the functional response of predatory consumption rates (Haddaway *et al.* 2012; Brachvogel *et al.* 2013; Dick *et al.* 2013). These difference need to be considered for the adequate prediction of impacts resulting from predator species loss, as this has implications for biodiversity conservation (Amarasekare *et al.*, 2004; Brachvogel *et al.* 2013; Haddaway *et al.* 2012; Dick *et al.* 2013; Heath *et al. in press*).

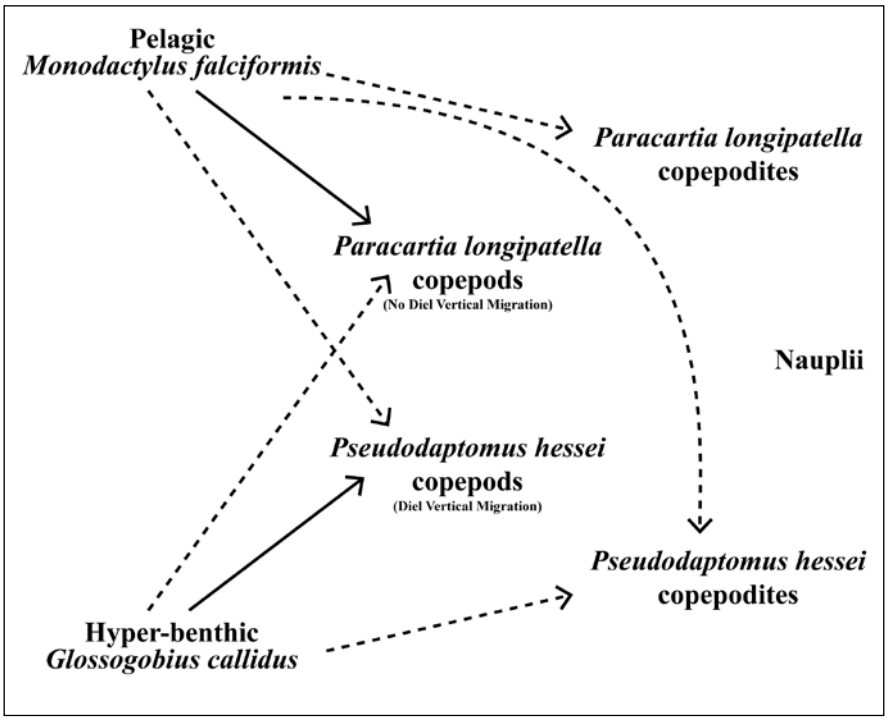

Figure 8.2: Conceptual model of trophic interactions between the two model predator species and the two dominant metazoan prey items (Chapter 7). Arrows depict direction of predatory pressure. Solid lines represent primary pressure and hatched lines represent secondary predation pressure.

Estuaries

Worldwide, estuaries are often highly modified (Morant and Quinn 1999; Nixon *et al.* 1986). South African estuaries are no exception. Despite the relatively low levels of industry along the coastline of South Africa, estuaries of the region are regarded as among the most ecologically threatened ecosystems (Morant and Quinn 1999; Mead *et al.* 2013). Estuarine perturbations of the region often arise as a result of alterations in freshwater inflow, which is likely to be exacerbated by population growth and the delivery of potable water (Schlacher and Wooldrige1996). These effects on estuaries are forecast to intensify with the onset of climate change (Christensen *et al.* 2007; Mead *et al.* 2013).

Climate-driven increases in wave action and reductions in freshwater inflow into estuaries are predicted with repercussions for the frequency and duration of mouth opening events in TOCEs (Adams and Bate 1994; New *et al.* 2006; Mead *et al.* 2013). While these predicted environmental changes will surely affect the estuarine biotic communities through various bottom-up processes (Schlacher and Wooldrige1996; Adams and Bate 1994), such changes are highly likely to have secondary effects on biological interactions. Since many marine fish species of the region access estuarine habitats during their vulnerable early-life history stages (Whitfield 1998), the facultative and obligatory utilisation of estuaries as nursery and refuge areas will be affected with connectivity changes between the marine and estuarine habitats (Mead *et al.* 2012). This will have trophic implications for planktonic communities as much of the zooplanktivorous pressure in estuaries is ascribed to the early life-history stages of fish (Griffiths 1999; Wisenden and Millard 2001). Such impacts are consistent with global predictions whereby habitat alteration associated with anthropogenic activities and climate change are forecast to have a greater impact on the higher trophic levels (Dulvy *et al.* 2004; Worsford *et al*. 2009 Estes *et al*. 2011). In estuarine environments the precise predictions of such outcomes on the lower trophic levels will be difficult to model given the paucity of information on biological interactions between specific species in these habitats (Heath *et al. in press*).

The relative scarcity of information pertaining to predator-prey interactions in the estuarine plankton, specifically in the southern African context, highlights the relevance of this thesis. Trophic interactions do indeed play an important role in structuring the estuarine plankton community, with apex predators affecting the prey populations as well as subsequent lower trophic levels as a result of trophic cascades. In addition, predators seem to select certain prey items over others, highlighting the importance of predator diversity in maintaining prey

communities. The various mechanisms by which apex predators control prey numbers include direct cropping of organisms, as well as non-consumptive effects associated with the mere presence of predation threat.

Future research

The greatest uncertainty when predicting repercussions of future perturbations on ecosystems is determining their effects on interactions among species (Stenseth and Mysterud 2002; Winder and Schindler 2004; Daufresne *et al.* 2009). This is particularly pertinent for those environments where limited baseline information is available regarding biological interactions. Additional studies on key biological interactions therefore need to be assessed in estuarine environments as information is generally lacking. Furthermore, future research on predator-prey interactions in the estuarine plankton should be conducted within the context of major perturbation predictions, linking climate change with other anthropogenic impacts on these ecosystems (Richardson 2008).

Mean atmospheric and water temperatures are predicted to be affected as climate change ensues (Mead *et al.* 2103). While temperatures largely determine global faunal distribution patterns (Krebs 1994; Richardson 2008), a more subtle effect of this important physical feature is its consequence on the body size of organisms. James' population body size shift hypothesis states that an increase in temperature should facilitate a decrease in the body size of organisms within a population (Daufresne *et al.* 2009). As body size is one of the most important characteristics for the determination of an individual's position and role in an ecosystem, shifts in body size in response to changing temperature are likely to affect community dynamics (Arendt 2007). Indeed, it has long since been recognised that predator-prey interactions in the plankton are largely size dependent (Hansen *et al.* 1994). Predicted

reductions in plankton body size will therefore likely have implications for biological interactions between planktonic organisms (Moore and Folt 1993; Richardson 2008; Daufresne *et al.* 2009). Such effects are, as of yet, largely unexplored in aquatic ecosystems.

In addition to the effect of climate change on global distributions of planktonic organisms, food-web structure could be dramatically altered if interacting species differ in phenological response to changing climate (Stenseth and Mysterud 2002; Winder and Schindler 2004). The effects of life-cycle shifts by key organisms in relation to changing environmental conditions have been shown to uncouple certain trophic relationships (Durant *et al.* 2007). The importance of phenological related trophic information within the context of climate change forecasts has received limited attention in terrestrial, freshwater and marine environments (Visser and Holleman 2001; Winder and Schindler 2004; Durant *et al.* 2007), and has never been assessed at all (to the best of the author's knowledge) in shallow estuarine habitats.

A further future environmental concern is the issue of coastal eutrophication (Gowen *et al.* 1992; Cloern 2001; Richardson 2008). Estuaries are natural nutrient traps (Morant and Quinn 1999; Nixon *et al.* 1986) with forecasts of increased eutrophication levels predicted for estuaries and coastal areas worldwide (Costanza *et al.* 1997; Mead *et al.* 2013). The effects of eutrophication on plankton predator-prey dynamics is well studied in freshwater environments (Pogozhev and Gerasimova 2001; Chase *et al.* 2003; Romo *et al.* 2004; Vakkilainen *et al.* 2004) but has received limited attention in estuarine habitats (Table 1.1). Through manipulative fertilisation of mesocosm enclosures, the relative strength of cascades could be measured and compared across productivity gradients, highlighting the relative contributions of top-down and bottom-up forces in structuring planktonic communities.

A better understanding of species specific information is required, as many current ecosystem models simplify complex problems by employing a functional group approach to defining trophic interactions (Richardson 2008). The assessment of potential shifts in biological interactions, in response to predicted anthropogenic and environmental perturbations will be invaluable for climate-change prediction models as little information is currently available within this context. Experimental field and laboratory studies will be crucial in this regard, allowing for simulation of scenarios representative of perturbation predictions (Lawton 1995; Benton *et al*. 2007).

LIST OF REFERENCES

Adams, J. B. (*in press*). A review of methods and frameworks used to determine the environmental water requirements of estuaries. *Hydrological Sciences Journal* - doi:10.1080/02626667.2013.816426.

Adams, J. B. and Bate, G. C. (1994). The tolerance to desiccation of the submerged macrophytes *Ruppia cirrhosa* Petagna (Grande) and *Zostera capensis* Setchell. *Journal of Experimental Marine Biology and Ecology*, **183**, 53-62.

Adams, J. B., Bate, G. C., Harrison, T. D., Huizinga, P., Taljaard, S., Van Niekerk, L., Plumstead, E.E., Whitfield, A.K. and Wooldridge, T. H. (2002). A method to assess the freshwater inflow requirements of estuaries and application to the Mtata Estuary, South Africa. *Estuaries*, **25**, 1382-1393.

Addicott, J. F (1974). Predation and prey community structure: an experimental study of the effect of mosquito larvae on the protozoan communities of pitcher plants. *Ecology*, **55**, 475-492.

Allan, E. L. (2008). Ecological role of free-living bacteria in the microbial food web of the temporarily open/closed East Kleinemonde Estuary, South Africa. PhD Thesis. Rhodes University, Grahamstown.

Allan, E. L. and Froneman, P. W. (2008). Trophic interactions amongst the plankton in the temporarily open/closed East Kleinemonde Estuary, South Africa. *African Journal of Aquatic Science*, **33**, 167-174.

Allanson, B. R. (2001). Some factors governing the water quality of microtidal estuaries in South Africa. *Water SA*, **27**, 373-386.

Allanson, B. R., Baird, D. and Heydorn, A. E. (1999). Perspectives. In Allanson, B.R. and Baird, D. (eds), *Estuaries of South Africa*. Cambridge University Press, Cambridge, pp. 321-327.

