Assessing dependence on groundwater of nearshore coastal habitats in south-east South Australia

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Abstract

The ecological function that groundwater has in influencing ecosystems in Australia is poorly understood and there is virtually no literature that gives evidence about the hypothesised dependence that nearshore coastal ecosystems may have on groundwater outflows. Specifically, nothing is known about what effects groundwater has on the ecological components of nearshore marine environments, nor the implications of any measurable dependence on groundwater that this may have for natural resource managers and water allocation planning. Thus, as a first step, this study set out to assess whether animal and plant assemblages in coastal waterbodies such as small estuaries, drains, creeks, ponds, springs, beaches and other nearshore marine environments in the South East Region of South Australia are influenced by groundwater discharge.

Waterbodies were first grouped by their type, regional location and observed flow regime. Analyses of salinity, temperature, [TIN], [NOx], [NO_3], [NO_2], and ^2H and ^{18}O isotopes found that: Eastern drains were different from Western Drains; seasonal-flow drains differed from continuous-flow drains; and Western drains were significantly different from Eastern drains, groundwater ponds and springs, which were all similar. Thus Eastern drains are predominantly maintained and fed from groundwater resources. Waterbodies associated with groundwater inputs tended to be fresher,

cooler, had more nitrogen (especially NO_3) and were more enriched with hydrogen and oxygen isotopes.

Continuous groundwater outflow in coastal creeks appeared to restrict any marine water influence so that estuarine gradients were non-existent. Macrofaunal total abundance and species richness was dominated by insects suggesting that continuous groundwater inputs might have a level of influence shaping macrofauna assemblages in these continuous-flow groundwater-fed streams, as opposed to a more estuarine character in intermittent streams.

Beach springs feature a sand slurry caused by constant outflow of groundwater. Meiofaunal abundance and species composition was lower in groundwater-driven beach springs than on marine intertidal (open coast) beaches. Animals unique to each sampling location were observed and two possibly new nematode species were collected from within the beach spring.

A locally common bivalve, *Paphies elongata,* was collected for $\delta^{15}N$ flesh analyses from an area under direct influence of discharging groundwater but showed no difference in the values from different sample groups. Thus groundwater-influenced ^{15}N accumulation was not evident in the tissue mass of *Paphies elongata*, suggesting that the spatially-removed individuals collected were from the same localised population. Nitrogen concentration in the flesh of *P. elongata* was approximately 10% of their dry weight; this is likely to be the first published data relating to N concentration in this species. Standing stock biomass of flora in coastal SE SA drains ranged on average from 0 to >263 g DW.m^{-2} and daily mean productivity of periphyton ranged from <1 to 18 g DW.m^{-2}.d^{-1}. Continuous-flow drains had about 10 times the average biomass of seasonal-flow drains, and mean productivity in seasonal-flow drains was approximately twice that of continuous-flow drains. Continuous- and seasonal-flow drains had approximately similar mean $\delta^{15}N$ values.

The findings and methodological learnings from this study can be built upon and applied by other researchers within this field as well as managers of these water resources.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference in made in the text.

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CHAPTER 1 – GENERAL INTRODUCTION

1 Why study groundwater dependent ecosystems in nearshore coastal ecosystems?

1.1 INTRODUCTION

"*Groundwater is water that has been present in pores and cracks of the saturated zone of soil or rock for sufficient time to undergo physical and chemical changes resulting from interactions with the aquifer environment.*

This functional definition extends the hydrogeological definition (*all water beneath the watertable in soils and geologic formations that are fully saturated) to one that is ecologically relevant*"

(Tomlinson and Boulton, 2008)

The function that groundwater has in influencing ecosystems in Australia is poorly understood (Hatton & Evans, 1998) and virtually no literature exists, giving evidence about the hypothesised dependence that nearshore coastal ecosystems may have on groundwater. As a poorly understood and studied resource, groundwater nonetheless may significantly influence some major marine ecosystems in Australia (Johannes 1980; Hatton & Evans, 1998). It is the main objective of this project to investigate the perceived influence that groundwater may, as a distinct water source, have on nearshore marine ecosystems and, in particular, upon the ecological processes that may influence the composition of plant and animal communities so that they may have some level of dependence upon groundwater inflow.

In this introductory first chapter, I will attempt to provide the reader with a basic understanding of this emerging and increasingly-important area of research by discussing published studies to outline the project's background, importance and general objectives. Specific detail relevant to particular objectives of the broader project will be discussed in more detail in subsequent chapters of this thesis.

1.2 BACKGROUND LITERATURE REVIEW

The following literature review has particular focus on those few ecological studies that have attempted to investigate impacts of groundwater on nearshore marine environments. It will also identify other areas of investigation that may have less direct reference to the present study but variously relate to: groundwater modelling and hydraulics; physical process and chemical tracking of groundwater entering the marine environment (e.g. via natural radioactive tracers); and the quality and quantity of groundwater entering the marine environment (e.g. via nutrient analyses). The present study does not intend to specifically investigate these components but comparisons with such references may be made later when discussing an ecological result.

Journal articles were sourced using the scientific databases Current Contents and Biological Abstracts between the years 1991 to 2006. Major search terms included 'groundwater', 'nearshore', 'marine', 'habitats', 'biota', 'seagrass', 'seepage', 'discharge', 'submarine', 'isotope', 'nitrogen', 'oxygen', 'hydrogen', 'deuteron' and '15N'. Terms were either used individually or in combinations. Where relevant papers were obtained, additional searches by the name(s) of author(s) of these papers were also conducted to see if they had done any similar studies.

Upon detailed review, many research papers obtained did not live up to expectations (due to either misleading titles or abstracts) or contained only one relevant statement about groundwater given as either an assertion or presumption but without data or any other evidence, especially from an ecological perspective.

1.2.1 Non-ecological studies

Many studies found have in some way investigated components of groundwater potentially entering or affecting marine environments. The studies identified below did not specifically attempt to determine whether nearshore coastal ecosystems are dependent on groundwater; instead they simply presented descriptively some likely influences of groundwater on nearshore marine environments. They are presented here for the purposes of this review and to reaffirm that there have been comparatively-limited ecological studies that attempted to directly describe and/or experiment with ecological relationships involving groundwater entering the sea.

1.2.1.1 Hydrological studies

Hydrological research that attempted to model groundwater entering the marine environment has been conducted (e.g. Todd 1959; Simmons 1992; Millham & Howes 1994; Portnoy *et al.* 1998; Li *et al.* 1999; Nowicki *et al.* 1999; Ranasinghe & Pattiaratchi 1999; Uchiyama *et al.* 2000; Ataie-Ashtiana *et al.* 2001; Hoefel & Evans 2001; Gobler & Boneillo 2003; Smith & Nield 2003; Michael *et al.* 2005).

Tobias *et al.* (2001a, 2001b) attempted to quantify groundwater inputs moving through fringing wetlands to estuaries and found that seasonal flushing by groundwater may be important in purging salt from wetlands and exporting other material such as nutrients to estuaries. Michael *et al.* (2005) considered the influence of seasonal oscillation of groundwater on the nearshore environment in Waquoit Bay, Massachusetts, USA. This study also trialled a novel non-submerged intertidal seepage meter (based on a Lee-type seepage meter, see Lee 1977). In an Australian example, Smith & Nield (2003) attempted to model general groundwater inflow into Cockburn Sound in Western Australia.

1.2.1.2 Chemical Tracers

Studies specifically tracking groundwater flow into the marine environment utilising tracers like CH_4 (Corbett *et al.* 1999, 2000) and Cl^- concentrations

(Bayari & Kurttas 2002; Garrison *et al.* 2003), or the isotopes ^{222}Rn (Corbett *et al.* 1999, 2000; Garrison *et al.* 2003; Blanco *et al.* 2011; de Sieyes *et al.* 2011) and ^{226}Ra (Corbett *et al.* 2000; Charette *et al.* 2001, Lamontagne *et al.* 2008), ^{223}Ra, ^{224}Ra, ^{226}Ra, ^{228}Ra (Lamontagne *et al.* 2008, Loveless *et al.* 2008), ^{3}H/ ^{3}He (Katz 1992; Toth & Katz 2006), have also been undertaken. These studies found, generally, that these tracking methods were satisfactory in estimating groundwater flux but the ability to routinely measure sometimes small amounts of particular chemicals may be limited and expensive.

In a South Australian paper, Lamontagne *et al.* (2008) used radon and radium tracers to estimate submarine groundwater discharge in coastal waters of the Adelaide region of Gulf St Vincent. Their study found that submarine groundwater discharge (SGD) flux in the nearshore marine environments was largely from seawater recirculation rather that continuous groundwater seepage per se. Other Australian examples include Loveless *et al.* (2008), who used four radon species to describe groundwater entering Cockburn Sound near Perth, Western Australia, and Stieglitz (2005), who used radon tracing and lower salinities combined to track groundwater discharge in the nearshore area of the Great Barrier Reef, Queensland. Stieglitz used the colloquial term ‘wonky holes’ to describe where point sources of groundwater discharge into the marine environment. In contrast, the term ‘pockmarks’ (Bussman *et al.* 2011) has been used to describe similar underwater point-source groundwater discharge areas in German (freshwater) lakes.

1.2.1.3 Nutrients

The impacts of nutrients entering the marine environment via groundwater have been better documented. Many studies have investigated the impact of anthropogenic nutrient (e.g. nitrogen, phosphorus, silicate) inputs via sewage, landfill leaching and/or fertiliser applications entering groundwater reserves and subsequently discharging into marine environments (e.g. Vanek & Lee 1991; Dollar & Atkinson 1992; Simmons 1992; Portnoy *et al.* 1998; Corbett *et al.* 1999; Laws *et al.* 1999; Nowicki *et al.* 1999; Uchiyama *et al.* 2000; Charette *et al.* 2001; Montlucon & Sanudo-Wilhelmy 2001; Valiela *et al.* 2001; Umezawa *et al.* 2002; Garrison *et al.* 2003; Gobler & Boneillo 2003; Sigua & Tweedale 2003; Ullman *et al.* 2003, Blanco *et al.* 2011). All these studies present comparable conclusions that human activities promote the amount of nutrients entering groundwater and that such nutrient inputs have been increasing over time.

Blanco *et al.* (2011) found that groundwater-associated nutrient inputs (*P*articularly nitrate) has the ability to change microalgal composition towards a specific classification (e.g. from diatom to cyanobacteria-dominated classes). Specifically, at Shiraho Reef (Okinawa, Japan) increased nutrients delivered via groundwater pathways induced a proliferation of cyanobacteria in nearshore marine waters.

1.2.2 Isotopic analyses of biota and water

1.2.2.1 Stable Isotope Analysis of $\delta^{15}N$ in Biota

Stable isotopes are alternative forms of chemical elements (Krebs 2001). The variations in the number of neutrons in the nucleus of an element provides for different atomic weights (masses) of a particular element (Clark & Fritz 1997). Nitrogen (N), for example, has two common isotopes ^{14}N and ^{15}N. The lighter ^{14}N isotope is more common (99.63%) where as only a much smaller fraction (0.37%) is the heavier ^{15}N isotope (Clark & Fritz 1997; Krebs 2001). The ratio of the isotopes in any material is expressed as δ (delta) values, which are parts per thousand (‰) (Krebs 2001) or permils (Clark & Fritz 1997), as departures from a standard substance. For nitrogen, that standard is air (Clark & Fritz 1997; Krebs 2001). Positive values indicate that the sample material is richer in the heavier ^{15}N isotope than the standard (Krebs 2001) (or 'enriched'); conversely, negative values indicate that the sample material is poorer in the heavier ^{15}N isotope than the standard or 'depleted'.

Nearshore studies involving $\delta^{15}N$ include Corbett *et al.* (1999), Laws *et al.* (1999), Valiela *et al.* (2001) and Kamermans *et al.* (2002). The main focus of these studies has been on anthropogenic nutrient inputs into the marine environment via sewage. Corbett *et al.* (1999) analysed $\delta^{15}N$ in attached macroalgae and tropical seagrass (Thalassia sp.) and suggested that the measured enrichment of $\delta^{15}N$ in macroalgae and seagrass is due to denitrification of $\delta^{15}N$-enriched groundwater in suboxic sediments.

Changes in osmotic conditions and increased supply of nutrients to primary producers are two main potential effects of submarine groundwater discharge (SGD) (Kamermans *et al.* 2002). In a study conducted in the tropical coastal lagoons of East Africa, Kamermans *et al.* (2002) tested an hypothesis that seagrass species diversity may be influenced by differences in groundwater outflow and that those seagrass species that tolerate or flourish in lower salinities may dominate in areas of known SGD. An objective of their study was to relate the rate of coastal groundwater discharge to the abundance and diversity of seagrasses. Their study suggested that the natural abundances of $\delta^{15}N$ in seagrass tissues can be used as a tracer of nitrogen inputs from groundwater and that $\delta^{15}N$ enrichment in these tissues may indicate denitrification in a suboxic zone or from wastewater.

Kamermans *et al.* (2002) used line transects and photographic methods to determine percent coverage of seagrasses. In addition, seagrasses were harvested for biomass calculations using $0.06m^2$ quadrats. Sediments and porewater samples were also collected at the same locations. Analysis of $\delta^{15}N$ was undertaken using Isotopic Ratio Mass Spectrometry (IRMS). Only one visit to each lagoon was conducted and thus the study yielded only a small sample size. As a consequence, a non-parametric test (Kendall's coefficient of rank correlation) was used in subsequent analyses.

Kamermans *et al.* (2002) found that, generally, the number of seagrass species found was low in areas of increased groundwater outflow and that

Thalassodendron ciliatum was the only seagrass species found at all sites with high groundwater outflow. *Thalassodendron* is from the same family (Cymodoceaceae) as, and morphologically resembles, *Amphibolis*, a genus found regionally in South Australia (Green & Short 2003). The Kamermans *et al.* (2002) study also found that a negative relationship was evident between predicted groundwater outflow and seagrass species diversity but a positive relationship between $\delta^{15}N$ concentrations in *T. ciliatum* and predicted groundwater outflow. A statistically-significant increase in ^{15}N concentration (mean +2 to +6‰ $\delta 1^{15}N$; maximum values +7 to +10‰ $\delta^{15}N$) in the leaves of *T. ciliatum* was found in areas with groundwater outflow (compared to those without groundwater outflow) suggesting that nitrogen is being absorbed from groundwater sources (Kamermans *et al.* 2002).

In other seagrass and groundwater studies, McClelland & Valiela (1998) reported 6‰ $\delta^{15}N$ in *Zostera marina* (Waquoit Bay near Cape Cod, USA) and Corbett *et al.* (1999) recorded 11-13‰ $\delta^{15}N$ in *Thalassia testudinium* (Florida). McClelland & Valiela (1998) recorded -0.9 to +14.9‰ NO_3–$\delta^{15}N$ in groundwater (4 – 86% of the total N pool) and Laws *et al.* (1999) found that the $\delta^{15}N$ of biologically-available nitrogen in groundwater was 10.4±0.7‰ $\delta^{15}N$. McClelland *et al.* (1997) suggested that generally NO_3^- -derived $\delta^{15}N$ values in groundwater coming from atmospheric deposition only has values ranging from +2 to +8‰ compared to +10 to +20‰ from human and animal wastes, and -3 to + 3‰ from synthetic NO_3^- fertilisers. McClelland *et al.* (1997) thus concluded that in Waquoit Bay, USA increased $\delta^{15}N$ in macroalgae and phytoplankton was due to anthropogenic wastewater entering groundwater resources.

It is anticipated that the analysis of $\delta^{15}N$ will be a major tool in initially assisting to determine groundwater signatures and later in the analysis of plants and animals to establish amounts of nitrogen uptake from groundwater sources.

1.2.2.2 Stable Isotope Analysis of $\delta^{2}H$ and $\delta^{18}O$ in waters

As discussed above, elements can vary in the number of neutrons which can result in different atomic weights of a particular element (Clark & Fritz 1997; Krebs 2001). The isotopic abundance for hydrogen is comprised predominantly of common hydrogen ^{1}H (>99.98%), with most of the remainder (<0.2%) made up of ^{2}H (also known as deuterium) and a very small percentage of ^{3}H. The isotopic abundance of oxygen is dominated by common oxygen ^{16}O (approximately 99.76%), with the remainder made up of about 0.2% heavy oxygen ^{18}O and the much rarer ^{17}O (Mazor 1991). The isotopic composition of water is expressed (in per mils) as a deviation from an internationally-agreed standard (i.e. the Vienna Standard Mean Ocean Water) (Clark & Fritz 1997).

Differences in the isotopic composition of ^{2}H and ^{18}O can be used as tracing methods and are commonly used in groundwater studies (Mazor 1991; Clark & Fritz 1997; Krebs 2001).

Groundwater values within the south east of South Australia have reportedly ranged between -33 and -12‰ for δ^2H, and between (approximately) -7 and -1‰ $\delta^{18}O$ (Love *et al.* 1993, Leaney & Herczeg 1995). In comparison, values in karst systems in Northern Mexico range between -68 and -32‰ for δ^2H, and between (approximately) -10 and +1‰ $\delta^{18}O$ (Ortego-Guerrero 2003).

1.2.3 Ecological Studies

Those few studies that have presented some discussion on identified marine species being affected by groundwater discharge have been classified, arguably, as 'ecological'. Many of these 'ecological' studies did not specifically discuss the phenomena and measurements that are intended to be undertaken in this study.

Kohout and Kolipinski (1967), undertaken in Biscayne Bay, Florida, stands as one of the oldest as well as the most relevant ecological studies of groundwater discharge to the marine environment. The study site included documented sites (mapped on nautical charts) where groundwater springs could be observed 'bubbling' out above the sea surface at low tide (in water depths of approximately 30 cm) via natural flow. The study was typically descriptive but did provide useful species lists of algae, seagrasses and fauna, and detailed their distribution and zonation in relation to salinity measurements. The list included species from major taxonomic groups of fauna including Porifera, Cnidaria, Annelida, Echinodermata, Mollusca, Crustacea and fishes (see Table 6 in Kohout & Kolipinski 1967). The methods used to sample them included trawling and visual assessment, with sampling undertaken across a relatively large spatial scale ranging from 15 to 2130 metres from shore.

The Kohout and Kolipinski (1967) study is, to date, the only one that has discussed in reasonable detail the distribution and zonation of multiple species of fauna and its relationship to salinity fluctuations related to SGD. Their discussion focussed mainly on the salinity preference of five species: the gastropod *Neritina virginea*, mysid *Taphomysis bowmani*, and rainwater killifish *Lucania parva* had a brackish-water preference; and the gastropod *Columbella rusticoides* and pinfish *Lagodon rhomboides* had a high-salinity preference. The former three species thus occurred in greater abundance closer to shore (i.e. nearer to groundwater sources) and the latter two species occurred in greatest abundance commencing from about 120 metres from shore (i.e. away from groundwater). Information was also presented on the distribution of an additional 13 species. Of the 10 species found commonly in the lower-salinity waters, seven were gastropods. While the Kohout and Kolipinski (1967) discussion pointed to tidal movements and hydrogen sulphide interactions in the nearshore region also affecting the movement of "shelled animals" (i.e. mainly gastropods), it may be possible that the higher nutrient values in groundwater (see discussion above) also benefited epiphytic growth on seagrass and algae, which in turn benefited some gastropod grazers. This observation could warrant a future investigation (not tested in this study, but see Chapter 6) to determine if any grazers take up

$\delta^{15}N$ via epiphytes enriched by groundwater sources of nitrogen in south east South Australia.

Apart from Kohout and Kolipinski (1967), only limited and mainly indirect faunal studies have been conducted in relation to groundwater outflow and these have been undertaken specifically on sandy beaches. Johannes and Rimmer (1984) investigated a possible relationship between salinity of sand moisture on northern Western Australian beaches and groundwater seepage as a possible cue for nesting sites for the green turtle, *Chelonia mydas* but no relationship could be found.

A series of studies conducted at Cape Henlopen, Delaware USA by Miller & Ullman (2004) and Dale & Miller (2007, 2008), found that groundwater seeps not only resulted in localised region of reduced salinity but also create environments of more stable temperatures. That is, areas under the influence of thermally-stable groundwater discharge displayed a reduced inter-annual temperature variation than adjacent marine habitats removed from groundwater influences.

Dale & Miller (2007) found that discharging groundwater in pore waters at intertidal depths of 1-6m was, on average, 3-4°C cooler in summer, and 5-6°C warmer in winter compared to nearby marine habitats This reduction in the net annual temperature variability range of 8-10°C was reported as being equivalent to a geographical shift of up to 250 km latitude north in summer (i.e. to cooler climes) and 380 km south in winter (to warmer climes) (Dale &

Miller 2007). The reader should note that as Cape Henlopen is a northern hemisphere site (USA), so the opposite range shift may apply to Australian sites at similar southern latitudes.

This reduced annual temperature variability range was found to provide a more relatively stable microhabitat, which may influence the community composition at these groundwater seeps (Dale & Miller 2008). Although total abundance did increase with increasing distance from groundwater sources, these groundwater-fed habitats seem to permit specialist species to exist in what is a primarily marine setting (Dale & Miller 2007, 2008).

The increased nutrients provided by this discharge were considered to exceed what is needed to support biomass at the Cape Henlopen site and thus influence productivity, abundance, composition of benthic, and/or benthic dependent species (Miller & Ullman 2004). For example, an increase in nutrients was found to increase microflora, thence deposit feeders (e.g. polychaete worms) and grazers up the food chain to crabs, fish and birds. For this reason, Miller & Ullman (2004) described these sites as “biological hotspots” for trophic transfer.

Miller & Ullman (2004) and Dale & Miller (2007, 2008) also found changes in polychaete composition with distance from groundwater sources. In another polychaete study conducted in an area of permanent discharge 50 – 200m offshore from Sylit Island in the German Wadden Sea, Zipperle & Reise (2005) found that polychaete dominance was switched by groundwater discharge.

Zipperle & Reise (2005) found that as salinity decreased towards the permanent discharge so did Arenicola marina (lugworm) abundance (to zero) whereas Nereis virens and N. diversicolor increased twelve-fold. It was observed that the distribution of both was scarce within the fresh (groundwater)-saline (marine) transition zone with the authors suggesting competitive interaction as the cause (Zipperle & Reise 2005).

Ardisson & Bourget (1997) investigated the effects of freshwater runoff as a source of imported energy for five dominant epibenthic species in the Gulf of St Lawrence estuary, Quebec, Canada. The study found, however, no significant relationships between runoff and abundance, biomass and mean weight. Similarly, Lercari and Defeo (1999) concluded that freshwater discharge across sandy beaches in Uruguay played a critical role in population dynamics of the mole crab *Emerita brasiliensis*. The studies did not state if the freshwater was of groundwater in origin.

Johannes' (1980) paper is probably one of the earliest and most cited in the submarine groundwater discharge and nearshore marine environment literature. Johannes (1980) posed the question "what effects does SGD have on the ecological components of nearshore marine environments?" The paper is somewhat of a review and/or conjecture and so does not directly test the hypothesis. Groundwater discharge to coastal waters north of Perth, Western Australia, contained higher nitrate levels than seawater (Johannes 1980). Perth is situated on a coastal sandplain thus extreme groundwater flows may be facilitated due to the sandy nature of the substrate. Johannes (1980) conservatively suggested that submarine groundwater discharge there might deliver up to 3 times as much nitrate as surface-water runoff, which may be important because nitrogen in coastal waters is often a limiting factor to marine plant growth (Johannes 1980).

In a related Australian study undertaken in Marmion Lagoon, Western Australia (north of Perth), Johannes & Hearn (1985) suggested that low salinities (as low as 2ppt, as opposed to the ~35ppt of seawater) close to shore and high nitrate concentrations (as high as 380µM) were due to submarine groundwater discharge. Johannes & Hearn (1985) discovered that diluted seawater due to submarine groundwater discharge was evident in a nearshore area 250 m wide and up to 3 m deep. The study also found a negative correlation between nitrate and salinity. Johannes & Hearn (1985) speculated that increased nutrients in groundwater were due to anthropogenic sewage and agricultural practices. The study also identified greater abundances of the opportunistic green algae *Ulva* spp. and *Enteromorpha* spp. near groundwater discharge sites.

In a study considering the effects of drainage discharge to *Posidonia sinuosa* seagrass beds in Geographe Bay, Western Australia, McMahon & Walker (1998) analysed dry weight of nitrogen in *P. sinuosa*, nutrients in sediments and the water column, and groundwater/seawater dilution factors. Their results suggested that the uptake of nutrients from groundwater was important in this location. McMahon & Walker (1998) found that nitrogen in *P. sinuosa* shoots was only 1.6% of dry weight and also reported that nitrogen within a seagrass system is limiting when nitrogen in shoots is less than 1.8% of dry weight.

Carruthers *et al.* (2005) investigated the influence of submarine groundwater springs and wastewater on seagrass meadows of the Yucatan Peninsula, Mexico. Their study found an increase of N in the tissues of the seagrass *Thalassia testudinum*. Stable isotope analyses revealed that the source of the elevated nitrogen is most likely to have originated from wastewater sources. Carruthers *et al.* (2005) also suggested that submarine groundwater springs can be a direct and important source of phosphorus and iron to otherwise iron-limited carbonate (karst) systems.

In a study involving groundwater flow into Carpinteria Saltmarsh east of Santa Barbara, California, Page (1995) suggested that the chenopod shrub *Salicornia virginica* had a nitrogen-uptake preference for NH_4-N over NO_3-N. NH_4 is likely to be labile in groundwater either being taken up by biota or quickly converted to NH_3.

Rutkowski *et al.* (1999) tested the hypothesis that a relationship exists between submarine groundwater discharge and the distribution of seagrass in nearshore habitats in the Gulf of Mexico. Their test was performed by contrasting 'sand' versus 'grass' areas. The study was unable to verify that a relationship existed with only 3 out of 7 sites having statistically-higher groundwater flow (measured with Lee-type seepage meters; see Lee 1977) in seagrass areas than sand areas. Their paper did not clearly state the sampling or statistical design employed and Kruskal-Wallis ANOVA (by ranks) was used for the statistical analysis. This type of non-parametric analysis is an interesting choice considering that the sample size (e.g. n = 42 - 143) seems adequate for parametric tests and there was no mention of attempts to transform data to meet standard statistical assumptions (Underwood 1997; Quinn & Keough 2002). Again, this is another 'ecological' paper which measured nutrients and flow within a groundwater seepage area, which included seagrass beds, but no direct (e.g. manipulative) experimental test of biota was applied; only indirect inferences via correlations were made.

Herrara-Silveira *et al.* (2002) suggested that coastal ecosystems are closely associated with groundwater discharge in a karst region of Yucatan, Mexico. Their study was fundamentally an 'indicator of health'-type investigation that measured inorganic nutrients, chlorophyll *a*, phytoplankton and macrophyte productivity. They found that nitrate concentrations suggested that the trophic status of these tropical lagoons were influenced by groundwater discharges and that the nitrogen (especially in the form of NO_3^-) cycle was clearly influenced by spring groundwater discharge (Herrara-Silveira *et al.* 2002). The study concluded that indices based upon nutrient concentration and phytoplankton biomass were useful as indicators of trophic status in these groundwater-influenced, tropical coastal lagoons by finding a high proportion of nitrates of groundwater origin in the metabolism of these systems (Herrara-Silveira *et al.* 2002).

In a study of *Zostera marina* seagrass in Long Island Sound Connecticut USA, Keser *et al.* (2003) suggested that the observed decrease in *Z. marina* distribution was not related to thermal input (from a nearby power station) but instead inferred that water-quality issues were a major contributing factor, including high nutrients from groundwater. In similar nutrient-based studies, Naim (1993) concluded that eutrophic stress from coastal development delivered through increased nutrients in groundwater was a main factor resulting in the transformation of coral-rich areas of tropical Reunion Island to algal-dominated ones. Lapointe (1997) also concluded that a bottom-up control of macroalgal biomass was a causal factor in a phase shift from corals to macroalgae on coral reefs in Jamaica and south-east Florida, with increased anthropogenic nutrient inputs into groundwater reported to be the major source. This study also discussed the effects of the loss of top-down controls of macroalgae due to loss of herbivory via over fishing.

It is notable that the majority of these studies view groundwater as primarily a nutrient-delivery mechanism from unnatural changes due to eutrophication. Any natural role for groundwater discharge into nearshore coastal ecosystems is hardly considered.

1.2.4 Management-based reviews: presumed effects and importance of groundwater?

A number of 'groundwater-dependent ecosystem' reviews have recently been published (e.g. Hatton & Evans 1998; Danielopol *et al.* 2003; Murray *et al.* 2003; Eamus *et al.* 2006a; CSIRO 2006; Tomlinson and Boulton, 2008), which discuss the importance of groundwater in terms of its social, economic and ecological importance within an Australian context (except Danielopol *et al.* 2003). In an Australian review, Murray *et al.* (2003) simply acknowledged that groundwater-dependent ecosystems do include estuarine and nearshore marine systems that 'use' groundwater discharge but no other reference or discussion was given to these systems. Danielopol *et al.* (2003) did not discuss groundwater-dependent coastal ecosystems at all in a global review. In fact, the word "marine" is only stated twice in the paper: once in a figure and once in a reference to that same figure. The term "nearshore" was not mentioned at all. It is interesting to note that, in their discussion on contaminants of groundwater, no reference is made to any of the studies listed above which investigated nutrients entering marine systems via groundwater. Their review paper "hope[d] to show the positive contribution that ecologists are able to offer within a domain that for a long period of time remained the speciality of hydrologists and water managers" (Danielopol *et al.* 2003, p105). In terms of marine ecologists, it has not.

In a report for the then Land and Water Resources Research and Development Corporation (now abolished), Hatton and Evans (1998) discussed the significance to Australia of groundwater-dependent ecosystems but, yet again, nearshore marine ecosystems barely rated a mention in the report. A statement was made to groundwater discharge maintaining stromatalite formations in Hamelin Pool, Shark Bay WA but further asserted that this dependence is essentially chemical.

Sinclair Knight Mertz (2009) produced a report for the Government of South Australia that looked the classification of groundwater-surface water interaction for water dependent ecosystems in the South East, South Australia. Nearshore interactions were not discussed.

In yet another SGD review, Moore (2010) summarised previous SGD studies including assessment techniques, locations, and modes of groundwater flow. Minimal biological information is contained in this review. Moore (2010) also discussed SGD at various scales from nearshore to oceanic.

Following on from Moore (2010), Bratton (2010) further discussed scale in his review, in particular the importance of SGD at three spatial scales: 1) nearshore scale, defined as 0-10m offshore and including unconfined (surface) aquifers; 2) embayment scale, ranging from 10m to 10km offshore and including confined aquifers; and 3) shelf scale, which includes aquifers of the entire continental shelf. A purpose of so distinguishing scale is to improve clarity in design and reporting of field and modelling results of SGD with a primary of objective of consensus amongst the SGD scientific community (Bratton 2010). In one of the only SGD review papers to do so, Bratton (2010) summarised studies of ecological significance and attempted to partition these studies according to his spatial scaling model.

Fleury *et al.* (2007) discussed submarine springs and coastal karst aquifers in their review. They summarised a number of karst groundwater systems in Greece, New Zealand, Libya, Croatia, France, Florida, Bahamas and Spain but Australian examples are not discussed.

Hayashi & Rosenberry (2002) looked at the effects of groundwater exchange on surface-water hydrology in freshwater systems. An interesting conclusion of this 2002 study was that the authors argued that ecology and hydrology do interact and that closer collaboration between these disciplines was required. It could be argued that ecologists have long known this and it is only over recent years that 'modern-thinking' hydrologists are beginning to consider ecological implications as well, and that previously hydrological-based management decisions are now made in conjunction with ecological information (e.g. for natural resource management and water allocation planning).

In a special edition of the Australian Journal of Botany that focused on groundwater dependent ecosystems (GDE), Eamus & Froend (2006) recommended a framework for future research and management of GDEs; Murray et al (2006) discuss functional methodology for GDE assessment that incorporates their ecosystem services. Although Eamus *et al.* (2006a) mention marine/nearshore/estuarine GDEs in their paper, there wasn't much more, simply stating that they exist and what they may be. A recommendation of the Eamus *et al.* (2006a) review was that a functional methodological approach should be adopted and that would determine ecosystem groundwater requirements. MacKay (2006) discussed management issues for the protection of GDEs, and highlighted emerging challenges, and potential approaches for policy and management. MacKay (2006) also discussed the importance of putting the science in place further suggesting tightening the science–policy– management cycle, concluding that such an action would lead to better protection and management of groundwater resources which should ultimately conserve GDE's.

The lack of discussion relating to groundwater-dependent marine ecosystems in these reviews is possibly due to two factors: 1) ignorance of any possible links; or 2) lack of published information or evidence. For the sake of marine ecologists and natural resource professionals, I hope it is merely the latter. There does appear to be an increasing awareness of groundwater-dependent ecosystems including marine environments and so it is hoped that marine groundwater-dependent ecosystems will attract more focus over the coming years.

Many of the reviews above have discussed GDE and SGD and their importance as a process and in relation to ecology. However much of the same issues have been restated over time and arguably little has been put into practice.

This is particularly pertinent to ecological studies and more so to marine-based studies.

In MacKay's (2006) concluding remarks (in specific reference to management and protection of GDEs), she suggested "start anywhere, just start". This present study, in specific reference to nearshore marine environments, adopts this philosophy.

1.2.5 South East Regional South Australian Studies

A number of studies (e.g. Turner *et al.* 1984, Dillon 1988, Leaney *et al.* 1995, Leaney & Herczeg 1995, Schmidt *et al.* 1999, Herczeg *et al.* 2003, Gouramanis *et al.* 2010) have been undertaken on groundwater in South East region of South Australia. Many of the studies have focused specifically on the regionally-important (i.e. for potable water supply) and geologically-significant Blue Lake in Mt Gambier, South Australia (Turner *et al.* 1984, Leaney *et al.* 1995, Herczeg *et al.* 2003, Gouramanis *et al.* 2010). The Blue Lake is a 28,000 year old extinct volcano and is located just south of the Mount Gambier township. The lake has a surface area of approximately 60 hectares, is approximately 74m deep and is predominately groundwater-fed from the karstic Gambier Limestone aquifer (Leaney *et al.* 1995, Herczeg *et al.* 2003).

The Dillon (1988) and Schmidt *et al.* (1999) studies investigated nitrate contamination in groundwater around Mount Gambier and the broader south east region. Turner *et al.* (1984) and Leaney *et al.* (1995) investigated isotopic (e.g. δ^2H, $\delta^{18}O$ and other elements) composition of groundwater whereas Gouramanis *et al.* (2010) conducted a palaeo-ecological study of ostracods and bivalves. The Leaney & Herczeg (1995) and Herczeg *et al.* 1997 studies investigated groundwater recharge and isotopic composition across sinkholes in the broader lower South East South Australia region..

1.2.6 Conclusions from the literature review

Whilst this literature review of ecological studies is interesting, none of the published studies have specifically examined, in detail, the ecological interaction of groundwater and nearshore coastal ecosystems. As such, the present study aims to take a new direction especially in sampling design, levels of replication and the variety of methods used to attempt to adequately test the hypothesis that the concept of groundwater-dependent ecosystems is relevant to nearshore coastal environments. Thus this study will be the first to critically and effectively examine (i.e. as a hypothesis) the perceived view that various nearshore marine ecosystems may be dependent on groundwater. To achieve this, the study will undertake an experiment-based approach rather than one that attempts to merely highlight observed phenomena via descriptive measurements. If dependence on groundwater of nearshore marine ecosystems can be biologically and statistically established, then this study will be the first conclusive study to do so.

The review above presents a summary of published studies, in some cases only remotely relevant to the objectives of this project (see Project

Background below). It is evident from the discussion presented above that no previous study has realistically or appropriately attempted to experimentally test the hypothesis that "nearshore marine ecosystems are dependent on groundwater resources". This project has attempted to set new standards in testing this hypothesis and is the first to develop stringent experimental and statistical designs from a purely ecological viewpoint.

1.3 PROJECT BACKGROUND AND OBJECTIVES

This project is a partnership between Flinders University and the South East Natural Resources Management Board (SENRMB, formerly known as South East Catchment Water Management Board or SECWMB). The project is the first assessment of groundwater outflow to the coastline of the South East region of South Australia and its influence on marine biota within ecosystems located in that area. The research aimed to combine field-based marine ecology with groundwater hydrology to assess the influence of groundwater outflow upon small estuaries and sandy beaches that exist along the coastline from near Kingston SE to the Victorian border (Figure 1.1).

Tackling such issues from two or more perspectives (e.g. hydrology and natural resource management from the land side, oceanography and ecology from the sea side) will be an important feature of 21st Century environmental research but quite challenging to facilitate (Burnett *et al.* 2001). This approach hopes to set a benchmark nationally and internationally for such applied research.

The benefits of such research go beyond merely increasing our understanding of coastal ecology because the regional setting of this research allows for widespread application of these findings in water allocation management. Indeed, this project arose from the needs that SENRMB had for guidance in water allocation planning. The SENRMB has statutory responsibility for managing water resources in the South East region in the Australian state of South Australia and were concerned that they (as at 2002) lacked any information about how dependent coastal marine ecosystems were upon groundwater. The SENRMB sought an understanding of perceived local dependencies on groundwater that nearshore coastal ecosystems have. This documented understanding could then be used as an information base as part of future water-allocation planning. At the start of this study, no data exist to inform these needs.

In some settings, groundwater outflow has been suspected for some time (e.g. Kohout & Kolipinski 1967; Johannes 1980) as having an important role in the ecological processes of nearshore coastal ecosystems but very few studies have examined this hypothesis. This potentially-important link has been overlooked by field biological research due to the inherent difficulty of measuring groundwater outflows, although this is starting to change (Burnett *et al.* 2001). Extensive literature searches have yielded only a handful of published studies exploring possible dependence of nearshore biota upon groundwater outflow in tropical places like Florida or Kenya (e.g. Corbett *et al.* 1999; Kamermans *et al.* 2002). It is also known that Western Australian seagrasses are affected by terrestrial nutrient inputs (McMahon & Walker 1998) and so it is likely that groundwater in the South East region of South Australia could be similarly linked to seagrass distribution and health. Currently there is not any known empirical research in South Australia into the dependence of nearshore ecosystems on groundwater.

In the past, groundwater-dependent ecosystems gained barely any public attention (but see Hatton & Evans 1998; Murray *et al.* 2003, for recent Australian reviews) and so hardly factored into current statutory water-allocation plans. Presently many state/territory jurisdictions are grappling with allocating water from groundwater reserves. The national significance of this issue relates to the many semi-arid or arid coastlines around Australia and our reliance on groundwater from the Great Artesian Basin and other artesian groundwater sources.

The South East region of South Australia is a semi-arid coastline with little runoff via coastal estuaries of any size but has an extensive system of subsurface aquifers. Current extraction of this regional groundwater resource supports extensive primary industries like grazing, plantation forests and wine grapes. Over-reliance on this extracted groundwater could threaten conservation parks that are located around spring-fed waters, ecotourism sites such as Ewens and Piccaninnie Ponds, or the coastal enterprises locally fishing for lobster, abalone, finfish, squid and giant crab.

Aquifers are a common feature near these karstic (limestone) coasts but not currently appreciated by many coastal biologists or natural resource managers. Wherever coasts abut aquifers there may be a natural dependency on groundwater linked to local biodiversity that needs consideration (roughly equivalent to environmental flows for surface waters). In semi-arid and arid regions, groundwater flow may be the most important water source for coastal creeks, estuaries and lagoons, especially in drier seasons (e.g. during summer) but this is impossible to estimate or appreciate at present.

Currently, the tools are not available to incorporate these considerations into water-allocation calculations and so awareness of these issues is also not widespread. Hence, a more thorough understanding of links between groundwater and nearshore coastal ecosystems will allow sensible planning of water allocation across a variety of interests, both of private industry and environmental public good, once this conceptual link between groundwater and coastal ecology can be forged.

This project potentially has a number of benefits including enhancing knowledge of ecological structures and functions of water-dependent ecosystems in line with the objectives of the South Australian State Government's State Water Plan 2000. The overall approach and methodology developed through this project may also assist in setting directions for further investigation of groundwater outflows and the dependence of nearshore marine ecosystems within other areas of South Australia and elsewhere in Australia where groundwater discharge to the ocean occurs.

Research to establish an understanding of the needs for water of groundwater-dependent ecosystems in Australia is therefore required to support future planning for sustainable management of the water resources and ecosystems of the catchment and its coastal marine area. For example, in Australia, we generally do not know: where groundwater accedes to the coast; how much flow there is; its capacity to replace or supplement surface flows; how much extraction for agriculture and forestry affects this accession; and which nearshore coastal ecosystems, if any, are dependent upon groundwater. Understanding how ecosystems depend on groundwater flows can give rise to a new way of viewing water allocation across Australia.

A variety of coastal ecosystems will be covered in this study, including sandy beaches, beach springs, coastal drains and other local nearshore ecosystems influenced by groundwater discharge. Information on groundwater influences upon these ecosystems within the south-east South Australian (SE SA) region is non-existent and rare at best.

Given the drivers and industry-based questions for this study, the study will be Australia-centric with comparisons of results discussed in a primarily Australian context. International comparisons will be limited and used only were Australian studies are non-existent.

More specific questions and hypotheses are posed, and relevant arguments and discussions are presented, in Chapters 3-7.

In order to address these questions it was necessary to (Fig. 1.2):

1. Describe the groundwater-related characteristics (e.g. flow, salinity, temperature, [NOx], [TIN], ^{2}H, ^{18}O) of various coastal water bodies (i.e. groundwater ponds, beach springs, drains, sea) affected by groundwater to various degrees (see Chapter 3);
2. Identify and describe the macrobiota found within seasonal (e.g. surface water runoff) and continuous-flowing (e.g. groundwater-fed) drains, streams and channels that are the small estuaries located along this coastline (Chapter 4);
3. Identify and describe the animals (especially the meiofauna) found living within sediments of beach springs and adjacent marine intertidal habitats (Chapter 5);
4. Determine the stable isotope ratio of nitrogen in the flesh of an intertidal bivalve population and compare those individuals found near beach springs with those from marine intertidal habitats removed from any measurable groundwater influence (Chapter 6); and
5. Compare standing stock biomass and productivity estimates for macroalgae and other biota living on and colonising hard substrata in seasonal versus continuous-flowing drains (Chapter 7).

The paucity of information about both the physico-chemical and biological environments of these south-east South Australian coastal (groundwater-influenced) water bodies necessitated that much of the study would at first be descriptive. Importantly, this descriptive information (e.g. Chapter 3) was viewed as a necessary first step in order to inform the subsequent formulation of hypotheses (Underwood *et al.* 2000), and thus a logical process and procedures focused on sequentially answering the key questions of the study. It is recognised that to elucidate the factors responsible for observed patterns and to test these hypotheses will require better targeted sampling (Chapters 4-6) and appropriate manipulative experiments (e.g. Chapter 7).

As a primary focus, comparisons against adjoining regional Australian settings (i.e. Victoria, and beyond) will be discussed in the first instance, across Chapters 3-7.

FIGURES

Figure 1.1: The coastal study region located within the South East of South Australia in relation to Australia, and South Australia.

Figure 1.2: Flowchart showing the relationships among key questions asked in the chapters of this thesis.

Figure 1.1:

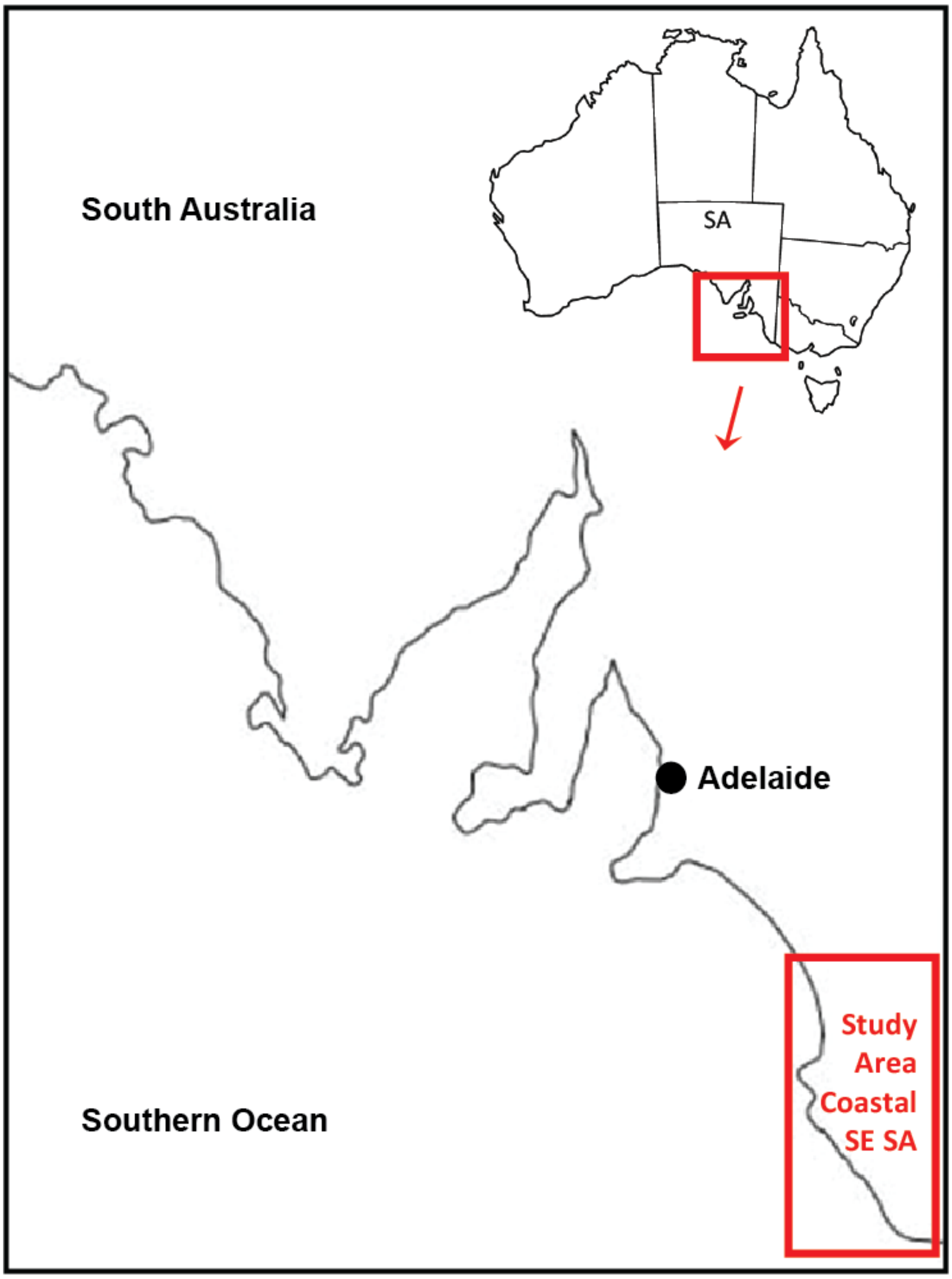
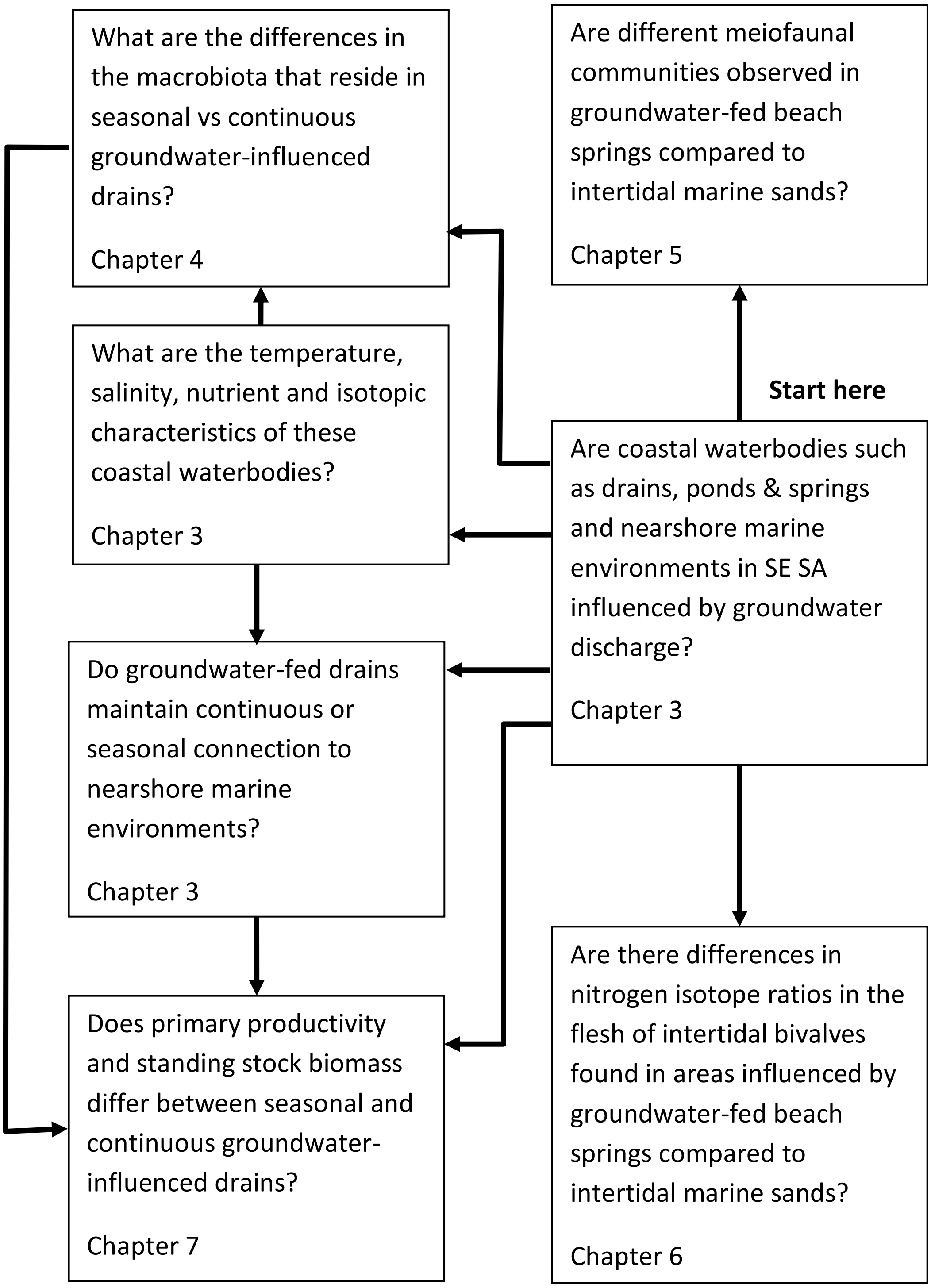

South Australia
SA
Adelaide
Southern Ocean
Study
Area
Coastal
SE SA

Figure 1.2:



What are the differences in the macrobiota that reside in seasonal vs continuous groundwater-influenced drains?
Chapter 4
Are different meiofaunal communities observed in groundwater-fed beach springs compared to intertidal marine sands?
Chapter 5
What are the temperature, salinity, nutrient and isotopic characteristics of these coastal waterbodies?
Chapter 3
Start here
Are coastal waterbodies such as drains, ponds & springs and nearshore marine environments in SE SA influenced by groundwater discharge?
Chapter 3
Do groundwater-fed drains maintain continuous or seasonal connection to nearshore marine environments?
Chapter 3
Does primary productivity and standing stock biomass differ between seasonal and continuous groundwater-influenced drains?
Chapter 7
Are there differences in nitrogen isotope ratios in the flesh of intertidal bivalves found in areas influenced by groundwater-fed beach springs compared to intertidal marine sands?
Chapter 6

CHAPTER 2 – GENERAL METHODS

2 Approach: Justification of sampling design used

2.1 PROGRESSIVE IMPLEMENTATION OF THE SAMPLING DESIGN

The present study was planned as a series of stages that logically progressed from one to the next in order to meet the main objectives of the project (see Fig. 1.2). Each stage is loosely based on each of the three years of the field component of the study

2.1.1 Year One: 2003-2004

The first stage of the project attempted to characterise permanent groundwater outflow versus seasonal freshwater flow at a local level by using in situ observations and measurements of salinity and chemical (nitrogen and isotopes of N, H and O) (Ch. 3), as well as considering invertebrate communities living therein (Ch. 4).

Starting with all irrigation drains and coastal creeks in the study region (the South East catchment of South Australia, see Figure 2.1) that appeared to discharge to the marine environment, their temporal connectedness to nearshore marine waters was assessed and salinity measured repeatedly. This enabled an important conclusion to be made regarding water flow in drains that may be dominated by surface-water runoff (e.g. derived from rainfall) versus groundwater-fed streams, versus estuarine mixing.

Macroinvertebrate benthic communities were also sampled and water samples were taken to test for concentrations of total nitrogen and nitrates (which tend to be elevated due to groundwater, Portnoy *et al.* 1998) and isotopes of H and O.

After characterising these drains and creeks, the study expanded to include outlets from known groundwater ponds, to make further comparisons with marine areas that were considered not to be influenced by groundwater outflow, and later beach springs, to look at localised effects of groundwater outflow.

2.1.2 Year Two: 2004-2005

Physico-chemical and ecological sampling continued during the second stage of the project. Creeks and drains that were observed to have stopped flowing (i.e. failed to maintain a permanent and direct connection to the sea) were no longer sampled routinely and so were eliminated from most future sampling events. Point source groundwater discharges or “beach springs” were increasingly identified during stage two and were subsequently sampled for salinity, nitrogen concentration, isotopes of H and O, and meiofauna. These measurements were later compared to other known groundwater sources to draw inferences about the water sources being discharged.

Basic sandy-beach and instream ecological sampling also continued during the second stage of the project to permit assessment of inter-annual and seasonal variability driven by environmental factors (e.g. rainfall patterns but this was not directly measured in this study) or regional physical oceanographic processes (e.g. coastal upwelling but again not directly measured in this study).

2.1.3 Year Three: 2005-2006

The third stage of the project had a key ecological focus and was based on all the findings collected during the previous two stages of the project. This part of the study was multifaceted and included direct testing of floral patterns (standing stock biomass and productivity). It also investigated faunal communities in terms of habitat effects and relationships (i.e. correlations) to groundwater inputs. A variety of experimental tests were conducted (not always successfully) and applied to a selection of faunal and vegetation communities in groundwater-fed waterbodies.

Comparing land-derived groundwater inputs (e.g. via coastal creeks and drains) with more direct (i.e. beach springs on sandy beaches, ponds) sources to nearshore coastal environments continued throughout stage three (Eamus *et al.* 2006a). Physico-chemical sampling continued but was reduced in intensity to encompass only a sub-set of study sites. Assessment of food web uptake of $\delta^{15}N$ (which tend to be enriched in groundwater, McClelland *et al.* 1997) sourced from groundwater by biota by analysing sources (e.g. total nitrogen in freshwater vegetation) and consumers (e.g. molluscs) were also undertaken during the third stage of the project. The nature and extent or magnitude of groundwater dependence requires further review, discussion and development as new data and knowledge are gained. Experiments to identify or confirm suspected groundwater dependence for individual species, community structure and/or other environmental variables were attempted.

2.2 DESIGN CONSIDERATIONS

As indicated above, the study aimed to have an unequivocally ecological and Australian focus and, as a consequence, the design limitations to the project are stated below.

2.2.1 An ecological approach as the prime focus

This study attempted to adopt an ecological viewpoint and so did not intentionally set out to replicate some of the chemical- and physical-based studies discussed in the literature review in Chapter 1. Chemical and physical samples were only collected when attempting to explain an ecological viewpoint or for the presentation of basic descriptive statistics to explain the local character of sampling sites in different waterbodies. Comments about and comparisons involving those will be made later when presenting an ecological result or observation. For example, chemical and nutrient measurement required consideration and methodological development to incorporate such issues as distance from point sources and dilution effects. Characterising no versus low versus high groundwater-input areas and times was used mainly to guide ecological sampling. Nitrogen contents, particularly nitrate (NO_3^-), was analysed in order to assess the uptake of groundwater-derived $\delta^{15}N$ by plants and animals (e.g. bivalves).

2.2.2 Groundwater matrix considerations

Location, volumes and hydrogeology of direct groundwater inputs to drains and drain-flow where beyond the scope of this study. The study did not consider directly any groundwater extraction issues nor concerns with saltwater intrusion to coastal aquifers. The study did not consider directly any terrestrial influences impacting on groundwater (i.e. increased nutrients, heavy metals, biocides etc.) *per se*. Those factors were considered to be beyond the scope of this study. Some general discussion of them may be presented later (especially in Chapter 8) merely as an overview of issues affecting regional groundwater quality and quantity. Groundwater composition, for the purpose of this study, will be determined as the characteristics of groundwater that discharged within the coastal or nearshore environment. I was not concerned with those factors directly impacting on the quality of such groundwater, whether naturally or anthropogenically enhanced. The emphasis was on the prevailing composition of groundwater (the groundwater matrix) that discharged into the marine environment because it was that composition which may have influenced nearshore communities. No prior information could be found on any changes in the quality of groundwater discharging into the marine environment within the designated study area. Where possible, direct historical comparisons with the present data were attempted for non-marine waterbodies (e.g. Scholz 1990 for Piccaninnie Ponds).

2.3 TERMINOLOGY CLARIFIED

Throughout this thesis, a number of terms are used that require a brief explanation about how they are to be interpreted. Here I attempt to explain my definition of a particular term and provide a brief argument about why and how I have applied such terminology. I have attempted to use published definitions from Australian reviews (e.g. Hatton and Evans 1998) to maintain, as a minimum, national consistency (see Hatton and Evans 1998 for a broader reasoning for their definitions). I also suggest in what habitats a particular term is most likely to be used. Types of habitats (e.g. drains, ponds, springs and marine) are also defined under their relevant sub-headings below (Section 2.5).

2.3.1 Groundwater

Groundwater in this study is defined as that water, having been below ground, which originates from an "extensive and persistent aquifer system" (Hatton & Evans 1998 p4). It is necessary to state this limitation on groundwater because soils in most terrestrial systems may be in a state of saturation at one time or another due to rainfall, flooding or other similar events (Hatton & Evans 1998), thereby clouding the argument about what may be groundwater-dependent ecosystems.

2.3.2 Groundwater Dependence

Groundwater dependence in this study is defined as where an ecosystem or component of it (e.g. a species or population) is in some way dependent on groundwater supply for its ongoing existence or survival and in particular may become threatened if that groundwater (as defined above) in the system were to become unavailable to plants and animals, e.g. if it were to be extracted by pumping or other means (*sensu* Hatton & Evans 1998 p4).

2.3.3 Surfacing Groundwater

Surfacing groundwater is defined as groundwater originating from an aquifer that surfaces through various pathways (including use for irrigation) and which may then flow via shallow sub-surface flow or (more likely) across the land surface and/or is channelled to the sea via drains or creeks. These pathways may include point sources of groundwater discharge such as ponds, springs, and in areas where the underlying bedrock has been anthropogenically fractured (e.g. as a means of sourcing water for stock) or diffuse sources where broader seepage through lowland areas occurs where the groundwater table is shallow and/or hydrological (head) pressure is high.

It has been necessary to develop this term because problems were encountered in discussions with natural resource managers generally and water managers specifically about differences amongst groundwater, fresh water, surface (i.e. rainfall-fed) runoff and drainage. The objective was to suggest that surfacing groundwater may have a different chemical signature and/or flow regimes from the other freshwater/surface-water entities, and that such differences for this surfacing groundwater may be important ecologically and need to be investigated.

2.3.4 Groundwater-fed

The definition of 'groundwater-fed' applies to those surface point sources (e.g. ponds, springs and some drains that have penetrated the underlying bedrock in their construction) which are known to be fed via direct connection to an aquifer, or by collecting groundwater after it has been used for irrigation purposes (e.g. mainly coastal drains that follow natural coastlines).

2.4 REGIONAL DESCRIPTION AND STUDY SITES

Sampling was conducted along the south-east coastal region (the so-called "Limestone Coast") of South Australia between The Granites (20 km north of Kingston SE, SA) and the Victorian border (23 km east of Port MacDonnell, SA), a linear coastline distance of approximately 210 km (see Fig. 2.1).

2.4.1 Physical Settings

The South East catchments cover an area of 28,120 km^2 and have a total coastline length of 378 km (SECWMB 2003). Geologically the majority of the region lies within the western boundary of the Otway Basin and the south-western boundary of the Murray Basin. Regionally-important geology dominates the underlying areas south of Mt Gambier and includes the Gambier Limestone, hence the region's karstic features (SECWMB 2003).

Major landscape changes have occurred in the South East region since European settlement in 1839 with only 13% of native vegetation remaining (Wells 2001). Historically the region was wet in winters with vast areas inundated by surface waters for long periods. Many permanent wetlands also existed. Natural drainage was slow, via either evaporation, or recharging to the subsurface unconfined aquifer, or slowly flowing in a north-north-westerly direction (parallel to the coast) towards the Coorong or eastward from Piccaninnie Ponds to the Glenelg River in Victoria. Artificial drainage of the region (to convert wetlands to pasture) commenced as early as 1864 with coastal diversion of surface waters commencing in 1911. Major catchment-wide drainage construction, modification and redirection occurred between the years 1950-1972. Extraction of groundwater for irrigation purposes from the unconfined aquifer commenced during the 1940s and from the confined aquifer in the north of the catchment in the 1970s (Tyler *et al.* 1983; SECWMB 2003).

An aquifer is a geological formation that holds groundwater within its structure and/or is sufficiently permeable to transmit water to surface wells and springs (Fetter 2001, Eamus *et al.* 2006b). A confined aquifer is overlain by a confining bed such as impermeable rock (Fetter 2001). Unconfined aquifers are not geologically confined from the surface (Fetter 2001) and the zone of saturation (or water table) is at atmospheric pressure and can rise and fall in relation to rainfall recharge (Fetter 2001, Eamus *et al.* 2006b). The region's main economic industries currently include agriculture (esp. grazing-related industries such as dairying and meat production), forestry, viticulture, tourism and fisheries (especially southern rock lobster). Agriculture, forestry, viticulture and nature conservation areas are the major land uses of the region.

2.4.2 Regional Climate

Mean coastal air temperatures (at Robe and Cape Northumberland [3 km west of Port MacDonnell]) generally range from 14°C (av. min) to 23°C (av. max) in summer (February) and between 7°C (av. min) and 14°C (av. max) in winter (July). Average annual coastal rainfall along the south-east coast ranges between 634 mm (Robe) to 704 mm (Cape Northumberland); Mt Gambier (at the Post Office gauging station) has a mean annual rainfall of 775 mm (BOM 2004).

2.5 SITE CLASSIFICATIONS

The information below briefly describes the different types of sites (in terms of their waterbodies) that were sampled during the project. More detailed information is provided in the subsequent relevant chapters of this thesis.

2.5.1 Drains

Drains are defined as all watercourses that originate inland from the coast and directly discharge water from inland floodplains to the sea. Drains included many natural creek lines. Most creeks within the region have been modified (e.g. channelised) to operate as irrigation drainage or manipulated (e.g. via floodgates or weirs) to regulate outflow of freshwater and/or prevent the inflow of marine water.

Drain sites were selected from a regional fire map (CFS 1999) and from regional 1:10,000 topographical maps. All drains and creeks that appeared to cross the coastline and have some connection to the sea were identified and their locations noted. Initially 26 such sites were identified for sampling. This was later reduced to 12 drains based on seasonal flow characteristics (Figure 2.2a-c, see Section 2.6.2 “Flow” for further discussion on final drain-site selection).

Most drains have been heavily modified and led to flow through agricultural land with some exceptions (e.g. the Piccaninnie Ponds outlet is located in a Conservation Park). Two drains, Blackford Drain (near Kingston SE) and Drain L outlet (Robe), each have a weir approximately 50 cm in height (apparent height varies subject to sand deposition and scouring around the weir, and with tidal and ocean swell heights) above the substratum in an approximate line with the foredune (Blackford) or cliffline (Drain L).

The decision to collect drain samples in line with the foredune was based on an argument to sample water immediately prior to the drain water discharging across the beach face and into the marine system. This was considered to be the most comparable description of the water matrix entering the marine system. That is, if samples were collected further upstream, any subsequent instream physical and chemical processes may not reflect, characteristically, the water that was entering the marine system. In contrast, any further downstream, the water was mixed with seawater at high tide or due to waves.

Drains were sampled for flow, salinity, temperature, concentration of nitrogen (total nitrogen, nitrate and nitrite), isotopes of O and H, and instream and epibenthic fauna and flora.

2.5.2 Groundwater Ponds ("ponds")

Three ponds or small lakes that were known (SECWMB 2003) to be groundwater-fed were chosen to be sampled. Sampling these ponds enabled direct comparisons of groundwater quality results (e.g. salinity, nitrogen concentrations) to be made with and statistically tested against other systems perceived to be groundwater-fed systems (e.g. some drains and beach springs). Two of these ponds (Ewens and Piccaninnie Ponds) are indirectly connected to the sea via their outlets (i.e. Eight Mile Creek and Piccaninnie Ponds outlet, respectively, treated as drains as above).

Piccaninnie Ponds is approximately 0.7 km from the coast, Ewens Ponds 2.5 km and Little Blue Lake approximately 14.5 km (Figure 2.3). Little Blue Lake has no connection to the sea and does not receive or discharge water via creeks or drains.

Ponds were sampled for salinity, temperature, concentration of nitrogen (total nitrogen, nitrate and nitrite) and isotopes of O and H.

2.5.3 Beach Springs (or "springs")

Over the duration of the study period, 12 beach springs were identified and sampled when possible (sampling was sometimes limited by tides and swell conditions). Most springs occurred within the lower intertidal zone of sandy beaches. Those springs identified were situated between Port MacDonnell and the Victorian border on the Discovery Bay Beach. Haphazard searches were also made whilst at drain and marine sites elsewhere and when in transit between sites travelling along a beach in relation to reports of beach springs elsewhere (e.g. Lacepede Bay north of Kingston) but few were found (e.g. one small spring near Blackford Drain). These contrasted with the more permanently visible and accessible beach springs located at the eastern end of Piccaninnie Ponds Conservation Park (Figure 2.4).

Most beach springs were small (approximately 20 cm diameter) except for those located in the Piccaninnie Ponds Conservation Park region. Outflow from springs at Piccaninnie Ponds Conservation Park could create craters up to 4 m in diameter due to the scouring effect of the discharging groundwater.

Beach springs were sampled for flow, salinity, temperature, concentration of nitrogen (total nitrogen, nitrate and nitrite, isotopes of O and H, and meiofauna.

2.5.4 Marine Sites ("marine")

Marine locations were selected randomly but based on accessibility and to span the whole study region (Figure 2.3). The marine sites chosen were sandy beaches (although one site, Little Dip Conservation Park, was a small sandy beach [approximately 500 m long] set within a rocky coastal landscape). The chosen marine locations were not known to be in proximity to any freshwater (i.e. low salinity, either surface runoff or groundwater-fed) inputs and were thus assumed to be representative of the regional "marine" nearshore habitats. Marine sites were generally sampled for only salinity and temperature.

2.6 SAMPLING DESIGN COMPONENTS

The information below summarises what sampling was undertaken throughout the duration of the project. Specific detail (e.g. instruments, processing methodologies) are provided in the subsequent relevant chapters of this thesis.

2.6.1 Sampling Period and Frequency

Sampling commenced in September 2003 and concluded in March 2006. Sites were sampled approximately monthly during summer months (December to March) and approximately every second month in other seasons (April to November) of each year.

2.6.2 Flow

On each sampling visit, drain sites were monitored for flow. This simply consisted of visually assessing if there was a direct connection of a drain to the sea and whether any water was flowing out. If a connection was not observed but others nearby were flowing, then the drain was eliminated as a possible site for groundwater-fed discharge and future sampling of the site was discontinued (but see assumption below).

An important assumption was made regarding continuity of outflow from drains due to groundwater. That is, it was assumed that those drains that had annual continuous flow were considered to have some proportion of that flow [baseflow] to be groundwater derived (i.e. groundwater-fed) (Eamus *et al.* 2006b). Many of the drains in the region are known to be solely used for drainage. It was argued that, when these drains ceased flowing after the winter/spring period (usually by November or December of each year), the majority of surface water inland had been drained or evaporated. It was also observed that groundwater ponds and beach springs maintained annual continuous flow during the project (albeit with some seasonal fluctuation).

It was therefore assumed that, once excess surface water had been drained from the inland areas adjoining the drains, then those remaining drains that maintained a continuity of flow could be apportioned to groundwater input. It was hoped that analyses of, say, isotopic signatures of hydrogen, oxygen and nitrogen would reveal to what extent groundwater contributed to such flow.

2.6.3 Salinity and Temperature

Triplicate salinity and temperature measurements were taken at each site on each sampling visit. Salinity (*P*pt) and temperature (degrees Celsius; °C) were measured using a handheld conductivity-salinity-temperature meter (WP-84; TPS Pty Ltd, Brisbane, Australia) using a k=1.0/ATC/temperature sensor. Calibration of the instrument was performed immediately before each field trip.

2.6.4 Water Quality

Water samples were collected on each sampling visit at drain, pond and spring sites for later determination of total nitrogen, nitrate and nitrite concentrations and oxygen and hydrogen isotopic signatures. Duplicate one-litre samples were filtered in the field using first 2.7µm Whatman paper filters (Grade 542) and later 0.45µm Millipore Durapore (*P*VDF) membranes (Millipore catalogue number HVLP04700) and then frozen.

Prior to freezing, 80mL of filtrate from each sample was collected and frozen separately for the later analyses of total nitrogen, nitrate and nitrite. Nitrogen analyses were conducted by the Water Studies Centre, Monash University, Victoria (a laboratory accredited by the National Association of Testing Authorities [NATA]).

2.6.5 Isotope Processing

Isotopes are different forms of a chemical element that vary in their mass. Stable isotope ratios are the ratio of the rare, heavy stable isotopes nitrogen to their lighter, more common forms. Isotopic signatures often persist, with varying levels of enrichment, across different trophic levels and may be used to match organisms with their source of organic material and determine their trophic level (Peterson & Fry 1987; Fry 1999).

Water samples for stable isotope analysis of δ^2H and δ^{18}O were collected when sampling for nitrogen (see methods for nitrogen above). Approximately 2mL of each sample was placed into a small glass vial and capped with a glass and rubber lid. Only one measurement (vial) was prepared for each stable isotope from each individual sample (i.e. a single value for each isotope from each sample – no procedural replication within each sample).

The $\delta^{15}N$ of consumers is enriched by 1-5‰ per trophic step (Davenport & Bax 2002) and is indicative of trophic level (e.g. up the food chain from groundwater nutrients to producers [phytoplankton], to primary consumers [meiofauna, filter feeding bivalves], to predators [such as fish]). A mean value of 3.4 ‰ $\delta^{15}N$ per trophic step can be applied to aquatic food webs (Post 2002). Thus, by determining the stable isotope ratios in groundwater (see Chapter 3) and the potential producers (see Chapter 7) or consumers (see Chapters 5 and 6), it may be possible to identify any trophic pathways between groundwater, and coastal and nearshore communities.

The incorporation of groundwater-derived nutrients into coastal and nearshore trophic webs was assessed using stable isotopes of N. I expected that fauna that rely directly or indirectly on groundwater would have stable isotope signatures that reflected both their trophic position and level of dependence on groundwater. I expected that $\delta^{15}N$ would be enriched by 1-5‰ (Davenport & Bax 2002) or an average of 3.4‰ (Post 2002) per trophic step.

Stable isotope ratios of $\delta^{15}N$ / $\delta^{14}N$ are expressed as the relative per mil (‰) difference between the sample and conventional standards (atmospheric nitrogen) given by the formula:

$$\delta X = (Rsample / Rstandard - 1) \times 1000 \ (‰)$$

where X = ^{15}N and R = $\delta^{15}N / \delta^{14}N$ (*P*eterson & Fry 1987). Instrumental precision was on average 0.03‰ for $\delta^{15}N$.

Stable isotope analysis was carried out in the Flinders Advanced Analytical Laboratory (FAAL, Flinders University, Adelaide, South Australia) using an Isoprime Isotope Ratio Mass Spectrometer (GV Instruments, Manchester, UK) and an elemental analyser (EuroVector, Milan, Italy). In-house standards, dummy samples, sample repeats and blanks were implemented by laboratory staff during analysis to ensure quality control of the analysis.

2.6.6 Fauna

Instream and epibenthic fauna were collected using a dip net (aperture area: 300 cm^2; mesh size: 500 µm) swept and bumped along the bottom for 30 seconds for each replicate (e.g. after Napier and Fairweather 1998). Specimens were first preserved in 10% formalin diluted in collection waters. Samples were later stored in a 70% ethanol in freshwater solution.

Meiofauna were collected from beach springs using a 20mL syringe and immediately preserved in a 10% formalin in seawater solution.

Sandy beach macroinfauna were collected using a 10cm internal-diameter corer to a depth of 15cm. Sediments were then sieved using a 1mm sieve with all animals retained on the mesh then counted and released. A number of each type of specimen was preserved in 70% ethanol for later identification.

The bivalve *Paphies elongata* was targeted for sampling in beach areas of groundwater discharge (Piccaninnie Ponds outlet and Piccaninnie Ponds beach springs) and analyses of ^{15}N in their tissues conducted. Samples were collected at areas directly under the influence of the discharging groundwater and in a control area further removed (distances >500m) from the influence of groundwater discharge. Other fauna (e.g. the pipi, *Donax deltoides*) were searched for using a variety of methods but only limited numbers were ever found.

2.6.7 Flora

Standing stock samples of algae, plants and detrital biomass were collected from a sub-set of drains using a 0.25m x 0.25m quadrat (area = 0.0625 m^2). Samples were first frozen and later dried at 60°C for 48h. Measurements of dry weight were recorded and a sub-sample from each sample was ground in a mortar and pestle for ^{15}N analyses.

Productivity samples were collected by placing household bricks on to the substrate in a subset of drains and collected approx. 3 to 4 weeks later. The top surface was then scraped and this periphyton sample frozen before later being dried at 60°C for 48h. Measurements of dry weight were recorded and a sub-sample from each sample was ground in a mortar and pestle for ^{15}N analyses.

2.7 GENERAL APPROACH TO STATISTICS AND INTERPRETATION

Various statistical approaches have been used in this study. Generally descriptive summaries have been presented first (e.g. ranges and mean values of measured variables by sites and groups). Statistical tests were then performed to test hypotheses (esp. about differences among groups of sites) and to further attempt to explain patterns, explore relationships and assess variability within the data.

Statistical analyses were performed using software packages that included SYSTAT v11 (SYSTAT 2004) for univariate statistics such as ANOVA, and PRIMER v5 or v6 (Clarke & Gorley 2006) with the PERMANOVA+ add-on (Anderson *et al.* 2008) for multivariate analyses.

FIGURES

Figure 2.1: South East Region of South Australia showing major townships.\

Figure 2.2: Location of drains across: a) the study region Australia; b) west; and b) east of Cape Northumberland. Blue dots are continuous-flow drains. Red dots are seasonal-flow drains. Green patches are conservation parks. A vertical line through Cape Northumberland separates and defines Western from Eastern drains. D = drain. See Table 3.1 for drain names.

Figure 2.3: Location of marine sites (green dots) and ponds (blue dots) within the study region. M = marine. P = pond. See Table 3.1 for marine site, and pond names.

Figure 2.4: Location of most often sampled springs (see Chapter 3). SP = spring

Maps produced using AQUAMAP

Figure 2.1:

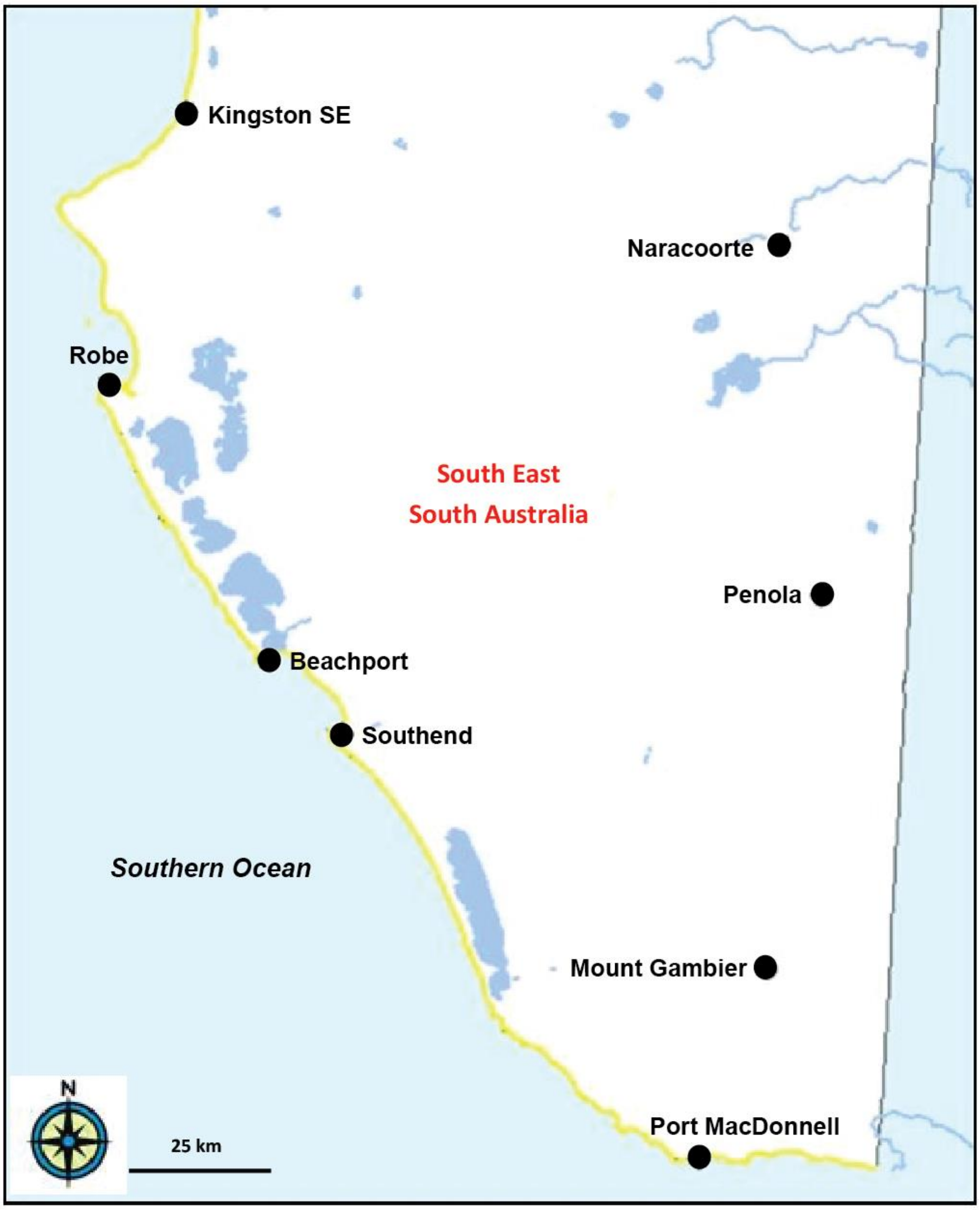

Kingston SE
Naracoorte
Robe
South East
South Australia
Penola
Beachport
Southend
Southern Ocean
Mount Gambier
N
25 km
Port MacDonnell

Figure 2.2a:

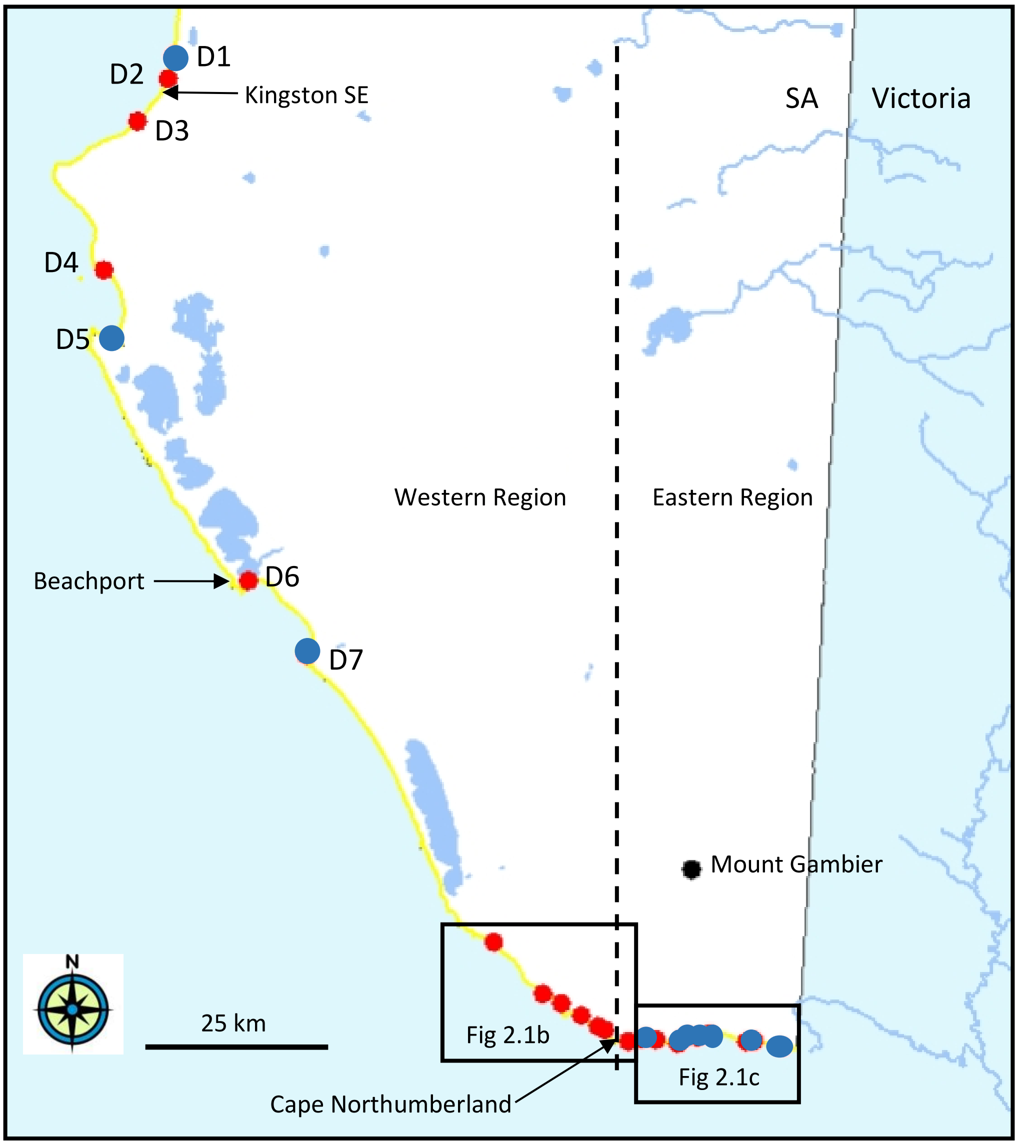

D1
D2
Kingston SE
D3
D4
D5
D6
D7
Beachport
SA
Victoria
Western Region
Eastern Region
Mount Gambier
N
25 km
Fig 2.1b
Fig 2.1c
Cape Northumberland

Figure 2.2b:

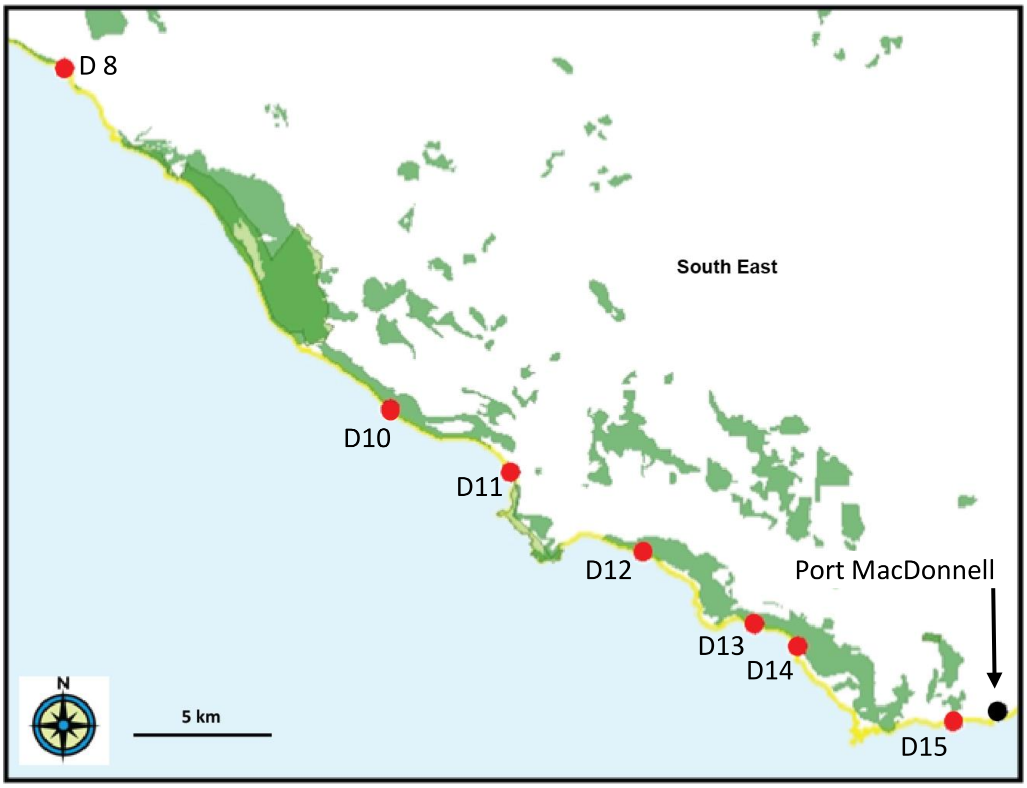


Figure 2.2c:

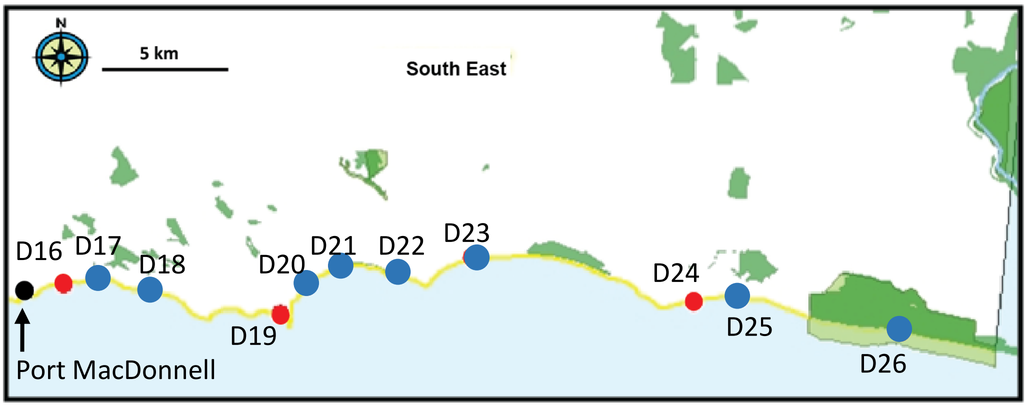
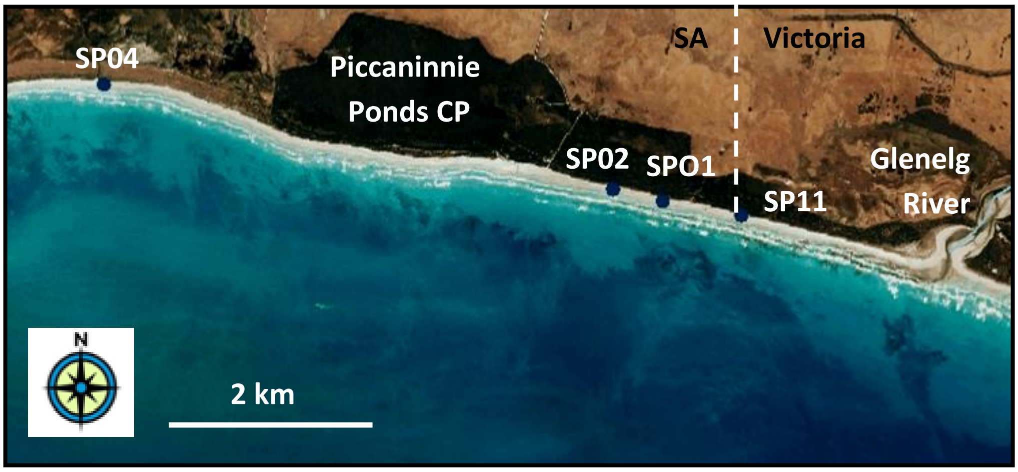


Figure 2.3:


M1
Kingston
M2
M3
South East
Beachport
M4
Southern Ocean
Mount Gambier
P1
M5
M6
P2
P3
Port MacDonnell
M7
N
25 km

Figure 2.4:


SP04
Piccaninnie
Ponds CP
SA
Victoria
SP02
SPO1
SP11
Glenelg
River
N
2 km

CHAPTER 3 – WATER QUALITY

3 Water-quality characteristics of coastal groundwater-fed drains versus other waterbodies in South East South Australia

3.1 INTRODUCTION

The characteristics of any waterbody may be compiled from many sources. For example, surface water may be a mixture of groundwater outflow and rainfall inputs. In attempting to determine what impact groundwater sources may have on nearshore coastal ecosystems, it is important to first understand what are the characteristics of any waterbody that discharges to these marine environments. I suspect that many of the coastal drains and streams that discharge to marine environment in the South East of South Australia are likely to contain some inputs from groundwater but how much groundwater contributes to each is unknown.

This chapter attempts to assess which coastal drains within the region maintain a permanent connection to the sea, and which others may only be seasonal. The seasonal weather patterns of the region mean that annual rainfall and thence surface water runoff results in wet conditions during winter-spring but a dry summer and autumn. This would have significant influence over the continuity of annual flow patterns of the coastal drains and creeks of the region.

It is plausible to suggest that those streams that maintain a permanent connection to the sea (i.e. discharge all year, including during the drier summer/autumn months) are more likely to have some contribution from groundwater. It is thus likely that groundwater inputs will be of significance in maintaining discharge through the drier, lower rainfall months of summer. Conversely, if connection is only seasonal (e.g. during the wetter winter/spring months of the year), then these streams are unlikely to have significant groundwater inputs even though regional groundwater recharge is likely to peak some time after winter rainfall (i.e. October/November).

Therefore we used a theoretical model of sub-surface groundwater, which is likely to have originated from a local unconfined aquifer, that is either mechanically extracted and used for irrigation purposes (with excess groundwater runoff entering drainage channels) or that local groundwater seeps naturally into drainage channels that directly connect, and discharge this extracted, surfacing groundwater to nearshore marine environments.

Physical and chemical water-quality parameters were chosen to further explore this argument. It was hypothesised that by sampling known groundwater sites of the region (e.g. groundwater ponds/lakes, groundwater springs), an understanding of the nature and characteristics of surfacing groundwater within the region could be obtained. The study would then be broadened to sample water quality parameters of coastal drains of the region, and to ascertain if flow is continuous or seasonal. By assessing the water quality (e.g. nutrients, isotopic signatures) in these regional drains, I could then infer what influence, if any, groundwater may be having on these waterbodies and finally infer what is the significance of this influence to the biota residing in them (see Chapters 4 – 7).

Specifically, I initially set out to attempt to: trace different sources of water (especially groundwater vs. surface runoff); understand any impacts the water quality of groundwater inputs (e.g. salinity, nutrients) may have on nearshore coastal habitats; and, infer information about characteristics of the local groundwater matrix (e.g. volumes and dilutions). For this study, I have used the term 'matrix' to describe that groundwater that enters the nearshore marine regardless of its history and composition including recharge area and method, origin, age, localised contaminants and geological, chemical and biological influences. That is, for the purposes of this study, the term groundwater describes the state of those waters that percolate or are expressed or artificially extracted from sub-surface origins.

Multiple simultaneous comparisons among ponds, springs, drains and marine sites were made over time. It is known that the regional ponds and springs are fed in large part by groundwater, so it was possible to establish regional groundwater characteristics by sampling these water bodies. It was also postulated that the marine sites sampled would approximate the marine conditions of the region.

Continuous-flow drains were likely to show predominantly diluted groundwater characteristics but may not be so often influenced by seawater, even though they were connected to the sea (i.e. salinities would be higher that groundwater but considerable less than seawater). If groundwater inputs were found to be significant then it is quite possible that any true estuarine extent in continuous-flowing drains is likely to be seasonal, spatially limited or non-existent.

For the purpose of statistical testing, the term 'region' was used to describe the spatial location of each subset of drains. That is, drains to the west of Cape Northumberland have been classified as 'western' drains and those to the east of Cape Northumberland have been classified as 'eastern' drains (see Figure 3.1). These regions also approximately match the two groundwater management areas of the SENRM region.

Drain sites were visited approximately eight times per year and on each sampling visit, drain sites were monitored for flow. This simply consisted of visually assessing if there was a direct connection of a drain to the sea and whether any water was flowing out. If a connection was not observed, but others nearby were flowing, then the drain was eliminated as a possible site for significant groundwater-fed discharge and future sampling, to monitor groundwater characteristics of the site, was discontinued.

It was assumed that those drains that had year-round continuous flow were considered to have some proportion of their flow (base flow) to be derived from groundwater (i.e. were groundwater-fed) and that drains also facilitate local mixing of groundwater with any surface runoff.

That is, all drains within the South East region of South Australia were assessed in November 2003 for continuity of flow. Those that displayed a connection with the sea on the first sampling event (November 2003) were classified as “continuous-flow” and thence regularly sampled for water quality over the duration of the project. Those drains that were dry or did not have a direct connection to the sea (i.e. had closed due to insufficient surface water/groundwater inputs) on the first sampling event (November 2003) were not revisited again except when specifically targeted for subsequent comparison tests (e.g. for macrofauna [see Chapter 4] or biomass and productivity [see Chapter 7]). Thus, the temporal aspect of these ‘seasonal-flow’ drains has not been considered in this study.

A fundamental objective of this chapter is to determine whether coastal creeks and agricultural irrigation drainage display groundwater characteristics. This objective will be achieved by the measurement of basic water-quality parameters including salinity, temperature, concentrations of nitrogenous nutrients (measured as TIN, NOx, NO3, NO3), and analyses of hydrogen (deuterium, δ^2H) and oxygen ($\delta^{18}O$) stable isotopes in the various waterbodies. The results will then be directly compared and statistically tested against known groundwater sources such as groundwater-fed ponds and beach springs, and thus inferences about the relative contribution of groundwater to the coastal drains will be drawn.

3.1.1 Drain – Ocean Connectivity

It was assumed that water bodies with lower salinity or with salinities matching those of known groundwater sources (e.g. groundwater ponds) are likely to be groundwater fed. Those that have higher salinities may also have some contributions from groundwater but may also be more influenced by other sources of water (e.g. surface water runoff from rainfall, brackish-water drainage, evaporation versus seawater inflow). Those with estuarine or higher salinities are likely to have only periodic inputs from groundwater sources and are also likely to be affected by marine inputs (e.g. tidal exchanges or large swells forcing waves into drains [pers. obs.]). Also the salinity of open-coast seawater is known (Lewis 1981; Schahinger 1987) to be around 35 ppt.

3.1.2 Salinity

Salinity was measured to provide information on any potential seasonal fluctuations in the salinity of different waterbodies. It was considered that, if salinity remained relatively constant and very low (particularly in continuous-flow drains and ponds), then a greater influence of groundwater contribution could be attributed to the maintenance of these systems. Continuous-flow drains that exhibited any seasonal fluctuation in salinity would be considered to still be influenced by groundwater inputs but to a lesser degree.

Salinity comparisons were made amongst known groundwater sources (e.g. groundwater ponds and springs), those suspected of having groundwater influence (e.g. continuous-flow drains) and those unlikely to be subjected to groundwater inputs (e.g. seasonal-flow drains and marine sites). The characteristics of such salinity regimes are likely to affect the types of biota that reside in them (see Chapter 4).

Specifically, salinity (in conjunction with other water-quality parameters) has been investigated to help characterise any influence groundwater may have on these systems and the impacts this may have on resident fauna. Thus, I predict that waterbodies will display lower salinity when under the influence of groundwater inputs.

3.1.3 Temperature

Groundwater expressed directly from underground aquifers might be cooler, or warmer, and display reduced annual variable over time than ambient surface waters (Dale & Miller 2007). The less-variable annual pattern may also influence seasonal surface water temperature differences. That is, groundwater discharges are likely to be cooler than ambient surface water temperatures in summer but also may be warmer than ambient water temperatures in winter months (Power *et al.* 1999; Hayashi & Rosenberry 2002; Ullman *et al.* 2003; Miller & Ullman 2004; Dale & Miller 2007). This value of the difference may be significant, particularly during summer months when many of the drains sampled in this study become shallower, slower flowing and may have less rain inputs (*P*ers. obs.). The biological significance of a reduced annual temperature range may influence ecology and tolerance of species that inhabit these niches.

It was hypothesised that, especially during summer, waterbodies with lower temperatures than those resembling surface waters are likely to have been influenced by groundwater sources, taking into account the time of day the samples were collected.

3.1.4 Nitrogen

In groundwater most nitrogen is in the form of nitrate (Fry 1999; Ullman *et al.* 2003). Groundwater is also known to be higher in nitrates because the depth and age of groundwater recharge accumulates nitrogen from detrital organic matter that is decomposed over long periods (e.g. as much as 10^2 – 10^5 years) to NO_x and thus is more biologically available than from other, more-complex organic compounds. Therefore I hypothesised that there is likely to be higher total nitrogen (TN) and higher nitrates (NO_3) contributing to NO_x (and thus TN) in samples collected from groundwater sources.

Any elevation in groundwater-originated N may also have some direct measurable effect via food chains (e.g. greater isotopic N enrichment incorporated into plants and then animals) in those individuals existing in groundwater-fed waterbodies.

3.1.5 Hydrogen and Oxygen Isotopes

Chemical elements can vary in the number of neutrons which can result in different atomic weights of a particular element (Krebs 2001). The isotopic abundance for hydrogen is comprised of predominantly by common hydrogen ^{1}H (>99.98%) with most of the remainder (<0.2%) made of ^{2}H (also known as deuterium) and a very small percentage of ^{3}H. The isotopic abundance of oxygen is of is dominated by common oxygen ^{16}O (approximately 99.76%) with the remainder made up of about 0.2% heavy oxygen ^{18}O and the much rarer ^{17}O (Mazor 1991). The isotopic composition of water is expressed (in per mils) as a deviation from an internationally-agreed standard (i.e. the Vienna Standard Mean Ocean Water) (Clark & Fritz 1997).

Our expectations from a literature review (Chapter 1) were that groundwater stable isotope signatures would differ from those of seawater. These stable isotope ratios of the hydrogen (H) and oxygen (O) atoms within the water can be indicative of groundwater sources. Isotopes of hydrogen (deuterium, $\delta^{2}H$) and oxygen ($\delta^{18}O$) stable isotopes in the various water bodies were thus also analysed to determine whether any of the different water masses sampled had signatures typical of groundwater.

Specifically I asked, are the isotopes of hydrogen (^{2}H) and oxygen (^{18}O) in groundwater-fed coastal drains comparable to known groundwater pond and spring sources?; and how quickly might it be used up and/or diluted or exported to the ocean? Answers to these questions will help further characterise the surfacing groundwater of the region and what influence they may have on coastal drains.

For each variable tested (i.e. salinity, temperature, TIN, NO_3, $\delta^{18}O$, δ^2H), the null hypotheses were stated as:

H_0 (1): No statistically-significant difference in mean salinity exists amongst location types (across all Drains, Ponds, Springs and Marine sites);

H_0 (2): No statistically-significant difference in mean temperature exists amongst location types (across all Drains, Ponds, Springs and Marine sites);

H_0 (3): No statistically-significant difference in mean TIN concentrations exists amongst location types (Drains, Ponds, and Springs only);

H_0 (4): No statistically-significant difference in mean NO_3 concentrations exists amongst location types (Drains, Ponds, and Springs only);

H_0 (5): No statistically-significant difference in mean $\delta^{18}O$ concentrations exists amongst location types (Drains, Ponds, and Springs only); and

H_0 (6): No statistically significant difference in mean δ^2H exists amongst location types (Drains, Ponds, and Springs only).

3.2 METHODS

Sampling was undertaken between September 2003 and March 2006. Sites were sampled approximately monthly during summer months (December to

March) and approximately every second month in other seasons (April to November) of each year.

Sampling sites were located within the south-east coastal region of South Australia between The Granites (north of Kingston SE) and the Victorian border (Figure 3.1). Sampling sites consisted of drains, ponds, springs and marine (= nearshore oceanic) sites. A brief description of drains ponds, springs and marine sites is presented below (see Definitions); more detail is presented in Chapter 2 “General Methods” (Section 2.5).

Drains were defined as all watercourses that originate inland from the coast and directly discharged to the sea. Drains included natural creek lines but most coastal creeks within the region have been modified so as to be channelised.

Groundwater ponds were large ponds and lakes that were known to be groundwater fed. These ponds provided background characteristics (e.g. H and O isotopes, TIN and NO_3 concentrations) and were used to describe generally the region’s groundwater. Ponds were typically located inland from the coast. The ponds themselves consisted of limestone walls and were fed directly upward by groundwater.

There are three main ponds as part of Piccaninnie Ponds system (First and Turtle Ponds, and The Chasm, which is 25m deep). The pond system has a total volume of 15,840 m^3, discharges at $1.24 m^3.s^{-1}$ and a turnover rate of <7 hours (Scholz 1990).

Beach springs are defined generally as small ponds or springs where water was observed to be flowing from out of the sand. This discharging water was assumed to originate from groundwater sources, i.e. a subterranean unconfined aquifer. Most springs were situated within the mid to low intertidal zone of the sandy beaches of the open coast. Most beach springs were small (approximately 20 cm diameter) but some larger (>1m diameter) ones were observed toward the eastern end of Piccaninnie Ponds Conservation Park.

Marine locations were selected based on accessibility but were otherwise haphazardly selected to span the whole study region along the coastline. The marine sites chosen were adjacent to sandy beaches. The chosen marine locations were not known to be in proximity to any permanent freshwater (i.e. lower salinity) inputs of either surface water or groundwater and were thus assumed to be representative of the regional nearshore marine habitats.

3.2.1 Salinity & Temperature

Salinity and temperature were measured using a handheld conductivity-salinity-temperature meter (WP-84; TPS Pty Ltd, Brisbane, Australia) using a k=1.0/ATC/temperature sensor. Calibration of the instrument was performed immediately before each field trip.

Three replicate measurements were collected from surface waters at each site on each sampling visit. Measurements in creeks/drains were haphazardly taken in the main channel of each waterbody and in an approximate line with the foredune (i.e. the most seaward vegetated dune) and in a water depth generally less than 60cm (but most were <30cm).

Measurements collected from marine sites were sampled directly in the lower intertidal area (when conditions allowed, in terms of safety) at water depths of approximately 30cm. When conditions were unfavourable (e.g. large seas resulting in dangerous conditions), a 15L bucket was used to collect water from waves crashing within the swash zone and running up the beach face. Measurements of salinity and temperature were then immediately taken from the bucket.

Due to the turbid nature (i.e. excessive sediment movement caused by the surfacing groundwater) and sometimes narrow diameter (<20cm) of the beach springs sampled, it was generally necessary to collect a small sample of discharging water (from the main point of discharge) using a 1L plastic beaker. This methodological modification allowed me to wait for any beach sediments collected in the sample to settle (usually in less than 10 seconds) and permitted a more rapid and stable measurement of salinity (*P*reliminary samples collected directly from springs showed small [±<0.5ppt] but continuous fluctuations in salinity but not temperature). Measurements were then immediately recorded. Some springs located in the swash zone of a beach were subjected to wave overtopping, thereby intermittently including seawater that would influence any measurements (particularly salinity). Timing was thus critical for the collection of a representative spring-water sample and, although much care was taken, it was likely that some measurements recorded have been influenced by this wave-driven process.

Measurements collected from ponds were taken from the most accessible location at each site (typically a floating pontoon or jetty).

3.2.2 Nitrogen

Duplicate 1L surface-water samples were collected from each site (excluding marine sites) on each sampling visit from March 2004. Marine sites, also known for this region from oceanography (see Lewis 1981; Schahinger 1987), were excluded because the large volume of sample required (due to low ambient N concentrations; Lewis 1981) to collect an amount of nitrogen (80-100µg total N) for reliable analysis was considered to be too great. Samples were collected from either the main water channel (drains) in line with the foredune, the most accessible location (*P*onds) or within the dominant discharging spring (beach springs). Samples were pre-filtered through Whatman filter papers (2.7µm) in the field and immediately refrigerated. Pre-filtering the samples using 2.7µm filters removed most of the organic/biological components likely to influence chemical processes of the sample without being overly time consuming whilst in the field. Samples were later filtered through Millipore (0.45µm) filters then frozen after dividing each sample. Approximately 80mL of the total sample was frozen separately for the analyses of total inorganic nitrogen (TIN) and NO_x (for nitrate [NO_3^-] and nitrite [NO_2^-]). The remainder of the sample was set aside for isotopic analysis (see “Isotopes” below).

Samples were analysed by a NATA-accredited laboratory (see Chapter 2 General Methods) for TIN and NO_x. Each sample was analysed in triplicate and values were corrected for sample salinity. A mean concentration was calculated from each set of triplicate measurements. This mean value was used as a replicate in all subsequent statistical analyses.