Allanson, B. R. and Read, G. H. L. (1995). Further comment on the response of Eastern Cape Province estuaries to variable freshwater inflows. *South African Journal of Aquatic Science*, **21**, 56-70.

Almeida, M.A., Cunha, M.A. and Alcântara, F. (2001). Factors influencing bacterial production in a shallow estuarine system. *Microbial Ecology*, **42**, 416-426.

Amarasekare, P., Hoopes, M.F., Mouquet, N. and Holyoak, M. (2004). Mechanisms of coexistence in competitive metacommunities. *The American Naturalist*, **164**, 310-26.

Amo, L., López, P. and Martín, J. (2004). Wall lizards combine chemical and visual cues of ambush snake predators to avoid overestimating risk inside refuges. *Animal Behaviour*, **67**, 647-653.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26**, 32-46.

Andersson, A. J., Kuffner, I. B., Mackenzie, F. T., Jokiel, P. L., Rodgers, K. S. and Tan, A. (2009). Net loss of $CaCO^3$ from a subtropical calcifying community due to seawater acidification: mesocosm-scale experimental evidence. *Biogeosciences*, **6**, 1811-1823.

Arendt, J. (2007). Ecological correlates of body size in relation to cell size and cell number: patterns in flies, fish, fruits and foliage. *Biological Reviews*, **82**, 241-256.

Bagøien, E. and Kiørboe, T. (2005). Blind dating-mate finding in planktonic copepods. I. Tracking the pheromone trail of *Centropages typicus*. *Marine Ecology Progress Series*, **300**, 105-15.

Banse, K. (1982). Experimental marine ecosystem enclosures in a historical perspective. In Grice, G. D. and Reeve, M. R. (eds), *Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems*. Springer-Verlag, New York, pp. 1-24.

Banse, K. (1995). Zooplankton: Pivotal role in the control of ocean production: I. Biomass and production. *ICES Journal of Marine Science: Journal du Conseil*, **52**, 265-277.

Bate, G. C. and Heelas, B. V. (1975). Studies on the nitrate nutrition of two indigenous Rhodesian grasses. *Journal of Applied Ecology*, **12**, 941-952.

Bate, G. C., Whitfield, A. K., Adams, J. B., Huizinga, P. and Wooldridge, T. H. (2004). The importance of the river-estuary interface (REI) zone in estuaries. *Water SA*, **28**, 271-280.

Beamish, C. A. (2002). The avian population dynamics of the Kasouga Estuary, with particular reference to the piscivores. Honours thesis, Rhodes University, South Africa.

Beddington, J. R., Free, C. A. and Lawton, J. H. (1976). Concepts of Stability and Resilience in Predator-Prey Models. *Journal of Animal Ecology*, **45**, 791-816.

Belmonte, G. and Puce, M. (1994). Morphological aspects of subitaneous and resting eggs from Acartia josephinae (Calanoida). In Ferrari, F. and Bradley, B. (eds), *Ecology and Morphology of Copepods Vol. 102*, Springer, Netherlands, pp. 131-135.

Benton, T. G., Solan, M., Travis, J. M. T. and Sait, S. M. (2007). Microcosm experiments can inform global ecological problems. *Trends in Ecology and Evolution*, **22**, 516-521.

Berlow, E. L., Navarrete, S. A., Briggs, C. J., Power, M. E. and Menge, B. A. (1999). Quantifying variation in the strengths of species interactions. *Ecology*, **80**, 2206-2224.

Blaustein, L. (1997). Nonconsumptive effects of larval *Salamandra* on its crustacean prey: can eggs detect predators? *Oecologia*, **110**, 212–217.

Blaustein, L., Friedman, J. and Fahima, T. (1996). Larval Salamandra drive temporary pool community dynamics: evidence from an artificial pool experiment. *Oikos*, **76**, 392-402.

Boltovskoy, D. (1999). *South Atlantic Zooplankton*. Backhuys, California.

Boonstra, R., Hik, D., Singleton, G. R. and Tinnikov, A. (1998). The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs*, **68**, 371-394.

Boothby, K. M. and Roberts, A. (1995). Effects of site of tactile stimulation on the escape swimming responses of hatchling *Xenopus laevis* embryos. *Journal of Zoology*, **235**,113-125.

Borer, E. T., Seabloom, E. W., Shurin, J. B., Anderson, K. E., Blanchette, C. A., Broitman, B., Cooper, S.D. and Halpern, B. S. (2005). What determines the strength of a trophic cascade? *Ecology*, **86**, 528-537.

Bornman, T. G., Adams, J. B. and Bate, G. C. (2002). Freshwater requirements of a semi-arid supratidal and floodplain salt marsh. *Estuaries*, **25**, 1394-1405.

Boveri, M. B. and Quiros, R. (2007). Cascading trophic effects in pampean shallow lakes: results of a mesocosm experiment using coexisting fish species with different feeding strategies. *Hydrobiologia*, **584**, 215-222.

Brachvogel, R., Meskendahl, L., Herrmann, J. P. and Temming, A. (2013). Functional responses of juvenile herring and sprat in relation to different prey types. *Marine biology*, **160**, 465-478.

Brett, M. T. and Goldman, C. R. (1996). A meta-analysis of the freshwater trophic cascade. *Proceedings of the National Academy of Sciences*, **93**, 7723-7726.

Brodie, E. D. and Brodie, E. D. (1999). Predator-prey arms races. *Bioscience*, **49**, 557-568.

Broglio, E., Saiz, E., Calbet, A., Trepat, I. and Alcaraz, M. (2004). Trophic impact and prey selection by crustacean zooplankton on the microbial communities of an oligotrophic coastal area (NW Mediterranean Sea). *Aquatic microbial ecology*, **35**, 65-78.

Brönmark, C. and Hansson, L. A. (2000). Chemical communication in aquatic systems: an introduction. *Oikos*, **88**, 103-109.

Brooks, J. L. and Dodson, S. I. (1965). Predation, body size, and composition of plankton. *Science*, **150**, 28-35.

Brown, G. E. (2003). Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries*, **4**, 227-234.

Brown, G. E. and Chivers, D. P. (2005). Learning as an adaptive response to predation. In Barbosa, P. and Castellanos, I. (eds), *Ecology of Predator-Prey Interactions.* Oxford University Press, Oxford, pp. 34-54.

Brown, G. E., Rive, A. C., Ferrari, M. C. and Chivers, D. P. (2006). The dynamic nature of antipredator behavior: prey fish integrate threat-sensitive antipredator responses within background levels of predation risk. *Behavioral Ecology and Sociobiology*, **61**, 9-16.

Brucet, B. S., Compte, C. J., Boix, M. D., López, F. R. and Quintana, P. X. (2008). Feeding of nauplii, copepodites and adults of *Calanipeda aquaedulcis* (Calanoida) in Mediterranean salt marshes. *Marine Ecology Progress Series*, **355**, 183-191.

Burks, R. L., Lodge, D. M., Jeppesen, E. and Lauridsen, T. L. (2002). Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. *Freshwater Biology*, **47**, 343-365.

Buskey, E. J. and Hartline, D. K. (2003). High-speed video analysis of the escape responses of the copepod *Acartia tonsa* to shadows. *The Biological Bulletin*, **204**, 28-37.

Buskey, E. J., Lenz, P. H. and Hartline D. K. (2002). Escape behavior of planktonic copepods in response to hydrodynamic disturbances: high speed video analysis. *Marine Ecology Progress Series*, **235**, 135-46.

Buskey, E. J., Lenz, P. H. and Hartline, D. K. (2011). Sensory perception, neurobiology, and behavioral adaptations for predator avoidance in planktonic copepods. *Adaptive Behavior*, **20**, 57-66.

Button, D. K. (1994). The physical base of marine bacterial ecology. *Microbial Ecology*, **28**, 273-285.

Cáceres, C. E. (1998). Seasonal dynamics and interspecific competition in Oneida Lake Daphnia. *Oecologia*, **115**, 233-244.

Calbet, A. and Landry, M. R. (1999). Mesozooplankton influences on the microbial food web: direct and indirect trophic interactions in the oligotrophic open ocean. *Limnology and Oceanography*, **44**, 1370-1380.

Calbet, A. and Saiz, E. (2005). The ciliate-copepod link in marine ecosystems. *Aquatic Microbial Ecology*, **38**, 157-167.

Carpenter, S. R., Kitchell, J. F. and Hodgson, J. R. (1985). Cascading trophic interactions and lake productivity. *BioScience*, **35**, 634-639.

Castilho-Noll, M. S. M. and Arcifa, M. S. (2007). Mesocosm experiment on the impact of invertebrate predation on zooplankton of a tropical lake. *Aquatic Ecology*, **41**, 587-598.

Chalcraft, D. R. and Resetarits, W. J. (2003). Mapping functional similarity of predators on the basis of trait similarities. *The American Naturalist*, **162**, 390-402.

Chang, K. H. and Hanazato, T. (2005). Impact of selective predation by *Mesocyclops pehpeiensis* on a zooplankton community: experimental analysis using mesocosms. *Ecological Research*, **20**, 726-732.

Chase, J. M. (2003). Strong and weak trophic cascades along a productivity gradient. *Oikos*, **101**, 187-195.

Chave, J., Muller-Landau, H. C. and Levin, S. A. (2002). Comparing classical community models: theoretical consequences for patterns of diversity. *The American Naturalist*, **159**, 1-23.

Christensen, J. H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Koli, R. K., Kwon, W. -T., Laprise, R., Rueda, V. M., Mearns, L., Menéndez, C. G., Räisänen, J., Rinke, A., Sarr, A. and Whetton, P. (2007). Regional Climate Projections. In Solomon, S., Qin, D., Manning, M., Chen, Z., Marqui,s M., Averyt, K. B., Tignor, M. and Miller, H. L. (eds), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, pp. 849-926.

Clark, B. M. (2006). Climate change: A looming challenge for fisheries management in southern Africa. *Marine Policy*, **30**, 84-95.

Clarke, K. R. and Warwick, R. M. (1994). Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. Plymouth Marine Laboratory, Plymouth, U.S.A.

Cloern, J. E. (2001). Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series*, **210**, 223-253.

Coetzee, J. C., Adams, J. B. and Bate, G. C. (1996). A botanical importance rating system for estuaries. *Journal of Coastal Conservation*, **2**, 131-138.

Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulam-Syed-Mohiddeen, A. S., McGarrel, D. M., Marsh, T., Garrity, G. M. and Tiedje, J. M. (2009). The Ribosomal Database Project: Improved alignments and new tools for rRNA analysis. *NAR*. **37** (Database Issue) D141-D145. URL: http://rdp.cme.msu.edu/classifier/classifier.jsp; http://pyro.cme.msu.edu/.

Connell, J. H. and Sousa, W. P. (1983). On the evidence needed to judge ecological stability or persistence. *American Naturalist*, **121**, 789-824.

Cornell, H. V. and Lawton, J. H. (1992). Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. *Journal of Animal Ecology*, **61**, 1-12.

Costanza, R., R. d'Arge, R. deGroot, S. Faber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton and M. vandenBelt (1997). The value of the world's ecosystem services and natural capital. *Nature*, **387**, 253-260.

Cottingham, K. L. and Schindler, D. E. (2000). Effects of grazer community structure on phytoplankton response to nutrient pulses. *Ecology*, **81**, 183-200.

Cottingham, K. L., Knight, S. E., Carpenter, S. R., Cole, J. J., Pace, M. L. and Wagner, A. E. (1997). Response of phytoplankton and bacteria to nutrients and zooplankton: a mesocosm experiment. *Journal of Plankton Research*, **19**, 995-1010.

Cottingham, K. L., Glaholt, S. and Brown, A. C. (2004). Zooplankton community structure affects how phytoplankton respond to nutrient pulses. *Ecology*, **85**, 158-171.

Cottrell, M. T. and Kirchman, D. L. (2000). Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **66**, 5116-5122.

Cox, P. A., Elmqvist, T., Pierson, E. D. and Rainey, W. E. (1991). Flying foxes as strong interactors in South Pacific island ecosystems: a conservation hypothesis. *Conservation Biology*, **5**, 448-454.

Creel, S. and Christianson, D. (2008). Relationships between direct predation and risk effects. *Trends in Ecology and Evolution*, **23**, 194–201.

Cyrus, D. P. (1991). Fish conservation in South African estuaries: pressures, problems and prospects. *Southern African Journal of Aquatic Sciences*, **17**, 19-27.

Daehler, C. C. and Strong, D. R. (1996). Can you bottle nature? The roles of microcosms in ecological research. *Ecology*, **77**, 663–664.

Daufresne, M., Lengfellner, K. and Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences*, **106**, 12788-12793.

Davis, M., Hodgkins, G. A. and Stoner, A. W. (1996). A mesocosm system for ecological research with marine invertebrate larvae. *Marine Ecology Progress Series*, **130**, 97-104.

Dawkins, R. and Krebs, J. R. (1979). Arms races between and within species. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **205**, 489-511.

DeLong, E. F., Franks, D. G. and Alldredge, A. L. (1993). Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnology and Oceanography*, **38**, 924-934.

Department of Water Affairs. (1986). *Management of Water Resources of the Republic of South Africa*. Government Printer, Pretoria, 488 pp.

Dewitt, T. J., Sih, A. and Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology and Evolution*, **13**, 77-81.

Deyzel, S. H. P. (2012). Mesozooplankton dynamics in a Biogeographical Transition Zone Estuary. PhD Thesis. Nelson Mandela Metropolitan University, Port Elizabeth.

Díaz, S., Fargione, J., Chapin III, F. S. and Tilman, D. (2006). Biodiversity loss threatens human well-being. *PLoS Biology*, **4**, 1300-1305.

Dick, J. T., Gallagher, K., Avlijas, S., Clarke, H. C., Lewis, S. E., Leung, S., Minchin, D., Caffrey, J., Alexander, M. E., Maguire, C., Harrod, C., Reid, N., Haddaway, N. R., Farnsworth, K. D., Penk, M. and Ricciardi, A. (2013). Ecological impacts of an invasive predator explained and predicted by comparative functional responses. *Biological invasions*, **15**, 837–846.

Diehl, S. (1993). Relative consumer sizes and the strengths of direct and indirect interactions in omnivorous feeding relationships. *Oikos*, **68**, 151-157.

Diehl, S. and Feissel, M. (2001). Intraguild prey suffer from enrichment of their resources: a microcosm experiment with ciliates. *Ecology*, **82**, 2977-2983.

Doak, D. F., Bigger, D., Harding, E. K., Marvier, M. A., O'malley, R. E. and Thomson, D. (1998). The statistical inevitability of stability-diversity relationships in community ecology. *The American Naturalist*, **151**, 264-276.

Dolan, J. R. (2005). An introduction to the biogeography of aquatic microbes. *Aquatic Microbial Ecology*, **41**, 39-48.

Duarte, C. M., Agusti, S. and Agawin, N. S. R. (2000). Response of a Mediterranean phytoplankton community to increased nutrient inputs: a mesocosm experiment. *Marine Ecology Progress Series*, **195**, 61-70.

Dulvy, N. K., Freckleton, R. P. and Polunin, N. V. (2004). Coral reef cascades and the indirect effects of predator removal by exploitation. *Ecology letters*, **7**, 410-416.

Durant, J. M., Hjermann, D. O., Ottersen, G. and Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Climate Research*, **33**, 271-283.

Dzialowski, A. R. and John O'Brien, W. (2004). Is competition important to arctic zooplankton community structure? *Freshwater Biology*, **49**, 1103-1111.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, **27**, 2194-2200.

Eklöv, P. and VanKooten, T. (2001). Facilitation among piscivorous predators: effects of prey habitat use. *Ecology*, **82**, 2486-2494.

Elser, J. J. and Goldman, C. R. (1991). Zooplankton effects on phytoplankton in lakes of contrasting trophic status. *Limnology and Oceanography*, **36**, 64-90.

Elser, J. J., Elser, M. M., MacKay, N. A. and Carpenter, S. R. (1988). Zooplankton-mediated transitions between N-and P-limited algal growth. *Limnology and Oceanography*, **33**, 1-14.

Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., Carpenter, S. R., Essington, T. E., Holt, R. D., Jackson, J. B. C., Marquis, R. J., Oksanen, T., Paine, R. T., Pikitch, E. K., Ripple, W. J., Sandin, S. A., Scheffer, M., Schroener, T. W., Shurin, J. B., Sinclair, A. R. E., Soule, M. E., Virtanen, R. and Wardle, D. A. (2011). Trophic downgrading of earth. *Science*, **333**, 301-306.

Eveleigh, E. S., McCann, K. S., McCarthy, P. C., Pollock, S. J., Lucarotti, C. J., Morin, B., McDougall, G. A., Strongman, D. B., Huber, J. T., Umbanowar, J. and Faria, L. D. (2007). Fluctuations in density of an outbreak species drive diversity cascades in food webs. *Proceedings of the National Academy of Sciences*, **104**, 16976-16981.

Fancett, M. S. and Kimmerer, W. J. (1985). Vertical migration of the demersal copepod *Pseudodiaptomus* as a means of predator avoidance. *Journal of Experimental Marine Biology and Ecology*, **88**, 31-43.

Fenchel, T. (1982). Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. *Marine Ecology Progress S*eries, **9**, 35-42.

Ferrari, M. C. O., Messier, F. and Chivers, D. P. (2007). Variable predation risk and the dynamic nature of mosquito antipredator responses to chemical alarm cues. *Chemoecology*, **17**, 223-9.

Ferrari, M. C. O., Messier, F. and Chivers, D. P. (2008). Can prey exhibit threat-sensitive generalization of predator recognition? Extending the predator recognition continuum hypothesis. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1811-1816.

Ferrari, M. C. O., Trowell, J. J., Brown, G. E. and Chivers, D. P. (2005). The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. *Animal Behaviour*, **70**, 777-784.

Ferrari, M. C. O., Wisenden, B. D. and Chivers, D. P. (2010). Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*, **88**, 698-724.

Feuchtmayr, H., Zöllner, E., Santer, B., Sommer, U. and Grey, J. (2004). Zooplankton interactions in an enclosure experiment: insights from stable isotope analyses. *Freshwater Biology*, **49**, 1495-1504.

Fey, K., Banks, P. B., Oksanen, L. and Korpimäki, E. (2009). Does removal of an alien predator from small islands in the Baltic Sea induce a trophic cascade? *Ecography*, **32**, 546-552.

Fischer, J. M., Klug, J. L., Ives, A. R. and Frost, T. M. (2001). Ecological history affects zooplankton community responses to acidification. *Ecology*, **82**, 2984-3000.

Fischer, J. M., Nicolai, J. L., Williamson, C. E., Persaud, A. D. and Lockwood, R. S. (2006). Effects of ultraviolet radiation on diel vertical migration of crustacean zooplankton: An in situ mesocosm experiment. *Hydrobiologia*, **563**, 217-224.

Flies, C. B., Jonkers, H. M., Beer, D., Bosselmann, K., Böttcher, M. E. and Schüler, D. (2005). Diversity and vertical distribution of magnetotactic bacteria along chemical gradients in freshwater microcosms. *FEMS Microbiology Ecology*, **52**, 185-195.

Forrest, J. and Arnott, S. E. (2006). Immigration and zooplankton community responses to nutrient enrichment: a mesocosm experiment. *Oecologia*, **150**, 119-131.

Fraser, D. F. and Gilliam, J. F. (1992). Nonlethal impacts of predator invasion: facultative suppression of growth and reproduction. *Ecology*, **73**, 959-970.

Fretwell, S. D. (1987). Food chain dynamics: the central theory of ecology? *Oikos*, **50**, 291-301.

Froneman, P. W. (2000). Feeding studies on selected zooplankton in a temperate estuary, South Africa. *Estuarine, Coastal and Shelf Science*, **51**, 543-552.

Froneman, P. W. (2002a). Food web structure in three contrasting estuaries determined using stable isotope (∂ 13C) analysis. *African Journal of Aquatic Science*, **27**, 107-115.

Froneman, P. W. (2002b). Response of the plankton to three different hydrological phases of the temporarily open/closed Kasouga Estuary, South Africa. *Estuarine, Coastal and Shelf Science*, **55**, 535-546.

Froneman, P. W. (2002c). Seasonal changes in selected physico-chemical and biological variables in the temporarily open/closed Kasouga Estuary, Eastern Cape, South Africa. *African Journal of Aquatic Science*, **27**, 117-123.

Froneman, P. W. (2004a). Food web dynamics in a temperate temporarily open/closed estuary (South Africa). *Estuarine, Coastal and Shelf Science*, **59,** 87-95.

Froneman, P. W. (2004b). Zooplankton community structure and biomass in a southern African temporarily open/closed estuary. *Estuarine, Coastal and Shelf Science*, **60**, 125-132.