3.2.3 Isotopes

Chemical elements can vary in the number of neutrons which can result in different atomic weights of a particular element (Krebs 2001). The isotopic abundance for hydrogen is comprised of predominantly by common hydrogen ^{1}H (>99.98%) with most of the remainder (<0.2%) made of ^{2}H (also known as deuterium) and a very small percentage of ^{3}H. The isotopic abundance of oxygen is of is dominated by common oxygen ^{16}O (approximately 99.76%) with the remainder made up of about 0.2% heavy oxygen ^{18}O and the much rarer ^{17}O (Mazor 1991). The isotopic composition of water is expressed (in per mils) as a deviation from an internationally-agreed standard (i.e. the Vienna Standard Mean Ocean Water) (Clark & Fritz 1997).

These stable isotope ratios of the hydrogen (H) and oxygen (O) atoms within the water can be indicative of groundwater sources. Isotopes of hydrogen (deuterium, $\delta^{2}H$) and oxygen ($\delta^{18}O$) stable isotopes in the various water bodies were thus also analysed to determine whether any of the different water masses sampled had signatures typical of groundwater. This was done using filtered water samples analysed at the Flinders Advanced Analytical Laboratory (FAAL, Flinders University, Adelaide) using an Isoprime Isotope Ratio Mass Spectrometer (GV Instruments, Manchester, UK) and an elemental analyser (EuroVector, Milan, Italy

Water samples for stable isotope analysis of δ^2H and $\delta^{18}O$ were collected when sampling for nitrogen (see methods for "Nitrogen" above).

Approximately 2mL of each sample was placed into a small glass vial and capped with a glass and rubber lid. Only one measurement (vial) was prepared for each stable isotope from each individual sample (i.e. a single value for each isotope from each sample, i.e. no sub-sampling replication within each sample).

Samples were analysed at the Flinders Advanced Analytical Laboratory (FAAL, Flinders University, Adelaide) using an Isoprime Isotope Ratio Mass Spectrometer (GV Instruments, Manchester, UK) and an elemental analyser (EuroVector, Milan, Italy).

3.2.4 Approach to these comparisons

A combination of low salinities, lower temperatures, higher nitrogen and larger hydrogen and oxygen isotopic values would suggest that most of the water has originated from groundwater sources. Those waterbodies with higher salinities, warmer temperatures, lower nitrogen and lower hydrogen and oxygen isotopic values are likely to have originated from non-groundwater sources. Those waterbodies displaying more partial characteristics are likely to have contributions from both groundwater and non-groundwater sources.

3.2.5 Statistical data analysis

Data were analysed using univariate (e.g. one-way Analysis of Variance [ANOVA]) and multivariate (e.g. principal components analysis [PCA} and non-metric Multi Dimensional Scaling [MDS] ordination) statistics using SYSTAT v11 and PRIMER v6 software packages, respectively. Due to some imbalance in the data sets collected, it has been necessary to analyse different configurations (subsets) of the data in order to present a result and/or argument. Assumptions of ANOVA were checked by inspecting graphs of residuals obtained when the ANOVA model was fitted (Quinn & Keough 2002). Some data sets were then transformed to meet the assumptions of ANOVA. The results of analyses for transformed data are shown below with the type of transformation stated accordingly.

Tukey post-hoc pairwise multiple comparisons were also applied to identify various groupings in data sets after any significant ANOVA result with >2 levels of a significant factor or interaction.

PCA using SYSTAT v11 (SYSTAT 2004) and PERMANOVA (Anderson *et al.* 2008) analyses were also performed. A series of PERMANOVA analyses on a selection of four data subsets were performed in an attempt to extract as much statistical information as possible from the dataset. Data subsets were necessary because of unplanned imbalance in data points collected over the duration of the study. Data within each subset were ‘trimmed’ for balance. Sub-sets included: 1) salinity and temperature only; 2) all water quality data except isotopes (i.e. salinity, temperature, TIN, NO_x, NO_3, NO_2); and 3) all water quality data (i.e. salinity, temperature, TIN, NO_x, NO_3, NO_2, δ^2H, $\delta^{18}O$) Data were normalised and subjected to 9999 permutations when performing PERMANOVA and PERMDISP analyses. PERMDISP is a comparison test of multivariate dispersions among groups and is based on a distance or dissimilarity measure (e.g. SIMPER/ANOSIM). Specifically, it calculates the distances from observations to their centroids and compares the average of these distances among groups (Anderson 2004, Anderson *et al.* 2008). PERMDISP was used to investigate whether the location type altered the degree of variability (i.e. between samples) of the water quality. Thus, any differences in variability may indicate some effect of location (e.g. influence of groundwater) on the water quality variables.

3.3 RESULTS

Those drains that were classified as continuous-flow (n = 12) during the first sampling event were observed to have a 100% connection to sea on each subsequent sampling event for the duration of the study.

3.3.1 Descriptive Summaries for each water quality variable & waterbody type

3.3.1.1 Salinity

Total sample replication (sample size at a single site) varied between *n* = 1 and *n* = 18 at Marine sites; *n* = 1 - 33 (at Pond sites); *n* = 1 - 48 (Drain sites); *n* = 1 - 57 (Springs). Salinity (grand mean ± s.e.) for each location type was 4.69 ± 0.38 ppt (all Drains, *n* = 465 measurements), 0.80 ± 0.07ppt (*P*onds, *n* = 87); 0.99 ± 0.09 ppt (Springs, *n* = 133); and 33.8 ± 0.2ppt (Marine sites, *n* = 102).

Salinity (mean ± s.e. per site using times as replicates) in coastal Drains ranged (Table 3.1) from 0.39 ± 0.03 ppt at Mt Benson Drain (*n* = 13) to 24.9 ± 3.4 ppt at Lake George Outlet (*n* = 22); similarly Ponds: 0.24 ± 0.01 ppt (Little Blue Lake, *n* = 21) to 1.56 ± 0.04 ppt (*P*iccaninnie Ponds, *n* = 33); Springs: 0.35 ± 0.01 ppt (Beach Spring 05, *n* = 3) to 2.86 ± 0.14 ppt (Beach Spring 12, *n* = 6); and Marine sites 31.6 ± 0.1ppt (Discovery Bay, *n* = 3) to 34.4 ± 0.5 ppt (Nene Valley, *n* = 15).

The greatest range (minimum – maximum) in salinity was observed (Table 3.1) in Drains at Lake George Outlet = 2.55 – 47.5 ppt (*n* = 22); Ponds = 1.26 – 2.07 ppt at Piccaninnie Ponds (*n* = 33); Springs = 0.14 – 4.91 ppt at Beach Spring 06 (*n* = 8); and Marine sites = 26.2 – 36.6 ppt at Pelican Point (*n* = 15).

Salinity (grand mean ± s.e.) for each location type (Table 3.1b) was 4.7 ± 0.2 ppt (Drains, *n* = 547); 0.8 ± 0.1 ppt (*P*onds, *n* = 87); 1.0 ± 0.1 ppt (Springs, *n* = 133); and 33.8 ± 0.2 ppt (Marine sites, *n* = 102).

Additional analyses were performed on the Drain data set in an attempt to identify the presence of any patterns in the assigned sub-groups (i.e. by flow status or region). The subsequent analyses (Table 3.1b) found that salinity (grand mean ± s.e.) in Continuous-flow Drains (3.5 ± 0.3 ppt, n = 466) were less salty than Seasonal-flow Drains (11.3 ± 1.6 ppt, n = 81) and that Eastern-region Drains (0.9 ± 0.1 ppt, n = 371) were less salty than Western-region Drains (12.6 ± 0.9 ppt, n = 176).

For completeness, Drain sites were then grouped by Flow and Region (combined) (Table 3.1b), which found that Seasonal-flow Drains in the Eastern region had the lowest salinity (0.7 ± 0.2 ppt, n = 15) similar to Continuous-flow Eastern Drains (0.9 ± 0.1 ppt, n = 356), followed by Continuous-flow Western Drains (12.0±1.0 ppt, n = 109) and Seasonal-flow Western Drains (13.6 ± 1.8 ppt, n = 67).

3.3.1.2 Temperature

Temperature (grand mean ± s.e.) across all sampling visits for each location type was 17.5 ± 0.2 ºC (all Drains, n = 465), 16.7 ± 0.2 ºC (*P*onds, n = 87); 15.8 ± 0.1 ºC (Springs, n = 133); and 17.2 ± 0.4 ºC (Marine sites, n = 102).

Temperature (mean ± s.e. per site) in Drains ranged from 10.9 °C (*P*ort MacDonnell Town Drain, n = 1) to 23.7 ± 0.1 °C (Spehrs Road Drain, n = 12); Ponds: 16.3 ± 0.1 °C (Ewens & Piccaninnie Ponds, both n = 33) to 18.0 ± 0.7 °C (Little Blue Lake, n = 21); Springs: 13.4 ± 0.2 °C (Beach Spring 04, n = 12) to

18.6 ± 0.1 °C (Beach Spring 12, *n* = 6); and Marine 15.7 ± 0.5 °C (Canunda NP, *n* = 18) to 19.5 ± 1.4 °C (*P*elican Point, *n* = 15) (Table 3.1).

The sites with the greatest temperature range (minimum – maximum) for each location type was observed in Drains at Blackford Drain 9.3 – 26.8 °C (*n* = 44); Ponds: 12.7 – 21.7 °C at Little Blue Lake (*n* = 21); Springs: 14.8 – 18.1°C at Beach Spring 02 (*n* = 9) and Marine: 9.8 – 24.8 °C at Pelican Point (*n* = 15) (Table 3.1).

Drain sub-grouping analyses (grand mean ± s.e.) found (Table 3.1b) that Continuous-flow Drains (17.3 ± 0.1 °C, *n* = 466) were slightly cooler than Seasonal-flow Drains (18.5 ± 0.5 °C, *n* = 81) and that Eastern region Drains (17.1 ± 0.2 °C, *n* = 371) were slightly cooler than Western region Drains (18.4 ± 0.3 °C, *n* = 176).

Drain temperatures by Flow and Region (combined) found (Table 3.1b) that Continuous-flow Eastern Drains were the coolest (17.0 ± 0.2 °C, *n* = 356), followed by Continuous-flow Western Drains (18.2 ± 0.4 °C, *n* = 109), Seasonal-flow Eastern Drains (18.3 ± 0.7 °C, *n* = 15) and Seasonal-flow Western Drains (18.5 ± 0.6 °C, *n* = 67).

3.3.1.2 <u>Total Inorganic Nitrogen (TIN)</u>

Total inorganic nitrogen (TIN) concentrations (grand mean ± s.e.) for each location type was 2.49 ± 0.01 $mg.L^{-1}$ TIN (all Drains, *n* = 248); Ponds (*n* = 58): 3.67 ± 0.21 $mg.L^{-1}$ TIN; and Springs (*n* = 38): 2.01 ± 0.12 $mg.L^{-1}$ TIN. Nutrient samples were not collected in marine waters.

TIN (mean ± s.e. per site) in Drains (Table 3.2) ranged from 0.74 ± 0.07 $mg.L^{-1}$ TIN (Clarke Park, *n* = 20) to 5.66 ± 0.18 $mg.L^{-1}$ TIN (Eight Mile Creek, *n* = 22); Ponds: 1.79±0.08 $mg.L^{-1}$ TIN (Little Blue Lake, *n* = 14) to 5.40 ± 0.18 $mg.L^{-1}$ TIN (Ewens Ponds, *n* = 22); and Springs: 0.93 ± 0.27 $mg.L^{-1}$ TIN (Beach Spring 12, *n* = 2) to 2.55 ± 0.95 $mg.L^{-1}$ TIN (Beach Spring 02, *n* = 2).

The greatest range (minimum – maximum) in TIN was observed (Table 3.2) in Drains at Lake George Outlet 0.22 – 6.80 $mg.L^{-1}$ TIN (*n* = 12); Ponds; 1.80 – 5.70 $mg.L^{-1}$ TIN at Piccaninnie Ponds (*n* = 22); and Springs: 1.10 – 3.30 $mg.L^{-1}$ TIN at Beach Spring 01 (*n* = 20).

Analyses of Drain sub-grouping (grand mean ± s.e.) found (Table 3.2b) that Continuous-flow Drains (2.5 ± 0.1 $mg.L^{-1}$ TIN, *n* = 218) were higher in TIN concentration than Seasonal-flow Drains (2.2 ± 0.3 $mg.L^{-1}$ TIN, *n* = 76) and that Eastern region Drains (2.8 ± 0.1 $mg.L^{-1}$ TIN, *n* = 172) were approximately 50% greater than Western region Drains (1.8 ± 0.2 $mg.L^{-1}$ TIN, *n* = 76).

TIN concentration (grand mean ± s.e.) by Flow and Region (combined) found (Table 3.2b) that Continuous-flow Eastern Drains (2.9 ± 0.1 mg L-1 TIN, *n* = 166) and Seasonal-flow Western Drains (2.5 ± 0.4 mg L-1 TIN, *n* = 24) had greater TIN concentrations than Continuous-flow Western Drains (1.5 ± 0.1 $mg.L^{-1}$ TIN, *n* = 52) and Seasonal-flow Eastern Drains (1.1 ± 0.1 $mg.L^{-1}$ TIN, *n* = 6).

3.3.1.3 Nitrate (NO3)

Nitrate (grand mean ± s.e.) for each location type was 1.66 ± 0.01 $mg.L^{-1}$ NO_3 (all Drains, *n* = 248); Ponds (*n* = 58): 3.05 ± 0.20 $mg.L^{-1}$ NO_3; and Springs (*n* = 38): 1.52 ± 0.11 $mg.L^{-1}$ NO_3.

NO_3 (mean ± s.e. per site) in Drains ranged (Table 3.2) from 0.04 ± 0.02 $mg.L^{-1}$ NO_3 (Spehrs Road, *n* = 6) to 5.00 ± 0.19 $mg.L^{-1}$ NO_3 (Eight Mile Creek, *n* = 22); Ponds: 1.34 ± 0.06 $mg.L^{-1}$ NO_3 (Little Blue Lake, *n* = 14) to 4.77 ± 0.16 $mg.L^{-1}$ NO_3 (Ewens Ponds, *n* = 22); and Springs: 0.50 ± 0.03 $mg.L^{-1}$ NO_3 (Beach Spring 12, *n* = 2) to 1.86 ± 0.14 $mg.L^{-1}$ NO_3 (Beach Spring 01, *n* = 20).

The greatest range (minimum – maximum) in NO3 was observed (Table 3.2) in Drains at Eight Mile Creek 2.40 - 5.98 $mg.L^{-1}$ NO_3 (*n* = 22); Ponds; 2.60 - 5.67 $mg.L^{-1}$ NO_3 at Ewens Ponds (*n* = 22); and Springs: 0.91 - 2.63 $mg.L^{-1}$ NO_3 at Beach Spring 01 (*n* = 20).

Continuous-flow Drains (grand mean ± s.e.) had more than twice as much NO_3 than Seasonal-flow Drains (1.8 ± 0.1 $mg.L^{-1}$ NO_3 and 0.7 ± 0.3 $mg.L^{-1}$ NO_3, respectively) (Table 3.2b). Eastern-region Drains were approximately four times greater than Western-region Drains (2.2 ± 0.1 $mg.L^{-1}$ NO_3 and 0.5±0.1 $mg.L^{-1}$ NO_3, respectively).

Drain sub-grouping analyses (grand mean ± s.e.) of nitrate by Flow and Region (combined) revealed (Table 3.2b) that Continuous-flow Eastern Drains (2.3 ± 0.1 $mg.L^{-1}$ NO_3, *n* = 166) had the highest concentration of NO_3 followed by Seasonal-flow Western Drains (0.9 ± 0.3 $mg.L^{-1}$ NO_3, *n* = 24). Seasonal-flow

Eastern Drains and Continuous-flow Western Drains were similar (0.3 ± 0.1 mg.L^{-1} NO_3) but total sample sizes were small and varied between them (*n* = 6 and *n* = 52, respectively).

Nitrate contribution (as a mean percentage) to TIN was approximately three times greater in Continuous-flow Eastern Drains (72% NO_3 to TIN) when compared to Seasonal-flow Eastern Drains (≈24%) and Seasonal-flow Western Drains (≈25%), and nearly six times greater than Continuous-flow Western Drains (≈13%) (Table 3.2b). Percent contribution of NO_3 to TIN was generally lower in seasonal-outflow Drains except Mt Benson Drain, which had a higher contribution than other Western coastal-region Drains.

NO_3 contributed >99.9% (mean value) to NO_x in all groundwater Ponds and Springs and slightly less (≈98% in Drains) whereas NO_3 contribution to NO_x was less (95%) in Western Drains (Table 3.2b).

3.3.1.4 Isotope ratios of hydrogen (δ^2H)

Stable isotope analyses found (Table 3.3) that δ^2H (mean ± s.e. per site) in Drains ranged from -26.3 ± 0.6 ‰ δ^2H (Cress Creek, *n* = 14) to 3.5 ± 3.2 ‰ δ^2H (Lake George Outlet, *n* = 10); Ponds: -28.3 ± 0.8 ‰ δ^2H (*P*iccaninnie Ponds, *n* = 16) to -17.9 ± 0.8 ‰ δ^2H (Little Blue Lake, *n* = 14); and Springs: -24.5 ± 1.4‰ δ^2H (Beach Spring 01, *n* = 12) to -19.4 ± 0.3 ‰ δ^2H (Beach Spring 04, *n* = 2).

The greatest site range (maximum - minimum) in δ^2H was observed (Table 3.3) in Drains at Lake George Outlet -12.3 to 15.0‰ δ^2H (*n* = 10); Ponds; -32.7

to -15.4 ‰ $\delta^{2}H$ (*P*iccaninnie Ponds, *n* = 16); and Springs: -30.1 to -15.4 ‰ $\delta^{2}H$ (Beach Spring 01, *n* = 14).

Analyses of stable isotopes in waterbodies found (Table 3.3b) that $\delta^{2}H$ (grand mean ± se, per mil) for each location type was -24.5 ± 0.8 ‰ $\delta^{2}H$ for Ponds (*n* = 46); -23.9 ± 1.2 ‰ $\delta^{2}H$ for Springs (*n* = 18); and -18.7 ± 0.8 ‰ $\delta^{2}H$ (all Drains, *n* = 188).

The δ2H (grand mean ± se) for continuous-flow Drains within the Western coastal region was -5.4 ± 1.0 ‰ $\delta^{2}H$ (*n* = 44) and -24.8 ± 0.3 ‰ $\delta^{2}H$ (*n* = 134) in the Eastern coastal region (Table 3.3b). Only one Seasonal-flow Drain (Lake George Outlet; 3.5 ± 3.2 ‰ $\delta^{2}H$, *n* = 10) was sampled for $\delta^{2}H$ in the aspect of the broader study, so sub-grouping analyses involving Seasonal-flow sites are not available.

3.3.1.5 Isotopes of oxygen ($\delta^{18}O$)

Stable isotope analyses found (Table 3.3) that $\delta^{18}O$ values (mean ± s.e. per site) in Drains ranged from -5.25 ± 0.15 ‰ $\delta^{18}O$ (Eight Mile Creek, *n* = 16) to - 1.43 ± 0.46 ‰ $\delta^{18}O$ (Drain L Outlet, *n* = 16); Ponds: -4.19 ± 0.33 ‰ $\delta^{18}O$ (Ewens Ponds, *n* = 16) to 5.03 ± 0.14 ‰ $\delta^{18}O$ (*P*iccaninnie Ponds, *n* = 16); and Springs: -5.04 ± 0.16 ‰ $\delta^{18}O$ (Beach Spring 01, *n* = 12) to -4.40 ± 0.65 ‰ $\delta^{18}O$ (Beach Spring 04, *n* = 2).

The greatest site range (maximum - minimum) in $\delta^{18}O$ values was observed (Table 3.3) in Drains at Clarke Park Reserve -5.26 to 1.93 ‰ $\delta^{18}O$ (*n* = 14);

Ponds: -6.41 to -1.95 ‰ $\delta^{18}O$ at Ewens Ponds (n = 16); and Springs: -5.92 to -3.79 ‰ $\delta^{18}O$ at Beach Spring 01 (n = 14).

Analyses of stable isotopes of $\delta^{18}O$ found (Table 3.3b) that, for each location type (grand mean ± se) was -3.89 ± 0.13 ‰ $\delta^{18}O$ (all Drains, n = 188); Ponds (n = 46): -4.62 ± 0.15 ‰ $\delta^{18}O$; and Springs (n = 18): -4.97 ± 0.14 ‰ $\delta^{18}O$.

The $\delta^{18}O$ value (grand mean ± se) for continuous flow Drains within the Western coastal region was -2.24 ± 0.30 ‰ $\delta^{18}O$ (n = 44) but -4.54 ± 0.12 ‰ $\delta^{18}O$ (n = 134) in the Eastern coastal region (Table 3.3b). Only one Seasonal-flow Drain (Lake George Outlet; -2.39 ± 0.22 ‰ $\delta^{18}O$, n = 10) was sampled for $\delta^{18}O$ so sub-grouping analyses involving Seasonal-flow sites are not available.

3.3.2 Outcomes of Statistical Analyses

3.3.2.1 Salinity

A one-way ANOVA found a statistically-significant difference (P <0.001) in mean salinity amongst location types (Drain, Pond, Spring, Marine). Tukey post-hoc multiple comparisons found significant differences (P <0.001) for all combinations of location type except for Spring vs. Pond (P = 0.831, NS) (Table 3.4). Marine values were much larger than for Drains, which were a little saltier than Ponds and Springs (Figure 3.1a).

When Drains were split into Eastern and Western coastal regions (see definition above), a statistically-significant difference (P <0.001) in mean salinity between regional location of Drain (Eastern n = 371; Western n = 176) was observed, with Western values being greater and fluctuated more than Eastern (Table 3.4, Figure 3.1b).

Further investigation of any possible relationship between the continuous-flow Drains, Springs and Ponds found a statistically-significant difference (*P* <0.001, F = 212.629) in mean salinity between location of Drain, Ponds and Springs (Eastern Drains: *n* = 369; Western Drains *n* = 109; Ponds: *n* = 87; Springs: *n* = 133). Tukey HSD post-hoc multiple comparisons found a statistically-significant difference in mean salinity for Location Type combinations of Western Drains and Eastern Drains, Ponds and Springs (all P <0.001) with Western Drains having the larger value. All combinations of Eastern Drains, Ponds and Springs were not statistically different (Table 3.4, Figure 3.1c).

3.3.2.2 Temperature

A one-way ANOVA found a statistically-significant difference (*P* <0.001) in mean temperature between location types (i.e. Drain, Pond, Spring, Marine). Tukey post-hoc multiple comparisons test found statistically-significant differences for Location Type pairs of Spring vs. Drain (*P* <0.001) and Spring vs. Marine (*P* = 0.003) (Table 3.4), with Springs having the smaller values (Figure 3.2a), hence being cooler.

A one-way ANOVA found a statistically-significant difference (*P* <0.001) in mean temperature between the two regional location of drains (Eastern: *n* =

371; Western: n = 176) (Table 3.4), with Western drains being warmer than Eastern ones (Figure 3.2b).

A one-way ANOVA found a statistically-significant difference (P <0.001, F = 17.531) in mean temperature between regional location of continuous-flow Drains, Ponds and Springs (Eastern Drains: n = 369; Western Drain n = 109; Ponds: n = 87; Springs: n = 133). Tukey post-hoc multiple comparisons found a statistically-significant difference in mean temperature for Location Type pairs of Western Drains vs. Spring and Western Drains vs. Pond (both P <0.001) and Western Drains v Eastern Drains (P = 0.002) (Table 3.4), with Western Drains being slightly warmer (Figure 3.2c).

3.3.2.3 Total Inorganic Nitrogen (TIN)

A one-way ANOVA found a statistically-significant difference between location type (Drain, Pond, Spring) in mean square-root TIN (P <0.001). Tukey post-hoc multiple comparisons found a statistically-significant difference in mean square-root TIN between pairwise comparisons for Drain vs. Pond and Pond vs. Spring (P <0.001) but Drain vs. Spring was non-significant (Table 3.5), with Ponds having smaller values (Figure 3.3a).

When Drains were split into Eastern and Western coastal regions, a statistically-significant difference (P <0.001) in mean square-root TIN between regional locations of Drains (Eastern n = 166; Western n = 52) was observed (Table 3.5), with Eastern drains having larger values than Western (Figure 3.3b).

Further investigation of any possible relationship between the Eastern coastal region Drains and Springs and Ponds found a statistically-significant difference (*P* <0.001) in mean square-root TIN between Eastern and Western coastal-region Drains, Ponds and Springs (Eastern Drains: *n* = 166; Western Drains: *n* = 52; Ponds: *n* = 58; Springs: *n* = 38). Tukey post-hoc multiple comparisons found statistically-significant difference in mean square-root TIN for all Location Type pairwise comparisons except Western Drains vs. Springs (Table 3.5), with Ponds having the greatest values (Figure 3.3c).

3.3.2.4 Nitrate (NO_3)

A one-way ANOVA found a statistically-significant difference between location type (Drain, Pond, Spring) in mean square-root NO_3 (*P* <0.001). Tukey post-hoc multiple comparisons found a statistically-significant difference in mean square-root NO_3 between pairwise comparisons for Drain vs. Pond (*P* <0.001) and Pond vs. Spring (*P* = 0.001) but Drain vs. Spring was non-significant (Table 3.5), with Ponds having the larger values (Figure 3.4a).

When Drains were split into Eastern and Western coastal regions, a statistically-significant difference (*P* <0.001) in mean square-root NO_3 between regional locations of Drains (Eastern *n* = 166; Western *n* = 52) was observed (Table 3.5), with Eastern drains having larger values (Figure 3.4b).

Further investigation of any possible relationship between the Eastern coastal region Drains, Springs and Ponds found a statistically-significant difference (*P* <0.001) in mean square-root NO_3 between Eastern and Western coastal region Drains, Ponds and Springs (Eastern Drains: $n = 166$; Western Drains: $n = 52$; Ponds: $n = 58$; Springs: $n = 38$). Tukey post-hoc multiple comparisons found a statistically-significant difference in mean square-root NO_3 for all Location Type pairwise comparisons except Eastern Drains vs. Springs (Table 3.5), with Ponds having the largest values (Figure 3.4c).

Statistical analysis of the percent contribution of NO_3 to TIN found a statistically-significant difference ($P < 0.001$) in mean arcsine %NO_3/TIN between Eastern and Western coastal region Drains, Ponds and Springs (Eastern Drains: $n = 166$; Western Drains: $n = 52$; Ponds: $n = 58$; Springs: $n = 38$). Tukey post-hoc multiple comparisons found statistically-significant differences in mean arcsine %NO_3/TIN for all pairwise comparisons of location type except Eastern Drains vs. Springs and Ponds vs. Springs (Table 3.5), with Western Drains having much smaller values at all times except during July of 2004 (Figure 3.5).

3.3.2.5 Isotopes of hydrogen (δ^2H)

A one-way ANOVA found a statistically-significant difference between location type (all Drains, Pond, Spring) in mean δ^2H ($P = 0.003$). Tukey post-hoc multiple comparisons found that all combinations of location type were not statistically significant for differences in mean δ^2H, except Drain vs. Spring ($P = 0.005$) (Table 3.6), with Drains having the lower values (Figure 3.6a).

When Drain sites were split into Eastern versus Western coastal regions (see "Statistics" in the Methods section above for definition) and excluding seasonal-flowing Drains (Table 3.3), a statistically-significant difference (*P* <0.001; *n* = 178) in mean δ^2H was observed between regional location of Drain (Eastern *n* = 134; Western *n* = 44) (Table 3.6), with Western values exceeding the Eastern (Figure 3.6b).

To further investigate any possible relationships between particularly the Eastern coastal-region Drains and Springs and Ponds, I then re-analysed these by location type (Eastern versus Western coastal-region Drains (for continuous-flowing drains only as these were assumed to be groundwater-fed), Ponds and Springs). A one-way ANOVA found that pairwise comparisons were not statistically-significant (*P* >0.05) for combinations of Eastern coastal region Drains, and Ponds and Springs. Statistically-significant differences were found for all pairwise comparisons involving Western coastal region Drains (all *P* <0.001) (Table 3.6, Figure 3.6c). That is there were two definitive groups: 1) Western drains versus 2) Eastern drains, Ponds and Springs, with the former group having the higher values.

3.3.2.6 Isotopes of oxygen (δ^{18}O)

A one-way ANOVA found a statistically-significant difference between location type (all Drains, Pond, Spring) in mean δ^{18}O (*P* = 0.007). Tukey post-hoc multiple comparisons found statistically-significant differences in pairwise combinations in mean δ^{18}O for location types Drain vs. Pond (*P* = 0.045) and Drain vs. Spring (*P* = 0.039, Table 3.6), with Drains having the higher values (Figure 3.7a).

When Drain sites were split into Eastern and Western coastal regions (see "Statistics" in the Methods section above for definition) and excluding seasonal-flowing Drains (Table 3.3), a statistically-significant difference (P <0.001; n = 178) in mean $\delta^{18}O$ was observed between regional locations of Drain (Eastern n = 134; Western n = 44) (Table 3.6), with Western values being larger (Figure 3.7b).

To further investigate any possible relationships between particularly the Eastern coastal region Drains and Springs and Ponds, I then re-analysed $\delta^{18}O$ data by location type (Eastern and Western coastal region Drains [for continuous-flowing drains only, as only one Seasonal-flow site was sampled in this aspect of the broader study], Ponds and Springs). A one-way ANOVA found that pairwise comparisons were not statistically-significant (P >0.05) for all combinations of Eastern coastal region Drains, Ponds and Springs. Statistically-significant differences were found for all pairwise comparisons involving Western coastal-region Drains (all P <0.001) (Table 3.6), with Western Drains having larger values than all others (Figure 3.7c).

3.3.3 Outcomes of Multivariate Analyses

A scatterplot of salinity against temperature found that groundwater influence of coastal waterbodies varies considerably across the study region (Figure 3.9). Western drains varied from fresh to hypersaline. Seasonal-flow western drains varied from fresh to hypersaline and continuous-flow western drains varied from brackish to marine (data not shown on Figure 3.9 for clarity). Ponds, Springs and Eastern Drains displayed a similar spread with minimal variation. The overlapping distribution of Spring, Pond, Eastern drains suggested they are all fresh (i.e. groundwater influenced).

Figure 3.10 displays again the consistent overlapping distribution of Spring, Pond, and continuous-flow eastern drains. Further inferring that these waterbodies were groundwater- influenced. Ponds and Springs showed a tighter grouping, suggesting less variation over the study period whereas continuous-flow Eastern drains were a little more spread (but also had more data points). Continuous-flow Western drains were the more removed and also showed some spread of values. Seasonal-flow Western drains were the furthest removed and had greater within spread but also had minimal points.

All water quality data suggested that Marine waters were different from all other groups, Western drains were different from Eastern drains, Continuous-flow drains were different from seasonal-flow drains, and Eastern continuous-flow drains ponds and springs were similar in their water quality characteristics, that is, predominantly groundwater-fed.

PERMANOVA results found a significant difference (*P*[perm] = 0.0001; Pseudo-*F* = 67.421; *df* = 6, 862) between groups for salinity and temperature analyses (*P*ERMANOVA sub-set 1, see Table 3.AA). Pairwise comparisons displayed significant differences [P(*P*erm) <<0.05) between most pairwise comparisons except Ponds vs Springs; Ponds vs Seasonal-flow Eastern Drains; Springs vs Seasonal-flow Eastern Drains; and Continuous-flow Eastern Drains vs

Seasonal-flow Eastern Drains (Table 3.7). Ranked (group average value) of PERMANOVA results showed that SW>CW>MAR>CE>SE>SPR=PO.

For PERMANOVA sub-set 2, a significant difference (*P*[perm] = 0.0001; Pseudo-*F* = 10.702; *df* = 5, 167) between groups for all water quality data (excluding δ^2H and $\delta^{18}O$ values). Pairwise comparisons (*P*ERMANOVA) displayed significant differences [P(*P*erm) <0.05) between most pairwise comparisons (see Table 3.7). Ranked (group average) values of PERMDISP results show that SW>CW>PO=CE>SPR>SE in terms of within-group variability.

For PERMANOVA sub-set 3, a significant difference (*P*[perm] = 0.0036; Pseudo-*F* = 4.292; *df* = 4,121) between groups for all water quality data values. Pairwise comparisons (*P*ERMANOVA) displayed significant differences [P(*P*erm)<0.05) between most pairwise comparisons (see Table 3.7). Ranked (group average value) of PERMDISP results show that SW>CW>CE>PO>SPR.

Thus the group average values (ranked) consistently suggest that western region drains were different from ponds, springs and eastern drains. The PCA analysis identified two principal components (PC1, PC2), which explained a total of >63% of the variance associated with a groundwater signal in the complete data set (Table 3.8). PC1 was positively correlated with nitrate and total nitrogen but negatively correlated with the stable isotope ratios. PC2 was positively correlated to both temperature and nitrite. The PCA suggested that salinity was only negatively related to PC1 but less strongly than N concentrations.

3.4 DISCUSSION

The term “seasonal-flow” was assigned to those drains (n = 13) that typically ceased flowing after the winter/spring period (usually by November or December of each year) and thus lost a direct connection with the sea. It was therefore assumed that, once excess surface water had been drained from the lands adjoining the drains, then those remaining drains that maintained a continuity of flow could be apportioned to having considerable groundwater inputs.

3.4.1 Salinity

The results suggest that salinity differences were apparent with the Marine group (ranging between 26 and 37ppt) suggesting some periodic influence of fresh water running off from the land may be occurring. The specific source of the fresh water was not identified (all sites were thought to be removed from known or observed point sources). Consistently lower salinities were observed in groundwater-fed Ponds (<2ppt) and Springs (<3ppt), which we know to have originated from groundwater sources. However some variability was also observed in the salinity of all drains sampled. Continuous-flow drains ranged from <1ppt to 23ppt and seasonal-flow drains from <1ppt to >35ppt.

When these continuous-flow drains were separated by region, a consistent pattern emerged. Western drain ranges displayed estuarine salinities (7-23 ppt) suggesting a mixed influence from surface and groundwater and from tidal exchange. It is worth noting that the upper reaches of Blackford Drain flows through a region of higher salinity groundwater (>6 ppt) and Blackford Drain maintained a relatively consistent salinity (6-9 ppt) over the duration of the study. This could suggest that most flow into Blackford Drain is surfacing groundwater but with periodic contributions from seasonal surface runoff from time to time.

The Eastern continuous-flow drains were consistently low in salinity (0.4-1.5 ppt), suggesting these drains could be influenced by groundwater-fed sources. When the Eastern drains were compared against groundwater Ponds, no significant differences in salinities were observed ($P > 0.05$). These salinity results suggest that Eastern drains are likely to be predominantly groundwater-fed but are also likely to be influenced from time to time by surface water runoff.

This pattern is potentially important for the resident biota. The consistent low salinities suggest that a dominant fresh-water habitat is likely in the Eastern drains whereas the biota of Western drains should display more estuarine to marine characteristics and so their species composition should be expected to differ, reflecting these fluctuating salinities (see Chapter 4).

Where seasonal-flow drains were sampled, salinity changes were inferred to have resulted from cessation of surface water inputs and increased evaporation and other losses.

The results of this study are comparable to those found in Schmidt *et al.* (1999), who suggested that the depth to the groundwater table within the

Port MacDonnell coastal region (*P*articularly to the east of Port MacDonnell) is less than 2m. This would further support my inference that the drains within this are (i.e. Drains/Creeks: Clarke Park, Cress, Jerusalem, Riddoch Bay, Deep, Eight Mile, Brown Bay, A.C.I Road, and Piccaninnie Ponds Outlet; Piccanninnie and Ewens Ponds; and beach springs; see Figure 3.1, Table 3.1) are predominantly groundwater fed.

Leaney & Herczeg (1995) reported regional groundwater salinity to range from 100 $mg.L^{-1}$ to greater than 2000 $mg.L^{-1}$ (i.e. approx. 0.1 to >2 ppt). Salinities of groundwaters close to point-source features (e.g. springs and ponds) were reported as ranging from 50 – 200 $mg.L^{-1}$ (i.e. approx. 0.05 to 0.2 ppt) with samples near large sinkholes having the lower salinities (Leaney & Herczeg 1995).

All continuous-flow Eastern region drains were observed to be similar to the regional salinity range for groundwater stated by Leaney & Herczeg (1995). Salinity of point-source (i.e. Little Blue Lake 0.24 ± 0.01 ppt, Ewens Ponds 0.39 ± 0.01 ppt, Beach Spring 01 0.43 ± 0.03 ppt) features were marginally above the value range (0.05 to 0.2 ppt) as reported by Leaney & Herczeg (1995). Piccaninnie Ponds (1.56 ± 0.04 ppt) was approximately 8 times the value but below the groundwater salinities typical for the region (i.e. 2 ppt, Leaney & Herczeg 1995).

Salinity of the Piccaninnie Ponds was 1.56 ± 0.04 ppt (this study n = 33) compared with 1.77 $mg.L^{-1}$ (~1.77 ppt) in Scholz (1990). Scholz (1990) also reported that the salinity of the local unconfined aquifer was only 0.39 mg.L^{-1} (0.39 ppt). This value is comparable to the observed salinity of the Piccanannie Ponds Conservation Park beach springs system of 0.43 ± 0.03 ppt (*n* = 57) (Beach Spring 01, see Table 3.1).

The mean salinity in the outlet of Piccaninnie Ponds was 1.27 ± 0.04 ppt (*n* = 48) which was slightly lower than the mean salinity of the Pond system itself (1.56 ± 0.04 ppt, *n* = 33). It may be possible that the outflow is being diluted by water from the unconfined aquifer as it exits the ponds and discharges to the sea. Scholz (1990) suggested that the majority of the system's water is fed from the underlying aquifer via a fracture in 'The Chasm' system of the ponds. The fracture descends at least 65m into the underlying limestone aquifer.

3.4.2 Temperature

Mean temperatures across all location types showed a typical annually-variable pattern with Marine and Drain sites having greater annual ranges of 15-20°C and 13-24°C, respectively. Springs did vary but the range of their means were somewhat less pronounced (approximately only 13-17°C). There was no observed difference between Western (mean temperature range = 17-19°C) and Eastern (15-19°C) Continuous-flow drains, suggesting that either surface water and groundwater may be rapidly mixed into the general waterbody to mask the influence of any groundwater contributions to temperature, or that any groundwater inputs may not be that significant in terms of volume.

Even though Springs were sampled at their discharge (surfacing) source, their lower temperatures were similar to those lower temperatures sampled in some Eastern drains. This may suggest that either groundwater may not be directly flowing from aquifers at any depth (and hence is no colder than surface waters) or that Eastern Drains are mainly groundwater-fed.

3.4.3 Nitrogen

Nitrogen concentration varied between location types with groundwater-fed Ponds having the highest values of TIN. Drains and Ponds had similar values but varied over time. Significant differences in TIN were observed when drains were analysed by region. Continuous-flow Eastern Drains displayed consistently higher concentrations of TIN whereas continuous-flow Western Drains were significantly lower. Eastern drains displayed consistent values (i.e. less within-site variability) whereas Western drains were generally lower but displayed more within-site variability. A similar pattern was observed for nitrate, except that Western drains had almost zero nitrate concentrations. This observation suggests that Western drains, whilst maintaining a continual discharge, do not have significant input from groundwater sources. The July 2004 anomaly (i.e significantly increased to the longer-term average) in nitrate may be related to a significant rainfall event that may have interacted with groundwater tables allowing expression of elevated nitrate concentrations to be observed.

Ponds, Springs and Eastern drains showed that between eighty and ninety percent of TIN was due to nitrates but in Western drains only ten percent of TIN was from nitrate (except for Mt Benson Drain). This would suggest that quite different sources of nitrogen are involved.

The ANZECC water quality guidelines (ANZECC 2000) define default trigger values for physical and chemical stressors for south central Australian aquatic ecosystems. Trigger values are used to assess risk of adverse effects due to nutrients, biodegradable organic matter and pH in various ecosystem types. The default trigger value for total nitrogen lowland rivers, freshwater lakes, estuarine and marine environments in South Australia is 1000 $\mu g.L^{-1}$ N. No data were available for defining default trigger values for wetlands (ANZECC 2000). These results suggest that for Drain and Spring sites, only Clarke Park Drain (0.74 ± 0.07 $mg.L^{-1}$ TIN, n = 20) and Beach Spring 12 (0.9 ± 0.3 $mg.L^{-1}$ TIN, n = 2), respectively, had mean TIN concentrations below the default trigger value for south central Australian aquatic ecosystems (ANZECC 2000). All Ponds were found to have exceeded the default trigger for TIN.

Schmidt *et al.* (1999) analysed nitrate data in regional SE SA bores (n = 1663 bore data points,) and suggested that elevated nitrate contamination (up to >20 $mg.L^{-1}$ NO_3 in areas around Mount Gambier) are due to nitrate leaching from agricultural practices. In comparison Turner *et al.* (1984) suggested that nitrate concentrations in Blue Lake, Mount Gambier were approaching 15 $mg.L^{-1}$ NO_3 in 1980.

Schmidt *et al.* (1999) further suggested that it may take decades or centuries for nitrate levels to achieve equilibrium due to low recharge rates, slow lateral movement and limited vertical mixing of regional groundwater. In terms of the south eastern sub-region (i.e. between Port MacDonnell and the Victorian border), the depth to local groundwater table (within this sub-region) is generally <2 m and nitrate levels are typically 2-10 mg.L^{-1} NO_3 in the coastal unconfined aquifer (Schmidt *et al.* 1999).

Schmidt *et al.* (1999) also reported nitrate in the unconfined aquifer within coastal areas of the Port MacDonnell study sub-region to be typically 2-10 mg.L^{-1} NO_3 but suggested elevated levels are due to agricultural practices. This study found that nitrate concentration within the same sub-region ranged between 0.10 ± 0.02 mg.L^{-1} NO_3 (Clarke Park Drain) and 5.00 ± 0.19 mg.L^{-1} NO_3 (Eight Mile Creek).

In specific reference to Piccaninnie Ponds, Scholz (1990), from one specific sampling event in July 1987, returned nitrate and total nitrogen concentrations of 0.74 mg.L^{-1} NO_3 and 2.46 mg.L^{-1} TN, respectivey. The longer-term mean values for this study found nitrate and total nitrogen concentrations (n = 22) of 2.41 ± 0.14 mg.L^{-1} NO_3 and 3.14 ± 0.17 mg.L^{-1} TN, respectively, thus a little higher. Nitrates contributed to approximately 78% of total nitrogen in this study whereas on 30% of total nitrogen was made up from nitrates.

3.4.4 Isotopes

Ponds and Beach Springs showed similar values of δ^2H. When Drains were separated by Flow and Region combined, continuous-flow Eastern drains reflected those values observed in groundwater-fed Ponds and Beach Springs (i.e. more enriched), whereas Western Drains generally were considerably lower in δ^2H (i.e. depleted).

A similar pattern was observed in location type and drain region for $\delta^{18}O$ with Western drains again showed smaller $\delta^{18}O$ values than Eastern drains, Ponds and Springs. This again suggests that Eastern drains are likely to be significantly groundwater-fed whereas Western drains are much less so, if at all.

Turner *et al.* (1984) previously reported δ^2H and $\delta^{18}O$ values (mean ± s.e.) to be -12.6 ± 0.5 ‰ δ^2H and -1.87 ± 0.03 ‰ $\delta^{18}O$ in water of the Blue Lake, Mount Gambier, whereas Leaney & Herczeg (1995) reported that more broader regional δ^2H and $\delta^{18}O$ values ranged from -10 to -25 ‰ δ^2H and from -1 to -6 ‰ $\delta^{18}O$.

In comparison, this study had smaller (i.e. more enriched) δ^2H values that ranged from -14.2 to -33.5 ‰ δ^2H for continuous-flow drains, +15.0 to -12.3 ‰ δ^2H (seasonal-flow drains), -12.5 to -32.7 ‰ $\delta^{18}O$ (*P*onds), and -15.4 to -30.1‰ $\delta^{18}O$ (Springs). In contrast $\delta^{18}O$ values fitted that range, i.e. from +1.9 to -6.9 ‰ $\delta^{18}O$ for continuous-flow drains, -1.3 to -3.3 ‰ $\delta^{18}O$ (seasonal-flow drains), -2.0 to -6.4 ‰ $\delta^{18}O$ (*P*onds), and -3.7 to -5.9 ‰ $\delta^{18}O$ (Springs) (Table 3.3a,b).

3.4.5 General Findings

The data anomaly observed in July 2004 in Western drains was displayed consistently across all components measured (i.e. TIN, NO3, % NO3, δ^2H and to a lesser extent $\delta^{18}O$). This anomaly may be explained by greater rainfall and surface-water runoff at that time. It is possible that rainfall interacted with groundwater to allow mixing with runoff in the drains. The observed values for TIN, NO_3, $\%NO_3$, ^2H, and ^{18}O at this time approached the values for Ponds, Eastern Drains and Springs, which have been clearly shown to be groundwater-fed.

How much groundwater contributes to each waterbody is unknown but would be valuable to explore and quantify further in another study using tracers, as per Chapter 1.

3.5 CONCLUSION

The results suggest that Western drains were significantly different from groundwater Ponds, Springs and Eastern drains and that Eastern drains are predominantly maintained and fed from groundwater resources. This study found that waterbodies associated with groundwater inputs tended to be fresher (low salinities), cooler, had more nitrogen (especially NO_x) and were more enriched with hydrogen and oxygen isotopes. It is probable that these water-quality characteristics of continuous groundwater inputs are likely to influence the species composition of the biota residing in them (see Chapter 4).

TABLES

Table 3.1a: Salinity and temperature summaries by location type and sites sampled. See Chapter 2 "General Methods" for additional site information including locations. ppt = parts per thousand; N = total number of replicate samples; Reg = Region: E = Eastern coastal region, W = Western coastal regional; Flow (see Chapter 2 Methods for definition of region and flow): C = Continuous outflow, S = Seasonal outflow; CP = Conservation Park; NP = National Park; Beach Spring 03 was intentionally omitted from table (i.e. it was observed but never sampled). - = not applicable

Site No.	Site Name	Reg	Flow	Salinity (ppt) mean±s.e.	min	max	Temperature (ºC) mean±s.e.	min	max	*N*
Drains:										
DR01	Blackford Drain	W	C	7.56±0.19	4.71	9.80	19.4±0.7	9.3	26.8	44
DR02	Maria Creek	W	S	35.4±0.20	34.8	35.6	12.9±0.3	12.3	13.8	4
DR03	Butchers Gap Drain	W	S	8.89±0.49	7.43	10.95	17.1±0.4	15.0	17.9	6
DR04	Mount Benson Drain	W	S	0.39±0.03	0.35	0.74	19.7±1.0	13.8	24.6	13
DR05	Drain L Outlet (Robe)	W	C	8.12±1.25	0.43	10.4	17.7±0.6	10.4	24.1	34
DR06	Lake George Outlet	W	S	24.9±3.40	2.55	47.5	16.8±1.2	9.4	25.8	22
DR07	Lake Frome Outlet	W	C	22.6±2.30	0.97	38.4	17.2±0.7	8.6	23.4	31
DR08	Pelican Point Road	W	S	0.74	-	-	18.3	-	-	1
DR10	Spehrs Road	W	S	11.1±2.80	0.60	23.4	23.7±0.1	22.8	24.6	12
DR11	Douglas Point CP	W	S	2.38±1.45	0.92	5.27	18.6±3.0	13.6	23.9	3
DR12	Cape Douglas Road	W	S	3.13±1.59	1.54	4.72	19.5±5.5	14.0	24.9	2
DR13	Blanche Bay	W	S	5.35±1.54	3.81	6.89	16.7±3.2	13.5	19.9	2
DR14	Finger Point	W	S	1.07±0.20	0.88	1.25	15.8±1.7	14.1	17.4	2

Table 3.1a (cont).

Site No.	Site Name	Reg	Flow	Salinity (ppt) mean±s.e.	min	max	Temperature (ºC) mean±s.e.	min	max	*N*
Drains (cont):										
DR15	Clarke Park Drain	E	C	0.56±0.02	0.40	0.84	16.3±0.7	9.5	22.5	31
DR16	Port MacDonnell Town	E	S	3.03	-	-	10.9	-	-	1
DR17	Cress Creek	E	C	0.92±0.01	0.78	1.17	17.6±0.3	14.1	20.6	43
DR18	Jerusalem Creek	E	C	0.40±0.02	0.25	0.77	18.7±0.8	10.6	25.5	31
DR19	Millstead Main Drain	E	S	1.00	-	-	15.2	-	-	1
DR20	Riddoch Bay	E	C	0.74±0.03	0.53	1.28	19.0±0.6	14.5	23.2	31
DR21	Deep Creek	E	C	1.52±0.02	1.19	1.98	17.5±0.3	15.1	20.4	43
DR22	Eight Mile Creek	E	C	0.67±0.08	0.34	3.84	16.9±0.2	14.6	19.0	43
DR23	Brown Bay	E	C	1.56±0.10	0.66	3.67	17.9±0.6	12.0	25.7	43
DR24	Green Point	E	S	0.52±0.07	0.32	0.94	19.1±0.4	17.2	21.3	13
DR25	A.C.I Road	E	C	0.47±0.01	0.34	0.61	14.9±0.4	9.3	18.6	43
DR26	Piccaninnie Ponds Outlet	E	C	1.27±0.04	0.94	2.04	15.8±0.3	12.0	20.0	48
Springs:										
SPR01	Beach Spring 01	E	C	0.43±0.03	0.22	1.21	15.5±0.1	14.8	17.9	57
SPR02	Beach Spring 02	E	C	1.94±0.32	0.98	3.06	16.3±0.4	14.8	18.1	9
SPR04	Beach Spring 04	E	C	1.08±0.21	0.29	2.55	13.4±0.2	12.9	14.2	12
SPR05	Beach Spring 05	E	C	0.35±0.01	0.34	0.35	14.4±0.1	14.3	14.4	3

Table 3.1a (cont).

Site No.	Site Name	Reg	Flow	Salinity (ppt) mean±s.e.	min	max	Temperature (ºC) mean±s.e.	min	max	*N*
Springs (cont):										
SPR06	Beach Spring 06	E	C	1.63±0.68	0.14	4.91	13.5±0.2	12.7	14.1	8
SPR07	Beach Spring 07	E	C	1.33±0.26	0.25	3.94	17.6±0.1	16.3	18.4	22
SPR08	Beach Spring 08	E	C	1.75±0.02	1.73	1.76	15.7±0.3	15.4	16.0	2
SPR09	Beach Spring 09	E	C	2.20	-	-	16.1	-	-	1
SPR10	Beach Spring 10	E	C	1.49±0.04	1.37	1.54	16.3±0.1	16.2	16.4	4
SPR11	Beach Spring 11	E	C	0.46±0.01	0.35	0.48	15.7±0.1	15.6	15.9	9
SPR12	Beach Spring 12	E	C	2.86±0.14	2.15	3.12	18.6±0.1	18.5	18.7	6
Ponds:										
PO1	Little Blue Lake	E[a]	-	0.24±0.01	0.21	0.42	18.0±0.7	12.7	21.7	21
PO2	Ewens Ponds	E[b]	-	0.39±0.01	0.36	0.53	16.3±0.1	15.2	17.7	33
PO3	Piccaninnie Ponds	E[c]	-	1.56±0.04	1.26	2.07	16.3±0.1	15.0	17.6	33
Marine Sites:										
MAR01	The Granites	W	-	34.3±0.3	31.6	35.7	16.4±0.8	10.8	19.8	18
MAR02	Wright Bay	W	-	32.8±0.5	30.0	35.5	18.2±1.0	10.8	24.8	15
MAR03	Little Dip CP	W	-	34.1±0.5	29.3	35.9	16.3±0.7	12.6	19.5	18
MAR04	Canunda NP	W	-	34.2±0.3	32.4	36.1	15.7±0.5	12.5	19.7	18

Table 3.1a (cont).

Site No.	Site Name	Reg	Flow	Salinity (ppt) mean±s.e.	min	max	Temperature (ºC) mean±s.e.	min	max	*N*
Marine Sites (cont):										
MAR05	Pelican Point	W	-	33.6±1.0	26.2	36.6	19.5±1.4	9.8	24.8	15
MAR06	NeneValley CP	W	-	34.4±0.5	29.0	35.7	18.2±0.9	12.6	22.1	15
MAR07	Discovery Bay	E	-	31.6±0.1	31.6	31.6	17.1±0.1	17.1	17.1	3

[a] = "Eastern" coastal region but approximately 13km inland from coast

[b] = "Eastern" coastal region but approximately 3km inland from coast

[c] = "Eastern" coastal region but approximately 1km inland from coast

Table 3.1b: Salinity and temperature summaries by location type (and three ways of drain sub-grouping). ppt = parts per thousand; *N* = total number of replicate samples.

Location Type	**Sites**	**Salinity (ppt)**			**Temperature (ºC)**			**Reps**
	N	**Mean±s.e**	**Min**	**Max**	**Mean±s.e**	**Min**	**Max**	***N***
Marine	7	33.8±0.2	26.2	36.6	17.2±0.4	9.8	24.8	102
Ponds	3	0.8±0.1	0.2	2.1	16.5±0.2	10.9	21.7	87
Beach Springs	15	1.0±0.1	0.1	4.9	15.5±0.1	10.4	18.7	133
Drains (All)	25	4.7±0.2	0.2	47.5	17.5±0.2	8.6	26.8	547
By Flow:								
Continuous	*12*	*3.5±0.3*	*0.2*	*38.4*	*17.3±0.1*	*8.6*	*26.8*	*466*
Seasonal	*13*	*11.3±1.6*	*0.3*	*47.5*	*18.5±0.5*	*9.4*	*25.8*	*81*
By Region:								
Eastern	*12*	*0.9±0.1*	*0.2*	*3.8*	*17.1±0.2*	*9.3*	*25.7*	*371*
Western	*13*	*12.6±0.9*	*0.3*	*47.5*	*18.4±0.3*	*8.6*	*26.8*	*176*
By Flow:Reg								
Cont. East	*9*	*0.9±0.1*	*0.2*	*3.8*	*17.0±0.2*	*9.3*	*25.7*	*356*
Cont. West	*3*	*12.0±1.0*	*0.4*	*38.4*	*18.2±0.4*	*8.6*	*26.8*	*109*
Seas. East	*3*	*0.7±0.2*	*0.3*	*3.0*	*18.3±0.7*	*10.9*	*21.3*	*15*
Seas. West	*10*	*13.6±1.8*	*0.3*	*47.5*	*18.5±0.6*	*9.4*	*25.8*	*67*

Table 3.2a: Concentration summaries by location type and site of various nitrogen-related water-quality parameters. TIN = total inorganic nitrogen; NO_x = oxygenated nitrogen species (e.g. nitrite, nitrate); NO_3 = Nitrate; CP = Conservation Park; NP = National Park; Reg = Region: W = Western coastal region, E = Eastern coastal region; Flow: C = continuous-flow; S = seasonal-flow.

Site Name	Reg	Flow	TIN (mg/L)			NO_x (mg/L)			NO_3 (mg/L)			%NO_3 of		*N*
			mean±SE	Range		mean±SE	Range		mean±SE	Range		TIN	NO_x	
				min	max		min	max		min	max	mean±SE	mean±SE	
<u>Drains:</u>														
Blackford Drain	W	C	1.47±0.11	0.62	2.72	0.20±0.11	<0.01	1.80	0.19±0.10	<0.01	1.71	9.5±4.3	96.6±1.7	22
Mount Benson Dr.	W	S	3.89±0.49	2.00	5.30	3.18±0.60	1.13	4.60	3.17±0.60	1.12	4.60	77.4±7.6	99.3±0.3	6
Drain L Outlet	W	C	1.51±0.35	0.53	5.04	0.54±0.32	0.02	3.87	0.53±0.32	0.01	3.82	15.6±6.2	96.1±1.7	16
Lake George Outlet	W	S	2.38±0.61	0.22	6.80	0.18±0.08	0.01	0.84	0.17±0.07	0.01	0.82	9.9±4.8	94.6±3.2	12
Lake Frome Outlet	W	C	1.57±0.24	0.35	3.30	0.36±0.16	<0.01	1.71	0.36±0.16	<0.01	1.69	14.7±5.5	91.1±6.3	14
Spehrs Road	W	S	1.27±0.20	0.79	2.20	0.04±0.02	<0.01	0.10	0.04±0.02	<0.01	0.10	3.4±1.4	99.6±0.4	6
Clarke Park Drain	E	C	0.74±0.07	0.46	1.33	0.11±0.02	0.02	0.29	0.10±0.02	0.02	0.28	14.9±3.3	98.2±0.8	14
Cress Creek	E	C	2.99±0.10	2.00	3.63	2.46±0.10	1.27	3.10	2.46±0.10	1.26	3.09	82.0±2.1	99.9±0.1	20
Jerusalem Creek	E	C	1.82±0.10	1.30	2.60	1.16±0.10	0.53	1.92	1.15±0.10	0.52	1.90	64.0±4.7	99.2±0.3	14
Riddoch Bay	E	C	2.92±0.08	2.30	3.43	2.23±0.18	0.19	2.93	2.23±0.18	0.19	2.92	75.4±5.7	99.8±0.1	14
Deep Creek	E	C	2.82±0.19	1.50	4.07	2.38±0.17	1.23	3.62	2.38±0.17	1.23	3.61	84.3±1.6	99.9±0.1	22
Eight Mile Creek	E	C	5.66±0.18	3.40	6.77	5.00±0.19	2.40	5.99	5.00±0.19	2.40	5.98	88.0±1.6	99.9±0.1	22
Brown Bay	E	C	3.40±0.19	2.17	4.93	2.63±0.15	1.47	3.90	2.60±0.15	1.44	3.90	77.1±2.5	98.8±0.3	20
Green Point	E	S	1.06±0.09	0.72	1.30	0.25±0.07	0.09	0.53	0.25±0.07	0.09	0.52	23.8±6.2	99.7±0.2	6
A.C.I Road	E	C	1.56±0.08	0.93	2.20	0.88±0.07	0.33	1.40	0.88±0.07	0.32	1.40	56.0±3.3	99.7±0.1	18
Picc. Ponds Outlet	E	C	2.42±0.10	1.60	3.23	1.94±0.07	1.30	2.50	1.94±0.07	1.30	2.50	81.0±1.9	99.9±0.1	22

Table 3.2 (cont).

Site Name	**Reg**	**Flow**	**TIN (mg/L)**			**NO_x (mg/L)**			**NO_3 (mg/L)**			**%NO_3 of**		***N***
			mean±SE	**Range**		**mean±SE**	**Range**		**mean±SE**	**Range**		**TIN**	**NO_x**	
				min	**max**		**min**	**max**		**min**	**max**	**mean±SE**	**mean±SE**	
<u>Springs:</u>														
Beach Spring 01	E	C	2.35±0.16	1.10	3.30	1.91±0.13	0.91	2.63	1.91±0.13	0.91	2.63	81.2±2.2	99.9±0.1	20
Beach Spring 02	E	C	2.55±0.95	1.60	3.50	1.63±0.43	1.20	2.07	1.63±0.43	1.20	2.07	67.0±8.0	100±0	2
Beach Spring 04	E	C	2.05±0.05	2.00	2.10	1.72±0.08	1.63	1.80	1.72±0.08	1.63	1.80	83.7±2.0	100±0	2
Beach Spring 05	E	C	2.17±0.13	2.03	2.30	1.40±0.10	1.30	1.50	1.40±0.10	1.30	1.50	65.2±8.6	100±0	2
Beach Spring 06	E	C	1.70±0.30	1.40	2.00	0.99±0.38	0.61	1.37	0.99±0.38	0.61	1.37	55.8±12.5	100±0	2
Beach Spring 07	E	C	1.16±0.09	0.95	1.40	0.72±0.14	0.36	0.97	0.72±0.14	0.36	0.97	61.6±11.1	100±0	4
Beach Spring 11	E	C	1.52±0.17	1.27	2.00	1.26±0.17	0.98	1.77	1.26±0.17	0.98	1.77	82.3±4.1	100±0	4
Beach Spring 12	E	C	0.93±0.27	0.66	1.20	0.50±0.03	0.48	0.53	0.50±0.03	0.48	0.53	60.3±20.4	100±0	2
<u>Ponds:</u>														
Little Blue Lake	E[a]	-	1.79±0.08	1.20	2.27	1.34±0.06	0.89	1.60	1.34±0.06	0.89	1.60	75.4±2.5	99.9±0.1	14
Ewens Ponds	E[b]	-	5.40±0.18	3.97	6.77	4.77±0.16	2.60	5.67	4.77±0.16	2.60	5.67	88.6±2.0	99.9±0.1	22
Piccaninnie Ponds	E[c]	-	3.14±0.17	1.80	5.70	2.41±0.14	1.03	3.40	2.41±0.14	1.03	3.40	77.7±3.8	99.9±0.1	22

Table 3.2b: Summary of nitrogen speciation in water samples by location type (and three ways of drain sub-grouping). TIN = total inorganic nitrogen. NO_3 = Nitrate. NO_x = nitrogen oxides (sum of nitrate [NO_2] and nitrate [NO_3]). N = total number of field replicate samples.

Location Type	**Sites**	**TIN (mg/L)**			**NO_3 (mg/L)**		**%NO_3 of**		**Reps**
							TIN	**NO_x**	
	N	**mean±se**	**min**	**max**	**mean±se**	**max**	**mean±se**	**mean±se**	**N =**
Ponds	3	3.7±0.2	1.2	6.8	3.1±0.2	5.7	81.3±1.9	99.9±0.1	58
Beach Springs	8	2.0±0.1	0.7	3.5	1.5±0.1	2.6	74.2±2.5	99.9±0.1	38
Drains (All)	16	2.5±0.1	0.2	6.8	1.7±0.1	6.0	53.8±2.2	98.3±0.4	248
<u>*By Flow:*</u>									
Continuous	*12*	*2.5±0.1*	*0.4*	*6.8*	*1.8±0.1*	*6.0*	*57.7±2.3*	*98.5±0.5*	*218*
Seasonal	*4*	*2.2±0.3*	*0.2*	*6.8*	*0.7±0.3*	*4.6*	*24.9±5.7*	*97.5±1.3*	*30*
<u>*By Region:*</u>									
Eastern	*10*	*2.8±0.1*	*0.5*	*6.8*	*2.2±0.1*	*6.0*	*70.1±1.9*	*99.6±0.1*	*172*
Western	*6*	*1.8±0.2*	*0.2*	*6.8*	*0.5±0.1*	*4.6*	*16.7±3.1*	*95.6±1.4*	*76*
<u>*By Flow:Reg*</u>									
Cont. East	*9*	*2.9±0.1*	*0.5*	*6.7*	*2.3±0.1*	*6.0*	*71.8±1.8*	*99.5±0.1*	*166*
Cont. West	*3*	*1.5±0.1*	*0.4*	*5.0*	*0.3±0.1*	*3.8*	*12.8±3.0*	*95.0±1.9*	*52*
Seas. East	*1*	*1.1±0.1*	*0.7*	*1.3*	*0.3±0.1*	*0.5*	*23.8±6.2*	*99.7±0.2*	*6*
Seas. West	*3*	*2.5±0.4*	*0.2*	*6.8*	*0.9±0.3*	*4.6*	*25.2±7.0*	*97.0±1.7*	*24*

Table 3.3a: Summary by location type and site of hydrogen and oxygen isotopes derived from water samples. ‰ = permil (see Chapter 2, General Methods for description); CP = Conservation Park; NP = National Park; Reg = Region: W = Western coastal region, E = Eastern coastal region; Flow: C = continuous outflow; S = seasonal outflow.

Site Name	Reg.	Flow	δ^2H (‰)			$\delta^{18}O$ (‰)			N
			mean±SE	min	max	mean±SE	min	max	
Drains:									
Blackford Drain	W	C	-3.0±1.9	-19.6	7.1	-3.58±0.44	-5.34	0.44	16
Drain L Outlet (Robe)	W	C	-9.5±1.2	-16.5	3.4	-1.43±0.46	-5.07	1.45	16
Lake George Outlet	W	S	3.5±3.2	-12.3	15.0	-2.39±0.22	-3.33	-1.31	10
Lake Frome Outlet	W	C	-3.2±1.8	-15.3	4.1	-1.52±0.49	-4.39	1.78	12
Clarke Park Drain	E	C	-23.6±0.6	-26.9	-19.2	-2.18±0.66	-5.26	1.93	14
Cress Creek	E	C	-26.3±0.9	-30.9	-20.6	-4.62±0.19	-5.87	-3.25	14
Jerusalem Creek	E	C	-24.8±1.0	-30.0	-16.9	-5.17±0.16	-6.20	-4.20	14
Riddoch Bay	E	C	-25.4±0.9	-28.7	-19.2	-4.88±0.38	-5.72	-3.50	14
Deep Creek	E	C	-24.6±1.1	-33.5	-14.2	-4.40±0.38	-6.10	-0.31	16
Eight Mile Creek	E	C	-25.1±0.7	-29.9	-19.9	-5.25±0.15	-6.94	-4.56	16
Brown Bay	E	C	-23.7±1.1	-30.4	-15.9	-4.66±0.20	-5.40	-2.57	14
A.C.I Road	E	C	-24.5±0.9	-28.9	-17.3	-4.75±0.16	-6.10	-3.87	16
Piccaninnie Ponds Outlet	E	C	-25.1±0.7	-29.6	-21.5	-4.86±0.10	-5.38	-4.13	16

Table 3.3a(cont.)

Site Name	**Reg.**	**Flow**	**δ^2H (‰)**			**$\delta^{18}O$ (‰)**			**N**
			mean±SE	**min**	**max**	**mean±SE**	**min**	**max**	
Springs:									
Beach Spring 01	E	-	-24.5±1.4	-30.1	-15.4	-5.04±0.16	-5.92	-3.73	14
Beach Spring 04	E	-	-19.4±0.3	-19.6	-19.1	-4.40±0.65	-5.05	-3.76	2
Beach Spring 07	E	-	-24.1±2.7	-26.9	-21.4	-5.03±0.02	-5.05	-5.01	2
Ponds:									
Little Blue Lake	E[a]	-	-17.9±0.8	-22.6	-12.5	-4.66±0.20	-6.03	-3.49	14
Ewens Ponds	E[b]	-	-26.4±0.6	-30.6	-22.8	-4.19±0.33	-6.41	-1.95	16
Piccaninnie Ponds	E[c]	-	-28.3±0.8	-32.7	-15.4	-5.03±0.14	-6.15	-4.22	16

[a] = "Eastern" coastal region but approximately 13km inland from coast

[b] = "Eastern" coastal region but approximately 3km inland from coast

[c] = "Eastern" coastal region but approximately 1km inland from coast

Table 3.3b: Summary of hydrogen and oxygen isotopes by location type (and three ways of drain sub-grouping). TIN = total inorganic nitrogen. NO_3 = Nitrate. NO_x = nitrogen oxides (sum of nitrate [NO_2] and nitrate [NO_3]). N = total number of replicate samples. '-' no samples for that group. See Chapter 2 for a description of flow and region.

Location Type	**Sites**	**δ^2H (‰)**			**δ^{18}O (‰)**			**Reps**
	N	**mean±s.e.**	**min**	**max**	**mean±s.e.**	**min**	**max**	**N=**
Ponds	3	-24.5±0.8	-12.5	-32.7	-4.6±0.2	-2.0	-6.4	46
Beach Springs	3	-23.9±1.2	-15.4	-30.1	-5.0±01	-3.7	-5.9	18
Drains (All)	13	-18.7±0.8	15.0	-33.5	-3.9±0.1	1.9	-6.9	188
By Flow:								
Continuous	*12*	*-24.8±0.3*	*-14.2*	*-33.5*	*-4.0±0.1*	*1.9*	*-6.9*	*178*
Seasonal	*1*	*3.5±3.2*	*15.0*	*-12.3*	*-2.4±0.2*	*-1.3*	*-3.3*	*10*
By Region:								
Eastern	*9*	*-24.8±0.3*	*-14.2*	*-33.5*	*-4.5±0.1*	*1.9*	*-6.9*	*134*
Western	*4*	*-3.8±1.1*	*15.0*	*-19.6*	*-2.3±0.2*	*1.8*	*-5.3*	*54*
By Flow/Reg:								
Cont. East	*9*	*-24.8±0.3*	*-14.2*	*-33.5*	*-4.5±0.1*	*1.9*	*-6.9*	*134*
Seas. East	*0*	-	-	-	-	-	-	-
Cont. West	*3*	*-5.4±1.0*	*7.1*	*-19.6*	*-2.2±0.3*	*1.8*	*-5.3*	*44*
Seas. West	*1*	*3.5±3.2*	*15.0*	*-12.3*	*-2.4±0.2*	*-1.3*	*-3.3*	*10*

Table 3.4: One-way ANOVA results for analyses of salinity and temperature data. *df* = degrees for *F* test; Sal = Salinity; Temp = temperature; log = $\log_{10}$ transformed data; *P* significant at ά=0.05; ns = statistically non-significant (*P*>0.05); N = number of replicates; DR = Drains; E = eastern region coastal drains; W = western region coastal drains; U = undistinguished (i.e. E and W combined); PO = Ponds; SPR = Springs; MAR = Marine.

Factor	*df*	Variable		Group (& *N*)						Tukey Pairwise Comparison
		Salinity	Temp	Drain			Pond	Spring	Marine	
		P	*P*	East	West	Undist.				
Location Type [a]	3,865	<0.001	<0.001	-	-	547	87	133	102	**Sal (log):** All sig (*P*<0.001) except PO v SPR (ns) **Temp:** All ns except SPR v DR (*P*<0.001); SPR v MAR (*P*=0.003)
Drain Region [b]	1,463	<0.001	0.002	356	109	-	-	-	-	-
Location Type [c]	3,681	<0.001	<0.001	356	109	-	87	133	-	**Sal:** All ns except WDR v EDR, WDR v SPR, WDR v PO (*P*<0.001) **Temp:** All ns except WDR v SPR, WDR v PO (*P*<0.001) WDR v EDR (*P*=0.002)

Table 3.4 (cont.)

[a] = Every site sampled across all sampling visits. Includes all groups (Drains: continuous and seasonal flow combined; Ponds; Springs; and Marine) sampled between September 2003 to February 2006.
[b] = Data for analyses derived from continuous-outflow drains only (see Methods) and separated into two sub-groups: "Eastern" and "Western" coastal regions (see Methods). That is, analyses did not include Drains observed to have seasonal outflow.
[c] = Includes location types Ponds, Springs, (as in [a] above) and continuous outflow Drains by Eastern and Western coastal region (as in [b] above)

Table 3.5: One-way ANOVA results for analyses of TIN and NO_3. *df* = degrees of freedom; TIN = total inorganic nitrogen; NO_3 = nitrate; %NO_3/TIN = percent contribution of NO_3 to TIN; sqrt = square-root transformed data; arcsin = arcsine-transformed data; *P* significant at ά=0.05; N=number of cases (samples); DR = Drains; E = eastern coastal region drains; W = western coastal region drains; U = undistinguished (i.e. E and W combined); PO = Ponds; SPR = Springs; MAR = Marine.

Factor	***df***	**Variable *P***			**Group *N***					**Tukey Pairwise Comparisons**
		TIN (sqrt)	**NO_3 (sqrt)**	**%NO_3/TIN (arcsin)**	**Drain East**	**Drain West**	**Undist.**	**Pond**	**Spring**	
Location Type [a]	2,341	<0.001	<0.001	-	-	-	248	58	38	**<u>TIN:</u>** PO v DR, & PO v SPR (*P*<0.001); DR v SPR (ns) **<u>NO_3:</u>** PO v DR (*P*<0.001); PO v SPR (*P*=0.001); DR v SPR (ns)
Drain Region [b]	1,216	<0.001	<0.001	-	166	52	-	-	-	-
Location Type [c]	3,310	<0.001	<0.001	<0.001	166	52	-	58	38	**<u>TIN</u>:** PO v SPR, PO v WDR, & EDR v WDR (*P*<0.001); EDR v PO (*P*=0.001); EDR v SPR (*P*=0.005); SPR v WDR (ns) **<u>NO_3:</u>** All sig (*P*<0.001) except EDR v SPR (ns) **<u>%NO_3/TIN:</u>** WDR v EDR, WDR v PO, & WDR v SPR (*P*<0.001); EDR v PO (*P*=0.004); EDR v SPR, & PO v SPR (ns)

[a] = Every site sampled across all sampling visits. Includes all groups (Drains: continuous and seasonal flow combined; Ponds; and Springs) sampled between March 2004 to February 2006. Water quality (nitrogen-related) data was not collected from Marine sites.
[b] = Data for analyses derived from continuous-outflow drains only (see Methods) and separated into two sub-groups: "Eastern" and "Western" coastal regions (see Methods). That is, analyses did not include Drains observed to have seasonal outflow.
[c] = Includes location types Ponds, Springs, (as in [a] above) and continuous outflow Drains by Eastern and Western coastal region (as in [b] above)

Table 3.6: One-way ANOVA results for analyses of hydrogen and oxygen stable isotope values. *df* = degrees of freedom; Sal = Salinity; Temp = temperature; log = log transformed data; *P* significant at ά=0.05; ns = statistically non-significant (*P*>0.05); N=number of cases (samples); DR = Drains; E = eastern region coastal drains; W = western region coastal drains; U = undistinguished (i.e. E and W combined); PO = Ponds; SPR = Springs; MAR = Marine.

Factor	***df***	**Variable (*P*=)**		**Group (*N*=)**					**Pairwise Comparisons (Tukey)**
		δ^2H	**δ^{18}O**		**DR**		**PO**	**SPR**	
				E	**W**	**U**			
Location Type [a]	2,239	0.003	0.007	-	-	178	46	18	**<u>δ^2H:</u>** All ns except DR v SPR (*P*=0.005) **<u>δ^{18}O:</u>** DR v PO (*P*=0.045) DR v SPR (*P*=0.039) Pond v SPR (ns)
Drain Region [b]	1,177	<0.001	<0.001	134	44	-	-	-	-
Location Type [c]	3,238	<0.001	<0.001	134	44	-	46	18	**<u>δ^2H:</u>** All ns except WDR & EDR, WDR v PO & WDR v SPR (*P*<0.001) **<u>δ^{18}O:</u>** All ns except WDR & EDR, WDR v PO & WDR v SPR (*P*<0.001)

[a] = Every site sampled across all sampling. Includes all groups (continuous outflow Drains, Ponds, and Springs) sampled between March 2004 and June 2005 inclusive. This data set excludes Lake George Outlet (the only seasonal outflow Drain sampled; see Table 3). Isotope-related data was not collected from Marine sites.
[b] = Data for analyses derived from continuous outflow drains only (see Methods) and separated into two sub-groups: "Eastern" and "Western" coastal regions (see Methods).

[c] = Includes location types Ponds, Springs, (as in [a] above) and continuous outflow Drains by Eastern and Western coastal region (as in [b] above).

Table 3.7: PERMANOVA results for pairwise comparisons between groups for data subsets 1, 2 and 3. Data Subset 1: salinity and temperature [all groups]. Subset 2: all water quality data excluding δ^2H and $\delta^{18}O$ values [excludes MAR]. Subset 3: all water quality data [excludes MAR and SE]. PO = Pond; SPR = spring; CE = continuous-flow eastern drain; SE = seasonal-flow eastern drain; CW = continuous-flow western drain; SW = seasonal-flow western drain; MAR = marine.

PermANOVA Results	**Data Subset**					
	1		**2**		**3**	
Deviation from centroid						
F	67.421		10.702		4.292	
df	6,862		5,167		4,121	
P(perm)	0.0001		0.0001		0.0036	
Pairwise Comparisons	*t*	*P(perm)*	*t*	*P(perm)*	*t*	*P(perm)*
PO,SPR	0.091	NS	4.511	0.0001	3.366	0.0027
PO,CE	4.540	0.0001	0.379	NS	0.865	NS
PO,SE	1.175	NS	2.411	0.0259	-	-
PO,CE	11.381	0.0001	0.719	NS	2.107	NS
PO,SW	14.323	0.0001	5.104	0.0001	2.981	0.0089
PO,MAR	7.091	0.0001	-	-	-	-
SPR,CE	5.597	0.0001	3.369	0.0011	3.041	0.0003
SPR,SE	1.547	NS	1.365	NS	-	-
SPR,CW	14.351	0.0001	4.541	0.0002	3.707	0.0057
SPR,SW	18.276	0.0001	11.100	0.0001	7.420	0.0005
SPR,MAR	8.981	0.0001	-	-	-	-

CE,SE	1.049	NS	1.789	NS	-	-

Table 3.7 (cont)

Pairwise Comparisons	*t*	*P(perm)*	*t*	*P(perm)*	*t*	*P(perm)*
CE,CW	9.762	0.0001	1.109	NS	1.549	NS
CE,SW	12.225	0.0001	4.841	0.0001	-	-
CE,MAR	3.537	0.0003	-	-	-	-
SE,CW	4.360	0.0001	2.297	0.0359	-	-
SE,SW	5.936	0.0001	5.234	0.0027	1.857	NS
SE,MAR	2.419	0.0218	-	-	-	-
CW,SW	3.045	0.0057	3.841	0.0023	0.863	NS
CW,MAR	4.708	0.0001	-	-	-	-
SW,MAR	7.466	0.0001	-	-	-	-

Table 3.8: This is a PCA output for the water quality data set. Strongest loadings (correlations) for each PC are in bold. n = 150

Principal Component	**1**	**2**
Rotated Variance (Initial Eigenvalue)	3.273 (3.310)	1.230 (1.192)
% variance explained	46.8	17.6
Raw Variable loadings (correlations)		
Nitrate	**0.890**	0.097
Total nitrogen	**0.834**	0.176
$\delta^{18}O$	**-0.887**	-0.146
$\delta^{2}H$	**-0.887**	0.054
Nitrite	0.146	**0.756**
Temperature	0.076	**-0.757**
Salinity	-0.551	0.140

FIGURES

Figure 3.1: Mean salinity across all sampling visits between September 2003 and February 2006 (inclusive) by a) Location Type (includes all Drains), b) Drain Region (continuous-flow only), and c) Location Type (incorporating Drain regionality & flow). Error bars are ± s.e. of mean. ppt: parts per thousand.

Figure 3.2: Mean temperature across all sampling visits between September 2003 and February 2006 (inclusive) by Location Type (incorporating Drain regionality & flow). Error bars are ± s.e. of mean. deg.C: degrees Celsius (ºC).

Figure 3.3: Mean total inorganic nitrogen (TIN) across all sampling visits between March 2004 and February 2006 (inclusive) by Location Type (incorporating Drain regionality & flow). Error bars are ± s.e. of mean. Dotted line is ANZECC Guideline = 1 mg N L^{-1}. Western Drains (are black); Eastern drains (blue); Ponds (green); Springs (red).

Figure 3.4: Mean nitrate (NO_3) across all sampling visits between March 2004 and February 2006 (inclusive) by Location Type (incorporating Drain regionality & flow). Error bars are ± s.e. of mean. Dotted line is ANZECC Guideline = 0.1 mg NO_x L^{-1}. Western Drains (black); Eastern drains (blue); Ponds (red); Springs (green).

Figure 3.5: Mean percentage contribution of nitrate (NO_3) to total inorganic nitrogen (TIN) for all location types including Springs, Ponds and continuous flow Drains by coastal region across all sampling visits between March 2004 and February 2006 (inclusive). Error bars are ± s.e. of mean. Western Drains (are black); Eastern drains (blue); Ponds (red); Springs (green).

Figure 3.6: Mean hydrogen (δ^2H) isotope ratios in water samples collected across all sampling visits between March 2004 and June 2005 (inclusive) by Location Type (incorporating Drain regionality & flow). No data was collected for marine sites. Error bars are ± s.e. of mean. Western Drains (are black); Eastern drains (blue); Ponds (green); Springs (red).

Figure 3.7: Mean oxygen (δ^{18}O) isotope ratios in water samples collected across all sampling visits between March 2004 and June 2005 (inclusive) by Location Type (incorporating Drain regionality & flow). No data was collected for marine sites. Error bars are ± s.e. of mean. Eastern Drains (black), Western drains (red), Ponds (green) and Springs (blue).

Figure 3.8: MDS ordination of δ^2H and δ^{18}O isotope ratios in water samples collected across all sampling visits between March 2004 and June 2005 (inclusive) by Location Type (incorporating Drain regionality & flow). No data

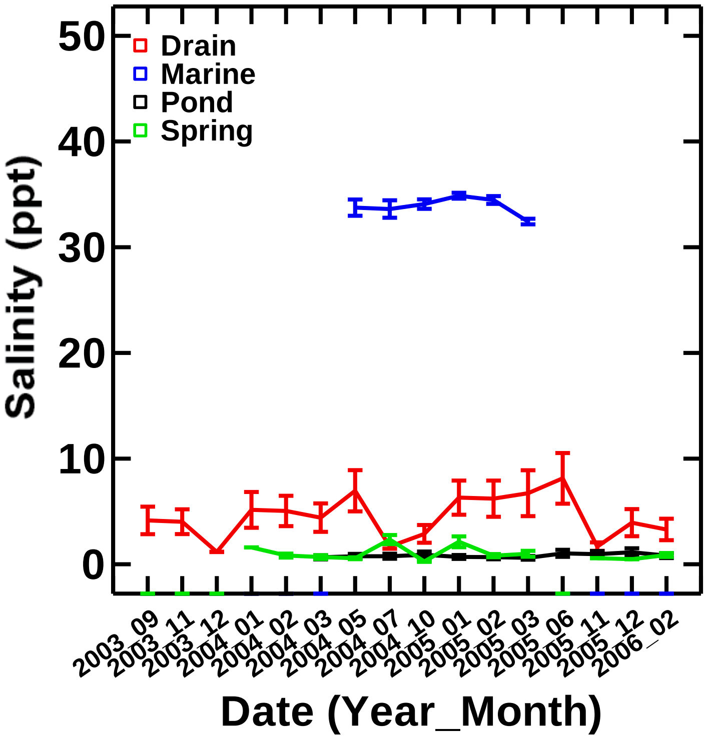
was collected for marine sites. Error bars are ± s.e. of mean. Eastern Drains (are red); Western drains (blue); Ponds (light blue); Springs (yellow)

Figure 3.9: Scatterplot of salinity and temperature for all main waterbody groups. Eastern Drains (are Blue); Western drains (green); Marine sites (yellow); Ponds (red); Springs (black).

Figure 3.10: MDS output of WQ data. Normalise Resemblance D1 Euclidean Distance. 2D Stress = 0.106

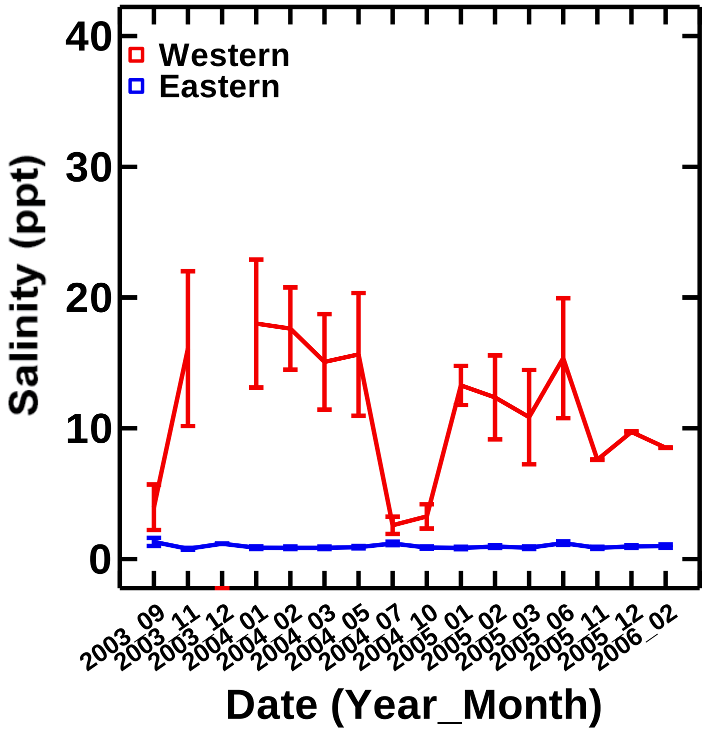
Figure 3.1:

a)



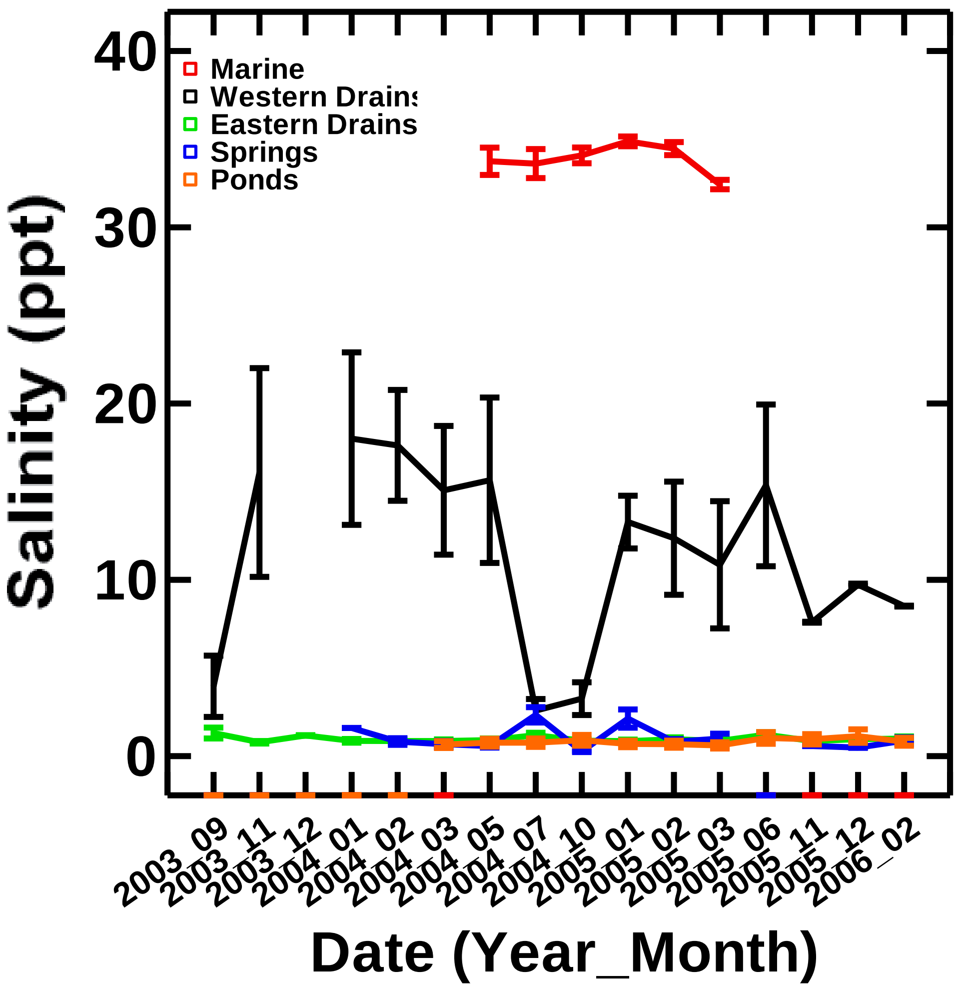
Drain
Marine
Pond
Spring
Salinity (ppt)
50
40
30
20
10
0
2003_09
2003_11
2003_12
2004_01
2004_02
2004_03
2004_05
2004_07
2004_10
2005_01
2005_02
2005_03
2005_06
2005_11
2005_12
2006_02
Date (Year_Month)

b)



Western
Eastern
Salinity (ppt)
40
30
20
10
0
2003_09
2003_11
2003_12
2004_01
2004_02
2004_03
2004_05
2004_07
2004_10
2005_01
2005_02
2005_03
2005_06
2005_11
2005_12
2006_02
Date (Year_Month)

c)



Salinity (ppt)
40
30
20
10
0
Marine
Western Drains
Eastern Drains
Springs
Ponds
2003_09
2003_11
2003_12
2004_01
2004_02
2004_03
2004_05
2004_07
2004_10
2005_01
2005_02
2005_03
2005_06
2005_11
2005_12
2006_02
Date (Year_Month)

Figure 3.2:

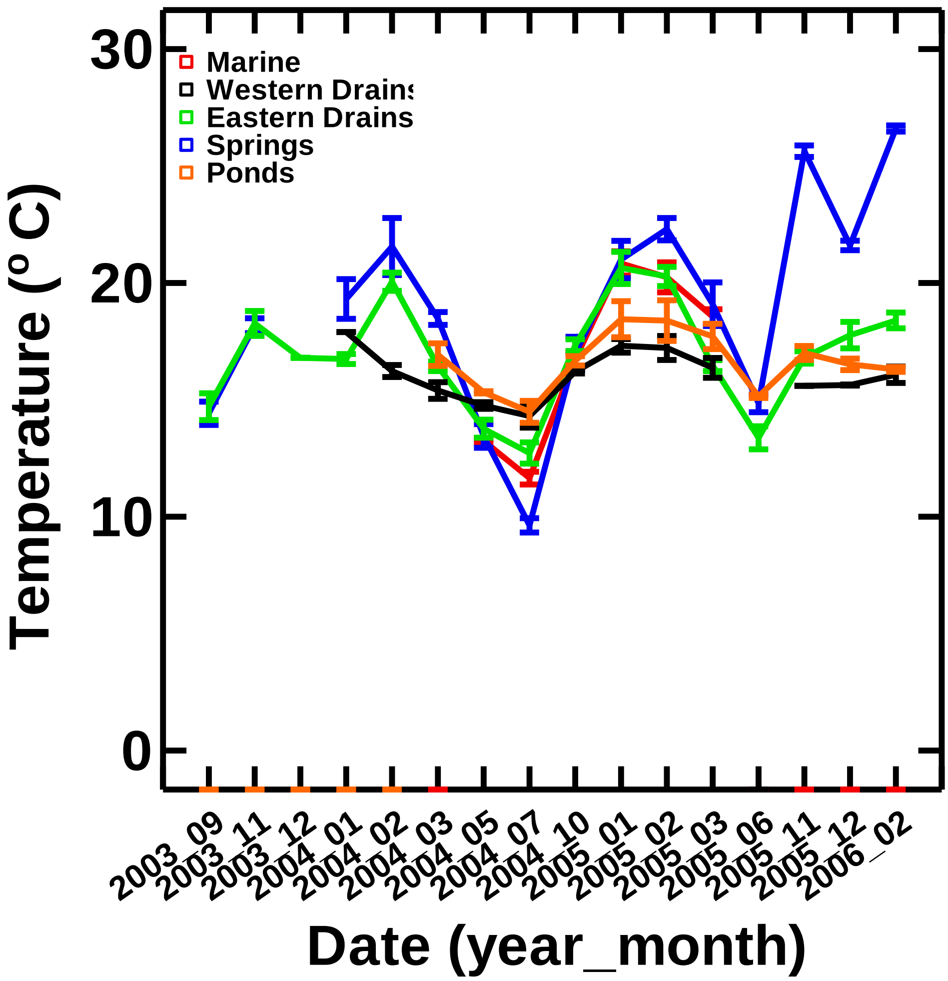

Marine
Western Drains
Eastern Drains
Springs
Ponds
30
20
10
0
Temperature (° C)
2003_09
2003_11
2003_12
2004_01
2004_02
2004_03
2004_05
2004_07
2004_10
2005_01
2005_02
2005_03
2005_06
2005_11
2005_12
2006_02
Date (year_month)

Figure 3.3:

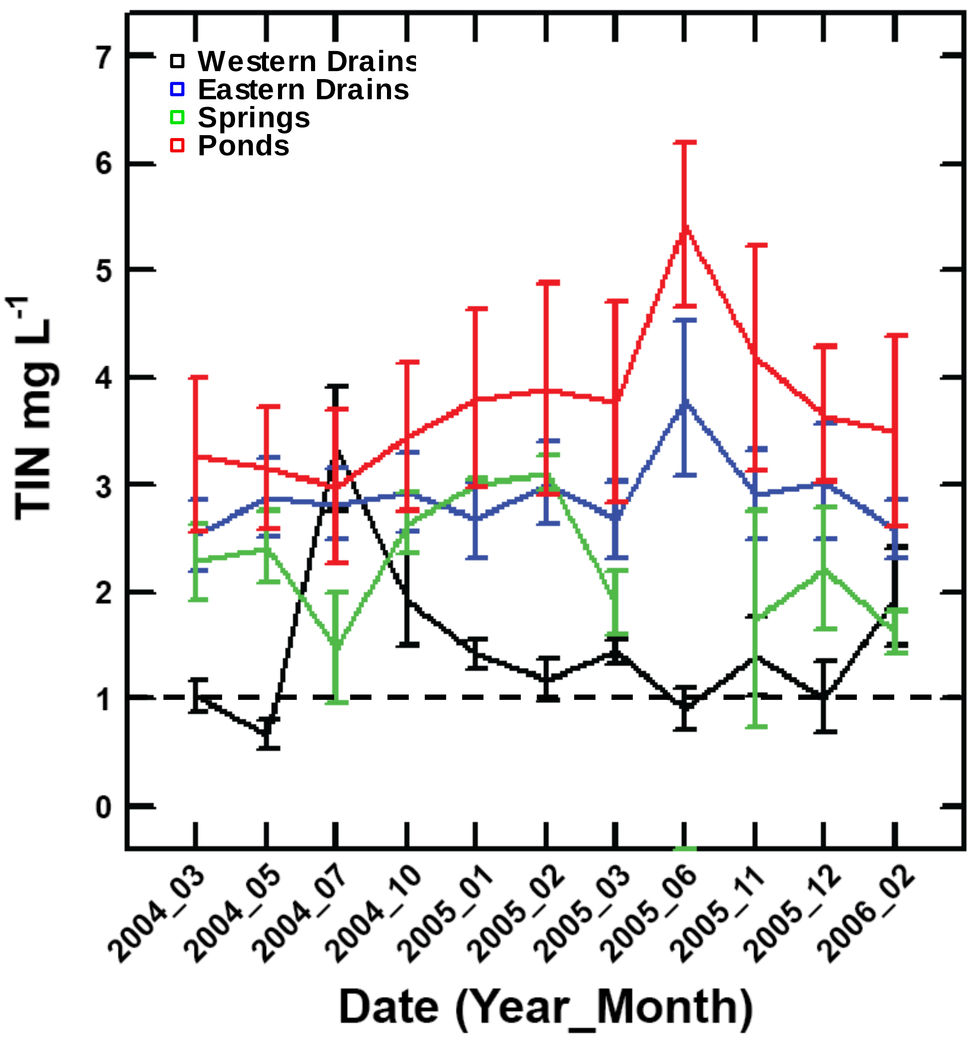

Western Drains
Eastern Drains
Springs
Ponds
TIN mg L-1
0
1
2
3
4
5
6
7
2004_03
2004_05
2004_07
2004_10
2005_01
2005_02
2005_03
2005_06
2005_11
2005_12
2006_02
Date (Year_Month)

Figure 3.4:

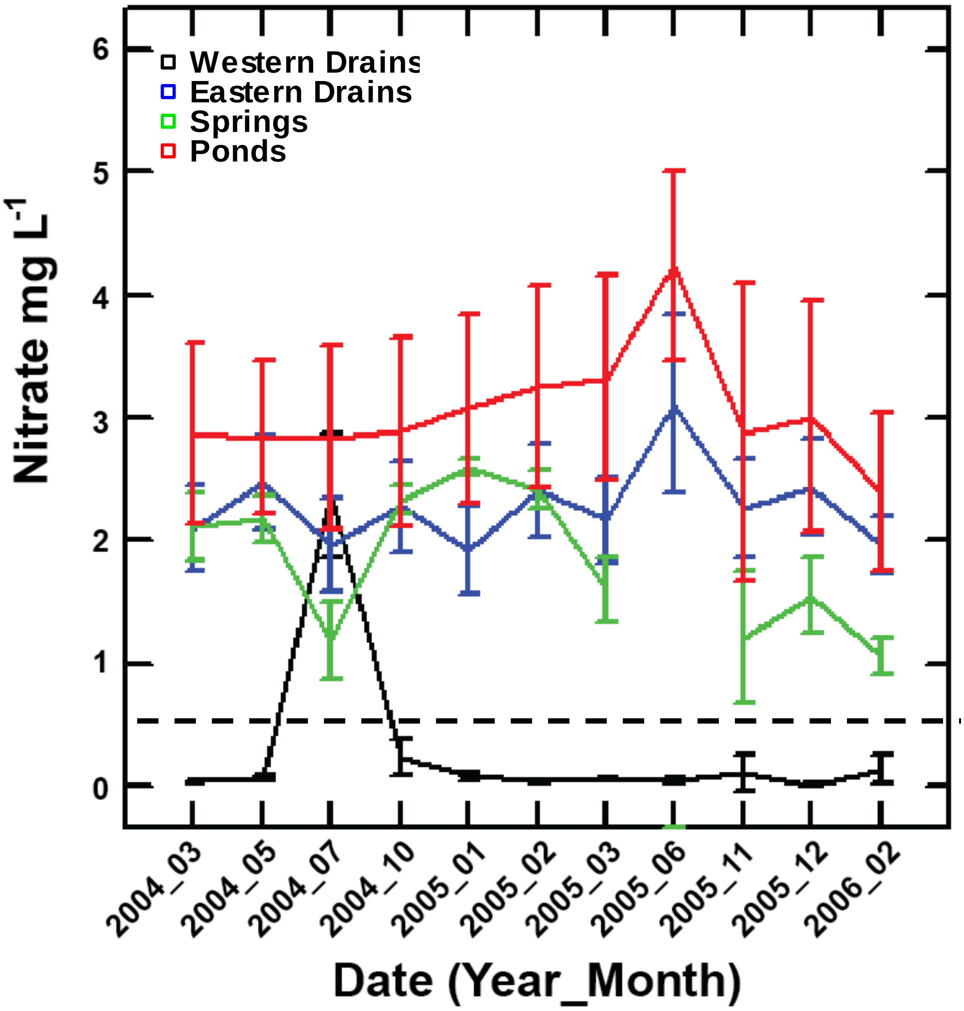

Western Drains
Eastern Drains
Springs
Ponds
Nitrate mg L-1
0
1
2
3
4
5
6
2004_03
2004_05
2004_07
2004_10
2005_01
2005_02
2005_03
2005_06
2005_11
2005_12
2006_02
Date (Year_Month)

Figure 3.5:

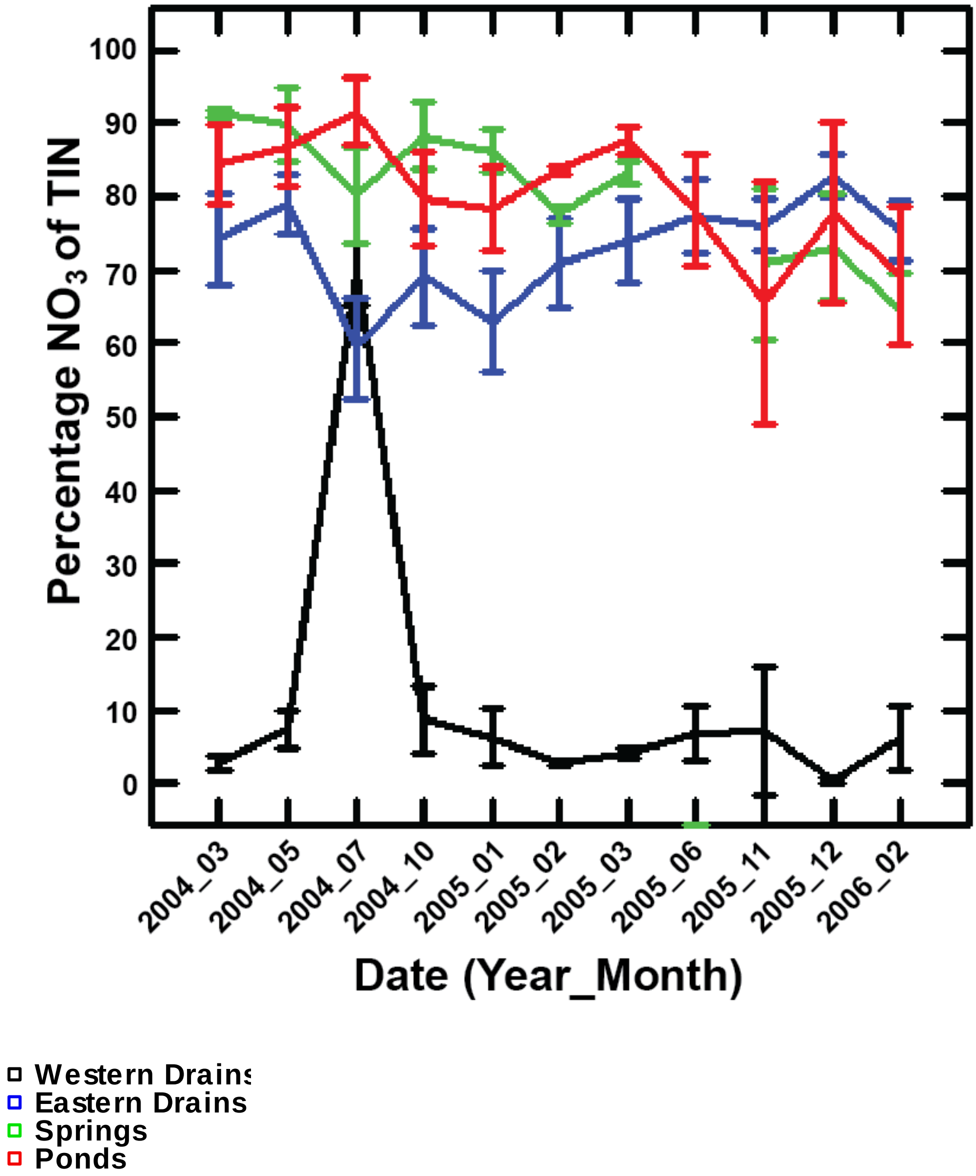
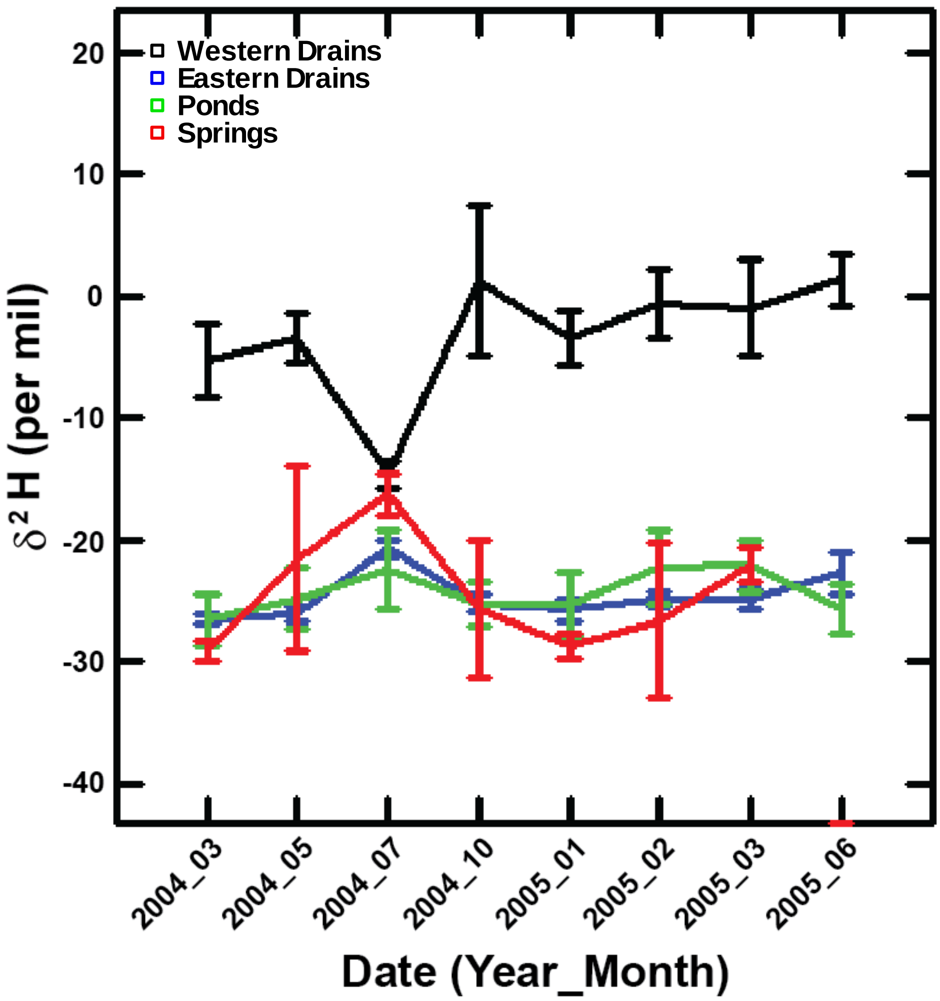
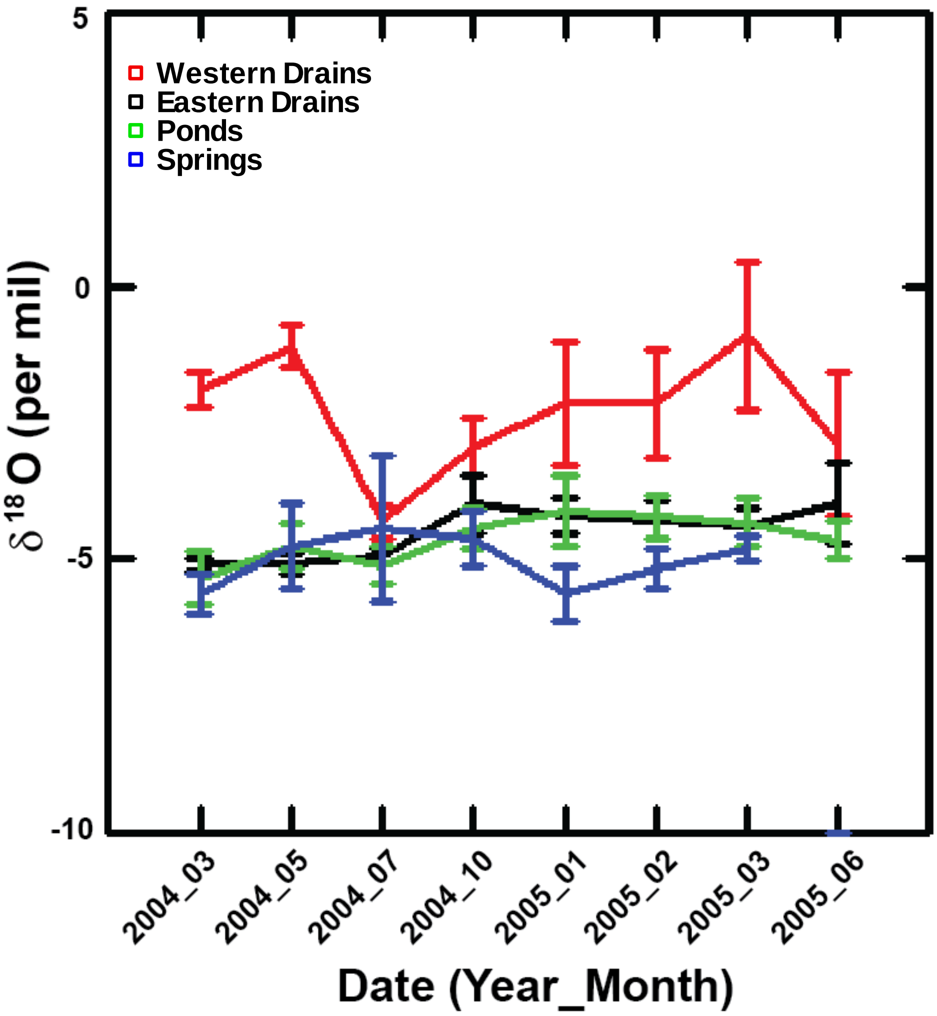