Froneman, P. W. (2004c). In situ feeding rates of the copepods, *Pseudodiaptomus hessei* and *Acartia longipatella*, in a temperate, temporarily open/closed Eastern Cape estuary. *South African Journal of Science*, **100**, 577-583.

Froneman, P. W. and Henninger, T. O. (2009). The influence of prolonged mouth closure on selected components of the hyperbenthos in the littoral zone of the temporarily open/closed Kasouga Estuary, South Africa. *Estuarine, Coastal and Shelf Science*, **83**, 326-332.

Gaston, K. J. and McArdle, B. H. (1994). The temporal variability of animal abundances: measures, methods and patterns. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **345**, 335-358.

Gibson, R. N., Barnes, M. and Atkinson, R. J. A. (1995). Impact of changes in flow of freshwater on estuarine and open coastal habitats and the associated organisms. *Oceanography and Marine Biology, An Annual Review*, **40**, 233.

Gilbert, B., Srivastava, D. S. and Kirby, K. R. (2008). Niche partitioning at multiple scales facilitates coexistence among mosquito larvae. *Oikos*, **117**, 944-950.

Glibert, P. M. (1997). Interactions of top-down and bottom-up control in planktonic nitrogen cycling. *Hydrobiologia*, **363**, 1-12.

Gobler, C. J. and Sañudo-Wilhelmy, S. A. (2001). Effects of organic carbon, organic nitrogen, inorganic nutrients, and iron additions on the growth of phytoplankton and bacteria during a brown tide bloom. *Marine Ecology Progress Series*, **209**, 19-34.

Gobler, C. J., Cullison, L. A., Koch, F., Harder, T. M. and Krause, J. W. (2005). Influence of freshwater flow, ocean exchange, and seasonal cycles of phytoplankton- nutrient dynamics in a temporary open estuary. *Estuarine, Coastal and Shelf Science*, **65**, 275-288.

Godin, J. G. J. and Smith, S. A. (1988). A fitness cost of foraging in the guppy. *Nature*, **333**, 69-71.

Gonzalo, A., Cabido, C. and López, P. (2012). Conspecific alarm cues, but not predator cues alone, determine antipredator behaviour of larval southern marbled newts, *Triturus pygmaeus*. *Acta Ethologica*, **15**, 211-216.

Gowen, R. J., Tett, P. and Jones, K. J. (1992). Predicting marine eutrophication: the yield of chlorophyll from nitrogen in Scottish coastal waters. *Marine Ecology Progress Series*, **85**, 153-161.

Granéli, E. and Turner, J. T. (2002). Top-down regulation in ctenophore-copepod-ciliate-diatom-phytoflagellate communities in coastal waters: a mesocosm study. *Marine Ecology Progress Series*, **239**, 57-68.

Griffen, B. D. (2006). Detecting emergent effects of multiple predator species. *Oecologia*, **148**, 702-709.

Griffiths, S. P. (1999). Consequences of artificially opening coastal lagoons on their fish assemblages. *International Journal of Salt Lake Research*, **8**, 307-327.

Grossart, H. P., LeVold, F., Allgaier, M., Simon, M. and Brinkhoff, T. (2005). Marine diatom species harbour distinct bacterial communities. *Environtal Microbiology*, **7**, 860-873.

Haddaway, N. R., Wilcox, R. H., Heptonstall, R. E., Griffiths, H. M., Mortimer, R. J., Christmas, M. and Dunn, A. M. (2012). Predatory functional response and prey

choice identify predation differences between native/invasive and parasitised/unparasitised crayfish. *PLoS ONE*, **7**, e32229.

Hairston, N. G. and Dillon, T. A. (1990). Fluctuating selection and response in a population of freshwater copepods. *Evolution*, **44**, 1796-805.

Hairston, N. G., van Brunt, R. A., Kearns, C. M. and Engstrom, D. R. (1995). Age and survivorship of diapausing eggs in a sediment egg bank. *Ecology*, **76**, 1706-1711.

Hall, D. J., Threlkeld, S. T., Burns, C. W. and Crowley, P. H. (1976). The size efficiency hypothesis and the size structure of zooplankton communities. *Annual Review of Ecology and Systematics*, **7**, 177-208.

Hamilton, J. D. (1994). *Time Series Analysis*. Princeton University Press, Princeton.

Hansen, B., Bjørnsen, P. K. and Hansen, P. J. (1994). The size ratio between planktonic predators and their prey. *Limnology and Oceanography*, **39**, 395-403.

Hansson, L. A. (1992.) The role of food chain composition and nutrient availability in shaping algal biomass development. *Ecology*, **73**, 241-247.

Hart, R. C. and Allanson, B. R. (1976). The distribution and diel vertical migration of *Pseudodiaptomus hessei* (Mrázek) (Calanoida: Copepoda) in a subtropical lake in southern Africa. *Freshwater Biology*, **6**, 183-198.

Hay, M. E. (2009). Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems. *Annual Review of Marine Science*, **1**, 193-212.

Heath, J. P. (2006). Quantifying temporal variability in population abundances. *Oikos*, **115**, 573-581.

Heath, M. R., Speirs, D. C. and Steele, J. H. (*in press*). Understanding patterns and processes in models of trophic cascades. *Ecology Letters* - doi: 10.1111/ele.12200.

Helfman, G. S. (1989). Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology*, **24**, 47-58.

Henninger, T.O. (2009). Aspects of the ecology and biology of the isopod, *Exosphaeroma hylocoetes*, (Barnard, 1940) in three temporarily open/closed southern African estuaries. PhD Thesis. Rhodes University, Grahamstown.

Henninger, T. O., Froneman, P. W. and Hodgson, A. N. (2008). Population dynamics of the estuarine isopod *Exosphaeroma hylocoetes* (Barnard, 1940) within three temporarily open/closed southern African estuaries. *African Zoology*, **43**, 202-217.

Heyns, E. and Froneman, P. W. (2010). Spatial and temporal patterns in the hyperbenthic community structure in a warm temperate southern African permanently open estuary. *Estuarine, Coastal and Shelf Science*, **88**, 105-115.

Hilmer, T. and Bate, G. G. (1991). Vertical migration of a flagellate-dominated bloom in a shallow South African estuary. *Botanica Marina*, **34**, 113-121.

Holling, C. S. (1973). Resilience and stability of ecological systems. *Annual Review of Ecology and Systematics*, **4**, 1-23.

Holzmueller, E. J., Gibson, D. J. and Suchecki, P. F. (2012). Accelerated succession following an intense wind storm in an oak-dominated forest. *Forest Ecology and Management*, **279**, 141-146.

Hooper, D. U., Chapin Iii, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A. J., Vandermeer, J. and Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3-35.

Houde, E. D. and Rutherford, E. S. (1993). Recent trends in estuarine fisheries: predictions of fish production and yield. *Estuaries*, **16**, 161-176.

Houde, E. D., Gamble, J. C., Dorsey, S. E. and Cowan, J. H. (1994). Drifting mesocosms: the influence of gelatinous zooplankton on mortality of bay anchovy, *Anchoa mitchilli*, eggs and yolk-sac larvae. *ICES Journal of Marine Science*, **51**, 383-394.

Hu, S. S. and Tessier, A. J. (1995). Seasonal succession and the strength of intra-and interspecific competition in a Daphnia assemblage. *Ecology*, **76**, 2278-2294.

Humes, A. G. (1994). How many copepods? *Hydrobiologia*, **292**, 1-7.

Hylander, S., Souza, M. S., Balseiro, E., Modenutti, B. and Hansson, L. A. (2012). Fish-mediated trait compensation in zooplankton. *Functional Ecology*, **26**, 608-15.

Iglesias, C., Goyenola, G., Mazzeo, N., Meerhoff, M., Rodó, E. and Jeppesen, E. (2007). Horizontal dynamics of zooplankton in subtropical Lake Blanca (Uruguay) hosting multiple zooplankton predators and aquatic plant refuges. *Hydrobiologia*, **584**, 179-189.

Isla, J. A. and Perissinotto, R. (2004). Effects of temperature, salinity and sex on the basal metabolic rate of the estuarine copepod *Pseudodiaptomus hessei*. *Journal of Plankton Research*, **26**, 579-583.

Ives, A. R., Dennis, B., Cottingham, K. L. and Carpenter, S. R. (2003). Estimating community stability and ecological interactions from time-series data. *Ecological Monographs*, **73**, 301-330.

Ivlev, V. S. (1966). The biological productivity of waters. *Journal of the Fisheries Board of Canada*, **23**, 1727-1759.

James, N. C. and Harrison, T. D. (2010). A preliminary survey of the estuaries on the southeast coast of South Africa, Cape Padrone–Great Fish River, with particular

reference to the fish fauna. *Transactions of the Royal Society of South Africa*, **65**, 149-164.

Jeong, H. J., Kim, J. S., Yoo, Y. D., Kim, S. T., Song, J. Y., Kim, T. H., Seong, K. A., Kang, N. S., Kim, M. S., Kim, J. H., Kim, S., Ryu, J., Lee, H. M. and Yih, W. H. (2008). Control of the harmful alga *Cochlodinium polykrikoides* by the naked ciliate *Strombidinopsis jeokjo* in mesocosm enclosures. *Harmful Algae*, **7**, 368-377.

Jerling, H. L. and Wooldridge, T. H. (1989). The developmental stages of *Pseudodiaptomus hessei* (Copepoda: Calanoida). *South African Journal of Zoology*, **24**, 139-145.

Jerling, H. L. and Wooldridge, T. H. (1991). Population dynamics and estimates of production for the calanoid copepod *Pseudodiaptomus hessei* in a warm temperate estuary. *Estuarine, Coastal and Shelf Scienc*e, **33**, 121-135.

Jerling, H. L. and Wooldridge, T. H. (1995a). Feeding of two mysid species on plankton in a temperate South African estuary. *Journal of Experimental Marine Biology and Ecology*, **188**, 243-259.

Jerling, H. L. and Wooldridge, T. H. (1995b). Plankton distribution and abundance in the Sundays River estuary, South Africa, with comments on potential feeding interactions. *South African Journal of Marine Science*, **15**, 169-184.

Jessup, C. M., Kassen, R., Forde, S. E., Kerr, B., Buckling, A., Rainey, P. B. and Bohannan, B. J. (2004). Big questions, small worlds: microbial model systems in ecology. *Trends in Ecology and Evolution*, **19**, 189-197.