100
90
80
70
60
50
40
30
20
10
0
Percentage NO_3 of TIN
2004_03
2004_05
2004_07
2004_10
2005_01
2005_02
2005_03
2005_06
2005_11
2005_12
2006_02
Date (Year_Month)
Western Drains
Eastern Drains
Springs
Ponds

Figure 3.6:



Western Drains
Eastern Drains
Ponds
Springs
20
10
0
-10
-20
-30
-40
δ²H (per mil)
2004_03
2004_05
2004_07
2004_10
2005_01
2005_02
2005_03
2005_06
Date (Year_Month)

Figure 3.7:



Western Drains
Eastern Drains
Ponds
Springs
5
0
-5
-10
δ18O (per mil)
2004_03
2004_05
2004_07
2004_10
2005_01
2005_02
2005_03
2005_06
Date (Year_Month)

Figure 3.8:

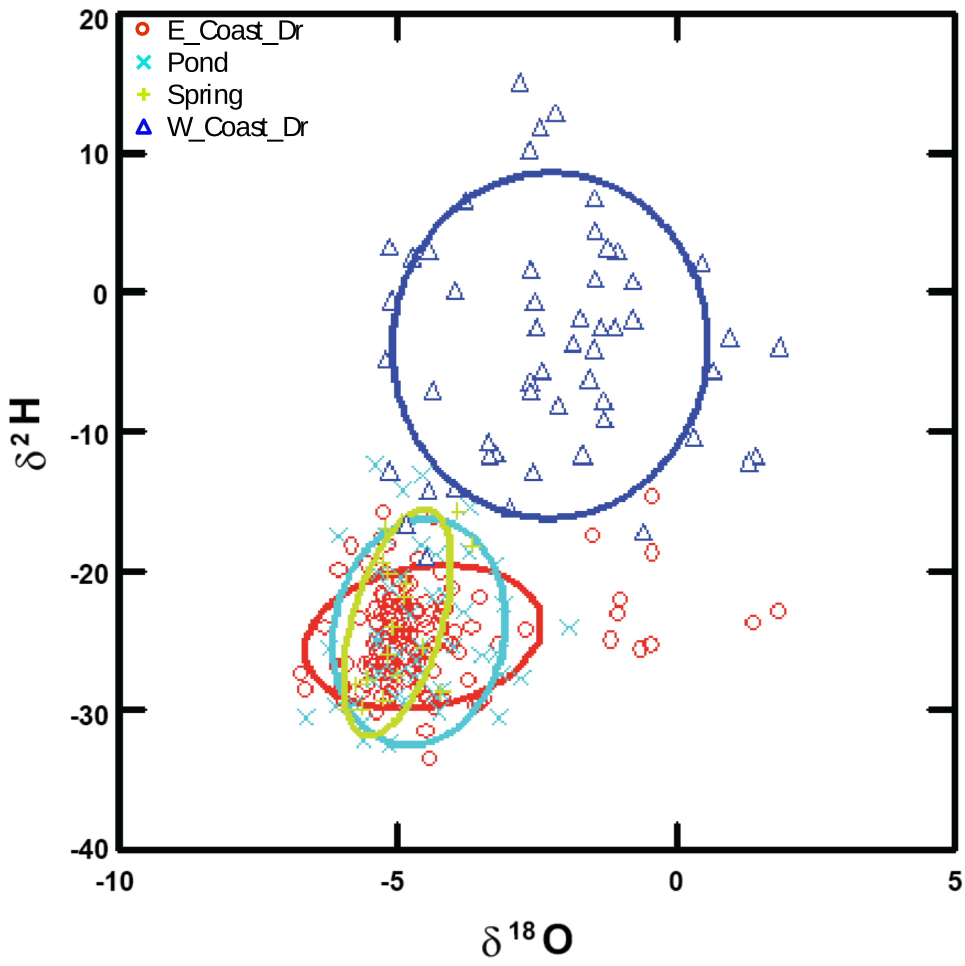

E_Coast_Dr
Pond
Spring
W_Coast_Dr
20
10
0
-10
-20
-30
-40
-10
-5
0
5
δ^2H
δ^{18}O

Figure 3.9:

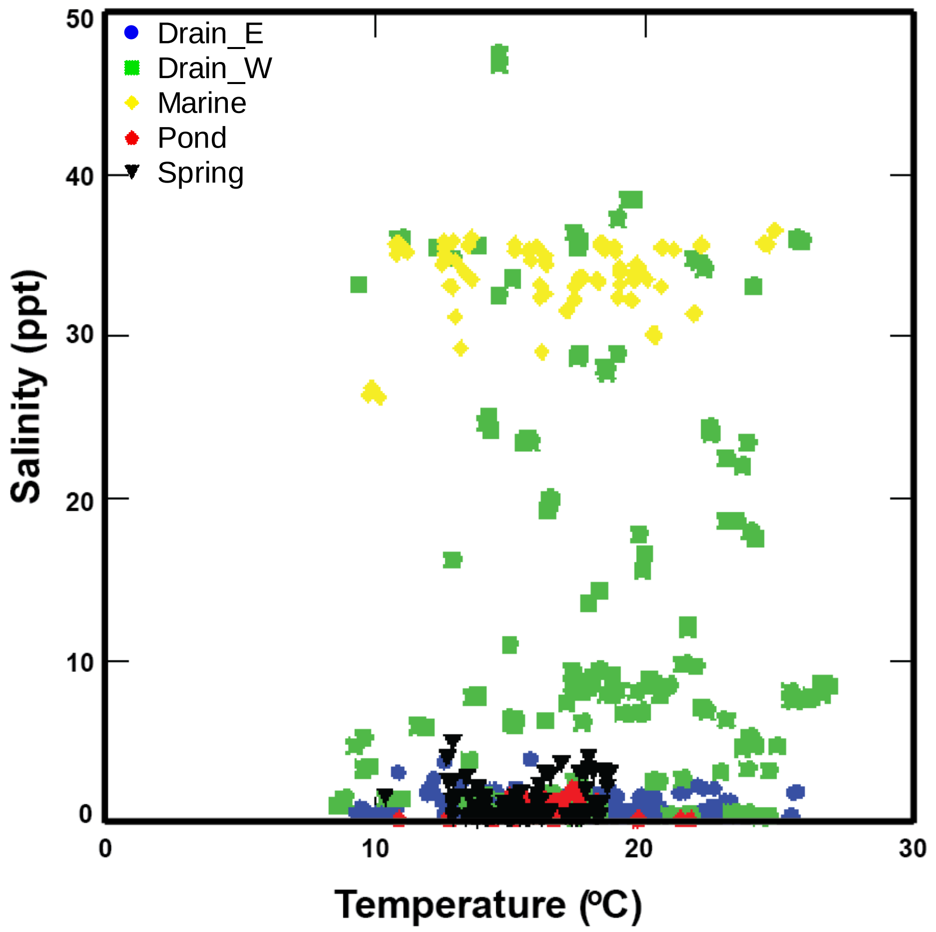
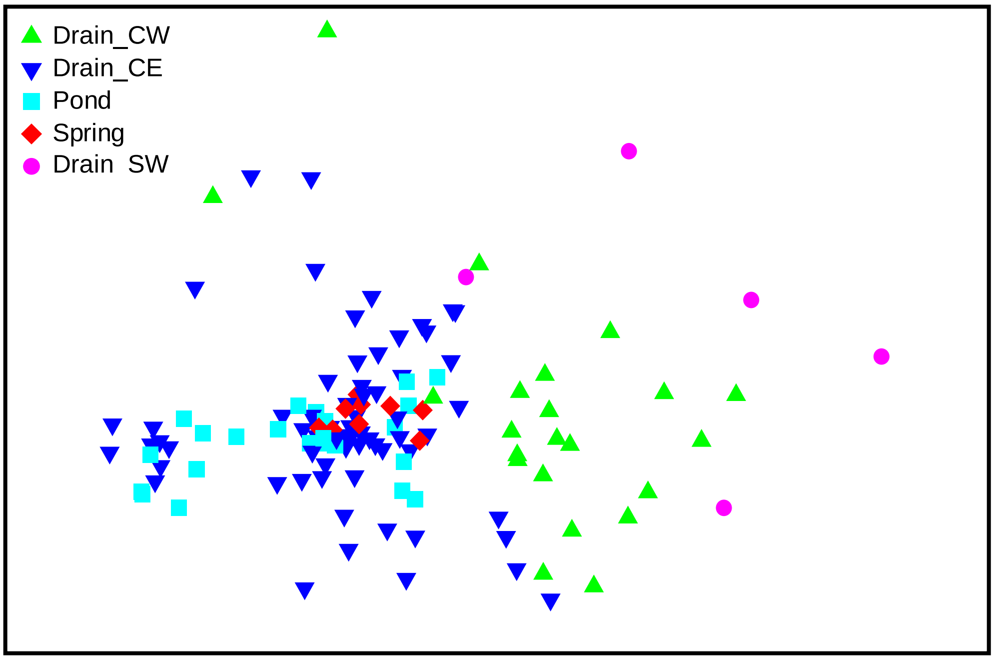

Drain_E
Drain_W
Marine
Pond
Spring
Salinity (ppt)
Temperature (ºC)
0
10
20
30
40
50
0
10
20
30

Figure 3.10:



CHAPTER 4 – MACROFAUNA ASSEMBLAGES

4 The association of groundwater discharge and macrofaunal assemblages in coastal waterways?

4.1 INTRODUCTION

Small coastal creeks in nearby south-west Victoria are naturally intermittent (Barton & Sherwood 2004; Becker *et al.* 2009; McKenzie *et al.* 2011). Typically, these creeks tend to run during winter and spring due to local rainfall within their catchments but can then potentially close in summer and autumn as rainfall and hence runoff is reduced. Also summertime south-easterly winds move sand to the west along the coastline with this longshore drift tending to infill estuary mouths. Thus these systems may also be classified as 'intermittently closed and open lakes and lagoons' (ICOLLs; Dye & Barros 2005; Dye 2006a,b).

This chapter attempts to address the consequences arising from observations made in Chapter 3 for the eastern part of South Australia of continuous-flowing drains being more groundwater-influenced than seasonally-flowing drains. It will also attempt to address the regional difference observed within continuous-flowing drains (i.e. the Eastern vs. Western comparison). That is, flow regime in these coastal drains is an important aspect but so too is salinity (or other aspects of water quality), particularly under the possibility of alterations to flows in the future (e.g. climate change-related impacts combined with water management actions, especially increased groundwater extraction that may result in reduced groundwater tables, reduced rainfall and surface water runoff).

In this chapter I ask "does the year-round influence of discharging groundwater affect the environmental character of these drains and thus alter the assemblage of resident macrofauna?" For example, in western Victoria we expect to get no flows in summer and so closure of smaller estuary mouths usually occurs. These estuaries are populated by a sparse but typically estuarine benthic fauna dominated by polychaete annelids, bivalve and gastropod molluscs, and a variety of crustaceans (*P*arreira 2000; Matthews 2000; Arundel 2003; Matthews & Fairweather 2003, 2004, 2006, 2008; Barton & Sherwood 2004; Becker 2007; Becker & Laurenson 2007, 2008; Barton *et al.* 2008; Nicholson *et al.* 2008; Becker *et al.* 2009). If water sources and/or continuity of flows are important in the groundwater-fed, continuous-flow systems in south-east South Australia, then we may expect to find more dominance of freshwater species but perhaps with some estuarine representatives (depending on any mixing of water sources).

4.1.1 Regional expectations

In Chapter 3, hydrological and water-quality commonalities within regional drains and some differences between regional drains were identified. For example, Eastern drains were consistently fresh (≈1-2ppt) when compared with Western drains. Western drains experienced a larger range of salinity (ranging from approximately 1 to 35ppt) and displayed regimes of salinities more similar to typically estuarine conditions.

Sampling of seasonal-flowing drains (sampled only in 2003 and 2005) displayed differences within salinity patterns as well (e.g. seasonal-flow drains had generally higher salinities than continuous-flow drains; see Chapter 3).

Macroinvertebrate assemblages in estuaries are known to vary along salinity gradients in southern Australia (Hirst 2004 [Victoria]; Edgar *et al.* 1999; Hirst & Kilpatrick 2007 [Tasmania]; Dye 2006b [NSW]; Potter & Hyndes 1999 [WA]). Any effects that salinity variation may have on the flora and fauna that reside in the drains of south-east South Australia are important to consider in order to understand what species may live in them. For example, in drains where low salinity is consistent throughout the year (i.e. Eastern drains), we may expect to find more species with freshwater affinities (e.g. insects). In those systems that display greater annual variability in salinity (i.e. Western drains), we may expect to find more estuarine species, at least at some times.

Water regime (i.e. seasonal runoff versus continuous groundwater flow) is likely to be a key factor influencing species composition in these drains but the physical characteristics of the water (e.g. salinity) is likely to be another primary influence on macrofaunal assemblages. Of particular interest is the influence of a consistent low salinity (i.e. <2ppt) profile from source to sea (e.g. Ewens Ponds via Eight Mile Creek, or Piccaninnie Ponds via Piccaninnie Ponds Outlet; see Chapter 3) in continuous-flow habitats. This type of salinity regime eliminates any estuarine transitional area. That is, the salinity abruptly changes from freshwater to marine at the point of outflow and thus is likely to limit the groups of animals that reside in or can utilise this type of habitat. In seasonal-flow environments, the duration of flow (e.g. days or months) is also likely to affect species composition (see also Chapter 7) but in a different way (i.e. determining the presence or absence of aquatic habitat available for colonisation).

Typically, freshwater habitats are dominated by representatives from a number of higher animal groups. In western Victoria, these macrofaunal representatives typically include insects (e.g. from the Orders Ephemeroptera, Diptera, Trichoptera, Hemiptera, Plecoptera), crustaceans (Decapoda, Amphipoda), freshwater fish, amphibians (including tadpoles), oligochaetes, leeches, and mites (Kefford *et al.* 2003, 2005; Robson & Clay 2005; Robson *et al.* 2005; Lester *et al.* 2007; Lind *et al.* 2007; Lind *et al.* 2009). Some smaller freshwater invertebrates are also likely to be found and these include micro-crustaceans like ostracods, copepods, and cladocerans (Robson & Clay 2005; Kefford *et al.* 2007) (see Table 4.1).

Fauna in marine habitats are composed of nematodes (rather ubiquitous), crustaceans, molluscs and polychaetes but the representatives present from these groups will be different from those freshwater examples given above (Underwood & Chapman 1995; Kingsford & Battershill 1998; Edgar 2001;

Connell & Gillanders 2007). Specialist marine groups also include echinoderms, sipunculids and nemerteans.

Under estuarine conditions we may expect to find crustaceans, molluscs and polychaetes but (some) insects and oligochaetes may appear in upper (i.e. lower salinity) reaches. The salinity regime in estuaries influences species composition via rapid and regular changes versus incremental change and a wider range of maximum-minimum values, as well as physical connectivity to both freshwater and marine inputs. Estuaries of Tasmania and Victoria are typically dominated numerically by infaunal species such as polychaetes and bivalves and epifauna such as gastropods, amphipods, shrimps and crabs (Edgar *et al.* 1999; Edgar & Barrett 2000; Hirst 2004; Hirst *et al.* 2006; Spruzen *et al.* 2008). Chironomid (midge) larvae are also common in some temperate estuaries (Edgar & Barrett 2000; Hastie & Smith 2006; Hirst *et al.* 2006).

The number of fish species in estuaries is generally less than in either freshwater or marine habitats and these estuarine species themselves may be specialised to lowered (i.e. brackish) salinities and other estuarine-specific conditions such as shelter from waves and fast water flows (Potter & Hyndes 1999; Becker 2007; Becker & Laurenson 2007, 2008; Wedderburn *et al.* 2007, 2008; Nicholson *et al.* 2008; Becker *et al.* 2009).

The aim of this chapter is to complement the physico-chemical information presented in Chapter 3 with biological data that will assist to further characterise these drains. In particular, I attempt to determine whether , if the continuous-flowing coastal drains of south-east South Australia are groundwater-fed, I can infer what effect this has on the compositions of resident faunal assemblages. The chapter will also discuss the implication for fauna under altered water regimes possibly in the future (e.g. reduced groundwater availability).

Specifically this chapter asks whether faunal communities in coastal creeks/drains that receive continuous groundwater differ from those that do not: does continuous discharge of used groundwater into coastal creeks as irrigation drainage change a faunal community from estuarine to freshwater in character?

4.2 Methods

A subset of continuous-flow drain sites were sampled in late spring (i.e. October/November) during 2003, 2004 and 2005 (Table 4.2). The three seasonal-flow drains were sampled additionally in 2005 only (Table 4.2). The sites were initially chosen for use in subsequent experiments (see Chapter 7) but are used for comparative purposes in this Chapter. Only eight or nine drains were sampled in any year (Table 4.2).

4.2.1 Sweep Netting

Instream and epibenthic fauna were collected using a triangular dip net (aperture area = 0.04m^2; mesh size = 1mm) swept for 30 seconds for each replicate (e.g. following Napier and Fairweather 1999). The method involved bumping the net off the bottom of the main waterbody of the drain whilst traversing across the drain (*P*erpendicular to the flow). The net was moved in an up-and-down motion through the water column to capture any fauna present. All material retained in the net was placed in a specimen jar and labelled. Specimens were first preserved in a 10% formalin and collection water solution. Samples were later stored in 70% ethanol in freshwater solution before being identified and counted.

4.2.2 Environmental Data

The environmental data for this chapter was collected as described in Chapter 3. The environmental data presented here are the same data as used in Chapter 3. Any exceptions and/or amendments to these statements have been highlighted and described.

4.2.3 Hypotheses tested

I compared univariate measures of total species abundance and species richness between type of flow (i.e. continuous versus seasonal), regions (Eastern versus Western), individual drains and years. Similarly I analysed the multispecies assemblages (in terms of taxonomic compositions and relative abundances across all species) for the same patterns of potential differences.

4.2.4 Statistics

Data were analysed using univariate (e.g. one- or two-factor Analysis of Variance [ANOVA]) and multivariate (e.g. Multi Dimensional Scaling [MDS] ordination, PERMANOVA) statistics using SYSTAT v11 and PRIMER v6 with the PERMANOVA+ add-on software packages, respectively. Assumptions of

ANOVA were checked by inspecting graphs of residuals obtained when the ANOVA model was fitted (Quinn & Keough 2002). Some data configurations have been transformed to meet the assumptions of ANOVA; the results for transformed data are shown below with the type of transformation stated accordingly. Additional tests on data were also performed using the PRIMER routines BIOENV (for correlations with environmental variables), RELATE (to see if biotic and environmental MDS were related) and SIMPER (to derive sets of indicator species) applications. The results for these analyses are discussed where appropriate.

Due to some imbalance in the data sets collected, it has been necessary to analyse different configurations of the data in order to present a comparison and/or argument. Sampling undertaken during 2003 was for exploratory purposes only (hence different numbers of samples were collected). Only samples from continuous-flow drains were sampled in 2003 and 2004. The data collected in 2004 was used primarily to compare between sampling years. One sample collected from ACI Road in 2004 was inadvertently lost. The addition of seasonal-flow drains in 2005 was primarily for the purposes of the manipulative experiments described in Chapter7. Whilst visiting these seasonal-flow sites, it was considered useful to also conduct sweep netting at these sites for later comparison against continuous-flow sites in order to assess their species compositions. This lack of continuity and consistency between sampling years resulted in an unbalanced sampling design. This is reflected in the statistical designs used to analyse the data collected. As a result of this imbalance, a number of subsets (*n* = 7) were assembled to conduct analyses on macrofaunal abundance and richness data (i.e. separate analyses for each subset). These subsets (see Table 4.3) include analysis of:

1. Site, and Region analyses for 2003
2. Site, and Region analyses for 2004
3. Site, and Region analyses 2005
4. Region-Flow (1-factor) analyses across all three years
5. Region*Flow (2-factor) for all 2005 sites only
6. Region*Flow (2-factor) across all three years (sites compared = 5)
7. Region*Flow (2-factor) across 2003 and 2004 only (sites compared = 8).

Analyses were conducted on the morphospecies data set but some analyses at a taxonomic Order level were conducted to further explain patterns and variation when morphospecies results returned non-significant outcomes.

4.3 RESULTS

4.3.1 Taxa Found

There were nine continuous-flow drains and three seasonal-flow drains sampled (Table 4.2). Sampling returned more than 3500 individuals from a total of 66 sweep nets; a total of 141 morphospecies were identified from 61 families and five phyla (Table 4.4).

Over two thirds (67.8%) of all individuals were collected from only one third (*N* = 4) of the sites sampled. The seven most-abundant taxa summed to 90.2%

of all individuals sampled. Insects were the most abundant major group (68.9% of all individuals) followed by crustaceans (14%), molluscs (11.9%), annelids (4.1%), chordates (0.5%) and others (0.6%) (Table 4.4).

Insects were the dominant group returning individuals from at least eight Orders (especially Diptera, Hemiptera, Coleoptera, Trichoptera) with dipterans (true flies) being the most abundant group. Crustaceans were represented by individuals from five higher groups but were dominated by amphipods and ostracods. Gastropod molluscs and oligochaete annelids were also commonly sampled.

Sixty-seven morphospecies returned only one individual (for 2% of all individuals combined). Nineteen morphospecies returned only two individuals (for 1% of all individuals). Another nineteen morphospecies contained between 3 and 5 individuals (inclusive), representing 2% (or n = 69) of all individuals. Twenty-nine morphospecies had between 6 and 100 individuals, representing 26.6% (or n = 944) of all individuals. Just six morphospecies comprised 2436 individuals (68.5% of all individuals collected). These six most-abundant species included: the dipterans Chironomidae sp.1 (n = 1191 individuals in total) and Simuliidae sp.1 (n = 347); Amphipoda sp.1 (n = 229); Gastropoda unknown sp.1 (n = 228) and the gastropod Pomatiopsidae sp. 1 (n = 185); and the trichopteran Leptoceridae sp.1 (n = 115).

4.3.2 Total Abundance

Individuals per sweep ranged from 1 (Brown Bay Drain 2005) to 455 (Mount Benson Drain 2005) with a grand mean (± s.e.) of 53.8 ± 9.2 individuals.sweep^{-1}. Macrofaunal total frequency (mean ± s.e.) per site and year ranged between 302.0 ± 81.1 individuals.sweep^{-1} (Mount Benson Drain 2005; n = 3) and 9.0 ± 3.5 individuals.sweep^{-1} (Spehrs Road Drain 2005; n = 3) for Seasonal-flowing drains, and between 93.7 ± 23.3 individuals.sweep^{-1} (Brown Bay Drain 2003; n = 3) and 8.0 ± 4.0 individuals.sweep^{-1} (ACI Road Drain 2003; n = 2) or 8.0 ± 2.0 individuals.sweep^{-1} (Brown Bay Drain 2004; n = 2) for Continuous-flowing drains (Table 4.5).

Drain sub-grouping summaries (Table 4.5) found that macrofaunal frequencies were generally larger in 2005 than 2004 but both were bigger than 2003. Seasonal-flow drains were approximately 4 times greater in macrofaunal frequency than Continuous-flow drains in 2004 and 2005 but approximately only half as numerous in 2003. Eastern versus Western region drains varied over sampling years with the groups approximately similar in 2003, Western drains more numerous in 2004 but Eastern drain were more numerous in 2005 (Table 4.6).

4.3.3 Morphospecies Richness

Species richness per sweep ranged from 1 (Lake Frome Drain 2003; Deep Creek 2003; Eight Mile Creek 2004; Brown Bay Drain 2005) to 24 (Mt Benson Drain 2005) with a grand mean (± s.e.) of 7.5 ± 0.6 species.sweep^{-1}.

Macrofaunal species richness (mean ± s.e.) per site and year ranged between 23.0 ± 0.6 species.sweep^{-1} (Mt. Benson Drain 2005; *n* = 3) and 3.0 ± 1.0 species.sweep^{-1} (Spehrs Road Drain 2005; *n* = 3) for Seasonal-flowing drains, and between 14.7 ± 1.5 species.sweep^{-1} (Blackford Drain 2003; *n* = 3) and 2.5 ± 0.5 species.sweep^{-1} (*P*iccaninnie Ponds Outlet 2003; *n* = 2) for Continuous-flowing drains (Table 4.5).

Drain sub-grouping summaries (Table 4.5) found that morphospecies richness was generally larger in 2005 than 2003 or 2004. Species richness was highest in Continuous-Western, Seasonal-Western (Flow*Region groups), Western (Region) and Seasonal (Flow) in 2005 (Table 4.6).

4.3.4 Statistical Analyses

4.3.4.1 <u>Univariate analyses of assemblages</u>

Data subsets 1,2 & 3: One-way ANOVA results suggested that a regional difference in morphospecies abundance was apparent in 2003 (P = 0.038; with Western Drains having a larger mean than Eastern drains) as were Site differences in 2005 (P <0.001) Tukey pairwise comparisons found statistically-significant (P <0.05) differences between the site Mt Benson versus all other sites except Green Point (NS), and also between Green Point versus Deep or Eight-Mile Creeks . All other pairwise comparisons by Site (in 2005) were not statistically significant (Table 4.7).

Statistically-significant (P <0.05) results for Region were observed for all analyses of species richness across 2003, 2004, and 2005 with Western drains typically having more species than Eastern Drains in these regional tests. Pairwise comparison of sites found statistically significant differences (P <0.05) between Blackford Drain versus Deep Creek in 2003, between Blackford Drain and Eight-Mile Creek or Piccaninnie Ponds Outlet in 2004, and between Mt Benson and all other sites in 2005, as well as between Blackford Drain and all other sites except Cress and Deep Creeks (Table 4.7).

Data subset #4: A statistically significant difference (P = 0.017) amongst Flow-Region groups for morphospecies abundance was observed. Only the pairwise comparison of Seasonal-Western versus Continuous-Eastern drains was significant (P <0.05) with Seasonal-Western having more individuals than Continuous-Eastern drains. The species richness test was all non-significant (Table 4.7).

Data subset #5: Two-way ANOVA results for the interaction of Flow and Region for 2005 only were not statistically significant for analyses of both total abundance and species richness of morphospecies. At the single factor level, Seasonal versus Continuous-flow drains were significantly different (P = 0.010) for total abundance, with seasonal drains having more individuals. Western region drains were statistically different from Eastern drain (P = 0.014) for species richness (Table 4.7).

Data subset #6: Two-factor analyses for the interaction of Year and Region was not statistically significant (NS) for total morphospecies abundance but Region alone was (P = 0.015), and also for species richness (P <0.001). The interaction of Year and Region for species richness was also non-significant but the main effect of Year alone was statistically significant (P <0.005). Tukey pairwise comparisons found a significant difference (P <0.05) between 2003 and 2004, and also for 2003 versus 2005 (Table 4.7).

Data subset #7: Year and Region (two-factor analyses)for data collected in 2003 and 2004 was not statistically significant (NS) for either total morphospecies abundance or species richness for main effects or the interaction, with the exception of Year for species richness (P = 0.046) (Table 4.7).

These results suggest that seasonal-flow drains had larger overall morphospecies abundance and total morphospecies richness than continuous- flow drains.

4.3.5 Multivariate analyses

The morphospecies MDS ordination showed a slight separation of groups with Continuous/East versus Seasonal/East drains displaying the larger spatial separation of data points. A high 2-D stress value of 0.22 means that we have to be cautious in interpreting this 2-D representation but spatial separation within the SW group was apparent (i.e. the lower centre cluster subset was the Mt Benson site) (Figure 4.2). Another ordination display, using raw data aggregated at the Order level, showed that there was some overlap of groups but that seasonal-flow drains fringed those that were continuous-flow in nature (figure not shown).

PERMANOVA analyses of the data set (4th-root transformed morphospecies data) showed a significant interaction of flow and region (Pseudo-F = 13.701, df = 1,62, P(Perm) = 0.0002). Pairwise comparisons of means were all significant (P(Perm) <0.015), except for the pair of Seasonal/Western and Continuous/Eastern (NS). Thus the groupings of means was SW=CE>CW>SE.

The interaction of Year and Flow was statistically significant (Pseudo-F = 3.515, df = 1,62, P(Perm) = 0.0065). Pairwise comparisons revealed statistically-significant differences between 2003 and 2004 year groups: Year groups 2005 versus 2003 t = 1.4868, P(Perm) = 0.0045; and 2005 versus 2004 t = 1.5242, P(Perm) = 0.0090.

Similarity percentages (SIMPER) analyses (Table 4.8) showed that average group dissimilarity was 79.7% between Western and Eastern drain groups with Western drains generally have a larger average abundance than Eastern drains. The average group dissimilarity between Continuous-flow and Seasonal-flow drains was of the same order at 81.0%, RELATE analyses were undertaken on water quality subsets using morphospecies and then Order-level data. Within-group comparison found that average group salinity ranged between 24.2% for Seasonal-flow drains, to 33.0% for Western Drains Chironomidae_1 was the morphospecies that contributed most to within group similarity (Table 4.8a). All results were statistically non-significant (P >0.05) suggesting that these data sets were unrelated. That is, there was no statistical association between environmental data and macrofauna relative abundances and taxonomic compositions.

Additional BIOENV analyses of the data set showed that environmental data explained only a small proportion of the variance in the biotic data with salinity having more influence, albeit very weak (correlation coefficient: *rho* = 0.1108), on abundances/compositions than other environmental (i.e. water quality) variables. BIOENV analyses at the Order level suggested that nitrite was most influential on abundance/composition, albeit also a weak association (correlation coefficient: *rho* = 0.128).

4.4 DISCUSSION

Generally, the 2004 sampling event was less speciose than 2003 and 2005 (see Table 4.6) but 2005 sampling event generally had a greater frequency of macrofauna individuals than the 2004 and 2003 sampling events.

The univariate statistical analyses suggest that, at a regional level, Western Drains had a greater frequency of macrofaunal individuals (and dominated by the Mt Benson Drain in 2005) than Eastern Drains. Seasonal-flow drains also showed generally a greater macrofaunal frequency than Continuous-flow drains.

Western drains had a greater morphospecies richness than Eastern drains. Species richness within Western drains was influenced most by large values from Blackford Drain in 2003 and 2004 and Mt Benson Drain in 2005. An interesting point to note here is that Blackford Drain is a continuous-flow site whereas Mt Benson is a seasonal-flow drain, and that Blackford Drain is more brackish in its salinity (but still groundwater-fed) whereas Mt Benson is fresh and not groundwater-fed. Generally, Seasonal-flow drains had larger morphospecies abundances and richnesses than Continuous-flow drains.

Statistical tests (BIOENV, RELATE) suggest that none of the water quality variables exerted a strong influence over macrofauna abundance/composition but salinity had a weak influence on patterns identified at the morphospecies level whereas nitrite had a weak influence at the Order level.

Salinity regimes in the majority of the drains have reduced the possibility of any true estuaries from occurring (see Chapter 3) and, as such, I did not find an abundance of dominant estuarine fauna such as polychaete worms, crustaceans, molluscs and fish. Nor were any true marine representative groups collected.

Because the results in Chapter 3 suggested that most of the drains sampled in this study were fresh and groundwater–fed, it is plausible to expect to find some of the freshwater faunal groups identified in Table 4.1 (and see 'Introduction'). This study did find small numbers of nematodes, leeches, polychaetes, mites and moderate numbers of oligochaetes, crustaceans and molluscs. Insect were by far the most dominant group numerically and in terms of diversity. Approximately 69% of total individuals and 73% of morphospecies sampled were from the insect class. The freshwater habitats that groundwater-fed systems provide are likely to be critical for insects to complete their life histories with many juvenile insects representative observed in the study such as larval dipterans (e.g. chironomids) as well as salinity-sensitive (Kefford *et al.* 2003) species such as trichopterans and ephemeropterans.

When considering each drain region, insects made up 82.2% of individuals collected in Eastern drains whereas only 56.4% of individuals were insects in the Western drain region (Table 4.9).

The percent of insect specimens collected across each sub-grouping ranged from 48.2% of total individuals in seasonal-flow Eastern drains to 75.9% of total individuals in continuous-flow Eastern drains (Table 4.9). Insect morphospecies observed across the sub-groups ranged 63.6% of total morphospecies in seasonal-flow eastern drains to 77.6% of total morphospecies in continuous-flow western drains. Generally, drains associated with a continuous-flow regime had larger percentage values of total insect individuals or total insect morphospecies than for season-flow drains (Table 4.9).

This generalised summary of insect associations with regional waterbodies suggests that this taxonomic group may be useful as a bio-indicator of such groundwater-fed (fresh) inputs to coastal waterbodies. The results here found that 75% of individuals sampled in continuous-flow drains belonged to Class Insecta, whereas Seasonal-flow drains had only a 59% contribution from insects to total individual abundance.

Another noteworthy summary was that from the total of 103 insect morphospecies sampled, 83% of these were observed in Continuous-flow drains. In comparison, only 41% of the total insect morphospecies were captured in Seasonal-flow drains. Given these results, I suggest that additional consideration be given to the possibility that insects could be used as a bioindicator of continuous freshwater influence. Preliminary data collected in this study warrants further investigation of this possibility.

It is plausible that insect richness may be influenced by continuous, freshwater (via groundwater) flow to coastal water bodies in the south east of South Australia. Many insect groups have aquatic larval strategies as part of their life histories and thus may be 'dependent' on water (with preferably low salinities) for success. A diverse and healthy instream macrofaunal community may provide benefits to other trophic levels within these coastal systems.

The insect results here just may suggest that continuous groundwater inputs have some level of influence on the local insect diversity within the south-east coastal region of South Australia. Information that concludes a relationship between continuous freshwater inflow and insect diversity may assist to inform natural resource managers in water conservation and allocation planning programs.

Similarly, in a recent northern hemisphere Mediterranean Sea study, Pavlidou et al. (2014) found that SGD had only a minor influence on the ecological quality of benthic macroinvertebrate communities compared to other freshwater discharges.

Most of the individuals sampled had freshwater affinities, particularly the insects including flies (with many at a larval stage) and aquatic beetles. The types (orders) of flies sampled included the true, caddis-, may-, stone-, damsel-, and black-flies. Freshwater representatives of gastropod molluscs were also collected (compare Tables 4.1, and 4.3).

Examples of potentially-estuarine fauna collected include polychaetes, shrimps, isopods, snails, fishes, amphipods, mites, and ostracods. It should be noted that gastropod snails, fish, amphipods, mites and ostracods have some freshwater representatives (but actually different species) as well. The arachnids, insects, copepods, other arthropods, annelids, molluscs and chordates found are all more characteristic of freshwater samples than either estuarine or (especially) marine samples.

Overall these results suggest that the drains sampled were dominated by freshwater assemblages rather than a marine / estuarine fauna. This study showed that the consistent low salinity of these groundwater-fed drains seem to support freshwater communities only.

It was interesting to note that a typically-estuarine fauna was not found in those creeks that dry up in summer. For example, in the estuaries of south-west Victoria, it is likely that specimens of *Australonereis ehlersi* and other polychaetes like *Simplesetia aesquitis*, *Soletellina alba* and *Arthritica semeni* bivalves, *Nassarius burchardi* and *Hydrococcus brazieri* snails, grapsid crabs and peracarid crustaceans would be present (e.g. Parreira 2000; Matthews 2000; Arundel 2003; Matthews & Fairweather 2003, 2004, 2006, 2008; Barton & Sherwood 2004; Barton *et al.* 2008; Nicholson *et al.* 2008). None of these estuarine species were observed in this study.

Commonly, estuarine studies adopt benthic (infaunal) and less commonly epibenthic sampling methodologies whereas this study adopted an approach which investigated epibenthic and water-column species. The use of fit-for-purpose (and habitat) sampling equipment should also be considered (see Discussion in Chapter 7 and 8) in the systems sampled here. The species collected in this study are more typical of those sampled in previous freshwater studies (e.g. Kefford *et al.* 2003, 2005; Robson & Clay 2005; Robson *et al.* 2005; Lester *et al.* 2007; Lind *et al.* 2007; Lind *et al.* 2009). Some smaller freshwater invertebrates are also likely to be found and these include micro-crustaceans like ostracods, copepods, and cladocerans (Robson & Clay 2005; Kefford *et al.* 2007) less so than estuarine (e.g. Edgar *et al.* 1999; Edgar & Barrett 2000; Parreira 2000; Matthews 2000; Matthews & Fairweather 2003, 2004, 2006, 2008; Barton & Sherwood 2004; Hirst 2004; Dye 2006b; Hastie & Smith 2006; Hirst *et al.* 2006; Hirst & Kilpatrick 2007; Barton *et al.* 2008; Nicholson *et al.* 2008; Spruzen *et al.* 2008) studies (Table 4.10).

4.4.1 Methodological improvements/shortfalls

This type of study could be better designed to capture in the future more relevant ecological information that could further compare the importance of groundwater-fed waterbodies with regional seasonal-flow ones. Such information could seek to capture data relating to colonisation of seasonal flow drains. For example, does instream and benthic fauna change over time (e.g. in relation to flow changes from pre inundation to post desiccation) in seasonal drains? What are the sources of faunal/algal colonisation (e.g. Robson 2000; Robson *et al.* 2005)? The collection of more-relevant environmental data to use as co-variates (e.g. DO, pH, sediment variables) might also be useful. Transplant manipulative studies could also be tried between seasonal- versus continuous-flow drains as well. Inclusion of multiple study sub-sites within seasonal and continuous-flow drains would look for within-drain variation (given the primary focus of this study, only areas in waterbodies in line with the most seaward coastal dune were sampled). Within stream transect linking physical-chemical and biological components could also prove beneficial as would information on stream elevation (above sea-level) and gradient (terrestrial coastal zone) as well as beach slope. Further investigation of adjacent nearshore environments that are influenced by seasonal-flow drains would also be warranted (Fairweather *et al.* 2011).

Other questions arising that are worth further study include:

- Do colonising macrofaunal species migrate to these areas or do they originate from a local source or from dormant cysts?
- Is there a difference between drains that flow through agricultural land compared to vegetated areas (this would be possibly for both seasonal and continuous flow areas?
- There are some very small (e.g. Jerusalem Creek, Clarke Park) continuous-flow drains that may be able to have their flows manipulated to observe ecological changes over time. How would their macrofauna respond?
- With appropriate management agency support, it may also be possible to manipulate seasonal- flow drains (e.g. change regimes by 6 months or to run continuously) to observe any ecological and/or behavioural changes over time.

4.5 CONCLUSION

These results suggest that most creeks were found to be fresh water in character displaying low salinity and typically lotic in its freshwater faunal characteristics.

Continuous groundwater outflow thus seems to restrict any marine influence in the coastal creeks investigated. Since anthropogenic intervention and modification, these small coastal creeks are groundwater-fed drains that have now been turned into coastal-plain freshwater streams running all the way to the sea. The fact that no estuarine gradient functionally exists in these streams may have had (and continues to have) impacts upon those species that rely on estuarine conditions as part of their life history and/or to migrate to historical spawning areas (e.g. Piccaninnie Ponds or Ewens Ponds). This may have significant impacts for resident fauna under a scenario of further rapid or altered (e.g. reduced) groundwater inputs and/or increasing sea level rise in the future by changing the ecological state of these systems from freshwater to more marine-dominated communities. Any rapid and/or permanent change in water quality, specifically salinity, is likely to result in a loss of freshwater species.

The insect patterns observed in this study could imply that continuous groundwater inputs might have some as yet undefined level of influence on local insect diversity within the south-east coastal region of South Australia. Additional research that seeks to explain and understand any significant relationships between continuous freshwater inflow and insect diversity may better assist and inform natural resource managers in water planning programs.

TABLES

Table 4.1: Typical faunal groups expected by habitat realm. Bold font indicates those taxa most expected.

Major taxon	Freshwater	Estuarine	Marine
Nematodes [a]	Yes	Yes	**Yes**
Nemerteans	Few	Few	**Yes**
Hirudine leeches	**Yes**	Few	Few
Oligochaetes	**Yes**	Yes – esp. in upper reaches	Few
Polychaetes	Few	**Yes**	**Yes**
Sipunculids	No	No	**Yes**
Hydracarine mites [a]	Yes	Few	Few
Insects	**Numerous** e.g. Orders:	Some e.g. Orders:	No
	Coleoptera	**Diptera**	
	Diptera	Coleoptera	
	Ephemeroptera	Hemiptera	
	Hemiptera		
	Odonata		
	Thysanoptera		
	Trichoptera		
Crustaceans [a]	Some	**Yes**	**Yes**
Molluscs [a]	**Yes**	**Yes**	**Yes**
Echinoderms	No	No	**Yes**
Amphibians	**Yes**	No	No
Fish	**Freshwater species**	**Freshwater- diadromous[b]-marine**	**Marine species**

[a] but different representative species for each habitat realm. [b] thus also transient in freshwater and marine habitats

Table 4.2: Summary of drain sites sampled for fauna by flow classification and region. *n* = number of sweep nets collected each sampling event. Salinity and temperature are grand mean ± s.e. Σ = total number of samples recorded for salinity and temperature (across all months, all visits – see Chapter 3 for more detail). "-" = not sampled during that year; a dash = zero (not sampled); @ = one replicate lost, so *n* = 3 became 2.

Flow	**Region**	**Site Name**	**Site No (Ch. 3)**	**2003 *n***	**2004 *n***	**2005 *n***	**Total *N***	**Salinity (ppt) mean ± s.e.**	**Temp (°C) mean ± s.e**	**Σ**
Continuous	Western	Blackford Drain	1	2	3	3	8	7.6±0.2	19.4±0.7	44
		Drain L	5	2	3	-	5	8.1±1.3	17.7±0.6	34
		Lake Frome Drain	7	2	3	-	5	22.6±2.3	17.2±0.7	31
	Eastern	Cress Creek	17	-	-	3	3	0.9±0.1	17.6±0.3	43
		Deep Creek	21	2	3	3	8	1.5±0.1	17.5±0.3	43
		Eight Mile Creek	22	2	3	3	8	0.7±0.1	16.9±0.2	43
		Brown Bay Drain	23	2	3	3	8	1.6±0.1	17.9±0.6	43
		ACI Road Drain	25	2	2@	-	4	0.5±0.1	14.9±0.4	43
		Piccaninnie Ponds Outlet	26	2	3	3	8	1.3±0.1	15.8±0.3	48
Seasonal	Western	Mount Benson Drain	4	-	-	3	3	0.4±0.1	19.7±1.0	13
		Sphers Road Drain	10	-	-	3	3	11.1±2.8	23.7±0.1	12
	Eastern	Green Point Drain	24	-	-	3	3	0.5±0.1	19.1±0.4	13
Total sites sampled			*12*	*8*	*8*	*9*	*12*			

Table 4.3: Summary of balanced statistical designs for data subsets used in ANOVA analyses. Each design was applied to the morphospecies-level data for the total abundance and species richness variables. See Table 4.2 for sites names. E = Eastern drains. W = Western drains. C = Continuous-flow. S = Seasonal-flow. Region-Flow is a single factor (i.e. levels = CE,CW,SE,SW).

Subset	**Factor**	**Cases Selected**
<u>**One-Way ANOVA**</u>		
1a	Site	2003 only. Data from *n* = 8 sites
1b	Region	
2a	Site	2004 only. Data from *n* = 8 sites
2b	Region	
3a	Site	2005 only. Data from *n* = 9 sites
3b	Region	
4	Region_Flow	2005 only. Data from *n* = 9 sites
<u>**Two-Way ANOVA**</u>		
5	Flow*Region	All years, Data from *n* = 12 sites [all sites]
6	Year*Region	All years, and only *n* = 5 sites.
7	Year*Region	2003, 2004, and only *n* = 8 sites

Table 4.4: Summary of fauna sampled at each site by higher taxonomic group (see Appendix 4A for more detail). Drain numbers: 01 = Blackford Drain; 04 = Mt Benson Drain; 05 = Drain L; 07 = Lake Frome Drain; 10 = Sphers Road Drain; 17 = Cress Creek; 21 = Deep Creek; 22 = Eight Mile Creek; 23 = Brown Bay Drain; 24 = Green Point Drain; 25 = ACI Rd Drain; 26 = Picaninnie Ponds Outlet. #MSpp = Number of morphospecies identified for that particular group. Rank: top 12 (of 26) groups only. %: percent of total individuals for groups contributing >0.5% only.

Phylum	Class or Sub-class	Order		Continuous-flow									Seasonal-flow			Total	Rank	%
		Drain number		01	05	07	17	21	22	23	25	26	04	10	24			
n =(across all years)			**#MSpp**	*8*	*5*	*5*	*3*	*8*	*8*	*8*	*4*	*8*	*3*	*3*	*3*	***66***		
Nematoda			1	0	0	0	0	0	0	0	0	2	0	0	0	**2**		
Annelida	Hirudinea		1	0	0	0	0	1	0	0	0	0	0	0	0	**1**		
	Oligochaeta		3	1	2	117	5	0	8	7	0	3	0	0	1	**144**	6	4.1
	Polychaeta	Canalipalpata	1	0	0	0	0	0	0	0	0	1	0	0	0	**1**		
Arthropoda	Arachnida	Acarina	6	0	1	3	0	0	0	1	0	0	3	0	1	**9**		
		Araneae	2	0	0	0	0	0	0	0	0	0	4	0	0	**4**		
	Diplopoda		1	0	0	0	0	0	0	0	0	0	1	0	0	**1**		
	Entognatha	Collembola	1	0	0	0	0	0	0	1	0	0	0	1	1	**3**		
	Insecta	Coleoptera	23	28	2	1	1	0	5	8	6	1	59	1	6	**118**	8	3.3
		Diptera	42	165	33	32	36	51	83	348	57	99	381	22	528	**1835**	1	51.6
		Ephemeroptera	5	5	0	0	1	1	6	1	0	82	36	0	0	**132**	7	3.7
		Hemiptera	9	24	12	1	0	0	2	0	1	1	3	0	0	**44**	10	1.2
		Hymenoptera	11	5	0	2	0	0	4	0	0	2	7	0	0	**20**	11	0.6
		Odonata	3	6	0	0	0	0	0	0	0	0	7	0	1	**14**		
		Thysanoptera	1	0	0	0	0	0	0	1	0	0	0	0	0	**1**		
		Trichoptera	8	59	8	0	23	11	2	39	0	9	130	0	3	**284**	3	8.0
		Unknown	1	1	0	0	0	0	0	0	0	0	0	0	0	**1**		

Table 4.4 (cont.)

Phylum	Class or Sub-class	Order Drain number	#MSpp	Continuous-flow 01	05	07	17	21	22	23	25	26	Seasonal-flow 04	10	24	Total	Rank	%
n =(across all years)				*8*	*5*	*5*	*3*	*8*	*8*	*8*	*4*	*8*	*3*	*3*	*3*	***66***		
	Malacostraca	Amphipoda	6	75	116	36	21	2	22	9	8	55	40	2	0	**386**	2	10.9
		Decapoda	1	0	0	0	12	1	1	0	0	0	0	0	0	**14**		
		Isopoda	2	0	0	2	0	0	0	0	0	0	0	0	0	**2**		
	Ostracoda	Ostracoda	1	85	9	0	0	0	0	0	0	0	0	1	0	**95**	9	2.7
	Copepoda	Cyclopoida	1	0	0	2	0	0	1	0	0	0	0	0	0	**3**		
Mollusca	Gastropoda	Mesogastropoda	2	52	4	0	32	31	14	30	0	9	22	0	0	**194**	5	5.5
		Pulmonata	1	0	0	0	2	3	5	2	2	1	213	0	0	**228**	4	6.4
Chordata	Actinopterygii		6	1	4	1	0	0	0	3	6	1	0	0	1	**17**	12	0.5
	Amphibia	(tadpoles)	1	0	0	0	0	0	0	0	1	0	0	0	0	**1**		
Total individuals				**507**	**191**	**197**	**133**	**101**	**153**	**450**	**81**	**266**	**906**	**27**	**542**	**3554**		
No. of Taxa Groups				13	10	10	9	8	12	11	7	14	10	5	8	26		100

Table 4.5: Summary by site and year. Sample sizes were n = 3 for each site in each year, except for n = 2 at ACI Road in 2003 and all sites in 2004. '-' Not sampled in that year. Σ = sum of all samples across all years.

Flow	Region	Site Name	Total Abundance			Richness			Σ
			2003	2004	2005	2003	2004	2005	
Continuous	Western	Blackford Drain	72.38±33.9	45.5±3.5	66.3±18.3	14.7±1.5	9.0±1.0	13.3±0.3	8
		Drain L	43.7±13.2	30.0±8.0	-	5.7±0.7	8.0±1.0	-	5
		Lake Frome Drain	18.7±10.8	70.5±64.5	-	6.3±3.2	3.5±0.5	-	5
	Eastern	Cress Creek	-	-	44.3±8.4	-	-	8.0±0.6	3
		Deep Creek	9.0±3.1	11.5±1.5	17.0±4.0	4.3±1.8	4.5±0.5	7.3±1.3	8
		Eight Mile Creek	21.3±3.1	11.5±1.5	22.0±9.5	9.7±1.2	3.0±2.0	5.7±0.7	8
		Brown Bay Drain	93.7±23.3	8.0±2.0	51.0±25.9	7.7±0.9	5.0±1.0	6.3±3.2	8
		ACI Road Drain	8.0±4.0	32.5±14.5	-	4.0±2.0	5.5±0.5	-	4
		Piccaninnie Ponds Outlet	53.0±21.0	10.0±8.0	29.0±11.6	8.0±2.5	2.5±0.5	6.3±1.3	8
Seasonal	Western	Mount Benson Drain	-	-	302.0±81.1	-	-	23.0±0.6	3
		Sphers Road Drain	-	-	9.0±3.5	-	-	3.0±1.0	3
	Eastern	Green Point Drain	-	-	180.7±26.0	-	-	5.7±0.3	3

Table 4.6: Mean (± s.e.per sweep) macrofaunal total abundance and richness summarised by flow classification and region. A dash indicates that combination was not sampled then.

Group	Total Abundance			Richness		
	2003	2004	2005	2003	2004	2005
By Flow:						
Continuous	44.8±9.2	21.3±4.2	38.3±6.6	7.9±0.9	5.3±0.7	7.8±0.8
Seasonal	18.7±10.8	70.5±64.5	163.9±49.1	6.3±3.1	3.5±0.5	10.6±3.2
By Region:						
Eastern	39.1±10.8	14.7±3.9	125.8±50.8	6.9±0.9	4.1±0.5	6.6±0.6
Western	44.9±13.4	48.7±18.3	57.3±14.8	8.9±1.8	6.8±1.1	13.1±2.9
By Flow:Reg						
Continuous Eastern	39.1±10.8	14.7±3.9	32.7±6.3	6.9±0.9	4.1±0.5	6.7±0.7
Continuous Western	58.0±17.5	37.8±5.7	66.3±18.2	10.2±2.1	8.5±0.6	13.3±0.3
Seasonal Eastern	-	-	180.7±26.0	-	-	5.7±0.3
Seasonal Western	18.7±10.8	70.5±64.5	155.5±74.9	6.3±3.2	3.5±0.5	13.0±4.5

Table 4.7: Region and Site ANOVA outcome summaries. = greater value. See Table 4.2X as to what sites were sampled in each year. Reg-Flow is one factor, with four groups. Region & Flow is two factors, each with 2 groups. Tukeys HSD post-hoc (significant results all *P* <0.05) '-" = no comparisons needed.

Subset	Analyses	Abundance *P*	Tukey Pairwise (where *P* <0.05)	Richness *P*	Tukey Pairwise (where *P* <0.05)
1	Site (2005 only)	<0.001	Mt Benson > all other sites except Green Point = NS Green Point > Deep, 8-Mile.	<0.001	Mt Benson > all other sites Blackford > all other sites except Cress, Deep = NS
2	Region-Flow (2005 only)	0.017	SW > CE	ns	-
3	Flow	0.010	Seasonal > Continuous	ns	-
	Region	ns	-	0.014	Western > Eastern
	Flow*Region	ns	-	ns	-
4	Year	ns	-	0.005	2003 > 2004; 2003 > 2005
	Region	0.015	Western > Eastern	<0.001	Western > Eastern
	Year*Region	ns	-	ns	-
5	Year	ns	-	0.046	2003 > 2004
	Region	ns	-	ns	-
	Year*Region	ns	-	ns	-

Table 4.8: Summary of SIMPER analyses for a) within-group similiarity, and b) between-group dissimilarity. Only those taxa with Diss/SD > 1 (i.e. reliable indicators) have been displayed. Ave = Average, Grp = Group, Diss = Dissimilarity, Sim = Similarity. SD = standard deviation. C =Continuous-flow. S = Seasonal-flow. W = Western region. E = Eastern Region.

a)

Within-group Comparison	***Ave Grp Sim %***	**Taxon contributing most to similarity**	**Ave Sim %**	**Diss/SD**	**% Contribution to Diss**
Continuous	26.61	Chironomidae_1	12.55	1.26	47.18
Seasonal	24.22	Chironomidae_1	18.93	1.08	78.15
Western	33.00	Chironomidae_1	13.53	1.14	41.00
Eastern	25.28	Chironomidae_1	12.88	1.22	50.94

b)

Between-group Comparison	***Ave Diss %***	**Taxon contributing most to dissimilarity**	**Ave Abundance Difference**	**Ave Diss %**	**Diss/SD**	**% Contribution to Diss**
Continuous vs Seasonal	81.0	Pomatiopsidae_1	C > S	4.31	1.09	5.33
Western vs Eastern	79.7	Amphipoda_1	W > E	8.09	1.52	10.14
		Corixidae_1	W > E	4.57	1.28	5.74
		Ostracoda_1	W > E	4.62	1.22	5.79
		Pomatiopsidae_1	W < E	3.83	1.05	4.80
		Leptoceridae_1	W > E	3.48	1.00	4.36

Table 4.9: Calculations of % insects as a bio-indicator index by location grouping, based on numbers of individuals or morphospecies from the Class Insecta versus all other taxonomic groups.

Drains Comparison	**Individuals**				**Morphospecies**				***Sample***
	Class Insecta	**Other**	**Total**	**% Insecta**	**Class Insecta**	**Other**	**Total**	**% Insecta**	***n***
Region:									
Eastern	1419	307	1726	82.2%	58	18	76	76.3%	42
Western	1030	798	1828	56.4%	70	27	97	72.2%	24
Flow:									
Continuous	1671	554	2225	75.1%	85	25	110	77.3%	52
Seasonal	778	551	1329	58.5%	42	23	65	64.6%	14
Flow*Region:									
CE	881	303	1184	74.4%	54	16	70	77.1%	39
CW	790	251	1041	75.9%	45	13	58	77.6%	13
SE	96	103	199	48.2%	7	4	11	63.6%	3
SW	682	448	1130	60.4%	36	20	56	64.3%	11
Total	2449	1105	3554	68.9%	103	38	141	73.1%	66

Table 4.10: Comparison of fauna observed in the present study against fauna observed in a subset of previous temperate Australian marine, estuarine and freshwater studies. EH = estuarine to hypermarine. N = New South Wales. Q = Queensland. T = Tasmania. V = Victoria. VN = Victoria and New South Wales. X = present, blank = absent

Water Regime	Freshwater									Estuarine									Marine		EH	**F-E**
State	V	V	V	V	V	V	N	Q	T	VN	N	N	N	N	N	Q	T	T	T	T	T	**SA**
Taxa Found	**Kefford et al. 2003**	**Kefford et al. 2005**	**Kefford et al. 2007**	**Marshall & Bailey 2004**	**Robson & Clay 2005**	**Thomson 2002**	**Scealy et al. 2007**	**Marshall et al. 2006**	**Edgar et al. 1999**	**Hirst 2004**	**Courtenay et al. 2004**	**Dye & Barros 2005**	**Dye 2006a**	**Dye 2006b**	**Hastie & Smith 2006**	**Currie & Small 2006**	**Edgar & Barrett 2000**	**Edgar et al. 1999**	**Edgar et al. 1999**	**Spruzen et al. 2008**	**Hirst et al. 2006**	**This Study**
Nematoda												X	X									**X**
Nemertea										X							X				X	
Hirudinea	X	X																				**X**
Oligochaeta	X	X		X				X				X	X	X								**X**
Polychaeta										X	X	X	X	X	X	X	X	X	X	X	X	**X**
Hydracarina	X	X		X				X														**X**
Crustacea											X					X						**X**
Amphipoda	X	X							X	X		X		X			X	X	X	X	X	**X**

Water Regime	Freshwater									Estuarine									Marine		EH	**F-E**
State	V	V	V	V	V	V	N	Q	T	VN	N	N	N	N	N	Q	T	T	T	T	T	**SA**
Taxa Found	**Kefford et al. 2003**	**Kefford et al. 2005**	**Kefford et al. 2007**	**Marshall & Bailey 2004**	**Robson & Clay 2005**	**Thomson 2002**	**Scealy et al. 2007**	**Marshall et al. 2006**	**Edgar et al. 1999**	**Hirst 2004**	**Courtenay et al. 2004**	**Dye & Barros 2005**	**Dye 2006a**	**Dye 2006b**	**Hastie & Smith 2006**	**Currie & Small 2006**	**Edgar & Barrett 2000**	**Edgar et al. 1999**	**Edgar et al. 1999**	**Spruzen et al. 2008**	**Hirst et al. 2006**	**This Study**
Cladocera			X					X														
Copepoda			X	X	X			X					X									**X**
Decapoda	X	X						X		X							X	X	X			**X**
Isopoda																	X	X	X			**X**
Notostraca					X																	
Ostracoda			X		X								X									**X**
Collembolla					X																	
Insecta	X	X																				**X**
Coleoptera				X		X	X	X														**X**
Diptera	X	X		X		X	X	X														**X**
Chironomidae				X	X	X									X		X				X	**X**
Ephemeroptera	X	X		X		X		X														**X**

Water Regime	Freshwater									Estuarine									Marine		EH	**F-E**
State	V	V	V	V	V	V	N	Q	T	VN	N	N	N	N	N	Q	T	T	T	T	T	**SA**
Taxa Found	**Kefford et al. 2003**	**Kefford et al. 2005**	**Kefford et al. 2007**	**Marshall & Bailey 2004**	**Robson & Clay 2005**	**Thomson 2002**	**Scealy et al. 2007**	**Marshall et al. 2006**	**Edgar et al. 1999**	**Hirst 2004**	**Courtenay et al. 2004**	**Dye & Barros 2005**	**Dye 2006a**	**Dye 2006b**	**Hastie & Smith 2006**	**Currie & Small 2006**	**Edgar & Barrett 2000**	**Edgar et al. 1999**	**Edgar et al. 1999**	**Spruzen et al. 2008**	**Hirst et al. 2006**	**This Study**
Hemiptera	X	X						X														**X**
Megaloptera				X		X																
Odonata								X														**X**
Plecoptera	X	X		X		X																
Trichoptera	X	X		X		X		X														**X**
Mollusca								X			X					X						**X**
Bivalvia										X		X		X	X		X	X	X	X	X	
Gastropoda	X	X		X					X	X				X	X		X	X	X	X	X	**X**
Total Groups	12	12	3	11	5	7	2	12	2	6	3	5	5	5	4	3	8	6	6	4	6	**21**

FIGURES

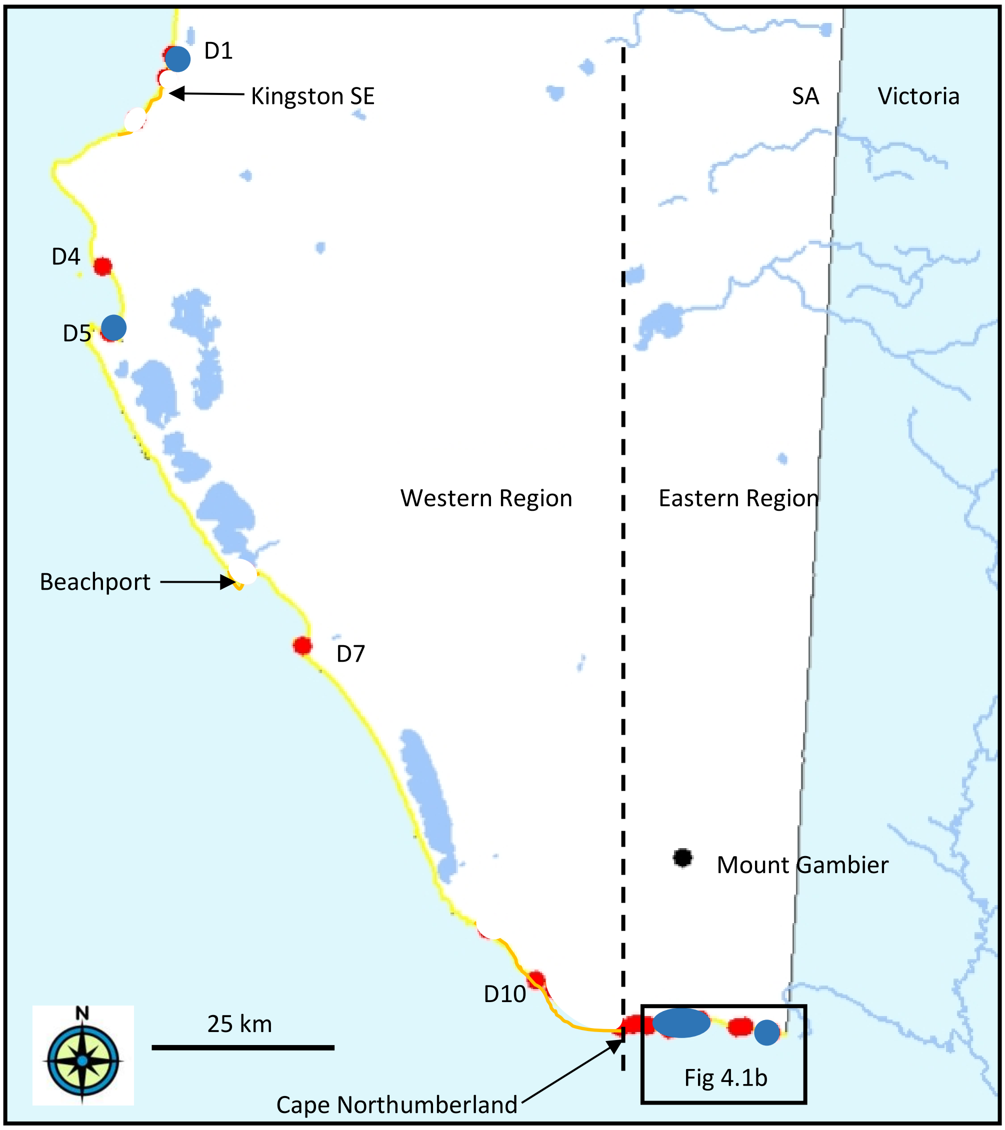
Figure 4.1: Map of sites sampled in this investigation a) regional; and b) enlargement of sites east of Cape Northumberland. Blue dots are continuous-flow drains. Red dots are seasonal-flow drains. Green patches are conservation parks. A vertical line through Cape Northumberland separates and defines Western from Eastern drains. D = drain. See Table 3.1 for drain names.

Figure 4.2: Mean morphospecies a) total abundance and b) richness by group and year. Error bars are standard errors for sample size n = 3 for each site in each year, except for n = 2 at ACI Road in 2003 and all sites in 2004. 'nd' = no data because that group was not sampled in that year. NB: the y-axis extends to the range of the raw data used in these bar charts.

Figure 4.3: MDS ordination for morphospecies-level data showing the four flow-region groups. PERMANOVA (4th root transform): Region-Flow interaction (2-factor) P <0.001

Figure 4.1

a)


D1
Kingston SE
SA
Victoria
D4
D5
Western Region
Eastern Region
Beachport
D7
Mount Gambier
D10
N
25 km
Fig 4.1b
Cape Northumberland

b)

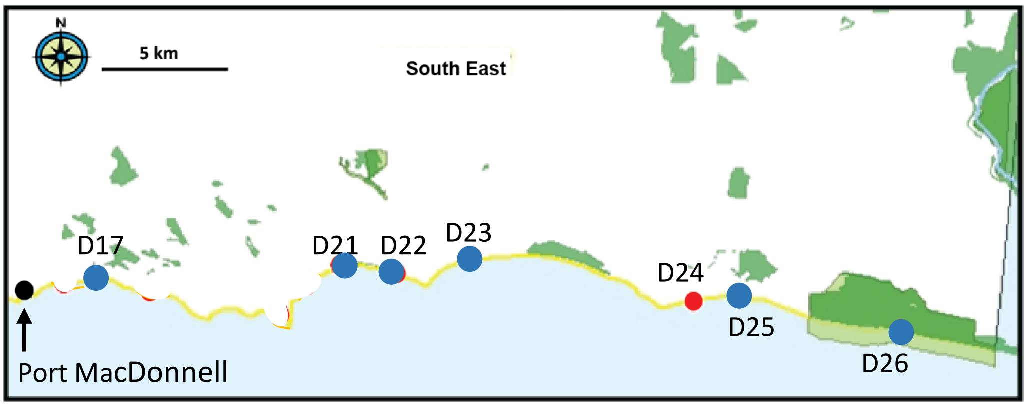
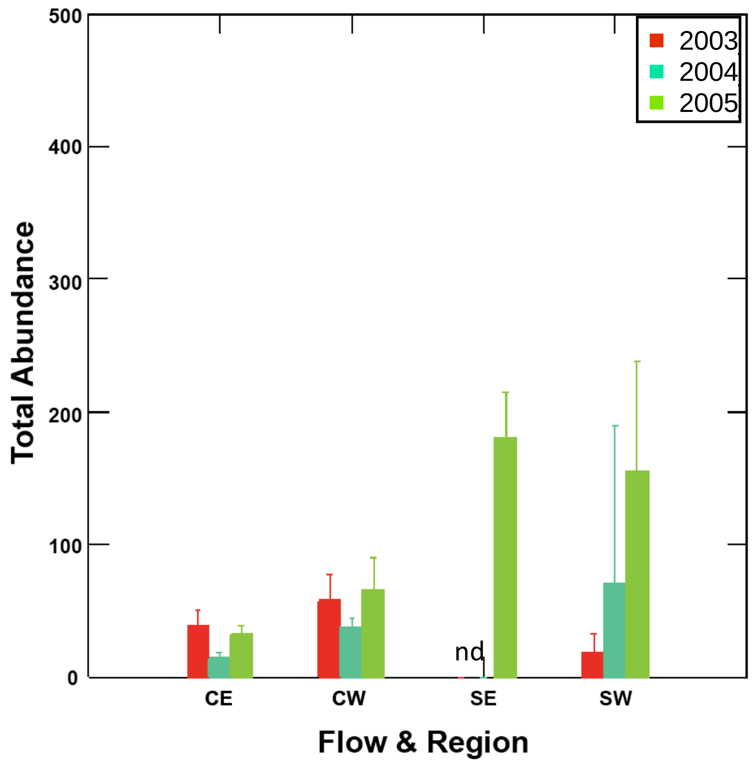

N
5 km
South East
D17
D21
D22
D23
D24
D25
D26
Port MacDonnell

Figure 4.2:

a)


500
400
300
200
100
0
Total Abundance
2003
2004
2005
nd
CE
CW
SE
SW
Flow & Region

b)

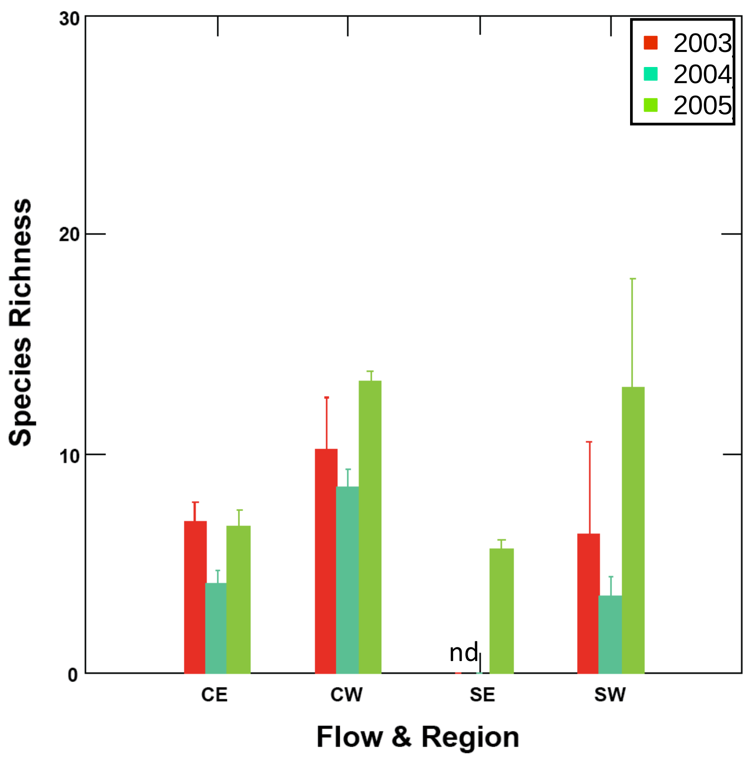
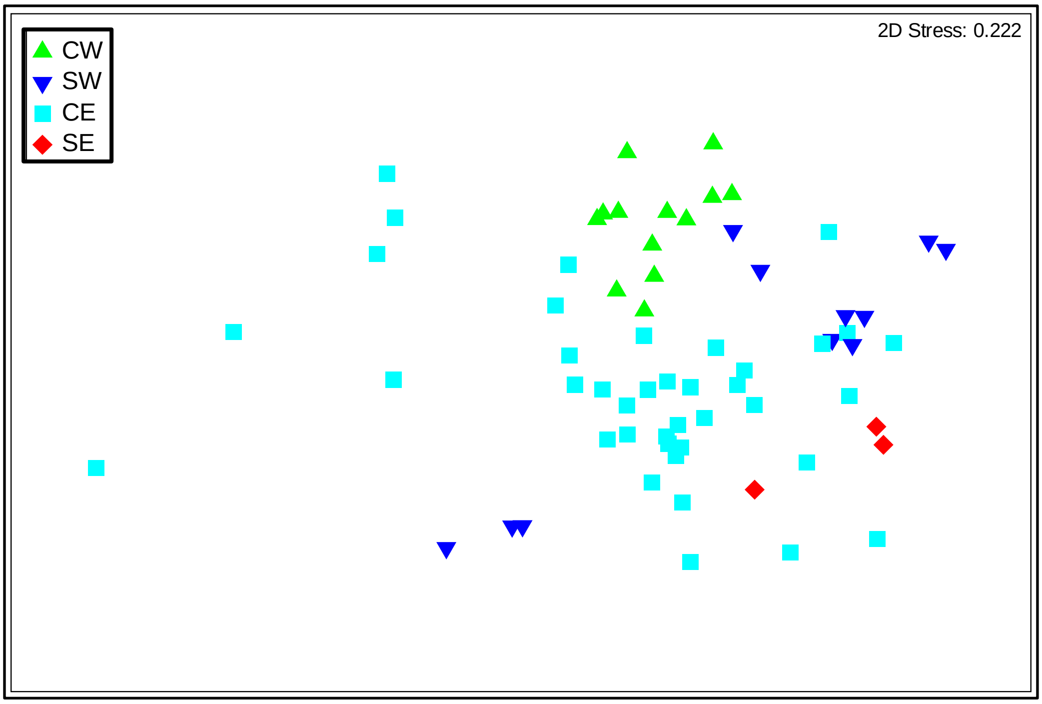

30
20
10
0
Species Richness
2003
2004
2005
nd
CE
CW
SE
SW
Flow & Region

Figure 4.3:



CHAPTER 5 - MEIOFAUNA

5 Does groundwater flow affect the animals living between sand grains on the beach?

5.1 INTRODUCTION

Meiofauna can be described as those animals that reside in the interstitial spaces of benthic sediments and which pass through a 2mm sieve (Nicholas & Hodda 1999) but are more commonly animals that pass through 0.5 to 1.0 mm screens and that get trapped on 30- to 100-µm screens (McLachlan & Brown 2006). Sandy beach meiofaunal communities can be diverse in taxonomic composition at the phyletic level (esp. the worm-like phyla, see below) and may have complex three-dimensional patterns (McLachlan & Brown 2006), which relate to physical conditions (e.g. oxygenation) within the sediments (Nicholas 2001; McLachlan & Brown 2006).

Meiofaunal representatives include many metazoans and protozoans from less-well known animal phyla such as Gnathostomulida, Gastrotricha, Kinorhyncha and Tardigrada (Hulings & Gray 1971) but also include some smaller species from other higher taxonomic groups such as Crustacea, Annelida and Platyhelminthes. Nematode worms are also a large component of the meiofaunal community containing in excess of 20,000 nominal species with approximately 4,000 of these being free-living marine forms (*P*latt & Warwick 1983).

Although there have been few studies on the meiofauna of sandy beaches (McLachlan & Brown 2006), some Australian studies have been conducted on general meiofaunal assemblages (e.g. McLachlan 1985; Dye & Barros 2005) whereas others have focused primarily on a particular meiofaunal group (i.e. the Nematoda: Nicholas & Hodda 1999; Nicholas 2001, 2002, 2004a,b; Nicholas & Trueman 2005). These Australian studies have been conducted in Western Australia (McLachlan 1985), Northern Territory and Victoria (Nicholas & Trueman 2005) but with most being undertaken in New South Wales (Nicholas & Hodda 1999; Nicholas 2001, 2002, 2004a,b; Dye & Barros 2005; Nicholas & Trueman 2005). To date, no studies have been published on the meiofauna of South Australian sandy beaches, nor on the meiofaunal assemblages associated with coastal groundwater-fed beach springs.

Meiofauna living between sand grains exposes them to groundwater sources (e.g. similar to freshwater hyporheos or stygofauna, Tomlinson and Boulton, 2008 see Boulton) because they have an intimate connection to their interstitial environment. These fauna are an often overlooked component of biodiversity, especially at the phyletic level (e.g. several unique animal phyla only live meiofaunally).

5.1.1 Description of Beach Springs

Groundwater springs on sandy beaches in South East South Australia are largely unstudied. An objective this chapter is to describe these unique habitats in terms of their physical characteristics, water quality and meiofauna communities. The beach spring (referred to as SPR_01 in Chapter 3) used in this assessment is situated within the Piccaninnie Ponds Conservation Park

These beach springs are dynamic environments. They are constant state of flux from the influence of water flow, waves, tides and wind. Discharging groundwater combines with waves, tides and wind to constantly erode beach sediments. Depending on tide level these springs would be temporarily inundated by individual waves or for full tidal cycles under high tide or storm conditions.

The changing nature of these habitats creates an arguably inhospitable environment for benthic infauna that might reside there. The variation in the flux result in ‘craters’ created by discharging groundwater ranging up to 8m in diameter at low tide. The groundwater discharge point itself may vary between 10cm to 1m at these springs. Discharging groundwater creates a sand-like slurry. The force of discharging groundwater was observed to burst through the ambient surface level of the springs (see Figure 5.1).

The harshness of these physical conditions result in sediments constantly changing thereby creating distinctive beach habitat. It is possible that any infauna that reside within these areas have adapted specialised life histories to withstand such conditions.

The aim of this chapter was to assess community composition of meiofauna in groundwater-fed beach springs and compare the abundance and composition of these uncommon habitats with those of more 'typical' intertidal beach habitats.

The main null hypothesis for these comparisons was that the meiofaunal community structure was the same across the three sample types (i.e. Away: Edge: Within) and that this situation did not differ over time.

5.2 METHODS

5.2.1 Description of study area

Sampling for this study concentrated on the beach springs to the east of Piccaninnie Ponds Conservation Park (Figure 5.2). The physical characteristics of these beach springs are quite energetic. Discharging groundwater creates a sand-slurry locally known as “boils”, which result in a saturated unconsolidated sedimentary environment in the centre of the spring. This unconsolidated microhabitat is bordered by more consolidated sediments that are subject to continual erosion from sub-surface water movement discharged from the springs (i.e. the groundwater) and also from waves and tides from the marine side.

These factors interact to alter the shape and physical conditions of the springs on a continual basis. Variations in tides and wave energy mean that the springs can be eroded, inundated, and backfilled on an hourly, tidal or daily cycle. The physical conditions of such a dynamic microhabitat may influence the abundance and composition of any fauna that may reside there.

The physical nature of the springs also facilitates the deposition of detached macroalgae and other biological or detrital matter to enter the springs (Figure 5.1a,c,d). Inert objects such as stones and shells may also be washed into the discharging spring.

5.2.2 Sampling protocol and timing

Sediment samples were collected from the groundwater-discharging beach springs (Figure 5.1) to the east of Piccaninnie Ponds Conservation Park (Figure 5.2). Sampling was conducted on five sampling events over 2004 (October) and 2005 (January, February, March, and November). Samples were collected from 'within' beach springs, immediately on the 'edge' of the beach spring and approximately 500m 'away' from the beach springs on the normal beach. Six replicate samples were collected from each location (within, edge and away) on each sampling event.

'Within' samples were collected directly from the unconsolidated slurry-like sand matrix within the centre or most energetic area of the discharging spring. 'Edge' samples were collected from the consolidated sand immediately on the edge of the spring and so may have had some groundwater influence. The distance between Within and Edge samples was dependent on the size of the discharging spring but was approximately 10 - 30 cm. 'Away' samples were collected from an area 500m to the west of the beach springs and thus were assumed to be removed from any [visible] influence of groundwater discharge (confirmed by salinity measurement of the water). Samples collected from the Away area were sourced from consolidated sediments within the mid-intertidal area of the beach face. This region of the beach face was subject to periodic inundation by waves during low tides but samples were collected when not directly under the influence of waves. The sediments sampled in this location (i.e. from the zone of retention where water movement occurs across and within the sand but where interstitial moisture is retained during low tides [McLachlan & Brown 2006]) were moist but not permanently wet. These samples were intended to be typical of the marine beach, especially the midshore intertidal habitat of this location, without the influence of groundwater.

5.2.3 Faunal Sampling

Meiofauna and associated sediments were collected from the sedimentary surface (to a depth of 10cm below the surface) of beach springs using a 20mL syringe (Somerfield & Warwíck 1996) and immediately preserved in a 10% formalin in natural background (i.e. seawater or spring water) solution.

Identification of samples was undertaken using a double-blind approach in an attempt to combat issues associated with systematic numbering of samples. Meiofauna were separated from sediments using the Ludox extraction method (Somerfield & Warwick 1996) with three extractions per sample undertaken. Ludox is an aqueous solution of colloidal silica that enables fauna to become suspended within the solution whereas the negatively-bouyant sediments sink enabling easier separation of fauna from within the sample matrix (Somerfield & Warwick 1996).

The Ludox extraction method involves removing silt/clay with freshwater and a 63µm mesh, followed by decantation of the material retained on the sieve to further separate larger fragments before subjecting the decanted material to the Ludox solution to separate out the meiofauna. Further washing and evaporation of the sample is conducted before the concentrated sample is mounted on a microscope slide, bordered with wax and sealed with a cover slip to preserve the sample before identification and longer-term storage (Somerfield & Warwick 1996).

Any meiofauna retained were identified to lowest possible taxonomic group (assisted by Dr Janet Gwyther, Deakin University, Waurn Ponds, Victoria) using taxonomic keys (Platt & Warwick 1983, 1988; Higgins & Thiel 1988; Warwick *et al.* 1998) and supplemental references (Dole-Olivier *et al.* 2000; Nicholas 2002,2004b) and then counted. It is likely that <2% of the longer turbellarians could actually be gnathostomulids based on finding 4 out of 200 specimens (upon subsequent re-identification). These two organisms are quite similar but can be differentiated by their jaws with gnathostomulids more easily identified (J. Gwyther pers. Comm..)

5.2.4 Statistics

Data were analysed for each time using univariate (e.g. one-way Analysis of Variance [ANOVA] with the single factor of sample location, or contingency tables) and multivariate statistics (e.g. MDS ordination, PERMANOVA (Anderson *et al.* 2008)) using SYSTAT v11 and PRIMER v6 with the PERMANOVA+ add-on software packages, respectively.

5.3 RESULTS

5.3.1 Taxa found

A total of 4483 multicellular organisms in 49 genera from 9 phyla were collected during the study (Table 5.1). Nematodes were easily the most dominant group of organisms (total individuals N = 3571, i.e. 83.7% of all collected), followed by turbellarians (N = 557, 12.4%), harpacticoid copepods (N = 196, 4.4%) and gastrotrichs (N = 97, 2.2%). Rarer taxa included a kinorhynch, nauplius larva, and ostracod # 20 (1 individual each); isopods (2 individuals); gnathostomulids (4 individuals minimum, see above) and halacarid mites (5 individuals) (Table 5.1).

Nematodes and turbellarians were collected at each type of site on each sampling event and harpacticoid copepods were collected from each group at every sampling event except in January 2005 (Within group) (Table 5.2). Nematodes, harpacticoids and gastrotrichs had greater within-group abundance in Away samples whereas turbellarians were more abundant in Edge samples (see rank in Table 5.3).

5.3.2 Total Abundance

The total number of individuals sampled in the Away group was 3787 (84.5% of all collected), versus 476 individuals (10.6%) (Edge group), and only 220 individuals (4.9%) (Within group). Statistically-significant difference in occurrence of total individuals between these groups was observed ($P <$ 0.001, Chi-square contingency tables).

A contingency table for number of individuals in the 4 main meiofaunal groups (i.e. in decreasing dominance: Nematoda, Turbellaria, Harpacticoida, Gastrotrichia) revealed that their distribution was not even across the groups (Chi-square: 1371.5, P <0.001 for df = 6), as expected by their relative frequencies (Table 5.3). For example, a general shift from dominance by nematodes (87%) on the Away beach, down to 20% at the edge of springs was seen, with relatively more turbellarians and others living in the groundwater (see also section 5.3.1).

Nematodes constituted 87.6% of the individuals collected in the Away group versus only 20.0% from the Edge, but 71.4% from within the springs. The most numerous nematode (= 90.6% of all nematodes collected) had characteristics of 2 genera and is thus described here as Catanema/Onyx (Table 5.3, see also Discussion).

Nematode abundance (mean + s.e. per core, all nematode species combined) varied from 345.2±126.6 individuals in January 2005 (Away) to only 0.7±0.3 individuals in October 2004 (Within) and.0.7±0.4 individuals in November

2005 (Within). A two-way contingency table gave a Chi-square value of 1208 ($P < 0.001$, $df = 2$), showing that nematodes were much more common away from groundwater springs.

5.3.3 Morphospecies Richness

The total number of morphospecies sampled in the Away group was 43 compared with only 17 morphospecies (Edge), and 19 morphospecies (Within) (Chi-square = 15.9, $P < 0.001$, $df = 2$).

There were a number of components unique to one or other type of sample. That is, there were two morphospecies each found in 'Within' (i.e. Isopoda, Nauplius) and ‘Edge' (i.e. Kinorhyncha, Unknown Nematoda sp. A) locations only. In contrast, twenty-one morphospecies were unique to the 'Away' location and included Gastrotrichia, Gnathostomulida, Ostracoda and 19 species of Nematoda. A total of 7 morphospecies (Oligochaeta, Harpacticoida, Turbellaria (2 species), Nematoda (3 species)) were found in all three sampling locations (Figure 5.3, and Table 5.1).

The two taxa found in both Within and Edge locations only were nematodes, as were the six taxa found in both Edge and Away locations only. Six of the eight taxa found only in Within and Away locations were also nematodes with the remaining two taxa being rotifers and halacarid mites (Figure 5.3 and Table 5.1).

5.3.4 Statistical Analyses

Two-way ANOVA revealed a statistically significant difference in mean total abundance for the interaction of time and location (P<0.001). Given the numerical dominance of nematodes, PERMANOVA was performed on 1) complete meiofauna data set; 2) with nematodes removed; and 3) nematodes only. Pairwise comparisons were additionally performed on each data set.

P(Perm)=0.0001 for all meiofaunal groups, and nematodes only by Year*Location. P(Perm)=0.0005 for all meiofaunal groups but without nematodes by Year*Location (Table 5.4). PERMANOVA analyses showed that meiofauna differed in all month comparisons except January 2005 and February 2005 (Table 5.5). Nematodes appear to have a larger influence results driven by greater abundances.

Average dissimilarity between groups (all meiofauna): W & A (83.5%) > E & A (77.6) = W & E (76.9%). Meiofauna causing most dissimilarity, typically Catanema/onyx, Turbellaria blob, Turbellaria-long, Harpacticoid copepod, and Theristus (Table 5.6).

The average dissimilarity between groups (with nematodes removed): W & A (84.3%) > W & E (78.4%) = E & A (76.3%). Meiofauna causing most dissimilarity Turbellarians, Harpacticoid copepod. Average dissimilarity between groups (nematodes only): W & A (84.7%) > W & E (81.0%) > E & A (79.8%). Nematodes causing most dissimilarity, typically Catanema/Onyx, Theristus, Unknown sp.D(pegtail), Unknown_sp.H(dotty), Cyatholaimid, and Daptonema.

Main MDS shows each group had outliers that influenced spatial separation. Away group slightly separated from Within and Edge group (particularly in expanded cluster) (Figure 5.4).

5.4 DISCUSSION

Overall, these results suggest that groundwater flow makes meiofaunal assemblages more sparse and less rich, probably because of the unconsolidated nature of the beach springs which are in a state of perpetual flux due to flow.

The most numerous nematode "Catanema/Onyx" (Table 5.1) has characteristics of 2 genera in the family Desmodoridae, Catanema (especially the muscular pharynx) and Onyx (especially the long stylet in the buccal cavity and pre-cloacal supplements) but it also has a multispiral amphid typical of neither (Janet Gwyther pers. comm.). It was difficult to assign these organisms to either genus and so we suspect it may be undescribed and, as a result, has been described here as "Catanema/Onyx" (see Table 5.1). There are several other putative identifications that also are tentative, as expected from the relative paucity of work on Australian meiofauna, but distinctions were made consistently and the most-likely genus assigned (even where it was probably a new species that thus requires description).

Although limited by the number of availabvle springs in which to sample, this study could have benefited from multiple springs in which to sample meiofauna assemblages. The study could be improved by collecting data a succession of increasing distance from a spring (e.g. 10, 20 50, 100 200, 500 m) at a similar beach height. Additional information such as the collection of oxygen or redox values may also assist. Sample of macrofauna associated with the springs should also be considered.

5.4.1 General Meiofaunal Community – compared with other temperate Australian studies

The results of this study can be compared with the taxa found in McLachlan (1985) and Dye and Barros (2005). These studies were undertaken in Western Australia (Scarborough and Quinns Rocks; McLachlan, 1985) and central NSW (Dye & Barros 2005). The purpose of this comparison was to attempt to explain any observed differences seen in the meiofaunal community of this study and attempt to infer (for future investigations) the role that surfacing groundwater (via beach springs) may have on the representative taxa of the community.

The study undertaken on the east coast of Australia (Dye & Barros 2005) returned 14 major taxonomic groups whereas the west-coast study (McLachlan 1985) revealed only 8 major taxa. Comparing only the Away group (as the other studies did not have "Edge-" and "Within-"defined samples), this study on the south coast of Australia returned 9 taxonomic groups in the

Away group (Table 5.7). Five taxa were found in all studies (Nematoda, Turbellaria, Harpacticoida, Gastrotricha and Oligochaeta). Nematoda was the most dominant taxon across all studies followed by the Harpacticoida, Turbellaria and Oligochaeta in various rank orders.

Six taxa (other Copepoda, Cumacea, Syncarida, Tanaidacea, Echiura and Tardigrada) were unique to Dye and Barros (2005b) and two (Rotifera and Gnathostomulida) to the present study but might overlap with the 'Other taxa' not described and unique to McLachlan (1985). With the exception of Harpacticoida, a relatively distinct difference in Crustacea representatives was observed with the study of Dye and

Barros (2005), who found additional Copepoda (other than harpacticoids), Cumacea, Syncarida, and Tanaidacea.

The study site contains numerous [macro-sized] polychaetes (unpublished data) but yet no meiofaunal representatives of that Class were observed. Meiofaunal polychaetes were present in both McLachlan (1985) and Dye and Barros (2005).

In a northern hemisphere comparison, Kotwicki et al. (2014) found that in a recent Baltic Sea study, SGD sites had generally a lower meiofaunal abundance than areas without SGD. The higher level taxa of the meiofaunal community (Kotwicki et al. 2014) was comparable with this study. Kotwicki et al. (2014) found, in rank order of abundance, samples dominated by Nematoda (order of magnitude higher) than Harpticoida, Tubellaria, Gastrotricha, Rotifera, Oligochaeta and Polychaeta. Rarer representations of Ostrocda, Tardigrada, Acari, Bivalvia, and Gastropoda were also observed. The salinity of the sediments (0-5cm layer) in which the samples were collected was approximately 7 PSU (Kotwicki *et al.* 2014), seven times higher than this study.

5.4.2 Nematode Community – compared with other temperate Australian studies

Nicholas and Trueman (2005) assessed the biodiversity of Australian nematodes across 3 states (Northern Territory, New South Wales and Victoria), 3 locations (across 5 sites) and various years. Their study identified a total of 126 nematode species from 102 genera (including one unnamed new genus) and 36 families from their three beach locations. Ninety-five species from 83 genera were found across 2 sites at Rapid Creek Beach, NT (sampled in 1988 and 1992); 59 species from 48 genera at Dolphin Beach (2 sites) in southern NSW (1995-1999); and 47 species from 44 genera at Lorne Beach, Victoria (one site sampled in 1991 only) (see Table 1 in Nicholas and Trueman 2005). In comparison, a total of 37 nematode morphospecies in at least 26 genera from a minimum of 16 families were identified in this study (see Table 5.1).

Excluding the Unknown Nematoda species (sp.A-I) observed in the present study, a comparison was then made against the species observed in Nicholas and Trueman (2005). Of the 27 genera from 16 families common to this study, only 2 genera (Synonchus and Thalassomonhystera or their families (Leptosomatidae and Monhystera, respectively) were not observed at any beach sampled by Nicholas and Trueman (2005). Another 7 morphospecies from the genera Steineridora, Nannolaimus, Adoncholaimus, Prooncholaimus, Para/Mesancanthion, Metadesmolaimus/Paramonhystera and Chromadorid? were also observed in this study but not in Nicholas and Trueman (2005), although their families (Chromadoridae, Ethmolaidmidae, Oncholaimidae, Thoracostomopsidae, and Xyalidae) were (Table 5.8).

5.5 CONCLUSION

Almost 50 species from 9 phyla were collected during the meiofaunal study. Nematodes were the most dominant and most speciose group of organisms collected. The Away group was more abundant and more speciose that the Within and Edge groups however a number of species were unique to the Within group.

The results suggest that groundwater or physical characteristics created by the combination of discharging groundwater and marine conditions may influence meiofaunal community composition on sandy beaches. The combination of these factors creates unique conditions that cause some species to drop out of the assemblage, resulting in an interesting mix of meiofaunal biodiversity that remain.

TABLES

Table 5.1: Total abundance of meiofaunal individuals collected by sampling location summed across all sample times and replicates.

Classification	Sub-group/Family	Genus	Within	Edge	Away	Total	Overall Rank
Annelida	Oligochaeta		5	24	1	30	=8
Gastrotricha			0	0	97	97	5
Gnathostomulida			0	0	4	4	
Kinorhyncha			0	1	0	1	
Rotifera			2	0	16	18	10
Acari	Halacaridae		3	0	2	5	
Platyhelminthes	Turbellaria Sp A		33	260	92	385	2
	Turbellaria Sp B		8	21	143	172	4
Crustacea	Harpacticoida copepods		9	75	112	196	3
	Isopoda		2	0	0	2	
	Ostracoda		0	0	1	1	
	Nauplius		1	0	0	1	
Nematoda	Axonolaimidae	*Ascolaimus*	0	0	1	1	
	Ceramonematidae	*Metadasmynoides*	0	0	1	1	
	Chromadoridae	Chromadorid	1	0	2	3	
		Steineridora	0	0	2	2	
	Desmodoridae	*Catanema/Onyx*	142	51	3041	3234	1
		Metachromadora	1	0	42	43	7

Classification	Sub-group/Family	Genus	Within	Edge	Away	Total	Overall Rank
		Red *Onyx* Sp A	0	1	10	11	=16
		Red *Onyx* Sp B	0	0	5	5	
	Enchelidiidae	*Symplocostoma?*	0	0	6	6	20
	Enoplidae	*Enoplus?*	0	3	2	5	
	Ethmolaidmidae	*Nannolaimus?*	0	0	2	2	
	Leptolaimidae	*Leptolaimus*	0	0	1	1	
	Leptosomatidae	*Synonchus?*	0	0	1	1	
	Linhomoeidae	*Eumorpholaimus*	0	0	2	2	
	Microlaimidae	*Microlaimus*	0	0	4	4	
	Monhysteridae	*Thalassomonhystera*	0	0	5	5	
	Oncholaimidae	*Adoncholaimus*	0	0	2	2	
		Metoncholaimus	1	0	9	10	18
		Oncholaimus	0	0	4	4	
		Prooncholaimus	0	0	3	3	
	Oxystominidae	*Halalaimus*	0	1	4	5	
		Viscosia	0	1	11	12	14
	Thoracostomopsidae	*Para/Mesancanthion?*	2	5	0	7	19
	Xyalidae	*Daptonema*	1	10	0	11	=16
		Gonionchus	1	0	4	5	
		Metadesmolaimus/Paramonhystera	0	1	17	18	=10
		Rhynconema	0	0	13	13	14

Classification	**Sub-group/Family**	**Genus**	**Within**	**Edge**	**Away**	**Total**	**Overall Rank**
		Theristus	3	4	70	77	6
	(Unknown)	Unkn. Nem. Sp. A	0	0	1	1	
		Unkn. Nem. Sp. B	0	0	1	1	
		Unkn. Nem. Sp. C	0	1	4	5	
		Unkn. Nem. Sp. D	3	0	12	15	=12
		Unkn. Nem. Sp. E	0	0	30	30	=8
		Unkn. Nem. Sp. F	0	0	5	5	
		Unkn. Nem. Sp. G	1	0	1	2	
		Unkn. Nem. Sp. H	1	2	1	4	
		Unkn. Nem. Sp. I	0	15	0	15	=12
	Total		**220**	**476**	**3787**	**4483**	
	Total species		***19***	***17***	***43***	***49***	

Table 5.2: Meiofaunal abundance (mean ± s.e. per sample) by sampling event and location for the 13 main taxonomic groups. W = within spring. E = at edge of spring. A = away from spring (see methods). The numerically largest values within each type and time are shown in bold.

Year Month	**Oct 2004**			**Jan 2005**			**Feb 2005**			**Mar 2005**			**Nov 2005**		
Location	**W**	**E**	**A**	**W**	**E**	**A**	**W**	**E**	**A**	**W**	**E**	**A**	**W**	**E**	**A**
Oligochaeta	0.3±0.2	0	0	0.3±0.2	0	0	0.2±0.2	0	0	0	4.0±2.2	0	0	0	0.2±0.2
Gastrotricha	0	0	12.5±2.3	0	0	1.7±1.1	0	0	1.7±1.0	0	0	0	0	0	0.3±0.3
Gnathostomulida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7±0.5
Kinorhyncha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<<0.1
Rotifera	0	0	1.8±1.6	0.3±0.2	0	0.5±0.3	0	0	0.3±0.2	0	0	0	0	0	<<0.1
Halacaridae	0.5±0.2	0	0.2±0.2	0	0	0	0	0	0.2±0.2	0	0	0	0	0	<<0.1
Turbellaria	**2.2±1.1**	0.7±0.3	14.2±4.2	0.7±0.5	**3.5±2.0**	3.2±0.8	0.7±0.3	6.3±1.4	1.0±0.5	2.7±1.6	**35.0±8.8**	13.7±6.9	**0.7±0.3**	1.3±0.6	5.0±1.9
Harpacticoida	0.3±0.2	0.2±0.2	6.7±2.0	0	1.2±0.4	1.3±0.7	0.5±0.2	**9.2±9.**0	9.0±6.3	0.5±0.5	1.3±0.6	1.2±0.8	0.2±0.2	0.7±0.3	0.5±0.3
Isopoda	0	0	0	0.3±0.3	0	0	0	0	0	0	0.2±0.2	0	0	0	<<0.1
Ostracoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2±0.2
Nauplius	0	0	0	0	0	0	0.2±0.2	0	0	0	0	0	0	0	<<0.1
Nematoda	0.7±0.3	**0.8±0.4**	**91.0±34.9**	**0.8±0.5**	2.2±0.6	**345.2±126.6**	**20.7±19.3**	7.3±1.7	**42.2±18.9**	**3.3±3.0**	3.9±1.9	**47.0±23.9**	**0.7±0.4**	**1.7±1.0**	**27.8±6.6**
Total Inds	24	10	771	15	41	2111	133	137	326	39	266	371	9	22	208
Total MSpp	7	4	14	8	5	17	10	11	13	6	9	12	5	6	26

Table 5.3: Contingency table for number of individuals in the four main meiofaunal groups plus all others combined. T = total individuals. % = percent of individuals. R = Rank for that particular group and sampling location.

Taxon	**Within**			**Edge**			**Away**			**Total**	**Rank**
	T	**%**	**R**	**T**	**%**	**R**	**T**	**%**	**R**		
Nematoda	157	71	1	95	20	2	3319	88	1	**3571**	**1**
Turbellaria	41	19	2	281	59	1	235	6	2	**557**	**2**
Harpacticoida	9	4	4	75	16	3	112	3	3	**196**	**3**
Gastrotricha	0	0	-	0	0	-	97	2.5	4	**97**	**4**
Others	13	6	3	25	5	4	121	0.5	5	**62**	**5**
Total	**220**	***4.9***	***3***	**476**	***10.6***	***2***	**3787**	***84.5***	***1***	**4483**	

Table 5.4: Summary of PERMANOVA results for the interaction of year month (fixed) and location (fixed) (Year-Month*Location). Data was 4th root transformed. 9999 permutations.

Analysis	***df***	**SS**	**MS**	**Pseudo-*F***	***P*(perm)**
All Meiofauna Groups					
Year	4	27598	6900	3.6718	0.0001
Location	2	33330	16665	8.8687	0.0001
Year*Location	8	38776	4847	2.5795	0.0001
Res	66	124020	1879		
Total	80	224600			
Without nematodes					
Year	4	23200	5800	3.9144	0.0002
Location	2	23956	11978	8.0836	0.0001
Year*Location	8	31285	3911	2.6392	0.0005
Res	53	78532	1482		
Total	67	157700			
Nematodes Only					
Year	4	26134	6534	4.6459	0.0001
Location	2	26369	13185	9.3753	0.0001
Year*Location	8	36421	4553	3.2373	0.0001
Res	53	74534	1406		
Total	67	170240			

Table 5.5: Pairwise comparison for meiofauna analyses. ns = not statistically significant

Pairwise Groups	All Meiofauna		Without Nematodes		Nematodes only	
	t	***P*(perm)**	***t***	***P*(perm)**	***t***	***P*(perm)**
Year						
2004_10, 2005_01	1.8255	0.0042	2.249	0.0008	2.455	0.0007
2004_10, 2005_02	1.6767	0.0206	1.087	ns	1.6628	0.0462
2004_10, 2005_03	1.6893	0.0185	1.6742	ns	1.5629	0.0484
2004_10, 2005_11	2.0257	0.0014	1.4398	ns	3.5125	0.0001
2005_01, 2005_02	1.6257	0.0190	2.3555	0.0020	1.3448	ns
2005_01, 2005_03	2.1212	0.0016	2.6128	0.0011	1.5098	ns
2005_01, 2005_11	2.2763	0.0002	2.5545	0.0003	3.0147	0.0001
2005_02, 2005_03	1.7379	0.0202	1.6638	ns	1.3791	ns
2005_02, 2005_11	2.1698	0.0009	1.0834	ns	3.165	0.0001
2005_03, 2005_11	1.8899	0.0049	1.4784	ns	2.2165	0.0002
Location						
Within, Edge	1.3516	ns	1.4575	ns	1.3635	ns
Within, Away	3.1983	0.0001	2.9996	0.0001	3.0345	0.0001
Edge, Away	4.0113	0.0001	3.6494	0.0001	3.9311	0.0001

Table 5.6: Comparative summary of abundances from SIMPER analyses of dissimilarity (all meiofauna groups) of top five groups contributing most to dissimilarity (top cumulative >50%) between sampling locations. W = within. A = away. E = edge. DG = dominant group for that comparison. Mag = difference in magnitude of average abundance between groups. '-' = that species not collected within one of the groups, thus no comparison possible. Cumm Diss >50% (% sum of the species listed that cumulatively contribute to >50% dissimilarity between groups).

	A & W (Diss %)	**DG (mag)**	**A & E (Diss %)**	**DG (mag)**	**E & W (Diss %)**	**DG (mag)**
Average Pairwise Group Dissimilarity	83.5		77.6		76.9	
Catanema/Onyx (N)	22.4	A >(x4) W	18.7	A >(x3) E	19.7	E > W
Turbellaria blob	9.0	A >(x4) W	8.6	A >(x3) E	-	-
Turbellaria-long	8.2	A > W	11.1	E >(x2) A	22.5	E >(x2) W
Harpacticoid copepod	8.1	A >(x3) W	8.6	A > E	14.7	E >(x2) W
Theristus (N)	6.1	A >(x6) W	6.0	A >(x4) E	-	-
Cumm Diss >50%	53.7		53.0		56.8	

Table 5.7: Comparative summary of higher order taxa found in recent Australian studies. Numbers identify the rank of a particular species in the identified study. P = present but not ranked highly (i.e. low numbers). * = Away group only

Phyla	**Sub-group**	**This Study***	**Dye & Barros 2005**	**McLachlan 1985**
Nematoda		1	1	1
Platyhelminthes	Turbellaria	2	3	4
Crustacea	Harpacticoida Copepoda	3	2	2
	Other Copepoda		4	
	Cumacea		P	
	Syncarida		P	
	Tanaidacea		P	
	Ostracoda	P	P	
Gastrotricha		4	P	P
Annelida	Oligochaeta	5	P	3
	Polychaeta		5	P
Rotifera		6		
Gnathostomulida		P		
Kinorhyncha			P	
Acari	Halacaridae	P		P
Echiura			P	
Tardigrada			P	
Other Taxa	Undefined			P
Total Groups		9	14	8

Table 5.8: Comparison of Nematoda found in this study and Nicholas and Trueman (2005). G = genus observed. F = family observed but not genus. * Incorporates data from Nicholas & Hodda (1999).

This Study SE SA		Nicholas & Trueman 2005*		
Family	**Genus**	**Dolphin Beach S NSW**	**Lorne Beach, SW Victoria**	**Rapid Creek Darwin NT**
Axonolaimidae	*Ascolaimus*	F	F	G
Ceramonematidae	*Metadasmynoides*	G	F	G
Chromadoridae	Chromadorid	F	F	F
	Steineridora	F	F	F
Desmodoridae	*Catanema/Onyx*	– / G	– / G	– / G
	Metachromadora	G	G	G
	Onyx	G	G	G
Enchelidiidae	*Symplocostoma?*	–	G	G
Enoplidae	*Enoplus?*	G	G	G
Ethmolaidmidae	*Nannolaimus?*	–	F	F
Leptolaimidae	*Leptolaimus*	F	G	G
Leptosomatidae	*Synonchus?*	–	–	–
Linhomoeidae	*Eumorpholaimus*	–	F	G
Microlaimidae	*Microlaimus*	G	G	G
Monhysteridae	*Thalassomonhystera*	–	–	–
Oncholaimidae	*Adoncholaimus*	F	F	F

	This Study SE SA	Nicholas & Trueman 2005*		
Family	**Genus**	**Dolphin Beach S NSW**	**Lorne Beach, SW Victoria**	**Rapid Creek Darwin NT**
	Metoncholaimus	G	F	G
	Oncholaimus	G	G	G
	Prooncholaimus	F	F	F
Oxystominidae	*Halalaimus*	G	G	G
	Viscosia	F	F	G
Thoracostomopsidae	*Para/Mesancanthion?*	F	F	F
Xyalidae	*Daptonema*	F	G	G
	Gonionchus	G	G	G
	Metadesmolaimus/Paramonhystera	F	F	F
	Rhynconema	G	G	G
	Theristus	G	G	G
Total Families	16	11	14	14
Total Genera	27	12	19	20

FIGURES

Figure 5.1: Image of a beach spring located at Piccaninnie Ponds Conservation Park showing a) where samples were collected, b) an example of the sand-like slurry boils referred to in the text, c) discharge for of small springsnature of springs), and d) how they can be a trap for marine-derived detritus. W = Within. E = Edge. A = Away (500 m further west). Scale bar is 1 m at lower edge of image only (in 'a'). Image captured at low tide. Note the erosion created by the springs. These craters can be formed and be backfilled on a daily (tidal) basis.