Jónasdóttir, S. H., Dutz, J., Koski, M., Yebra, L., Jakobsen, H. H., Vidoudez, C., Pohnert G. and Nejstgaard, J. C. (2011). Extensive cross-disciplinary analysis of biological and chemical control of *Calanus finmarchicus* reproduction during an aldehyde forming diatom bloom in mesocosms. *Marine Biology*, **158**, 1943-1963.

Jones, J. I. and Sayer, C. D. (2003). Does the fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes? *Ecology*, **84**, 2155-2167.

Jubb, R. A. (1979). Cichlid fish mortality: Kasouga River estuary. *Lammergeyer*, **27**, 51.

Kabacoff, R. I. (2011). *R in Action: Data Analysis and Graphics with R.* Manning Publications Co., Shelter Island.

Kats, L. B. and Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience*, **5**, 361-394.

Keller, A. A. and Klein-MacPhee, G. (2000). Impact of elevated temperature on the growth, survival, and trophic dynamics of winter flounder larvae: a mesocosm study. *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 2382-2392.

Kesavaraju, B., Damal, K. and Juliano, S. A. (2007). Threat-sensitive behavioral responses to concentrations of water-borne cues from predation. *Ethology*, **113**, 199-206.

Kibirige, I. and Perissinotto, R. (2003). The zooplankton community of the Mpenjati Estuary, a South African temporarily open/closed system. *Estuarine, Coastal and Shelf Science*, **58**, 727-741.

Kirchman, D. L. (1994). The uptake of inorganic nutrients by heterotrophic bacteria. *Microbial Ecology*, **28**, 255-271.

Kirchman, D. L. (2002). The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiology Ecol*ogy, **39**, 91-100.

Klug, J. L. and Fischer, J. M. (2000). Factors influencing the growth of Mougeotia in experimentally acidified mesocosms. *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 538-547.

Kouassi, E., Pagano, M., Saint-Jean, L., Arfi, R. and Bouvy, M. (2001). Vertical migrations and feeding rhythms of *Acartia clausi* and *Pseudodiaptomus hessei* (Copepoda: Calanoida) in a tropical lagoon (Ebrié, Côte d'Ivoire). *Estuarine, Coastal and Shelf Science*, **52**, 715-728.

Krebs, C. J. (1994). *Ecology: the experimental analysis of distribution and abundance, Vol. 4*. Harper Collins College Publishers, New York.

Kreutzweiser, D. P., Back, R. C., Sutton, T. M., Thompson, D. G. and Scarr, T. A. (2002). Community-level disruptions among zooplankton of pond mesocosms treated with a neem (azadirachtin) insecticide. *Aquatic toxicology*, **56**, 257-273.

Krupa, J. J. and Sih, A. (1998). Fishing spiders, green sunfish, and a stream-dwelling water strider: male-female conflict and prey responses to single versus multiple predator environments. *Oecologia*, **117**, 258-265.

Kusch, R. C., Mirza, R. S. and Chivers, D. P. (2004). Making sense of predator scents: investigating the sophistication of predator assessment abilities of fathead minnows. *Behavioral Ecology and Sociobiology*, **55**, 551-555.

Lafontaine, Y. D. and Leggett, W. C. (1987). Evaluation of *in situ* enclosures for larval fish studies. *Canadian Journal of Fisheries and Aquatic Sciences*, **44**, 54-65.

Lampert, W. (1989). The adaptive significance of diel vertical migration of zooplankton. *Functional Ecology*, **3**, 21-27.

Lauth, J. R., Scott, G. I., Cherry, D. S. and Buikema, A. L. (1996). A modular estuarine mesocosm. *Environmental Toxicology and Chemistry*, **15**, 630-637.

Lawton, J. H. (1995). Ecological experiments with model systems. *Science*, **269**, 328-331.

Leibold, M. A. (1996). A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence, and diversity patterns in communities. *The American Naturalist*, **147**, 784-812.

Leibold, M. A., Chase, J. M., Shurin, J. B. and Downing, A. (1997). Species turnover and the regulation of trophic structure. *Annual review of ecology and systematics*, **28**, 467-494

Letnic, M. and Symon, A. D. (2011). Does a top predator reduce the predatory impact of an invasive mesopredator on an endangered rodent? *Ecography*, **34**, 827-835.

Lima, S. L. (1998). Nonlethal effects in the ecology of predator- prey interactions: what are the ecological effects of anti-predator decision-making? *Bioscience*, **48**, 25-35.

Lindeman, R. L. (1942). The trophic-dynamic aspect of ecology. *Ecology*, **23**, 399-417.

Link, W. A. and Nichols, J. D. (1994). On the importance of sampling variance to investigations of temporal variation in animal population size. *Oikos*, **69**, 539-544.

Liu, Z., Lozupone, C., Hamady, M., Bushman, F. D. and Knight, R. (2007). Short pyrosequencing reads suffice for accurate microbial community analysis. *Nucleic Acids Research*, **35**, e120.

Löder, M. G., Meunier, C., Wiltshire, K. H., Boersma, M. and Aberle, N. (2011). The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea. *Marine biology*, **158**, 1551-1580.

Lorenzen, C. J. (1966). A method for the continuous measurement of *in vivo* chlorophyll concentration. *Deep Sea Research and Oceanographic Abstracts*, **13**, 223-227.

Losey, J. E. and Vaughan, M. (2006). The economic value of ecological services provided by insects. *BioScience*, **56**, 311-323.

Lynch, M. and Shapiro, J. (1981). Predation, enrichment, and phytoplankton community structure. *Limnology and Oceanography*, **26**, 86-102.

MacArthur, R. (1955). Fluctuations of animal populations and a measure of community stability. *Ecology*, **36**, 533-536.

MacKenzie, B. R., Leggett, W. C. and Peters, R. H. (1990). Estimating larval fish ingestion rates: Can laboratory derived values be reliably extrapolated to the wild? *Marine Ecology Progress Series*, **67**, 209-225.

Maier, G. (1989). Variable life cycles in the freshwater copepod *Cyclops vicinis* (Uljanin 1875): support for the predator avoidance hypothesis? *Archives of Hydrobiology*, **115**, 203- 219.

Maier, G., Berger, I., Burghard, W. and Nassal, B. (2000). Is mating of copepods associated with increased risk of predation? *Journal of Plankton Research*, **22**, 1977-1987.

Marino, R., Chan, F., Howarth, R. W., Pace, M. and Likens, G. E. (2002). Ecological and biogeochemical interactions constrain planktonic nitrogen fixation in estuaries. *Ecosystems*, **5**, 0719-0725.

Martin, D., Pinedo, S. and Sardá, R. (1996). Grazing by meroplanktonic polychaete larvae may contribute to control the nanoplankton in the NW Mediterranean littoral: In situ experimental evidence. *Marine Ecology Progress Series*, **143**, 239-246.

Matcher, G. F., Dorrington, R. A., Henninger, T. O. and Froneman, P. W. (2011). Insights into the bacterial diversity in a freshwater-deprived permanently open Eastern Cape estuary, using 16S rRNA pyrosequencing analysis. *Water SA*, **37**, 381-390.

May, R. M. (1976). Simple mathematical models with very complicated dynamics. *Nature*, **261**, 459.

McArdle, B. H., Gaston, K. J. and Lawton, J. H. (1990). Variation in the size of animal populations: patterns, problems and artefacts. *The Journal of Animal Ecology*, **59**, 439-454.

McCormick, M. I. and Kerrigan, B. A. (1996). Predation and its influence on the condition of a newly settled tropical demersal fish. *Marine and Freshwater Research*, **47**, 557-562.

Mead, A., Griffiths, C. L., Branch, G. M., McQuaid, C. D., Blamey, L. K., Bolton, J. J., Anderson, R. J., Dufois, F., Rouault, M., Froneman, P. W., Whitfield, A. K., Harris, L. R., Pillay, D. and Adams, J. B. (2013). Human-mediated drivers of change - impacts on coastal ecosystems and marine biota of South Africa. *African Journal of Marine Science*, **35**, 403-425.

Mehner, T. and Thiel, R. (1999). A review of predation impact by 0+ fish on zooplankton in fresh and brackish waters of the temperate northern hemisphere. *Environmental Biology of Fishes*, **56**, 169-181.

Mills, L. S., Soulé, M. E. and Doak, D. F. (1993). The keystone-species concept in ecology and conservation. *BioScience*, **43**, 219-224.

Mirza, R. S., Ferrari, M. C. O., Kiesecker, J. M. and Chivers, D. P. (2006). Responses of American toad tadpoles to predation cues: behavioural response thresholds, threat-sensitivity and acquired predation recognition. *Behaviour*, **143**, 877-889.

Møller, E. F. (2005). Sloppy feeding in marine copepods: prey-size-dependent production of dissolved organic carbon. *Journal of Plankton Research*, **27**, 27-35.

Møller, E. F. (2007). Production of dissolved organic carbon by sloppy feeding in the copepods *Acartia tonsa*, *Centropages typicus*, and *Temora longicornis*. *Limnology and oceanography*, **52**, 79-84.

Montoya-Maya, P. H. and Strydom, N. A. (2009). Description of larval fish composition, abundance and distribution in nine south and west coast estuaries of South Africa. *African Zoology*, **44**, 75-92.

Moore, M. and Folt, C. (1993). Zooplankton body size and community structure: effects of thermal and toxicant stress. *Trends in Ecology and Evolution*, **8**, 178-183.

Morant, P. D. and Quinn, N. W. (1999). Influence of man and management on South African estuaries. In Allanson, B. R. and Baird, D. (eds), *Estuaries of South Africa*. Cambridge University Press, Cambridge, pp. 289-320.

Morin, P. (1999). Productivity, intraguild predation, and population dynamics in experimental food webs. *Ecology*, **80**, 752-760.

Mos, B., Cowden, K. L., Nielsen, S. J. and Dworjanyn, S. A. (2011). Do cues matter? Highly inductive settlement cues don't ensure high post-settlement survival in sea urchin aquaculture. *PLoS ONE*, **6**, e28054.

Moss, B., Stephen, D., Balayla, D. M., Becares, E., Collings, S. E., Fernandez-Alaez, C., Fernandez-Alaez, M., Ferriol, C., Garcia, P., Goma, J., Gyllstrom, M., Hansson, L.A., Hietala, J., Kairesalo, T., Miracle, M. R., Romo, S., Rueda, J., Russell, V., Stahl-Delbanco, A., Svensson, M., Vakkilainen, K., Valentin, M., Van De Bund, W. J., Van Donk, E., Vicente, E. and Villena, M. J. (2004). Continental-scale patterns of nutrient and fish effects on shallow lakes: synthesis of a pan-European mesocosm experiment. *Freshwater Biology*, **49**, 1633-1649.