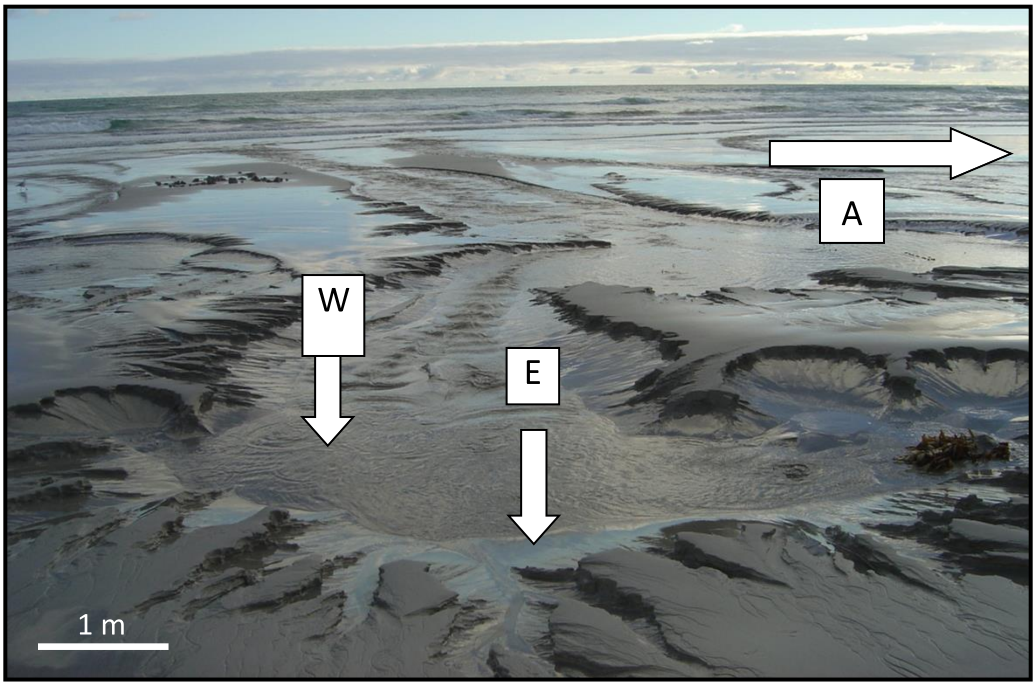
Figure 5.2: Aerial image of the location of the groundwater beach spring (SPR01) with Pinccaninnie Ponds Conservation Park (CP) where meiofauna sampling was undertaken. A = Away. E = Edge. W = Within

Figure 5.3: Number of taxa sampled across sample types shown by a Venn digram of overlapping locational subsets. Numbers in square brackets are the totals for each type of sample.

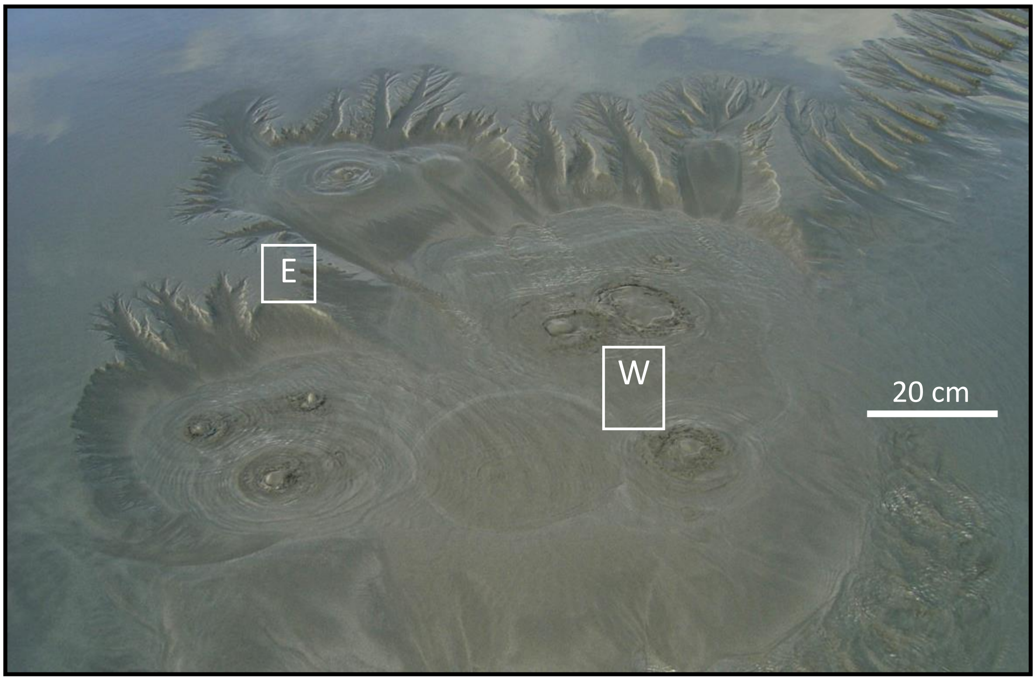
Figure 5.4: MDS ordination of meiofauna by sampling location; a) all data, and b) data contained within the central cluster. Based on Bray-Curtis similarity measures. Data have been 4th root-transformed. Stress values as stated.

Figure 5.1:

a)



b)



c)

d)

Figure 5.2

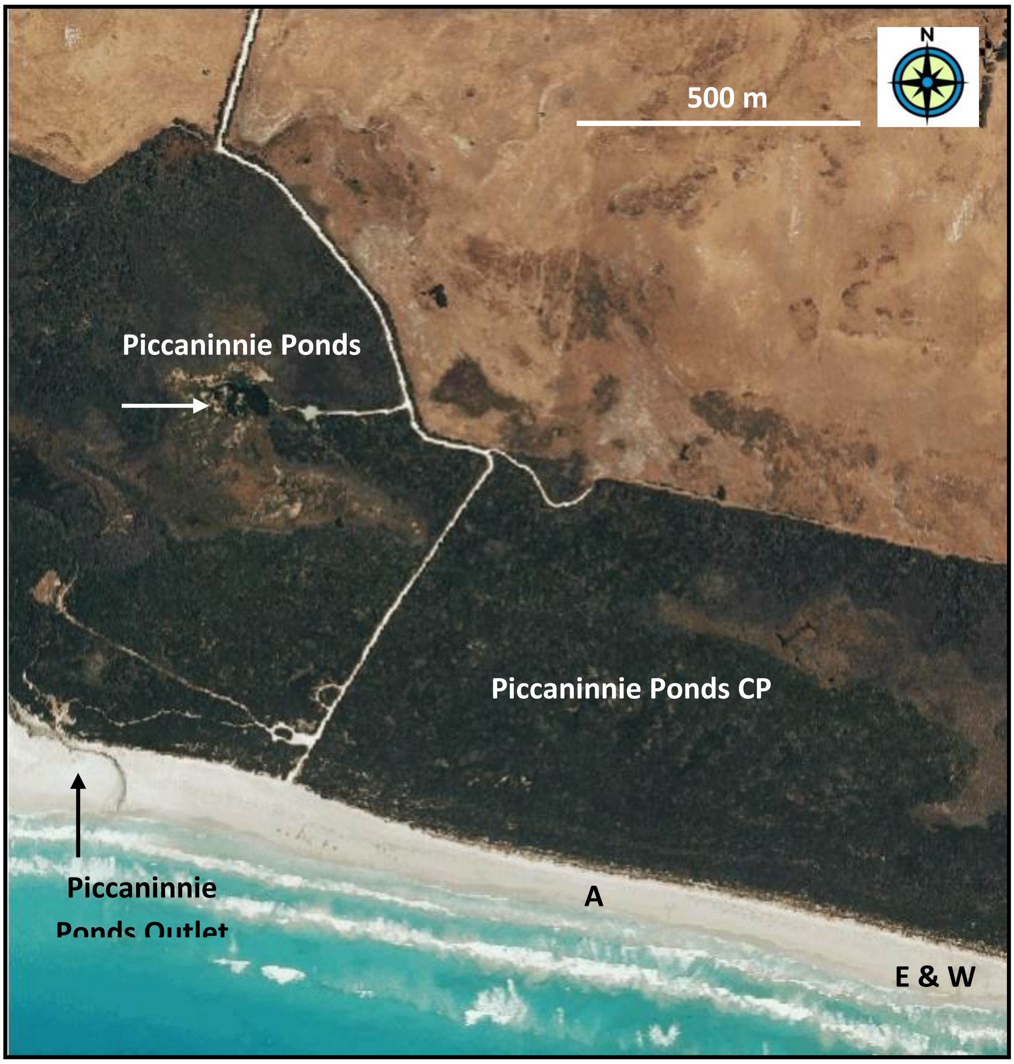

N
500 m
Piccaninnie Ponds
Piccaninnie Ponds CP
Piccaninnie
Ponds Outlet
A
E & W

Figure 5.3:

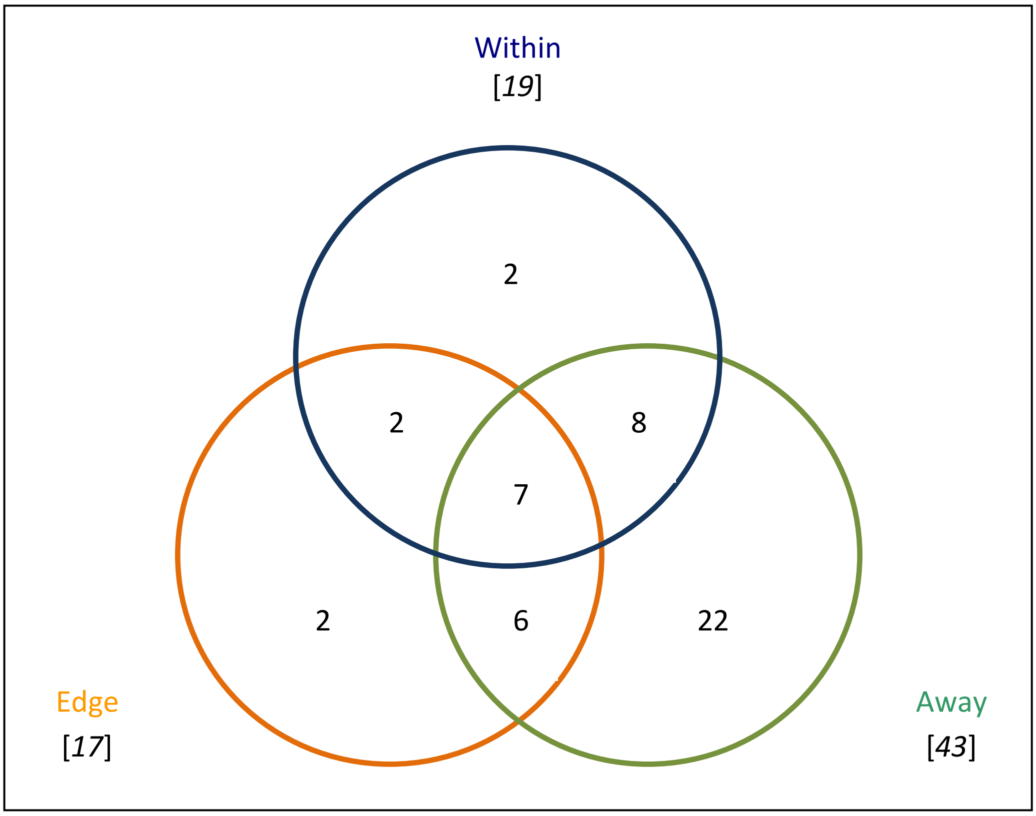
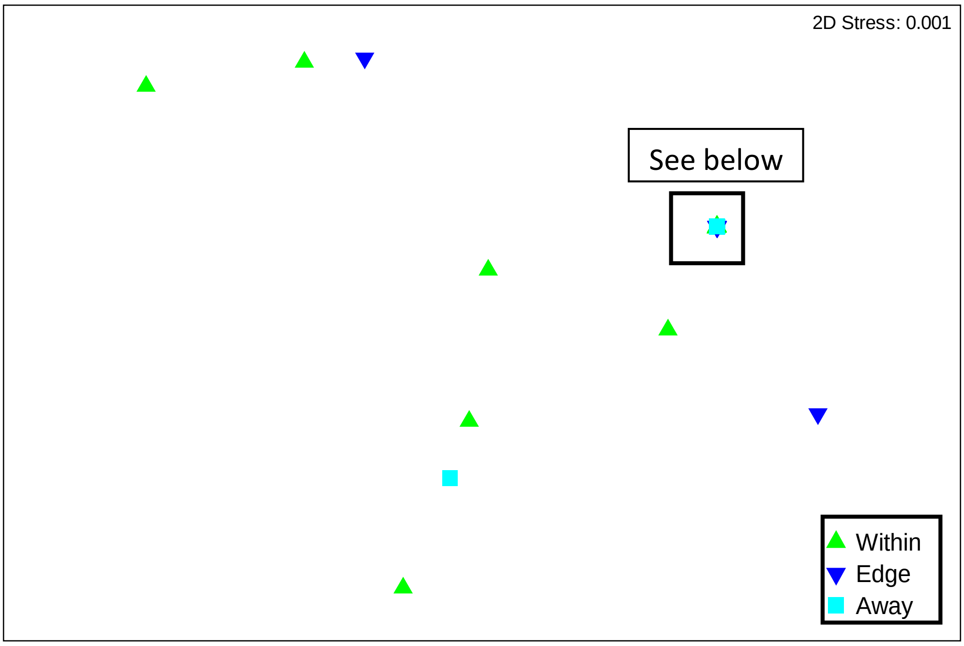
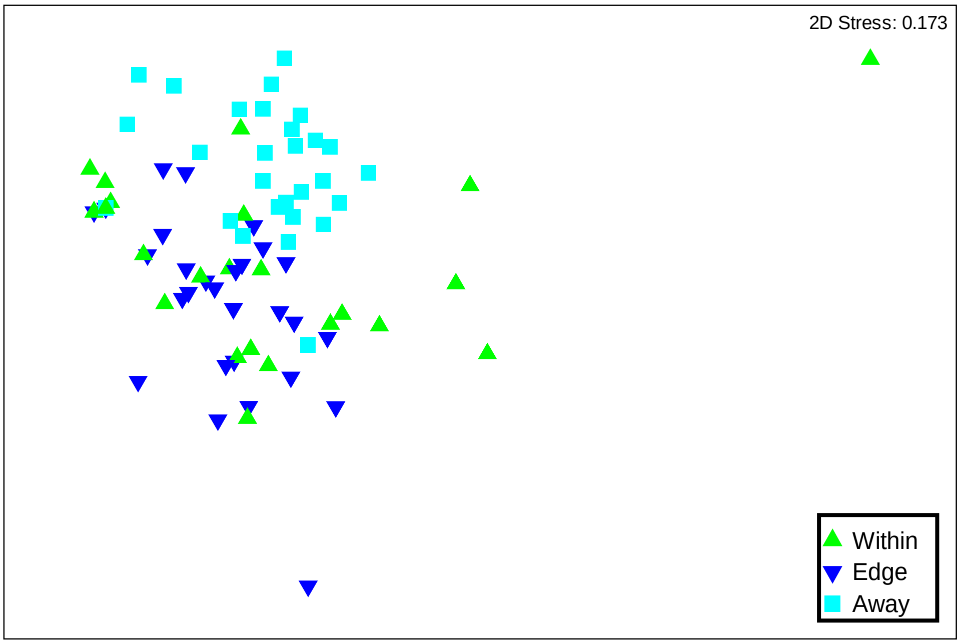


Figure 5.4:

a)


2D Stress: 0.001
See below
Within
Edge
Away

b)


2D Stress: 0.173
Within
Edge
Away

CHAPTER 6 – BIVALVES

6 Tracking groundwater signals in an aquatic organism: the sandy-beach bivalve *Paphies elongata*

6.1 INTRODUCTION

This chapter attempts to describe a different approach for assessing groundwater-dependence in coastal organisms (e.g. molluscs) to complement water quality (Chapter 3) and invertebrate faunal (Chapters 4 & 5) information presented earlier.

Assessing dependence on groundwater of coastal organisms is useful from a management perspective in that it helps ascertain if there are ecological linkages between groundwater discharge and nearshore ecosystems.

Thus, there was a need to determine any groundwater dependence or association if SENRMB or other water authorities are to know if groundwater-dependent fauna need specific water allocations for habitat and community protection.

Assessing isotopic ratios in the flesh of organisms helps to assess connection to groundwater resources and enables researchers to establish at what trophic level(s) such an interaction is occurring (see Chapter 2 for description of transfer of nutrients and isotopes between trophic levels).

This small-scale investigation asks the question; will individuals collected from areas under direct groundwater influence have elevated levels of $\delta^{15}N$ in body tissues when compared to individuals collected in areas away from groundwater influence? That is, if the local *P. elongata* population feed on groundwater-derived food sources then we would expect to find elevated $\delta^{15}N$ ratios in these bivalves associated with groundwater discharge.

6.1.1 Stable Isotope Analysis of $\delta^{15}N$

Stable isotopes and their use in ecological studies have been discussed in Chapter 1. Organisms take up nitrogen in different ways so that the isotopic composition of plants and animals varies (Krebs 2001). On average, $\delta^{15}N$ increases by an average of 3.4‰ in animals relative to their diet (Krebs 2001). This information plays a practical role when investigating trophic structure of an ecological community and its accumulation of $\delta^{15}N$ from a specific source (e.g. groundwater) by allowing counting of trophic levels. Thus, filter-feeding bivalves should show enrichment compared to their food (*P*hytoplankton) or the water they both grow in, especially close to groundwater sources.

Thus, it may also be possible to try to use $\delta^{15}N$ as a "tracer" where enrichment levels in the flesh of a bivalve like *Paphies elongata* might be proportional to the proximity of a groundwater source.

As described above and in Chapter 1 of this thesis, stable isotope analyses can be used to infer uptake of N from groundwater sources by plants and up through food chains to animals (consumers) to describe the trophic structure of ecosystems. This chapter adopts this theory to assess $\delta^{15}N$ ratios in the flesh of the common southern Australian intertidal bivalve *P. elongata*.

6.1.2 Paphies elongata

The wedge shell (a bivalve of the Family Mesodesmatidae), *Paphies elongata* (Reeve, 1854), is found commonly in the mid- to low-intertidal zone of exposed sandy beaches (*P*eterson & Wells 1998; Edgar 2000). Their Australian distribution ranges from temperate (south-west WA, SA, Victoria, Tasmania, NSW; Edgar 2000) to tropical (Queensland to Torres Strait; Lamprell & Whitehead 1992) regions. *P. elongata* grow to a maximum length of between 25mm (Edgar 2000) and 30mm (Lamprell& Whitehead 1992). *P. elongata* use both suspension and deposit feeding strategies.

Descriptive biological information on Australian mesodesmatids is provided in Allan (1959), Cotton (1961), Roberts (1984), Lamprell & Whitehead (1992), and Healy & Lamprell (1998a,b) but specific research within the group is limited and much remains to be learned (Healy & Lamprell 1998a,b; Peterson & Wells 1998).

Database searches (e.g. via Current Contents, Biological Abstracts) using the terms "*Paphies*", "Mesodesmatidae" or previous generic names (e.g. *Donacilla*) returned only limited recent studies. No recent Australian studies concentrating on *P. elongata* specifically were found (but see Roberts 1984).

In comparison, a larger number of studies involving four New Zealand *Paphies* species have been published. These four species include: the common New Zealand pipi, *P. australis* (Hooker, 1997; Hull et al., 1998; Cole et al., 2000; Cummings & Thrush 2004; Norkko et al., 2005; Norkko et al., 2006a, 2006b; Hewitt &Norkko, 2007); the tuatua, *P. donacina* (Marsden, 1999a, 1999b, 2000, 2002; Cranfield& Michael, 2001); *P. subtriangulata* (Grant et al., 1998); and the toheroa, *P. ventricosa* (Akroyd et al., 2002; Beentjes et al., 2006). Both *P. donacina* and *P. subtriangulata* are known as tuatua in New Zealand; the former is predominantly subtidal in its distribution while the latter is intertidal (Leach et al., 2001). These New Zealand studies have considered influences on the distribution and abundance (e.g. Akroyd et al. 2002; Beentjes 2006) and recruitment and growth dynamics (e.g. Marsden 2000, 2002; Cranfield & Michael 2001) of *Paphies* spp. populations.

6.2 METHODS

6.2.1 Collection of Specimens and Timing

For this investigation, the larger and regionally recreationally- and commercially-important bivalve *Donax deltoides* (Goolwa cockle or pipi) was initially targeted. Preliminary investigations (using methods of James & Fairweather 1995) returned only limited numbers (<5 individuals) from sampling areas on the SE beaches during the October 2004 sampling visit. During this visit, it was observed that there were numerous *P. elongata* at the ‘away’ area (and so they were sampled) but none were observed at the ‘within’ area.

Only a limited number of *D. deltoides* were observed during November 2005 but *P. elongata* were again present in suitable numbers at both locations, and so were once more targeted for collection.

The bivalve, *Paphies elongata*, was eventually collected from areas ‘within’ and ‘away’ from the influence of discharging groundwater for the analysis and comparison of their $\delta^{15}N$ ratios.

Samples were collected in October 2004 and November 2005, near Piccaninnie Ponds Conservation Park, South East South Australia from an area under the influence of the discharging beach springs (‘within’ samples) and from an area removed from groundwater/springs influence (‘away’ samples) (Figure 6.1, but see also Figures 5.1 for Spring images). The distance between the within and away sampling areas was approximately 500m.

Beach Spring 01 was used for the ‘within’ group as this spring appeared to be reasonably static with respect to its location, size and discharge. Beach Spring 01 had a salinity (mean ± s.e.) of 0.43 ± 0.03 ppt (*n*=57) (see Chapter 3 Water Quality) and ranged (min. – max.) between 0.22 ppt and 1.21 ppt, whereas a nearby Marine site (Discovery Bay further east of Beach Spring 01) had mean salinity of 31.6 ± 0.1 ppt.

Individuals were collected from both areas during the November 2005 visit using a spade to turn over sediments and expose *P. elongata* individuals where present. Sampling continued until a cumulative volume of approximately 250 mL of individuals (in two 125 mL containers) were collected from each sampling area (see Results for final sample sizes) and then these were immediately refrigerated. Samples were later drained of excess water before freezing. Samples were not given time in water to purge stomach contents (depurate). Samples remained frozen (< 2 weeks) until laboratory analysis.

6.2.2 Sample processing

Each individual bivalve was measured for shell length and height, and wet and dry weights. Complete individuals were dried at low temperature (50°C) until constant weight was achieved (approximately 72 hours). Maintaining a low constant temperature was considered to be essential to prevent proteins from denaturing, which would result in a potential loss of nitrogen from the sample (Fry 1999).

The dried flesh from each individual was separated from the shell and the flesh then ground to a fine consistency using a mortar and pestle.

6.2.3 Sample analyses

Sufficient dry, ground sample (approximately 2.0 ± 0.5g) was transferred to small tin cups for isotopic analysis of $\delta^{15}N$. While a precise mass of a sample is not required for isotope ratio calculations, precise mass measurements (to 6 decimal places) were required for the calculation of accurate elemental analyses (e.g. percentage total nitrogen) in a sample. Insufficient precision (i.e. not enough decimal places) was used when measuring the mass of individuals in the Away_2004 samples, so precise nitrogen values for this group were unable to be calculated. Approximately 80 – 100 µg of nitrogen (total) was required for the valid analysis of $\delta^{15}N$ (Daniel Jardine pers. comm.).

No studies stating the nitrogen content of *P. elongata* were found in the literature, so pilot studies were first undertaken to estimate the percentage of nitrogen [% N] in these *P. elongata*. Initial sample mass was based on published values of percent protein for *Crassostrea gigas* (*P*acific oysters). A subsequent conversion calculation was applied to estimate a sample mass range to permit meaningful instrumental analysis in an attempt to obtain a valid estimate of percent nitrogen, and therefore a correct mass of nitrogen in the flesh of *P. elongata*. The calculation was based on a range of published values that stated the percent nitrogen in protein of molluscs.

Samples were analysed by Flinders Advanced Analytical Laboratory (Flinders University, Bedford Park, South Australia) using a GV Instruments (Manchester, UK) IsoPrime Stable Isotope Ratio Mass Spectrometer and an elemental analyser (EuroVector, Milan, Italy). In-house standards, dummy samples, sample repeats and blanks were implemented by laboratory staff during analysis to ensure quality control of the analysis. Instrumental precision was 0.03 ‰ for $\delta^{15}N$ on average.

Stable isotope ratios of $\delta^{15}N$ / $\delta^{14}N$ are expressed as the relative per mil (‰) difference between the sample and conventional standards (atmospheric nitrogen used as the standard reference for nitrogen) and given by the formula (*P*eterson & Fry 1987):

$$\delta^{15}N = [\ (Rsample / Rreference) - 1\ ] \times 1000\ (‰)$$

where R is $^{15}N/^{14}N$ and the reference is atmospheric nitrogen.

6.2.4 Statistics

Data were analysed using one-way analysis of variance (ANOVA) (SYSTAT v11). Samples were analysed by combining location (either 'within' or 'away') with year of collection (2004 or 2005), which resulted in three separate groups (Away_2004, Away_2005 and Within_2005). These data were set out as three independent groups to permit multiple comparisons (rather than do two separate analyses of away vs. within and 2004 vs. 2005) for each variable (i.e. for more statistical power).

6.3 RESULTS

6.3.1 Shell Length

The 'away' group of *Paphies elongata* specimens were slightly larger with shell length (mean ± s.e.) 20.2 ± 0.6 mm (n = 49) for the Away_2004 group, 22.4 ± 0.3 mm (n = 48) for Away_2005, and 19.6 ± 0.6mm (n = 48) for Within_2005 samples (Table 6.1; Figure 6.2a).

The Away_2005 group had a longer shell length on average than the other two groups. A one-way ANOVA found a statistically-significant difference (P = 0.001) in mean shell length between groups. Tukey post-hoc test for multiple comparisons found a statistically-significant difference (P = 0.010) between Away_2004 and Away_2005, and also between Away_2005 and Within_2005 (P = 0.001) groups, with the Away_2005 being larger in both cases. The pairwise comparison between Away_2004 and Within_2005 was not statistically significant (Table 6.2).

6.3.2 Dry Weight

Mean dry weights (per bivalve including shell) were 765 ± 64 mg (mean ± s.e.) (n = 39) for Away 2004, 1131 ± 60 mg (n = 48) for Away_2005, and 847 ± 66 mm (n = 48) for Within_2005 (Table 6.1; Figure 6.1a).

A one-way ANOVA found a statistically-significant difference ($P < 0.001$) in mean dry weight between groups. Tukey post-hoc multiple comparisons found a statistically significant difference ($P < 0.001$) between Away_2004 and Away_2005; and between Away_2005 and Within_2005 (P = 0.003). The pairwise comparison between Away_2004 and Within_2005 was statistically non-significant (Table 6.2). Thus the Away_2005 group were, on average, larger and heavier than the other two sample groups.

6.3.3 Nitrogen

Stable isotope ratio values ($\delta^{15}N$) in the flesh of *P. elongata* were 8.30 ± 0.06 ‰ $\delta^{15}N$ (mean ± s.e.) (n = 48) for Away_2004, 8.45 ± 0.04 ‰ $\delta^{15}N$ (n = 48) for

Away 2005, and 8.41 ± 0.06 ‰ $\delta^{15}N$ (n = 45) for Within_2005 samples (Table 6.1; Figure 6.2c) but the mean $\delta^{15}N$ between location-year groups was not statistically different (P = 0.151) (Table 6.2).

No percent nitrogen measurements could be calculated for Away_2004 samples (see Methods above). The percentage of nitrogen (dry flesh only) was 10.2 ± 0.1 %N (mean ± s.e.) (n = 48) for Away 2005 and 9.3 ± 0.4 %N (n = 45) for Within_2005 samples (Table 6.1; Figure 6.2c). A one-way ANOVA found a statistically-significant difference (P = 0.025) in mean percent nitrogen between these two groups, with less N content associated with groundwater sources.

6.4 DISCUSSION

The results for this preliminary investigation found no real biological difference between the groups in regard to $\delta^{15}N$, whereas some statistical differences were observed for bivalve morphometrics (i.e. shell length and dry weight). This study is the first to analyse the $\delta^{15}N$ content in the flesh of this common sandy beach bivalve, *Paphies elongata*.

These results suggest that, whilst the chosen statistical analyses revealed significant P values (with the exception of ^{15}N), these differences were relatively inconsequential in a biological sense. That is, the groundwater-influenced "within" samples were only slightly smaller in length, height, weight and percent nitrogen content but there was no difference in ^{15}N (Table

6.1). ANOVA results (Table 6.2) suggest no ^{15}N enrichment in the tissue of *P. elongata* as a result of their proximity to groundwater sources.

Generally the Within_2005 data displayed the greatest range of values across each of the selected variables (except dry weight). The greater range is likely to have been manifested as by the number of outliers (most evident in Figure 6.2c) within this group.

Whilst 'within' samples were collected from areas where groundwater was discharging (i.e. with 'freshwater' salinities, see Chapter 3), the continuity of influence from the seawater varied (e.g. due to tidal inundation, wave, and longshore current effects) caused fluctuation in the salinity experienced and so the realised delivery of organic matter from the groundwater source is unknown. There is possibly a need to assess the organic content in groundwater at its source (see Chapter 3 for NO_x values). It is possible that *P. elongata* are likely to opportunistically feed more often on marine-derived organic matter (e.g. surf-zone diatoms) rather than on the groundwater/spring-derived organic content. That is, particulate organic matter may be more abundant and readily available from marine sources than from groundwater sources. Also much of the macroscopic organic content within the beach springs were observed to be marine in origin (e.g. kelp wrack). This material is likely to have been washed into the springs during high tides by wave action, where it remains within/beneath the sand slurry crater created by the discharging groundwater. Any specific groundwater-derived organic matter is likely to be small in size (e.g. < 0.5 mm) as a result of the porosity of the substrate through which the groundwater moves. The nature of the subterranean (e.g. interstitial spaces) is likely to influence the size of flora (e.g. to microflora) or fauna (e.g. meiofauna size or smaller, see Chapter 5) that exist there.

Although individuals were collected from an area under the influence of groundwater, it remains very probable that these individuals are likely to feed on marine-derived organic matter. Subsequent studies should incorporate the analyses of organic content in both the water column and any surf-zone algae growing in the groundwater from the springs. Analyses should consider the type (e.g. phytoplankton, seagrass, macroalgae, terrestrial) and origin (e.g. groundwater, freshwater, marine, terrestrial detritus) of organic matter type as additional factors (e.g. live or detrital material) when attempting to explain ^{15}N concentrations resulting from groundwater influence.

Thus it may be more appropriate for future studies to sample organic matter discharging with the groundwater from springs and compare that to organic content derived from predominantly marine sources.

Whilst it may be possible that individuals collected from the "within" area may be able to tolerate (or avoid) fluctuations in salinity due to variable groundwater influences (not tested in this study), ^{15}N values were not elevated in individuals collected from this area when compared to individuals collected away from groundwater influence. They were slightly smaller from

Within and this could suggest that some association with groundwater influence energy sources may exist.

No evidence of a difference in shell length against dry weight (Figure 6.2a) and only slightly greater variation in ^{15}N (Figure 6.2b) and percentage N (Figure 6.2c) between the groups was apparent. These results suggest that the individuals collected are from the same local population although from the two separate locations.

Sedimentary disturbance from discharging groundwater may also affect the distribution of infauna such as *P. elongata*. It was observed that discharging groundwater created a "quicksand" effect that can turn firm-packed sediments into a mobile sand-slurry. These effects may create less-than-favourable conditions for bivalves to reside in and may have a greater effect than that of reduced or variable salinity conditions. This may be why sampling attempts to collect individuals from the 'within' area during the 2004 sampling returned only very low numbers of small individuals. That is, these groundwater spring areas and areas of confluence with marine waters may, in general, be more variable through time, resulting in less-than-ideal conditions for macroscopic individuals such as bivalves to reside.

This point-source sedimentary disturbance effect (from discharging groundwater that scours away the beach sand) may, in conjunction with wave action, release any trapped interstitial organic matter from below the sand surface. This may create an environment of overall increased particulate matter for swash-zone inhabitants. This consideration is testable in future work if sufficient fauna to work with could be collected. Further studies could thus assess if swash zone fauna in areas under direct influence from discharging groundwater gain most of their energy from organic matter released from within the beach rather than from incoming offshore sources directly (the argument could be that organic matter released from beach was initially from offshore sources).

It is possible that diffuse submarine groundwater discharge (Moore 2010) along this coastline could affect all sites but further investigation into the volume, spatial and temporal extent of this processes needs to be identified and quantified to answer this question (e.g. Herpich 2010).

It should be noted that this was a very preliminary study that involved only two time periods and only two locations. The information gained and lessons learnt through this study can be used to build a more relevant hypothesis that is valid and robust and that can inform the development of a specific controlled experimental test that investigates the influence of groundwater sources on, and ^{15}N uptake by, biota that reside in nearshore coastal habitats.

Although *Paphies elongata* were not the preferred species for using in this study (explained in the Methods section of this Chapter), their presence, abundance and distribution within the study area meant that they were suitable for use when attempting to track any groundwater signals in biota associated with groundwater in nearshore coastal habitats. Originally other approaches, such as transplants of infaunal communities had been planned (inconjuction with those suggestions proposed in Section 4.4.1) but no distinctive assemblages were found on the beach in sufficient prevalence to warrant the effort on such experiments.

6.5 CONCLUSION

The study revealed no difference in ^{15}N in the flesh of *Paphies elongata* between sample groups suggesting that the individuals collected were from the same localised population. Thus difference in ^{15}N accumulation as a result of groundwater influences was not evident in the tissue mass of *P. elongata* sampled in this study. These filter-feeding bivalves did not appear to be strongly influenced nutritionally by organic matter derived from groundwater sources. Thus, these results argue against any general groundwater dependence of this taxon.

This study did find that N concentration is the flesh of *P. elongata* was approximately 10 %. It is likely that this is the first published data relating to N concentration in this species. This may assist other research with future studies of N uptake.

The information gained through this preliminary study can guide prospective researchers to better define and design future studies that test hypotheses involving groundwater interactions with nearshore coastal marine biota.

TABLES

Table 6.1: Summary (mean±se (n)) of selected variables for *Paphies elongata* by sample group. Wet and dry weights include the shell. $\delta^{15}N$ and nitrogen concentrations [N] are shell-free (i.e. flesh only) values. '-' no results for this sample group (see Methods for explanation). DW = dry weight.

Location_Year	Shell Length mm	Shell Height mm	Wet Weight mg	Dry Weight mg	$\delta^{15}N$ ‰	[N] % DW
Away_2004	20.2±0.6 (49)	11.1±0.4 (39)	1027±86 (39)	765±64 (39)	8.30±0.06 (48)	-
Away_2005	22.4±0.3 (48)	13.2±0.2 (48)	1669±73 (48)	1154±56 (48)	8.45±0.04 (48)	10.2±0.1 (48)
Within_2005	19.6±0.6 (48)	11.6±0.4 (48)	1271±97 (48)	838±68 (48)	8.41±0.06 (45)	9.3±0.4 (45)

Table 6.2: One-way ANOVA results for selected variables for *Paphieselongata* by sample group. A'04 = Away 2004; A'05 = Away 2005; W'05 = Within 2005 (see Methods for explanation of these three sample groups). Bold *P* values are significant at α=0.05; ns: statistically not significant (*P* >0.05). "na" = not applicable because pairwise comparison for %[N] has only two sample groups (see Methods). See Table 6.1 for group means.

Variable	df	P	Pattern of Significance	Pairwise Comparison (Tukey) Group & Direction (*P*)
Shell Length	2,142	0.001	A'05 > A'04 = W'05	A'05 > W'05 (*P* = 0.001) A'05 > A'04 (*P* = 0.010) A'04 = W'05 (ns)
Dry Weight	2,132	<0.001	A'05 > A'04 = W'05	A'05 > A'04 (*P* <0.001); A'05 > W'05 (*P* = 0.001); A'04 = W'05 (ns)
δ^{15}N	2,138	0.151	A'04 = A'05 = W'05	All ns
%N	1,91	0.025	A'05 > W'05	Na

FIGURES

Figure 6.1: Aerial image of the location of the groundwater beach spring (SPR01) with Pinccaninnie Ponds Conservation Park (CP) where bivalve sampling was undertaken. A = Away. W = Within

Figure 6.2: Scatterplots of *Paphies elongata* shell length against a) dry weight; b) delta $\delta^{15}N$; and c) percent nitrogen (no Away_2004 group for this variable).

Figure 6.1:

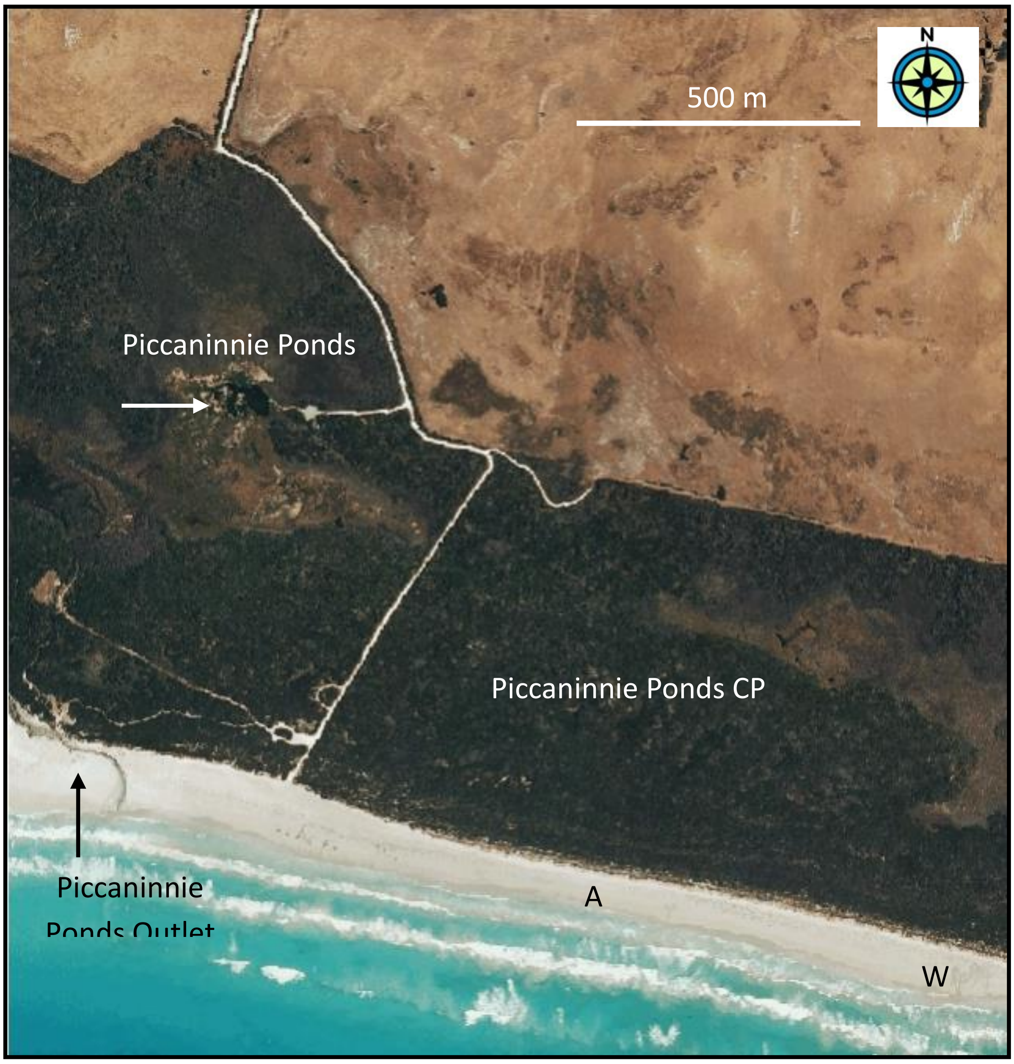
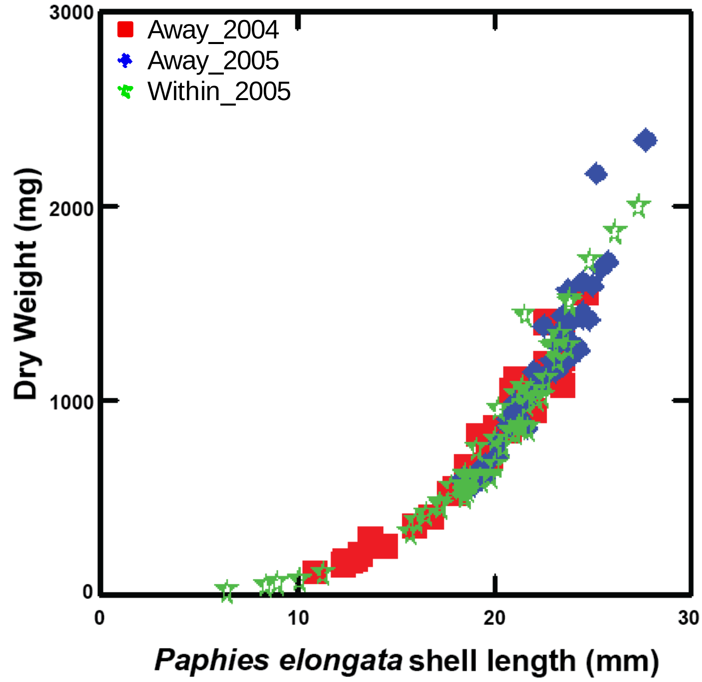
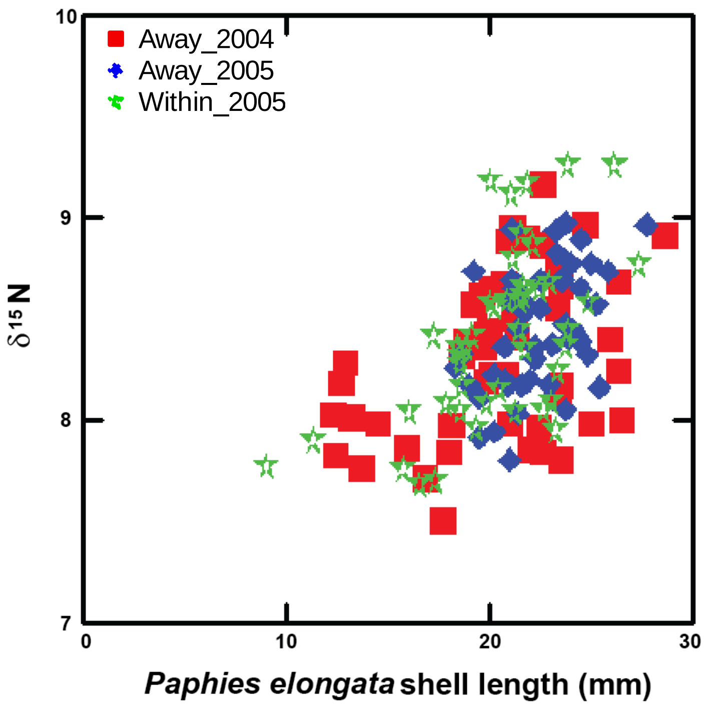

500 m
N
Piccaninnie Ponds
Piccaninnie Ponds CP
Piccaninnie
Ponds Outlet
A
W

Figure 6.2

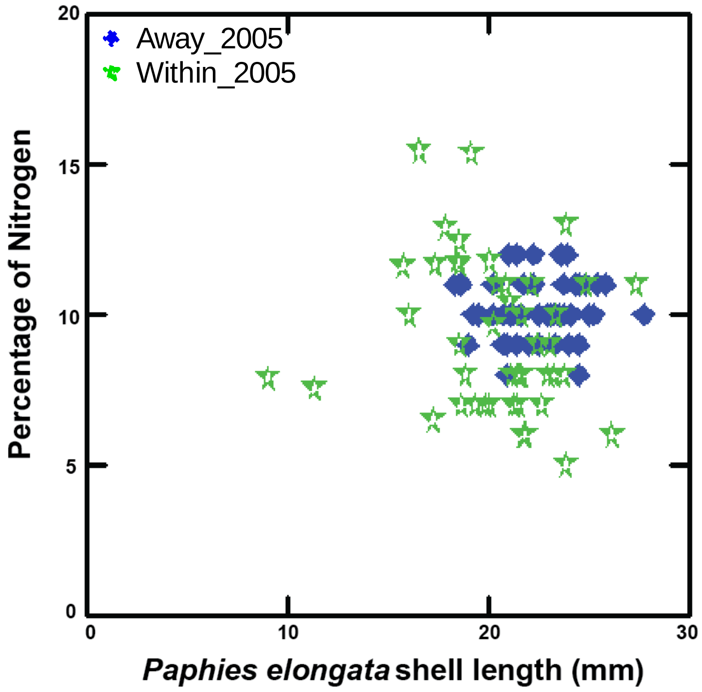
a)



b)



c)


Away_2005
Within_2005
Percentage of Nitrogen
0
5
10
15
20
0
10
20
30
Paphies elongata shell length (mm)

CHAPTER 7 – FIELD EXPERIMENTS

7 Biomass and productivity within groundwater-fed coastal creeks

7.1 INTRODUCTION

It is likely that continuous-flow drains provide a more stable environment for the flora and fauna that reside in them when compared to seasonal-flowing drains, which may only contain water for 1 to 6 months of the year. Chapter 3 has shown that water quality varies between seasonal- and continuous-flow drains. Chapter 4 has shown that, on average, seasonal-flowing drains had greater abundances than continuous-flowing drains but that species richness was approximately in the same in each year.

As a result, we may also expect to find differences in biomass and productivity of periphyton, macrophytic plants and algae that may then influence fauna abundances and overall communities (e.g. via different nutrient and food sources for them). Direct inputs of groundwater with potentially higher nitrogen levels may also contribute to an increase in production in these drains. This additional information would then be used to assist in the assessment of perceived dependence upon groundwater of ecological communities within these environments.

The purpose of this chapter was to experimentally test some of the knowledge gained in earlier chapters using field-based experiments. To do this, I set out to compare the standing stock biomass and productivity of aquatic plants and algae in a subset of the continuous-flow versus seasonal-flow drains. I hoped that the results would further complement the work undertaken in earlier chapters (e.g. Chapter 3: Water Quality, and Chapter 4: Macrofauna) by providing additional information regarding environmental processes operating in groundwater-fed coastal streams and drains in south-east South Australia.

7.1.1 Standing Stock Biomass

Standing stock (also described as standing crop or biomass) is a term used to describe the weight of a biotic population (e.g. a single species) or a community (e.g. aquatic flora generally) at a particular moment in time. In most cases, standing stock (Cronk & Siobhan 2001) refers to plant material above ground (i.e. does not include the mass of a plants' roots) and is usually represented as a measure of dry weight per unit area (i.e. g $DW.m^{-2}$). The standing crop of an aquatic ecosystem can be influenced by a variety of factors including hydrology, nutrients and substrate type (Cronk & Siobhan 2001).

In this chapter I ask whether flow regime might influence the standing stock biomass within the coastal drains of south-east South Australia. I have previously described (see Chapter 3) water quality and flow characteristics of continuous-flow (i.e. probably groundwater-fed) and seasonal-flow (*P*redominantly driven by surface-water run-off) drains. It is plausible that the delivery of water (constant versus variable) and other inputs (e.g. nutrients) into these systems may influence aquatic floral and algal biomass. Continuous-flow drains are likely to have a consistent delivery of nutrients and water movement that can support instream aquatic plant communities (especially rooted plants, pers. obs.) whereas seasonal-flow drains have episodic water flow and variable nutrient delivery which may support more boom-and-bust types of communities such as microphytobenthos and other algal communities.

The continuous-flow drains sampled in the broader study are permanently inundated (i.e. flow year-round) and have a relatively consistent delivery of nutrients (dominated by groundwater inputs with some periodic influence from additional surface water runoff) (see Chapter 3). This type of environment is likely to provide a more stable environment for permanent plant communities (*P*ers. obs.).

Seasonal-flow drains are, in contrast, typically driven by local climatic conditions and thus are only seasonally inundated (e.g. during winter/spring months) but inundation of an individual drain may vary by weeks to months. Nutrient delivery is also likely to vary and may involve significant first-flow inputs (e.g. a first flush from initial agricultural run-off and re-wetting of instream soils, comparable to the ‘flood pulse concept’, Miller *et al.* 2009). Plants in these systems may be short-lived and may re-generate from soil seed banks (e.g. macrophytic plants) or longer-lived (e.g. *Typha* spp., swamp tea tree) but be able to withstand periods of inundation. Algal recolonisation in seasonal-flowing streams in Grampian Ranges in western Victoria, Australia has been shown to originate from dry biofilms or drift provided from upstream drought refuges (Robson 2000, Robson & Matthews 2004, Robson *et al.* 2008).

Once seasonal run-off has ceased, inflow stops (typically after spring), the connection to the sea is lost, and these systems fundamentally become isolated, closed and much more like lentic systems. The longevity of aquatic flora and fauna is then controlled by a number of factors influenced by ambient temperature. Ambient temperature controls evaporation and thereby water quality factors such as salinity and dissolved oxygen, as the system changes under increased water temperatures. Thus, flora and fauna in these systems may live by quite different life-history strategies to those that reside in continuous-flow ones.

7.1.2 Productivity

Productivity, in this instance, is defined as an accumulation of biomass over time. Using those arguments presented above (see 7.1.1 Standing Stock), I suggest that differences in net productivity are also possible between the two flow regimes. It is possible that productivity in seasonal-flow drains could vary at any particular time of the wet season. For example, preliminary seasonal run-off and flows may trigger the onset of instream processes and initiate germination and establishment of aquatic plants and algae. Peak-season flows may allow plants to grow and increase their biomass; cessation of flow results in changes to water quality, which may reduce plant health and productivity. Peak-season flow may also remove some biomass through scour and habitat disturbances.

As water evaporates during summer closures and water quality decreases, plant and algae begin to die and thereby releasing nutrients and organic matter (i.e. carbon) back into the water column and sediments for uptake by a new cohort to utilise in the follow winter/spring season (Robson 2000, Robson & Matthews 2004, Robson *et al.* 2008).

7.1.3 Stable Isotope Analysis of $\delta^{15}N$ in periphyton and other plant matter

As discussed in Chapters 1 (General Introduction), 3 (Water Quality) and Chapter 6 (*Paphies elongata*), stable isotope analyses can be used to infer uptake and transfer of elements like nitrogen (e.g. groundwater versus surface water) through food webs.

Organisms take up nitrogen in different ways so that the isotopic composition of plants and animals varies (Krebs 2001) and so should their accumulation of $\delta^{15}N$ from a specific source (e.g. groundwater in this case).

What we expect to find is potentially a greater enrichment of ^{15}N in continuous-flow drains because of higher, on average, nitrogen concentrations in the water column. The higher nitrogen concentrations are inferred to be a result of the groundwater sources that contribute to the continuous water flow of these drains.

The analysis of $\delta^{15}N$ in plants and periphyton will complement the work undertaken in earlier chapters and help groundwater influences in the coastal streams in south-east South Australia.

Thus, I seek to discern whether continuous-flow (i.e. groundwater-fed) coastal drains are an important source of nitrogen for aquatic plants and indirectly animals, in areas under the influence of groundwater discharges via a stable isotope comparison.

Specifically, in this chapter I ask:

1. What is the standing stock biomass of aquatic flora in coastal streams in south-east South Australia?
2. Is there a difference in the mean standing stock biomass of aquatic flora between continuous-flow and seasonal-flow drains?
3. What is the productivity of aquatic flora in coastal streams in south-east South Australia?
4. Is there a difference in the mean productivity of aquatic flora between continuous-flow and seasonal-flow drains?

5. What are the $\delta^{15}N$ ratios in aquatic flora in coastal streams in south-east South Australia?
6. Is there a difference in the mean $\delta^{15}N$ ratios of aquatic flora between continuous-flow and seasonal-flow drains?

7.2 METHODS

A subset of coastal drains was selected to be used as experimental sites for assessing biomass and productivity, including stable isotope contents. Initially 12 sites were targeted for both studies, 6 continuous-flowing (i.e. groundwater-fed) drains and 6 seasonally-flowing drains. The 6 seasonal-flow drains chosen were postulated to have the best opportunity of maintaining their seasonal connection to the sea, at least for the duration of the productivity experiment, planned for 6 weeks of warm weather (i.e. spring/summer) from early November 2005 to mid December 2005. The study duration of six weeks was thought, based on comparable experiments in other waterbodies (P. Fairweather, pers. comm.) to be adequate for colonisation and growth to occur on each of the experimental treatments

Below-average winter rainfall was experienced across SE SA during the 2005 drought conditions (www.bom.gov.au). As a result of these conditions, three of the six seasonal-flow drains selected for use in this study were either closed (i.e. had no connection to the sea) and/or the physical conditions of the drains (i.e. too shallow a water depth) were such that it would not permit the layout and positioning of the experimental units at the commencement of the study (i.e. November 2005). As a consequence, only 3 seasonally-flowing sites were ultimately used. The seasonal-flow sites thus chosen were Mt Benson Drain, Sphers Road Drain and Green Point Drain (see Chapter 2 for descriptions). The continuous-flow (i.e. groundwater-fed) sites chosen were Blackford Drain, Cress Creek, Deep Creek, Eight-Mile Creek, Brown Bay Drain, and Piccaninnie Ponds Outlet, i.e. covering the entire study area and exhibiting a wide range of sizes and other characteristics (see Chapter 2).