Mowitt, W. P., Houde, E. D., Hinkle, D. C. and Sanford, A. (2006). Growth of planktivorous bay anchovy *Anchoa mitchilli*, top-down control, and scale-dependence in estuarine mesocosms. *Marine Ecology Progress Series*, **308**, 255-269.

Muška, M., Tušer, M., Frouzová, J., Draštík, V., Čech, M., Jůza, T., Katochvíl, M., Mrkvička, T., Peterka, J., Prchalová, M., Říha, M., Vašek, M. and Kubečka, J. (2013). To migrate, or not to migrate: partial diel horizontal migration of fish in a temperate freshwater reservoir. *Hydrobiologia*, **707**, 1-12.

Nagata, T. and Hanazato, T. (2006). Different predation impacts of two cyclopoid species on a small-sized zooplankton community: an experimental analysis with mesocosms. *Hydrobiologia*, **556**, 233-242.

Navarrete, S. A. and Menge, B. A. (1996). Keystone predation and interaction strength: interactive effects of predators on their main prey. *Ecological Monographs*, **66**, 409-429.

Nejstgaard, J. C., Hygum, B. H., Naustvoll, L. J. and Bamstedt, U. (2001). Zooplankton growth, diet and reproductive success compared in simultaneous diatom- and flagellate-microzooplankton-dominated plankton blooms. *Marine Ecology Progress Series*, **221**, 77-91.

Nelson, E. H., Matthews, C. E. and Rosenheim, J. A. (2004). Predators reduce prey population growth by inducing changes in prey behaviour. *Ecology*, **85**, 1853-1858.

Nemec, A. F. L. (1996). *Analysis of Repeated Measures and Time Series: An Introduction with Forestry Examples* (6th edition). Ministry of Forests Research Program, Province of British Columbia.

New, M., Hewitson, B., Stephenson, D. B., Tsiga, A., Kruger, A., Manhique, A., Gomez, B., Coelho, C. A. S., Masisi, D. N., Kululanga, E., Mbambalala, E., Adesina, F., Saleh, H., Kanyanga, J., Adosi, J., Bulane, L., Fortunata, L., Mdoka, M. L. and Lajoie, R. (2006). Evidence of trends in daily climate extremes over southern and west Africa. *Journal of Geophysical Research: Atmospheres (1984-2012)*, **111,** D14.

Newton, A. and Mudge, S.M. (2005). Lagoon-sea exchanges, nutrient dynamics and water quality management of the Ria Formosa (Portugal). *Estuarine, Coastal and Shelf Science*, **62**, 405-414.

Nishino, S.F. (1986). Direct acridine orange counting of bacteria preserved with acidified lugol iodine. *Applied and Environmental Microbiology*, **52**, 602-604.

Nixon, S. W., Oviatt, C. A., Frithsen, J. and Sullivan, B. (1986). Nutrients and the productivity of estuarine and coastal marine ecosystems. *Journal of the Limnological Society of Southern Africa*, **12**, 43-71.

Nixon, S. W., Ammerman, J. W., Atkinson, P., Berounsky, V. M., Billen, G., Boicourt, W. C., Boynton, W. R., Church, T. M., Ditoro, D. M., Elmgren, R., Garber, J. H., Giblin, A. E., Jahnke, R. A., Owens, N. J. P., Pilson, M. E. Q. and Seitzinger, S. P. (1996). The fate of nitrogen and phosphorus at the land-sea margin of the North Atlantic Ocean. *Biogeochemistry*, **35**, 141-180.

Nogueira, M. G., Oliveira, P. C. R. and de Britto, Y. T. (2008). Zooplankton assemblages (Copepoda and Cladocera) in a cascade of reservoirs of a large tropical river (SE Brazil). *Limnetica*, **27**, 151-169.

Nowicki, B. L. and Oviatt, C. A. (1990). Are estuaries traps for anthropogenic nutrients? Evidence for estuarine mesocosms. *Marine Ecology Progress Series*, **66**, 131-146.

O'Connor, N. E., Grabowski, J. H., Ladwig, L. M. and Bruno, J. F. (2008). Simulated predator extinctions: predator identity affects survival and recruitment of oysters. *Ecology*, **89**, 428-438.

O'Keeffe, J. H. and De Moor, F. C. (1988). Changes in the physico-chemistry and benthic invertebrates of the Great Fish River, South Africa, following an interbasin transfer of water. *Regulated Rivers: Research and Management*, **2**, 39-55.

Odum, E. P. (1984). The mesocosm. *Bioscience*, **34**, 558-562.

Odum, H. T. (1957). Trophic structure and productivity of Silver Springs, Florida. *Ecological Monographs*, **27**, 55-112.

Ogbebo, F. E. and Ochs, C. (2008). Bacterioplankton and phytoplankton production rates compared at different levels of solar ultraviolet radiation and limiting nutrient ratios. *Journal of Plankton Research*, **30**, 1271-1284.

Olsen, Y., Agustí, S., Andersen, T., Duarte, C. M., Gasol, J. M., Gismervik, I., Heiskanen, A. S., Hoell, E., Kuuppo, P., Lignell, R., Reinertsen, H., Sommer, U., Stibor, H.,

Tamminen, T., Vadstein, O., Vaqué, D. and Vidal, M. (2006). A comparative study of responses in planktonic food web structure and function in contrasting European coastal waters exposed to experimental nutrient addition. *Limnology and Oceanography*, **51**, 488-503.

Oviatt, C. A., Pilson, M. E. Q, Nixon, S. W., Frithsen, J. B., Rudnick, D. T., Kelly, J. R., Grassle, J. F. and Grassle, J. P. (1984). Recovery of a polluted estuarine system: A mesocosm experiment. *Marine Ecology Progress Series*, **16**, 203-217.

Pace, M. L. and Cole, J. J. (1994). Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. *Microbial Ecology*, **28**, 181-193.

Pagano, M., Kouassi, E., Saint-Jean, L., Arfi, R. and Bouvy, M. (2003). Feeding of *Acartia clausi* and *Pseudodiaptomus hessei* (Copepoda: Calanoida) on natural particles in a tropical lagoon (Ebrié, Côte d'Ivoire). *Estuarine, Coastal and Shelf Science*, **56**, 433-445.

Paine, R. T. (1966). Food web complexity and species diversity. *The American Naturalist*, **100**, 65-75.

Paine, R. T. (1980). Food webs: linkage, interaction strength and community infrastructure. *Journal of Animal Ecology*, **49**, 667-685.

Painting, S. J., Devlin, M. J., Malcolm, S. J., Parker, E. R., Mills, D. K., Mills, C., Tett, P., Wither, A., Burt, J., Jones, R. and Winpenny, K. (2007). Assessing the impact of nutrient enrichment in estuaries: susceptibility to eutrophication. *Marine Pollution Bulletin*, **55**, 74-90.

Pangle, K. L., Peacor, S. D. and Johannsson, O. E. (2007). Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. *Ecology*, **88**, 402-412.

Parsons, T. R., Maita, Y. and Lalli, C. M. (1984). *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, New York.

Pearson, R. G. and Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography*, **12**, 361-371.

Peckarsky, B. L., Cowan, C. A., Penton, M. A. and Anderson, C. (1993). Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. *Ecology*, **74**, 1836–1846.

Perissinotto, R., Nozais, C., Kibirige, I. and Anandraj, A. (2003). Planktonic food webs and benthic-pelagic coupling in three South African temporarily-open estuaries. *Acta Oecologica*, **24**, S307-S316.

Perissinotto, R., Walker, D. R., Webb, P., Wooldridge, T. H. and Bally, R. (2000). Relationships between zoo- and phytoplankton in a warm temperate, semi-

permanently closed estuary, South Africa. *Estuarine, Coastal and Shelf Science,* **51**, 1-11.

Persson, L. (1999). Trophic cascades: abiding heterogeneity and the trophic level concept at the end of the road. *Oikos*, **85**, 385-397.

Persson, L., Diehl, S., Johansson, L., Andersson, G. and Hamrin, S. F. (1992). Trophic interactions in temperate lake ecosystems: a test of food chain theory. *American Naturalist*, **140**, 59-84.

Pimm, S. L. (1984). The complexity and stability of ecosystems. *Nature,* **307**, 321-326.

Pogozhev, P. I. and Gerasimova, T. N. (2001). The effect of zooplankton on microalgae blooming and water eutrophication. *Water Resources*, **28**, 420-427.

Polis, G. A. (1991). Complex trophic interactions in deserts: an empirical critique of food-web theory. *American Naturalist*, **138**, 123-155.

Polis, G. A. (1994). Food webs, trophic cascades and community structure. *Australian Journal of Ecology*, **19**, 121-136.

Polis, G. A. and Holt, R. D. (1992). Intraguild predation: the dynamics of complex trophic interactions. *Trends in Ecology and Evolution*, **7**, 151-154.

Polis, G. A., Myers, C. A. and Holt, R. D. (1989). The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology and Systematics*, **20**, 297-330.

Polis, G. A., Sears. A. L. W., Huxel, G. R., Strong, D. R. and Maron, J. (2000). When is a trophic cascade a trophic cascade? *Trends in Ecology and Evolution*, **15**, 473-475.

Porter, K. G. (1996). Integrating the microbial loop and the classic food chain into a realistic planktonic food web. In Polis, G. A. and Winemiller, K. O. (eds), *Food Webs*: integration of patterns and dynamics. Chapman and Hall, New York, pp. 51-59.

Porter, K. G., Sherr, E. B., Sherr, B. F., Pace, M. and Sanders, R. W. (1985). Protozoa in Planktonic Food Webs. *Journal of Eukaryotic Microbiology*, **32**, 409-415.

Preisser, E. L., Bolnick, D. I. and Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, **86**, 501–509.

Purvis, A., Gittleman, J. L., Cowlishaw, G. and Mace, G. M. (2000). Predicting extinction risk in declining species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **267**, 1947–1952.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, E. and Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, **41**, D590-D596.

R Development Core Team (2013). R: A language and environment for statistical computing. Available at: http://www.R-project.org/ [accessed 25 August 2013].

Reid, F. M. H. (1983). Biomass estimation of components of the marine nanoplankton and picoplankton by the Utermöhl settling technique. *Journal of Plankton Research*, **5**, 235-252.

Richardson, A. J. (2008). In hot water: zooplankton and climate change. *ICES Journal of Marine Science*, **65**, 279-295.

Riedel, G. F. and Sanders, J. G. (2003). The interrelationships among trace element cycling, nutrient loading, and system complexity in estuaries: A mesocosm study. *Estuaries*, **26**, 339-351.

Ripple, W. J., Rooney, T. P. and Beschta, R. L. (2010). Large predators, deer, and trophic cascades in boreal and temperate ecosystems. In Terborgh, J. and Estes, J. A. (eds), *Trophic cascades: predators, prey, and the changing dynamics of nature.* Island Press, Washington, pp. 141-161.

Romo, S., Miracle, M. R., Villena, M. J., Rueda, J., Ferriol, C. and Vicente, E. (2004). Mesocosm experiments on nutrient and fish effects on shallow lake food webs in a Mediterranean climate. *Freshwater Biology*, **49**, 1593-1607.

Rondel, C., Arfi, R., Corbin, D., Le Bihan, F., Ndour, E. H. and Lazzaro, X. (2008). A cyanobacterial bloom prevents fish trophic cascades. *Freshwater Biology*, **53**, 637-651.

Ruxton, G. D. and Lima, S. L. (1997). Predator–induced breeding suppression and its consequences for predator–prey population dynamics. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **264**, 409-415.

Saba, G. K., Steinberg, D. K., and Bronk, D. A. (2011). The relative importance of sloppy feeding, excretion, and fecal pellet leaching in the release of dissolved carbon and nitrogen by *Acartia tonsa* copepods. *Journal of Experimental Marine Biology and Ecology*, **404**, 47-56.

Salo, P., Banks, P. B., Dickman, C. R. and Korpimäki, E. (2010). Predator manipulation experiments: impacts on populations of terrestrial vertebrate prey. *Ecological Monographs*, **80**, 531-546.

Sameoto, D., Wiebe, P., Runga, J., Postel, L., Dunn, J., Miller, C. and Coombs, S. (2000). Collecting zooplankton. In Harris R. P., Wiebe P. H., Lenz J., Skjoldal H. R. and Huntley, M. (eds), *ICES Zooplankton Methodology Manual*. Academic Press, San Diego, pp. 55-81.

Sampson, R. N., Wright, L. L., Winjum, J. K., Kinsman, J. D., Benneman, J., Kürsten, E. and Scurlock, J. M. (1993). Biomass management and energy. *Water, Air, and Soil Pollution*, **70**, 139-159.

Sanford, A., Morgan, J., Evans, D. and Ducklow, H. (2001). Bacterioplankton dynamics in estuarine mesocosms: Effects of tank shape and size. *Microbial Ecology*, **41**, 45-55.

Santangelo, J. M., Bozelli, R. L., Esteves, F. D. A. and Tollrian, R. (2010). Predation cues do not affect the induction and termination of diapause in small-bodied cladocerans. *Freshwater Biology*, **55**, 1577-1586.

Schlacher, T. A. and Wooldridge, T. H. (1996). Ecological response to reductions in freshwater supply and quality in South Africa's estuaries: lessons for management and conservation. *Journal of Coastal Conservation*, **2**, 115-130.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B., Parks, D., Robinson, C., Sahl, J., Stres, B., Thallinger, G., Van Horn, D. and Weber, C. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied Environmental Microbiology*, **75**, 7537-7541.

Schmitz, O. J. (2007). Predator diversity and trophic interactions. *Ecology*, **88**, 2415-2426.

Schmitz, O. J., Beckerman, A. P. and O'Brien, K. M. (1997). Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology*, **78**, 1388–1399.

Schmitz, O. J., Krivan, V. and Ovadia, O. (2004). Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters*, **7**, 153-163.

Schmitz, O. J., Grabowski, J. H., Peckarsky, B. L., Preisser, E. L., Trussell, G. C. and Vonesh, J. R. (2008). From individuals to ecosystem function: toward an integration of evolutionary and ecosystem ecology. *Ecology*, **89**, 2436-2445.

Schoener, T. W., Shurin, J. B., Sinclair, A. R., Soule, M. E., Virtanen, R. and Wardle, D. A. (2011). Trophic downgrading of planet Earth. *Science* **333**, 301-306.

Schumann, E. H. and Pearce, M. W. (1997). Freshwater inflow and estuarine variability in the Gamtoos Estuary, South Africa. *Estuaries*, **20**, 124-133.

Shapiro, J. and Wright, D. I. (1984). Lake restoration by biomanipulation. Round Lake, Minnesota, them first two years. *Freshwater Biology*, **14**, 371-383.

Short, F. T. (1987). Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. *Aquatic Botany*, **27**, 41-57.

Sih, A. (1986). Antipredator responses and the perception of danger by mosquito larvae. *Ecology*, **67**, 434-441.

Sih, A., Englund, G. and Wooster, D. (1998). Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution*, **13**, 350-355.

Ślusarczyk, M. (1999). Predator-induced diapause in *Daphnia magna* may require two chemical cues. *Oecologia*, **119**, 159-65.

Ślusarczyk, M., Dawidowicz, P. and Rygielska, E. (2005). Hide, rest or die: a light-mediated diapause response in *Daphnia magna* to the threat of fish predation. *Freshwater Biology*, **50**, 141-146.

Smetacek, V., Assmy, P. and Henjes, J. (2004). The role of grazing in structuring Southern Ocean pelagic ecosystems and biogeochemical cycles. *Antarctic Science*, **16**, 541-558.

Smith, V. H. (2007). Microbial diversity productivity relationships in aquatic ecosystems. *FEMS Microbiology Ecology*, **62**, 181-186.

Snow, G. C., Adams, J. B. and Bate, G. C. (2000). Effect of river flow on estuarine microalgal biomass and distribution. *Estuarine, Coastal and Shelf Science*, **51**, 255-266.

Sokol-Hessner, L. and Schmitz, O. J. (2002). Aggregate effects of multiple predator species on a shared prey. *Ecology*, **83**, 2367-2372.

Sommer, U. (2008). Trophic cascades in marine and freshwater plankton. *International Review of Hydrobiologia*, **93**, 506-516.

Sommer, U. (2009). Copepod growth and diatoms: insensitivity of *Acartia tonsa* to the composition of semi-natural plankton mixtures manipulated by silicon: nitrogen ratios in mesocosms. *Oecologia*, **159**, 207-215.

Sommer, U. and Sommer, F. (2006). Cladocerans versus copepods: the cause of contrasting top-down controls on freshwater and marine phytoplankton. *Oecologia*, **147**, 183-194.

Sommer, U., Hansen, T., Blum, O., Holzner, N., Vadstein, O. and Stibor, H. (2005). Copepod and microzooplankton grazing in mesocosms fertilised with different Si: N ratios: no overlap between food spectra and Si: N influence on zooplankton trophic level. *Oecologia*, **142**, 274-283.

Sommer, U., Aberle, N., Engel, A., Hansen, T., Lengfellner, K., Sandow, M., Wohlers, T, Zollner, E. and Riebesell, U. (2007). An indoor mesocosm system to study the effect of climate change on the late winter and spring succession of Baltic Sea phyto- and zooplankton. *Oecologia*, **150**, 655-667.

Spiller, D. A. and Schoener, T. W. (1988). An experimental study of the effect of lizards on web-spider communities. *Ecological Monographs*, **58**, 57-77.

Spiller, D. A. and Schoener, T. W. (1998). Lizards reduce spider species richness by excluding rare species. *Ecology*, **79**, 503–516.

Spivak, A. C., Vanni, M. J. and Mette, E. M. (2011). Moving on up: can results from simple aquatic mesocosm experiments be applied across broad spatial scales? *Freshwater Biology*, **56**, 279-291.

Stenseth, N. C. and Mysterud, A. (2002). Climate, changing phenology, and other life history traits: nonlinearity and match–mismatch to the environment. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 13379-13381.

Stibor, H., Vadstein, O., Diehl, S., Gelzleichter, A., Hansen, T., Hantzsche, F., Katechakis, A., Lippert, B., Loseth, K., Peters, C., Roederer, W. Sandow, M., Sundt-Hansen, L. and Olsen, Y. (2004a). Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. *Ecology Letters*, **7**, 321-328.

Stibor, H., Vadstein, O., Lippert, B., Roederer, W. and Olsen, Y. (2004b). Calanoid copepods and nutrient enrichment determine population dynamics of the appendicularian *Oikopleura dioica*: a mesocosm experiment. *Marine Ecology Progress Series*, **270**, 209-215.

Stoecker, D. K. and Capuzzo, J. M. (1990). Predation on protozoa: its importance to zooplankton. *Journal of Plankton Research*, **12**, 891-908.

Strong, D. R. (1992). Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. *Ecology*, **73**, 747-754.

Strydom, N. A., Whitfield, A. K. and Wooldridge, T. H. (2003). The role of estuarine type in characterizing early stage fish assemblages in warm temperate estuaries, South Africa. *African Zoology*, **38**, 29-43.

Suzuki, K., Handa, N., Nishida, T. and Wong, C. S. (1997). Estimation of phytoplankton succession in a fertilized mesocosm during summer using high-performance liquid chromatographic analysis of pigments. *Journal of Experimental Marine Biology and Ecology*, **214**, 1-17.

Svensson, J. E. (1997). Fish predation on *Eudiaptomus gracilis* in relation to clutch size, body size, and sex: a field experiment. *Hydrobiologia*, **344**, 155-161.

Taylor, D. I., Nixon, S. W., Granger, S. L., Buckley, B. A., McMahon, J. P. and Lin, H. J. (1995). Responses of coastal lagoon plant communities to different forms of nutrient enrichment- a mesocosm experiment. *Aquatic Botany*, **52**, 19-34.

Teira, E., Gasol, J. M., Aranguren-Gassis, M., Fernandez, A., Gonzalez, J., Lekunberri, I. and Alvarez-Salgado, X. A. (2008). Linkages between bacterioplankton community composition, heterotrophic carbon cycling and environmental condition in a highly dynamic coastal system. *Environmental Microbiology*, **10,** 906-917.

Teplitsky, C., Plénet, S. and Joly, P. (2003). Tadpoles' responses to risk of fish introduction. *Oecologia*, **134**, 270-277.

Tilman, D. and Downing, J. A. (1996). Biodiversity and stability in grasslands. *Ecosystem Management: Selected Readings*, **367**, 363-365.

Tranvik, L. J. and Hansson, L. A. (1997). Predator regulation of aquatic microbial abundance in simple food webs of sub-antarctic lakes. *Oikos*, **79**, 347-356.

Troussellier, M., Got, P., Mboup, M., Corbin, D., Giuliano, L., Cappello, S. and Bouvy, M. (2005). Daily bacterioplankton dynamics in a sub-Saharan estuary (Senegal River, West Africa): a mesocosm study. *Aquatic Microbial Ecology*, **40**, 13-24.

Turley, C. M. (1993). Direct estimates of bacterial numbers in seawater samples without incurring cell loss due to sample storage. In Kemp, P. F., Sherr, B. F., Sherr, E. B. and Cole, J.J. (eds), *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Boston Raton, pp. 143-147.

Turpie, J. K., Adams, J. B., Joubert, A., Harrison, T. D., Colloty, B. M., Maree, R. C., Whitfield, A. K., Wooldridge, T. H., Lamberth, S. J., Taljaard, S. and Van Niekerk, L. (2004). Assessment of the conservation priority status of South African estuaries for use in management and water allocation. *Water SA*, **28**, 191-206.

Tweddle, G. P. (2005). Population dynamics of selected ichthyofaunal components in the temperate, temporarily open/closed Kasouga Estuary, South Africa. MSc thesis, Rhodes University, Grahamstown.

Vakkilainen, K., Kairesalo, T., Hietala, J., Balayla, D. M., Becares, E., Van de Bund, W. J., Van Donk E., Fernández-Aláez, M., Gyllstrom, M., Hansson, L. A., Miracle, M. R., Moss, B., Romo, S., Rueda, J. and Stephen, D. (2004). Response of zooplankton to nutrient enrichment and fish in shallow lakes: a pan-European mesocosm experiment. *Freshwater Biology*, **49**, 1619-1632.

Vanni, M. J. (1987). Effects of nutrients and zooplankton size on the structure of a phytoplankton community. *Ecology*, **68**, 624-635.

Verity, P. G. and Smetacek, V. (1996). Organism life cycles, predation, and the structure of marine pelagic ecosystems. *Marine Ecology Progress Series*, **130**, 277-293.

Visser, M. E. and Holleman, L. J. (2001). Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **268**, 289-294.

von Rückert, G. and Giani, A. (2008). Biological interactions in the plankton community of a tropical eutrophic reservoir: is the phytoplankton controlled by zooplankton? *Journal of Plankton Research*, **30**, 1157-1168.

Vumazonke, L. U., Mainoane, T. S., Bushula, T. and Pakhomov, E. A. (2008). A preliminary investigation of winter daily food intake by four small teleost fish species from the Igoda Estuary, Eastern Cape, South Africa. *African Journal of Aquatic Science*, **33**, 83-86.

Walker, D. R., Perissinotto, R. and Bally, R. P. A. (2001). Phytoplankton/protozoan dynamics in the Nyara Estuary, a small temporarily open system in the Eastern Cape (South Africa). *Southern African Journal of Aquatic Sciences*, **26**, 31-38.

Walter, T. C. (1987). Review of the taxonomy and distribution of the demersal copepod genus *Pseudodiaptomus* (Calanoida: Pseudodiaptomidae) from southern Indo-West Pacific waters. *Marine and Freshwater Research*, **38**, 363-396.

Wang, Y. and Qian, P. (2009). Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS ONE*, **4**, e7401.

Wang, Q., Garrity, G. M., Tiedje, J. M. and Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, **73**, 5261-5267.

Ward, P. I. (1986). A comparative field study of the breeding behaviour of a stream and a pond population of *Gammarus pulex* (Amphipoda). *Oikos*, **46**, 29-36.

Warfe, D. M. and Barmuta, L. A. (2004). Habitat structural complexity mediates the foraging success of multiple predator species. *Oecologia*, **141**, 171-178.

Warlen, S. M. and Burke, J. S. (1990). Immigration of larvae of fall/winter spawning marine fishes into a North Carolina estuary. *Estuaries*, **13**, 453-461.

Wasserman, R. J. (2012). Feeding ecology of the early life-history stages of two dominant gobiid species in the headwaters of a warm-temperate estuary. *Estuarine Coastal Shelf Science*, **109**, 11-19.

Wasserman, R. J. and Mostert, B. P. (*in press*). Intertidal crab burrows as a low-tide refuge habitat for a specific gobiid: preliminary evidence for commensalism. *Marine and Freshwater Research* - doi.org/10.1071/mf13081.

Wasserman, R. J. and Strydom, N. A. (2011). The importance of estuary head waters as nursery areas for young estuary- and marine-spawned fishes in temperate South Africa. *Estuarine, Coastal and Shelf Science*, **94**, 56-67.

Wasserman, R. J., Strydom, N. A. and Wooldridge, T. H. (2010). Larval fish dynamics in the Nxaxo-Ngqusi Estuary complex in the warm temperate-subtropical transition zone of South Africa. *African Zoology* **45**, 63-77.

Weissenberger, J. (1998). Arctic Sea ice biota: design and evaluation of a mesocosm experiment. *Polar Biology*, **19**, 151-159.

Welton, N. J., McNamara, J. M. and Houston, A. I. (2003). Assessing predation risk: optimal behaviour and rules of thumb. *Theoretical Population Biology*, **64**, 417-430.

Westman, W. E. (1978). Measuring the inertia and resilience of ecosystems. *BioScience*, **28**, 705-710.

Wheeler, B. (2010a). *lmPerm: Permutation tests for linear models. R package version 1.1-2*. Available at: http://CRAN.R-project.org/package=lmPerm [accessed 25 August 2013].

Wheeler, R. E. (2010b). *Permutation tests for linear models in R.* Available at: http://cran.r-project.org/web/packages/lmPerm/vignettes/lmPerm.pdf [accessed 25 August 2013].

Whitfield, A. K. (1983). Factors influencing the utilisation of southern African estuaries by fishes. *South African Journal of Science*, **79**, 362-365.

Whitfield, A. K. (1992). A characterization of southern African estuarine systems. S*outhern African Journal of Aquatic Sciences*, **18**, 89-103.

Whitfield, A. K. (1998). Biology and Ecology of Fishes in South African estuaries. Ichthyological Monographs of the J.L.B. Smith Institute of Ichthyology 2.

Whitfield, A. K. (2000). *Available scientific information on individual South African estuarine systems.* WRC Report No. 577/3/00.

Whitfield, A. K. (2005). Fishes and freshwater in southern African estuaries - a review. *Aquatic Living Resources* **18**, 275-289.

Whitfield, A. K. and Baliwe, N. G. (2013). *A century of science in South African estuaries: Bibliography and review of research trends*. SANCOR Occasional Report No. 7.

Whitfield, A. K. and Bruton, M. N. (1989). Some biological implications of reduced fresh water inflow into eastern Cape estuaries: a preliminary assessment. *South African Journal of Science*, **85**, 691-694.

Whitfield, A. K. and Harrison, T. D. (1996). *Gilchristella aestuaria* (Pisces: Clupeidae) biomass and consumption of zooplankton in the Sundays Estuary. *South African Journal of Marine Science* **17**, 49-53.

Whitfield, A. K., Adams, J. B., Bate, G. C., Bezuidenhout, K., Bornman, T. G., Cowley, P. D., Froneman, P. W., Gama, P. T., James, N. C., MacKenzie, B., Riddin, T., Snow, G. C., Strydom, N. A., Taljaard, S. S., Terode, A. I., Theron, A. K., Turpie, J. K., van Niekerk, L., Vorwerk, P. D. and Wooldridge, T. H. (2008). A multidisciplinary study of a small intermittently open South African estuary with particular emphasis on the influence of mouth state on the ecology of the system. *African Journal of Marine Science*, **30**, 463-473.

Williams, A. E., Moss, B. and Eaton, J. (2002). Fish induced macrophyte loss in shallow lakes: top–down and bottom–up processes in mesocosm experiments. *Freshwater Biology*, **47**, 2216-2232.

Winder, M. and Schindler, D. E. (2004). Climate change uncouples trophic interactions in an aquatic ecosystem. *Ecology*, **85**, 2100-2106.

Wisenden, B. D. and Millard, M. C. (2001). Aquatic flatworms use chemical cues from injured conspecifics to assess predation risk and to associate risk with novel cues. *Animal Behaviour*, **62**, 761-766.

Wooldridge, T. H. (2007). Biotic response to altered freshwater inflow patterns to the Kromme River Estuary, South Africa. *WIT Transactions on Ecology and the Environment*, **103**, 687-696.

Wooldridge, T. and Bailey, C. (1982). Euryhaline zooplankton of the Sundays Estuary and notes on trophic relations. *South African Journal of Zoology*, **17**, 151-163.

Wooldridge, T. H. and Callahan, R. (2000). The effects of a single freshwater release into the Kromme Estuary. 3. Estuarine zooplankton response. *Water SA*, **26**, 311-318.

Wooldridge, T. H. and Deyzel, S. H. P. (2012). Variability in estuarine water temperature gradients and influence on the distribution of zooplankton: a biogeographical perspective. *African Journal of Marine Science*, **34**, 465-477.

Wooldridge, T. H. and Melville-Smith, R. (1979). Copepod succession in two South African estuaries. *Journal of Plankton Research*, **1**, 329-341.

Wooldridge, T. H. and Webb, P. (1988). Predator-prey interactions between two species of estuarine mysid shrimps. *Marine Ecology Progress Series.* **50**, 21-28.

Worsfold, N. T., Warren, P. H. and Petchey, O. L. (2009). Context-dependent effects of predator removal from experimental microcosm communities. *Oikos*, **118**, 1319-1326.

Worthen, W. B. (1989). Predator-mediated coexistence in laboratory communities of mycophagous *Drosophila* (Diptera: Drosophilidae). *Ecological Entomology*, **14**, 117–126.

Zhao, X. and Chivers, D. P. (2005). Response of juvenile goldfish to chemical alarm cues: relationship between response intensity, response duration and the level of predation risk. In Mason, R. T., LeMaster, M. P. and Müller-Schwartze, D. (eds), *Chemical Signals in Vertebrates, Vol. 10*. Springer Verlag, New York, pp. 334-341.

Zimmer, R. K. and Butman, C. A. (2000). Chemical signaling processes in the marine environment. *The Biological Bulletin*, **198**, 168-187.

Zöllner, E., Hoppe, H. G., Sommer, U. and Jürgens, K. (2009). Effect of zooplankton-mediated trophic cascades on marine microbial food web components (bacteria, nanoflagellates, ciliates). *Limnology and Oceanography*, **54**, 262-275.