The predictive model applied to these experiments was that drains that were groundwater-fed flowed continuously and also had more nutrients in them. Continuous flows thus provide longer periods each growing season for standing stocks of mainly algal biomass to accumulate. This biomass may result in different species composition with less opportunistic characteristics when compared to seasonal-flowing drains.

The higher nitrogen concentration, in combination with elevated ^{2}H and ^{18}O ratio in continuous-flow drains (see Chapter 3), led to the hypothesis that, because these drains were found to be groundwater-fed, ^{15}N in the tissue of plants would also be elevated.

7.2.1 Standing Stock Biomass Sampling

In early November 2005, 8 replicate quadrats (each 0.25m × 0.25m = 0.0625m^2 in area) were randomly positioned at each site. Within each quadrat, all biotic material (i.e. vegetation, epiphytes, algae within sediments, but with any visible animals removed) above the substrate was harvested, bagged and frozen. Only plants originating in the quadrat were harvested to get estimates of biomass attached per unit area. Entire plants were harvested even if a particular plant extended beyond the limit of the quadrat. Conversely, if a quadrat contained fronds, leaves, or other biotic material that did not originate within that particular quadrat, that material was not harvested.

If there was no material available for collection in a particular quadrat (i.e. there was zero cover), then a zero measurement was recorded but a targeted replacement sample was collected nearby to get some material for ^{15}N analyses only. These extra targeted samples have not been used when reporting mean biomass (as dry weight per unit area).

Samples were bagged and refrigerated for later transport for processing and analysis. Upon return to laboratory, samples were first weighed (wet weight) then dried at 50°C until constant weight was established (24 to 120 hours depending on sample volume), then re-weighed (dry weight).

Dry material was analysed for organic matter (OM) and ^{15}N contents. Due to the length and/or mass of some of the vegetative material collected, it was first necessary to fragment (to approximately 0.5cm lengths) and homogenise some sub-samples in order to obtain a representative measure for the sample. Smaller (total dry weight <5g) samples were homogenised using a mortar and pestle. Larger (total dry weight >5g) samples were first homogenised using a laboratory blender before sub-sampling approximately

5g for further processing. All dried samples and sub-samples were ground to a fine consistency using a mortar and pestle.

Approximately 1g of the finely-ground material was further sub-sampled and used to estimate organic content using loss on ignition (LOI) analyses. Some samples collected had total dry weights of <1g. Where this was the case, the entire sample up to 1g was used. Each sample was weighed into pre-ashed crucibles. Due to the time exposed to air during sample preparation, all samples were placed in a drying oven at 50ºC for approximately 2 hours to remove any atmospheric-derived moisture. Samples were then placed in a desiccator until cool and re-weighed. It was this last before-ashing measurement that was used to calculate %OM as lost on ignition (LOI).

Samples were then placed into a muffle furnace for 3 hours (allowing one hour to reach the programmed burn temperature set at 550°C). Upon completion of the burn, samples were removed from the muffle furnace and transferred to a desiccator until cool. Each crucible was again weighed. This after-ashing value was then used to calculate OM mass per sample (as %LOI) for later use in statistical analyses.

With each burn, a control (1g of laboratory-grade sucrose) was used to assess for burn and measurement errors. An acceptable error rate of 0.2% was calculated across all burns (*N*=11). That is, the pre- and post-ashing measurements resulted in, on average, an additional 0.2% of material LOI.

Due to the matrix of material collected (i.e. both organic and inorganic materials), it was important to estimate %OM (as ash-free dry weight, AFDW) in order to calculate the mass of each sample required for δ15N analysis (for best results, the IRMS instrumentation required between 80 and 110µg of nitrogen to be present in a sample). It was necessary to ensure total N was within the above-stated range in order to permit optimum analysis of ^{15}N. Sample sizes ranged from 3.1mg DW (97.36%OM) to 118.7mg DW (2.53%OM). These values were based on an assumption that the total nitrogen in plants is usually approximately 1.2-1.8% (dry weight) of the organic component of the total sample.

The appropriate mass of sample was transferred to small tin cups, rolled lightly into small balls, placed in a 96-well plate, and then analysed using an Isoprime Isotope Ratio Mass Spectrometer (GV Instruments, Manchester, UK) and an elemental analyser (EuroVector, Milan, Italy). Stable isotope analysis was carried out in the Flinders Advanced Analytical Laboratory at Flinders University in Adelaide, South Australia.

Unfortunately some problems were encountered during the ^{15}N analyses resulting in a few missing values. The primary cause for the missing values related to the sizes (in terms of mass and therefore volume) of the samples. Where the organic content of the samples was calculated to be low, a greater mass of sample was required to enable the appropriate levels of nitrogen to be present in the sample analysed. The size (volume) of these samples led to unreliable results and in some cases, leaking (splitting of tin cups and release of material) of samples during analysis. A total number of 31 samples were thus lost (see Tables 7.1; 7.2). As a consequence, some sites have no data whereas others have limited values (see Tables 7.1; 7.2 for number of samples used in each analysis; the implications of this variation are dealt with in the "Discussion").

7.2.2 Productivity Experiment

At each of the 9 sites stated above, 6 replicate house bricks (experimental surface area = 0.0179 m^2) were haphazardly positioned to measure growth of periphyton on this hard surface as an index of productivity over a period of 27-29 days commencing mid-November 2005. All experimental bricks had been "conditioned" prior to positioning in drains. That is, all bricks had been rinsed, the experimental surface scrubbed and re-rinsed before then soaking the bricks in clean domestic tap water for approximately 14 days. Bricks were conditioned as an attempt to permit the leaching of any chemical compounds that may have been present within the bricks.

Bricks were oriented parallel to the channel and both the leading edge and upper (experimental) surface were identified using a cable tie. Measurements of brick depth (measured from the surface of water to the upper surface of brick) were collected as well as estimates of water flow. Flow was measured by recording the time taken for an orange to pass 10m marked along the bank

(Hauer & Lamberti 2006). Channel dimensions were also measured to estimate discharge volume.

All sites were revisited in mid-December and any growth on the bricks at that time was removed using a paint scraper. All scraped material (i.e. including all vegetation, epiphytes, and algae within any sediments, but with all visible animals removed) collected was bagged and refrigerated. Bricks were then repositioned to be opportunistically sampled again in February 2006. Samples were later dried and analysed for dry weight, %LOI and ^{15}N content using the same methods used in the biomass study above.

On both sampling occasions (December 2005 and February 2006), some of the bricks could not be located (see '*n*' in last column of Table 7.3); some had been repositioned and/or overturned, presumably through water movement, some were buried by sediments and some had become exposed because of falling water levels. Observations were recorded regarding a brick's position at each time of collection. Only a very limited number of bricks were observed during February 2006 and, as a consequence, these data are not presented here nor have they been included in any of the statistical analyses.

7.2.3 Statistical Analyses

Data were analysed using univariate (e.g. one-way Analysis of Variance [ANOVA]) statistics using SYSTAT v11. Due to some missing bricks and hence imbalance in the data collected, it has been necessary to analyse different configurations of the data in order to test the hypotheses. It was necessary to transform some data configurations after examination of residuals to meet the assumptions of ANOVA (as reported below).

Due to the nature of the samples collected (see Methods above), an unbalanced design resulted in many of the analyses. Statements are made (below) where an unbalanced design has occurred.

7.3 RESULTS

7.3.1 Environmental data

The environmental data measured during this study (i.e. in November and December 2005) were comparable with the longer-term (i.e. 3 years of data) recorded for these sites. Differences between environmental data in this study compared to the longer-term results are summarised in Table 7.4. Generally salinities were very similar with only Brown Bay Drain (half the longer-term salinity) and Spehrs Road Drain (a third [November only]) showing observable differences. Temperatures were approximately ±1 to 2°C different from longer-term results except for Blackford Drain, which was approximately 6°C greater (or 30%) (November only) than the longer-term average (see Table 7.4).

Nitrate and total nitrogen in the water were more variable with differences in concentration of up to ±25% observed (see Table 7.4).

7.3.2 Standing Stock Biomass Estimates

Standing stock biomass (mean ± s.e.) in Drains ranged between 0 (Green Point, *n* = 8) and 262.86 ± 148.54 g DW m^{-2} (*P*iccaninnie Ponds Outlet, *n* = 8) (Table 7.2). A one-way ANOVA found a statistically-significant difference (P <0.024) in mean square-root biomass between types of Drains. Tukey post-hoc multiple comparisons found a significant difference (P = 0.013) between mean square root biomass for Green Point Drain and Piccaninnie Ponds Outlet only (Table 7.2).

Regional standing stock biomass (mean ± s.e.) was approximately three times greater in the Eastern coastal region (95.76 ± 30.21 g DW m^{-2}, *n* = 48) than the Western coastal region (30.88 ± 14.09 g DW m^{-2}, *n* = 24, Table 7.2, Figure 7.1a). Mean square-root biomass was not significantly different (P = 0.098) between regional locations of Drain.

Standing stock biomass (mean ± s.e.) in continuous-flow Drains (105.94 ± 30.32 g DW m^{-2}, *n* = 48) was approximately one order of magnitude greater than the standing stock biomass in seasonal-flow Drains (10.52 ± 5.42 g DW m^{-2}, *n* = 24, Table 7.2, Figure 7.1b). A statistically-significant difference (P = 0.001) in mean square-root biomass was found when Drains were analysed by flow state.

7.3.2.1 Flow data during the Standing Stock Biomass study (i.e. mean November values only)

Mean (± s.e.) flow velocity at the time of standing-stock biomass collection (November 2005) in Drains ranged by an approximate magnitude of eight (0.10 ± 0.01 $m.s^{-1}$ [Spehrs Road Drain, *n* = 3] and 0.81 ± 0.06 $m.s^{-1}$ [Eight Mile Creek, *n* = 3]) (Table 7.1).

Flow velocity (mean ± s.e.) in the Eastern coastal region was approximately twice that of the Western coastal region (0.48 ± 0.5 $m.s^{-1}$, *n* = 18 versus 0.21 ± 0.02 $m.s^{-1}$, *n* = 9, respectively, Table 7.1, Figure 7.2).

Similarly, flow velocity (mean± s.e.) at continuous-flow Drains (0.48 ± 0.05 $m.s^{-1}$, *n* =18) was twice that of seasonal-flow Drains (0.21 ± 0.03 $m.s^{-1}$, *n* = 9, Table 7.1, Figure 7.2).

7.3.2.2 Isotopes of Nitrogen ($\delta^{15}N$) in biomass

Due to analytical issues relating to sample volumes (see Methods), no $\delta^{15}N$ results were possible for Spehrs Road Drain and Piccaninnie Ponds Outlet (see Table 7.2). Nitrogen isotope ratio values (mean ± s.e.) ranged from 4.0 ± 0.2 ‰ $\delta^{15}N$ (Eight Mile Creek, *n* = 8) to 9.6 ± 0.2 ‰ $\delta^{15}N$ (Blackford Drain, *n* = 7) (Table 7.2).

A one-way ANOVA found a statistically-significant difference (*P* <0.001) in mean $\delta^{15}N$ amongst Drains. Tukey post-hoc multiple comparisons for mean ⍰15N found significant differences between Blackford Drain versus Deep Creek, and Eight-Mile Creek versus Blackford Drain, Cress Creek, and Green

Point Drain (P <0.001); Brown Bay Drain versus Blackford Drain, and Eight-Mile Creek(P = 0.001); Mt Benson Drain versus Eight-Mile Creek (P = 0.008); Blackford Drain versus Mt Benson Drain (P = 0.012); Deep Creek versus Green Point Drain (P = 0.013); and Deep Creek versus Eight-Mile Creek (P = 0.035) (Figure 7.3a).

Nitrogen isotope ratio values (mean ± s.e.) were closer between the regional groups (Western: 6.3 ± 0.4 ‰ $\delta^{15}N$, n = 30 versus Eastern: 8.6 ± 0.5 ‰ $\delta^{15}N$, n = 11, Table 7.2) but the difference was statistically significant (P = 0.002).

Nitrogen isotope ratio values (mean ± s.e.) in continuous-flow Drains (6.7 ± 0.4 ‰ $\delta^{15}N$, n = 32) were approximately equal to seasonal-flow Drains (7.8 ± 0.4 ‰ $\delta^{15}N$, n = 9, Table 7.2, Figure 7.3b) and were found to be not statistically significant (P = 0.165).

7.3.3 Productivity Experiment

Daily productivity (mean ± s.e.) in Drains ranged from 0.09±0.04 $gDW.m^{-2}.d^{-1}$ (Blackford Drain, n = 4) to 18.38 ± 2.20 $gDW.m^{-2}.d^{-1}$ (Eight Mile Creek, n = 6) (Table 7.4), an approximate magnitude of 200 times greater.

A one-way ANOVA found a statistically-significant difference (P <0.001) in mean daily productivity ($gDW.m^{-2}.d^{-1}$) amongst Drains. Tukey post-hoc multiple comparisons for mean daily productivity found significant differences between Eight-Mile Creek versus Blackford Drain, Spehrs Road Drain, Cress Creek, Deep Creek, Brown Bay Drain, and Piccaninnie Ponds Outlet (P <0.001); Eight-Mile Creek versus Mt Benson Drain, and Deep Creek versus Green Point

Drain (P = 0.001); Green Point Drain versus Blackford Drain, and Piccaninnie Ponds Outlet (P = 0.002); Mt Benson Drain versus Eight-Mile Creek (P = 0.008); Green Point Drain versus Eight-Mile Creek (P = 0.005); Green Point Drain versus Cress Creek (P = 0.032); and Green Point Drain versus Spehrs Road Drain (P = 0.038) (Figure 7.4a).

Regional productivity (mean ± s.e.) in Eastern coastal-region Drains (6.66 ± 1.42 $gDW.m^{-2}.d^{-1}$, n = 29) was approximately twice that of Western coastal-region Drains (3.69 ± 1.19 $gDW.m^{-2}.d^{-1}$, n = 14) (Table 7.4) but this difference in mean daily productivity was not statistically significant (P = 0.189) between regional locations of Drains.

Productivity (mean ± s.e.) in seasonal-flow Drains was approximately two times that of continuous-flow Drains (7.006 ± 1.199 $gDW.m^{-2}.d^{-1}$, n = 16) and 4.911 ± 1.510 $gDW.m^{-2}.d^{-1}$, n = 27, respectively) (Table 7.4). This mean daily productivity difference was not statistically significant (P = 0.341) when Drains were analysed by flow type (Figure 7.4b).

7.3.3.1 Productivity Experiment Flow Data (i.e. comparison between November & December)

Mean (± s.e.) flow velocity in Drains at the end of the experiment ranged by approximately 18 times from 0.05 ± 0.02 $m.s^{-1}$ (Spehrs Road Drain, n = 6) to 0.93 ± 0.06 $m.s^{-1}$ (Eight Mile Creek, n = 6) (Table 7.4).

Regional flow velocity (mean ± s.e.) ranged by a factor of three from 0.12 ± 0.03 $m.s^{-1}$ (n = 18) for the Western coastal region to 0.41 ± 0.5 $m.s^{-1}$ (n = 36) for the Eastern coastal region (Table 7.4).

Flow velocity (mean ± s.e.) at continuous-flow Drains (0.42 ± 0.05 $m.s^{-1}$, n = 36) was approximately four times greater than seasonal-flow Drains (0.11 ± 0.03 $m.s^{-1}$, n = 18) (Table 7.4).

Additional statistical tests (i.e. ANOVA) using salinity, temperature, nitrate, total nitrogen, flow velocity and volume as covariates were performed on both the Productivity and Biomass data; all results were statistically non-significant (P >0.05). Thus, these individual results have not been presented here.

7.4 DISCUSSION

Environmental values (e.g. salinity, temperature, TN, NO_3) experienced during this sampling and experiment were comparable and all fell within the longer-term data ranges for these individual drains. Variation in values at the individual drain level was also observed for biomass, productivity, $\delta^{15}N$, and flow velocity and volume.

When drains were grouped by region (i.e. Eastern versus Western drains), Eastern drains were found to have three times greater standing-stock biomass and two times greater productivity than Western region drains. Flow velocity (and volume) was also greater in Eastern drains as were TN and NO_3 but $\delta^{15}N$ values were marginally higher in Western drains.

In contrast, when grouped by flow regime (i.e. continuous- versus seasonal-flow drains), continuous-flow drains were found to have ten times more standing stock biomass but only half the productivity rate than those of Seasonal-flow drains. Flow velocity (and volume) was also greater in continuous-flow drains as were TN and NO_3 in seasonal drains but $\delta^{15}N$ values were reasonably similar amongst both groups.

The results of this chapter follow a similar theme to those of earlier chapters (e.g. Chapter 3). That is, the groundwater-influenced Eastern and continuous-flowing drains had greater water flow and nitrogen concentrations. Some seasonal drains had closed as a result of the reduction and/or cessation of surface water inflows. This resulted in these drains closing over therefore losing their connection to the sea. This was evident in the lower flows and increased temperatures and the end of the experiment.

Vegetation was not individually sorted and identified in this study to a particular species when sampling for biomass values. That is, the 'community' mix of species that was collected from within a quadrat was treated as simply 'biomass'. This material was not separated and/or individually weighed by species. The necessity to freeze material for handling when away in the field made later identification impossible.

I recommend a detailed presence/absence inventory of vegetation taxa be mandatory for any similar future study. That is, from within in each sampling unit (e.g. quadrat) each species collected (be it terrestrial, aquatic, or algal) be treated separately during processing and analysis as an attempt to better explain or extrapolate patterns between the sample groups. In the case of this study, such a methodological modification would allow for an improved discrimination between the effects of continuous versus seasonal flow and the effect of other parameters (e.g. high versus low N).

Nevertheless the samples collected here did contain a mix of algae, and aquatic and some terrestrial plants (i.e. grasses) that were submerged at time of sampling. How much the influence of terrestrial vegetation had on the global ^{15}N result is unknown (i.e. did it significantly increase or decrease the value?). Thus it could also be worth comparing $\delta^{15}N$ in the biomass of terrestrial versus aquatic plants in any future study. Terrestrial species were more commonly observed in some samples collected in seasonal drains. Most of the observed biomass (i.e. typically aquatic plants in continuous drains, and some inundated terrestrial grasses in seasonal drains) may have further influenced differences in $\delta^{15}N$ values measured (i.e. land-based vs aquatic life histories and nutrient uptake processes).

It is likely that the biomass in the continuous-flow drains fluctuates over the year. Periodically these drains would contain dense stands of the green alga *Enteromorpha* sp. whereas at other times, it was evident that growth in aquatic plants had been excessive such that long branches and sometimes whole plants could be seen flowing downstream. The ultimate fate of this material ends up in the nearshore environment. It was not uncommon to see adjacent sandy beach drift lines littered with (freshwater) aquatic plants.

To maximise differences, this study was undertaken in late spring/early summer when seasonal-flow drains were becoming or had just become disconnected as a result of cessation of freshwater inputs. The results presented here may not truly reflect average productivity under peak seasonal conditions, at other times of the year or year-round patterns.

In terms of the productivity, most of the observed production was by periphyton, as expected upon a hard substratum. Periphyton ranged from a simple, sediment-free, thin biofilm layer to other samples containing filamentous algae interspersed amongst sediments. Sample collection involved the collection of the sediment in addition to the organic component. This embedded sediment made %N and $\delta^{15}N$ analyses difficult because of the need to have a minimum N concentration in the sample. The bigger the ratio of sediment to organic material, the bigger the sample volume needed to be for analysis. Most difficulties were experienced by the auto sampler associated with the IRMS. Some sample capsules split and leaked and so could not be measured. This method would need to be fine-tuned for future studies (e.g. using some collecting surface elevated from sediments, perhaps floated instead of placed on the benthos, or with a mesh structure with would allow sediments to be dislodged by water movement). This may help to eliminate excess sediments on the experimental unit before collection allowing for a greater organic to sediment ratio in the sample analysed. This would reduce the overall size (volume) of the sample mass to be analysed thus enabling accurate %N and $\delta^{15}N$ values to be returned from all replicates and providing valuable data to be captured (i.e. a more positive return on sampling effort).

Continuous-flow drains provide a continual source of primary production and standing stock biomass for animals that reside in these systems. It is likely that productivity and standing stock biomass change seasonally over a year but none-the-less an energy source is available all year round. In comparison seasonal-flow drains provide a source of energy (*P*rimary production) only when inundated and there is sufficient water inflows to sustain the system. The impacts this has on faunal communities in terms of their composition and resilience is likely to be significant.

As we have seen in Chapter 4, the macrofaunal abundance and richness was different between continuous- and seasonal-flow drains (with seasonal drains displaying a greater total abundance and species richness when compared to continuous-flow drains). The results could also suggest that groundwater dependence may constrain variability. That is, continuous flow may provide more uniform conditions for plant growth and animal colonisation as compared to seasonal flows that may or may not become lentic as inputs and outputs (as a physical connection to the sea is lost) cease, thereby having significant biological effects. Further investigations could be warranted that consider the role of boom-bust versus more continuous life histories of the fauna found in these opposing water-flow regimes.

For continuous-flow drains should groundwater discharge be reduced substantially or stop (e.g. due to excessive draw-down of the local groundwater table), it is possible that the present flora and fauna communities that have adapted to continuous-flow regime will be unlikely to persist under a rapid transition to a seasonal flow regime. Initially, as groundwater levels decrease so will groundwater inputs to these drains. Consequently, it is likely that seawater infiltration to these drains would increase as groundwater input decreases. For example, it was observed during the January 2005 sampling visit that excessively high tides and large seas had penetrated upstream in Deep Creek (continuous-flow drain). It was observed that instream salinity changed from 1ppt (i.e. freshwater) to 35ppt (i.e. marine) concentrations overnight. This resulted (*P*ers. obs.) in a significant fish kill of Yarra pygmy perch. This suggests that any rapid change from a permanent freshwater system in which resident flora and fauna have adapted to something other may have significant impacts on the fauna that reside there. This observation may also occur as any historically-continuous groundwater input is eliminated.

In comparison, flora and fauna communities within seasonal flow drains have adapted over time to flow variability. Regeneration of in stream vegetation and/or algal communities has been shown in rivers nearby to be driven by upstream refuge pools and propagules within the sediments (Robson 2000, Robson & Matthews 2004, Robson *et al.* 2008).

There may be some lag time before once continuous-flow systems permanently connected to the sea transition to be seasonal-flow regimes that facilitate different plant and animal assemblages. Performance of species during such a lag phase would be expected to suffer and so be measurable.

7.5 CONCLUSION

In general the results suggest that there are differences amongst the main groupings of drains (i.e. continuous-flow versus seasonal-flow drains) but not for both experiments. That is one group does not have both a greater biomass and production rate but rather one or the other. Specifically, continuous-flow drains had generally higher biomass but lower productivity than seasonal-flow drains. The higher biomass and lower productivity in continuous-flow drains suggests that these permanent water bodies provide ongoing suitable conditions for aquatic plant growth and that their permanence is strongly influenced by groundwater discharge throughout the dry months. The relatively-continuous groundwater discharge into these drains provides permanency to the flora and fauna of these systems and, as such, a different type of community has established (i.e. periphyton in seasonal drains compared to aquatic plants and macroalgae in continuous-flow drains)

The lower biomass and higher productivity in seasonal-flow drains suggests that food webs in these temporary water bodies are algal driven and are strongly influenced by seasonal weather patterns (i.e. winter and spring rainfall and subsequent surface water runoff) that stimulate periphyton growth (Robson 2000; Robson & Matthews 2004; Robson *et al.* 2008).

It is also possible that there may be a greater turnover in seasonal drains (e.g. maybe due to animals grazing off the production and so keeping biomass very low) despite such high observed productivity values, as suggested by Figure 7.5. Grazing-rate experiments would be needed to test this idea.

In relation to the questions raised at the start of this chapter, the present study found that in coastal drains of SE SA:

1. Standing stock biomass ranged on average from 0 to >263 $gDW.m^{-2}$
2. Continuous-flow drains had about 10 times the average standing stock biomass than seasonal-flow drains
3. Daily mean periphyton productivity in these drains ranged from <1 to 18 $g\ DW.m^{-2}.d^{-1}$
4. Mean periphyton productivity in seasonal-flow drains was approximately twice that of continuous-flow drains (but statistically non-significant)
5. Nitrogen isotopic ratios in flora ranged, on average, between 4 and >9 ‰ $\delta^{15}N$
6. Continuous- and seasonal-flow drains had approximately similar mean ^{15}N values

TABLES

Table 7.1: Standing stock biomass density (mean ± se gm^{-2} DW) and other water quality measures (mean ± se) at harvest in November 2005. See Chapter 2 "General Methods" for additional site information and location. See Chapter 3 "Results" for additional site data. E = Eastern coastal region, W = Western coastal regional; Flow (see Chapter 3 Methods for definition of "flow"): C = Continuous-flow, S = Seasonal-flow; ppt = parts per thousand.

Region	Flow	Site Name	Site no.	Salinity (ppt)	Temp (°C)	Flow Velocity (ms^{-1})	Flow Volume (m^3s^{-1})	NO_3 (mgL^{-1})	TN (mgL^{-1})
		***n* =**		**3**	**3**	**3**	**3**	**2**	**2**
W	C	Blackford Drain	1	7.60±0.02	25.6±0.19	0.26±0.02	0.23±0.12	0.11±0.08	1.40±0.20
	S	Mount Benson Drain	4	0.39±0.01	17.6±0.03	0.27±0.01	0.03±0.01	4.10±0.50	4.60±0.70
	S	Spehrs Road	10	3.22±0.05	23.7±0.52	0.10±0.01	0.01±0	0.07±0.03	0.90±0.10
E	C	Cress Creek	17	0.84±0.01	16.1±0.03	0.42±0.02	0.24±0.01	1.84±0.58	2.52±0.52
	C	Deep Creek	21	1.39±0.01	16.8±0	0.68±0.06	1.14±0.24	1.32±0.02	1.82±0.02
	C	Eight Mile Creek	22	0.56±0.01	16.8±0.03	0.81±0.06	2.76±0.30	4.30±0.10	4.90±0.37
	C	Brown Bay	23	0.67±0.01	18.4±0.18	0.32±0.01	0.09±0.01	2.50±0.40	3.43±0.30
	S	Green Point	24	0.39±0.01	21.3±0	0.25±0.01	0.01±0.01	0.45±0.08	1.30±0
	C	Piccaninnie Ponds Outlet	26	1.24±0.01	17.8±0.09	0.39±0.01	0.62±0.01	1.38±0.08	1.87±0.23

Table 7.2: Standing stock biomass density (mean ± se gm^{-2} DW, n = 8) and biomass isotopic ratio values for $\delta^{15}N$ (mean $\pm$ s.e.) and organic mass (mean $\pm$ s.e.) per quadrat (0.0625 m^2) at harvest in November 2005. E = Eastern coastal region, W = Western coastal regional; Flow: C = Continuous-flow, S = Seasonal-flow; ‰: per mil; Organic Mass: n = 8 for all sites. $\delta^{15}N$: n as stated but see methods for explanation. "-" samples unable to be analysed (see Methods for explanation).

Region	Flow	Site Name	Organic Mass (g DW per sample)	$\delta^{15}N$ (‰) [n]	Measured Biomass Density (gm^{-2} DW)	# quadrats returning no cover [out of 8]
W	C	Blackford Drain	3.98±2.39	9.6±0.2 [7]	61.08±38.67	1
	S	Mount Benson Drain	1.64±0.42	6.8±0.5 [4]	15.90±5.89	2
	S	Spehrs Road	7.70±2.71	-	15.67±15.21	6
E	C	Cress Creek	5.50±2.74	8.8±0.2 [2]	88.02±43.86	0
	C	Deep Creek	7.82±4.61	6.0±0.8 [7]	121.24±74.56	3
	C	Eight Mile Creek	4.11±1.83	4.0±0.2 [8]	65.72±29.31	0
	C	Brown Bay	3.06±1.04	6.7±0.4 [8]	36.71±18.03	2
	S	Green Point	8.15±5.83	8.6±0.5 [5]	0	8
	C	Piccaninnie Ponds Outlet	16.43±9.28	-	262.9±148.5	0

Table 7.3: Differencing in environmental data (mean values) between the productivity, and the broader longer-term study. All environmental data are reported as productivity study (November and December 2005) minus the longer-term (September 2003 to February 2006, inclusive) results. E = Eastern coastal region, W = Western coastal regional; Flow: C = Continuous outflow, S = Seasonal outflow; psu = practical salinity units. ΔSS = difference in sample size. See Chapter 3 for additional site data.

Region	**Flow**	**Site Name**	**Salinity (ppt)**	**%**	**Temp (ºC)**	**%**	**ΔSS**	**NO_3 (mgL^{-1})**	**%**	**TN (mgL^{-1})**	**%**	**ΔSS**
W	C	Blackford Drain	1.04	13.8	4.2	21.6	-38	-0.13	-68.4	-0.26	-17.7	-18
	S	Mount Benson Drain	-0.01	-2.6	-0.2	-1.0	-7	0.92	29.0	0.69	17.7	-2
	S	Spehrs Road	1.83	16.5	-0.1	-0.4	-6	0	0	-0.27	-21.3	-2
E	C	Cress Creek	-0.05	-5.4	0.6	3.4	-37	-0.49	-19.9	-0.48	-16.1	-16
	C	Deep Creek	0	0	0.9	5.1	-37	-1.00	-42.0	-1.09	-38.7	-18
	C	Eight Mile Creek	-0.09	-13.4	0.4	2.4	-37	-0.66	-13.2	-0.44	-7.8	-18
	C	Brown Bay	-0.85	-54.5	0.7	3.9	-37	0.02	0.8	0.13	3.8	-16
	S	Green Point	0.11	21.2	0.2	1.0	-7	0.08	32.0	0	0	-2
	C	Piccaninnie Ponds Outlet	0.06	4.7	0.4	2.5	-42	-0.49	-25.3	-0.56	-23.1	-18

Table 7.4: Productivity (mean ± SE) and water quality measures (mean±se) for the growth experiment conducted November 2005 to December 2005. E = Eastern coastal region, W = Western coastal regional; Flow (see Chapter 3 Methods for definition of "flow"): C = Continuous outflow, S = Seasonal outflow; psu = practical salinity units; *n* = 4 for nitrate (NO_3) and total nitrogen (TN); *n* = 6 for salinity, temperature, flow velocity and flow volume; with sample size (experimental units [bricks] recovered) *n* for Productivity as stated; Days = duration of growth experiment. Salinity, temperature, NO3 and TN all n=6.

Region	Flow	Site Name	Salinity (psu)	Temp (ºC)	Flow Velocity (ms^{-1})	Flow Volume (m^3s^{-1})	NO_3 (mgL^{-1})	TN (mgL^{-1})	Productivity ($gm^{-2}d^{-1}$ DW)	Days	*n*
W	C	Blackford Drain	8.66±0.48	23.6±0.9	0.16±0.05	0.14±0.07	0.06±0.04	1.21±0.16	0.09±0.04	26	4
	S	Mount Benson Dr.	0.38±0.01	19.5±0.9	0.15±0.05	0.02±0.01	4.09±0.23	4.58±0.29	7.77±3.24	27	4
	S	Spehrs Road	12.93±4.4	23.6±0.3	0.05±0.02	0.01±0.01	0.04±0.02	1.00±0.08	3.37±0.67	27	6
E	C	Cress Creek	0.87±0.01	18.2±1.0	0.41±0.01	0.23±0.01	1.97±0.26	2.51±0.24	1.69±1.55	28	3
	C	Deep Creek	1.52±0.06	18.4±0.7	0.53±0.07	0.88±0.16	1.38±0.07	1.73±0.08	0.96±0.72	28	6
	C	Eight Mile Creek	0.58±0.01	17.3±0.3	0.93±0.06	3.13±0.23	4.34±0.14	5.22±0.47	18.38±2.20	28	6
	C	Brown Bay	0.71±0.02	18.6±0.1	0.21±0.05	0.06±0.02	2.62±0.19	3.53±0.33	2.28±0.78	28	6
	S	Green Point	0.63±0.11	19.3±0.9	0.13±0.06	0.01±0.01	0.33±0.09	1.06±0.14	10.13±1.47	28	6
	C	Picc.Ponds Outlet	1.33±0.04	16.2±0.7	0.26±0.06	0.43±0.09	1.45±0.07	1.86±0.14	1.09±0.73	28	6

FIGURES

Figure 7.1: Mean biomass density at a Drain sites sampled in November 2005 and grouped by Flow state and Region for a) individual drains and b) means for each type. State: C = Continuous outflow, S = Seasonal outflow; Region: E = Eastern region, W = Western regional. Continuous-flow (black solid dot), seasonal-flow (open square)

Figure 7.2: Scatterplot of mean velocity and flow at Drain sites sampled in November 2005 and grouped by flow regime. State: C = Continuous outflow, S = Seasonal outflow; Region: E = Eastern coastal region, W = Western coastal regional. Continuous-flow (black solid dot), seasonal-flow (open square)

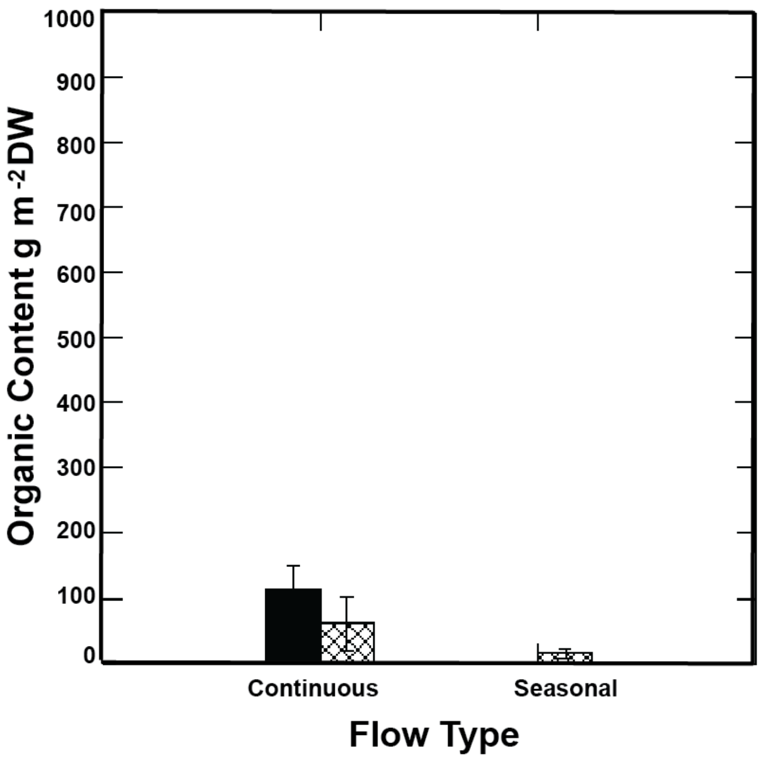
Figure 7.3: Mean $\delta^{15}N$ by Drain. Site No = DR_01: Blackford Drain; DR_04: Mount Benson Drain; DR_17: Cress Creek; DR_21: Deep Creek; DR_22: Eight Mile Creek; DR_23: Brown Bay; DR_24: Green Point. No data was available for site 10 (Spehrs Road Drain. See methods for explanation. Continuous-flow (black solid dot), seasonal-flow (open square)

Figure 7.4: Mean daily productivity by Drain. Site No = DR_01: Blackford Drain; DR_04: Mount Benson Drain; DR_10: Spehrs Road; DR_17: Cress Creek; DR_21: Deep Creek; DR_22: Eight Mile Creek; DR_23: Brown Bay; DR_24: Green Point; DR_26: Piccaninnie Ponds Outlet. Continuous-flow (black solid dot), seasonal-flow (open square)

Figure 7.5: Continuous-flow (black solid dot), seasonal-flow (open square)

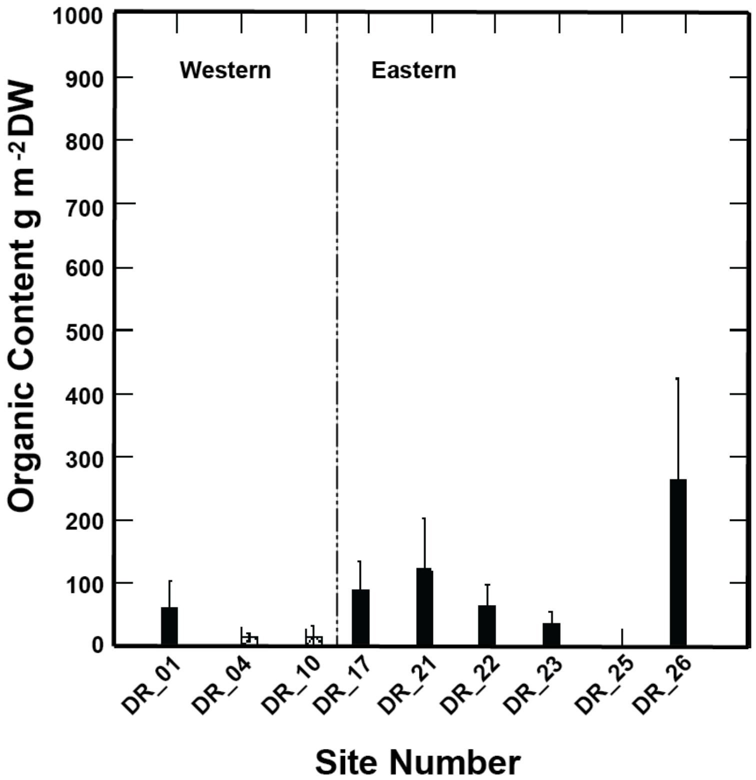
Figure 7.1

a)



Organic Content g m -2DW
1000
900
800
700
600
500
400
300
200
100
0
Continuous
Seasonal
Flow Type

b)



Organic Content g m -2DW
1000
900
800
700
600
500
400
300
200
100
0
Western
Eastern
DR_01
DR_04
DR_10
DR_17
DR_21
DR_22
DR_23
DR_25
DR_26
Site Number

Figure 7.2

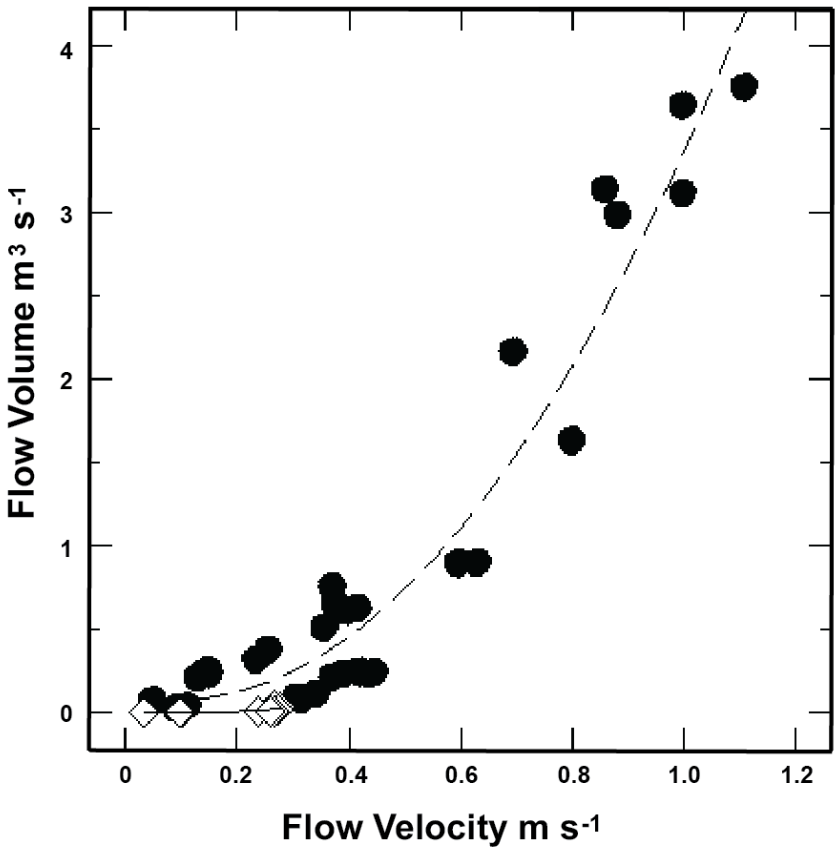


Figure 7.3

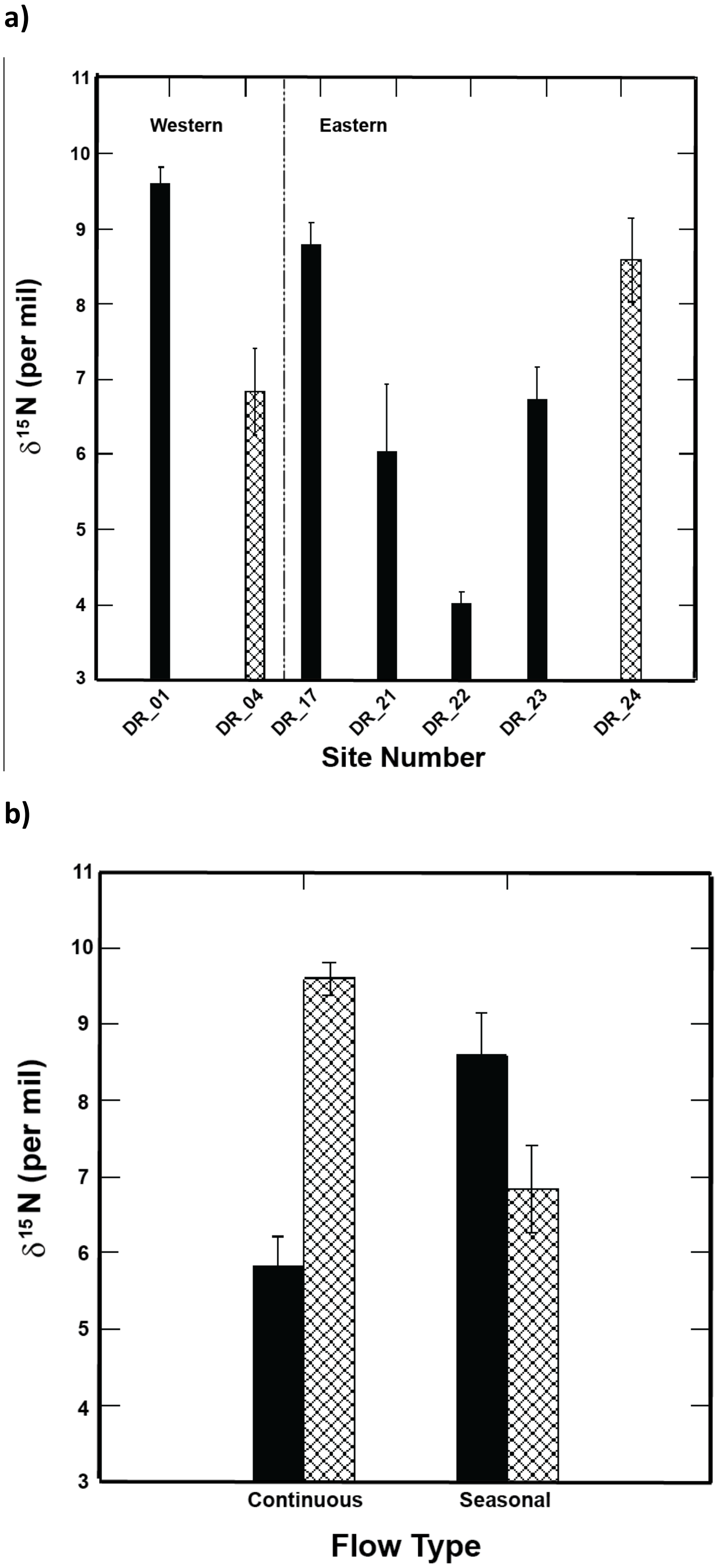
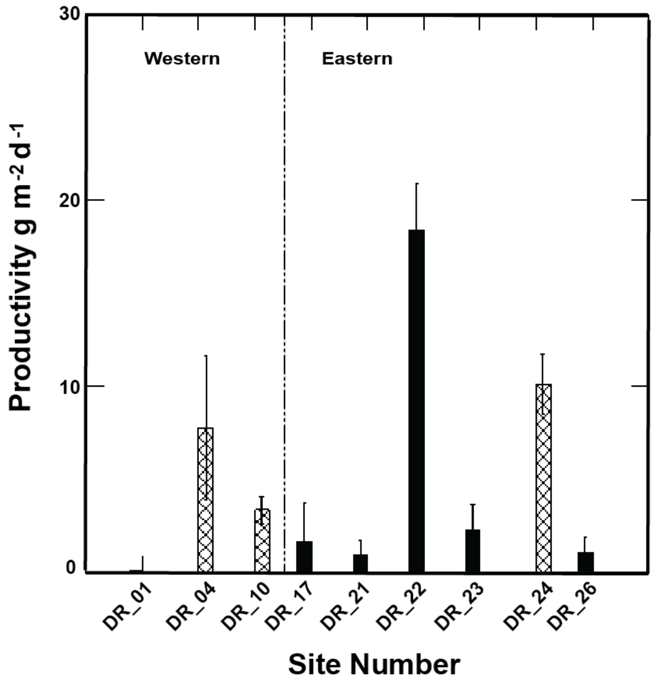

a)
11
10
9
8
7
6
5
4
3
Western
Eastern
δ15N (per mil)
DR_01
DR_04
DR_17
DR_21
DR_22
DR_23
DR_24
Site Number
b)
11
10
9
8
7
6
5
4
3
δ15N (per mil)
Continuous
Seasonal
Flow Type

Figure 7.4

a)


Western
Eastern
Productivity g m-2 d-1
30
20
10
0
DR_01
DR_04
DR_10
DR_17
DR_21
DR_22
DR_23
DR_24
DR_26
Site Number

b)

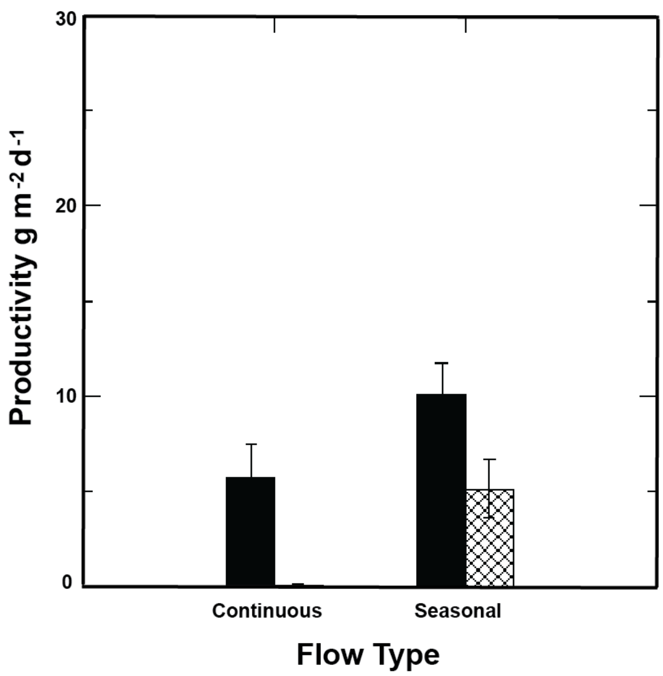
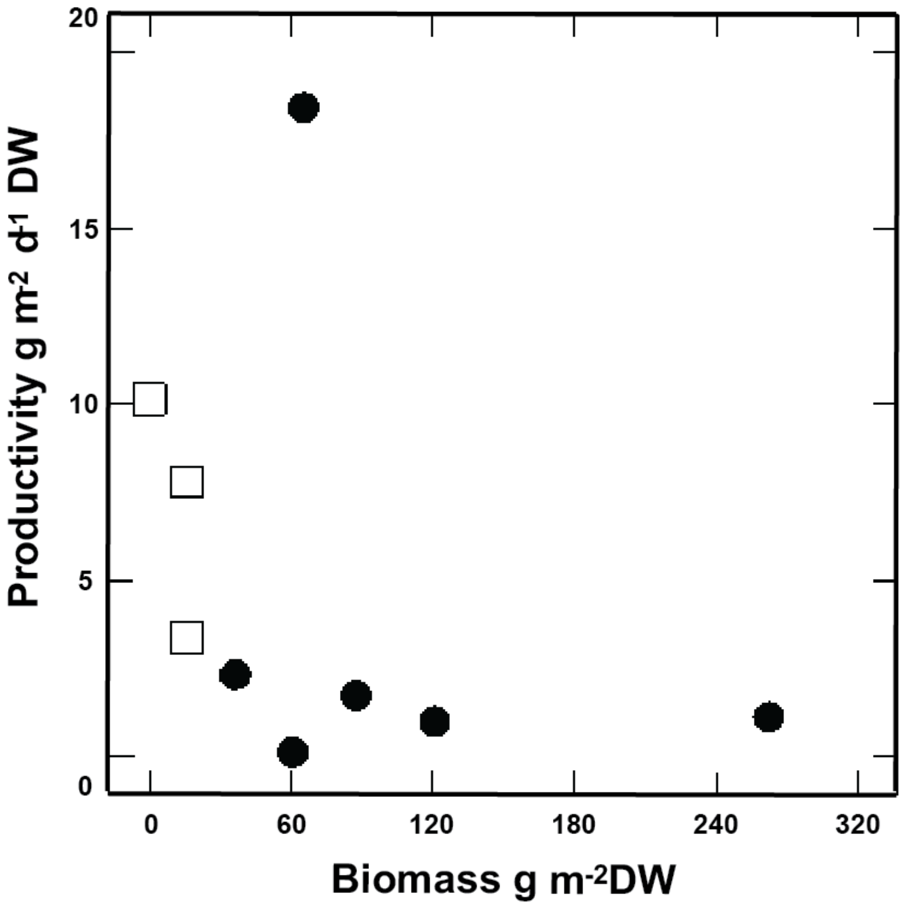

Productivity g m-2 d-1
30
20
10
0
Continuous
Seasonal
Flow Type

Figure 7.5:


20
15
10
5
0
0
60
120
180
240
320
Productivity g m-2 d-1 DW
Biomass g m-2DW

CHAPTER 8 – GENERAL DISCUSSION

8 Synthesis of the Physical, Chemical and Biological Characteristics of the Waterbodies of coastal South East South Australia

This final chapter aims to summarise the outcomes of each research component undertaken over the life of the study (Section 8.1, but see Figure 8.1). General implications of these outcomes and observations to regional marine ecology are then discussed (8.2), before answering specifically, those key ecological questions posed in Chapter 1 (see Table 1.2).

8.1 MAIN FINDINGS

Investigation of water quality characteristics in this study suggested that regional groundwater has a significant influence in maintaining annual water flow, and thus ecological communities, within some of the coastal creeks and drains within the South East of South Australia. Regular intra-annual sampling events revealed that some drains flowed all year (and so were termed Continuous-flow Drains) whereas others were intermittent (termed Seasonal-flow Drains) and typically flowed during the wetter parts of the year. Preliminary observations revealed that there were some similarities and also differences where drains were located. Arbitrarily, this border between drain regions was set as east (Eastern Region) versus west (Western Region) of Cape Northumberland (approximately 3 km west of the Port MacDonnell township). Both regions contained examples of Continuous- and Seasonal-flow Drains.

A total of 25 coastal creeks were initially visited in this study (13 in the western versus 12 in the eastern region). Of these 25 coastal drains sampled 12 had continuous-flow and 13 had seasonal flow (see Chapter 3). Nine of the Continuous-flow drains were located east of Cape Northumberland between Port MacDonnell and the Victorian border.

8.1.1 Review of regional waterbody characterisation and patterns

Water characterisation results (Chapter 3) suggested that salinity differences were apparent between the four main waterbody groups (marine waters, drains and creeks, groundwater ponds and beach springs). Marine waters, as expected, showed no influences of either surface water or groundwater. Consistently low salinities were observed in ponds (<2 ppt) and beach springs (<3 ppt), which I infer to have originated from groundwater sources (i.e. described above as groundwater-fed). Salinity was observed to vary (range: <1 to >47 ppt) between drains of the broader study region which, in part, are likely to be attributed to the quality and quantity of water inputs (groundwater, rainfall and agricultural run-off, and periodic marine influences from tides and waves) and the mixing and hence dilutions thereof.

A further, consistent salinity pattern emerged when Continuous-flow Drains were separated by region. Typically, Western Continuous-flow Drains displayed fresh, brackish and sometime marine salinities (ranging between 1 and 31 ppt). This observation suggests a mixed influence from surface and/or groundwater and also from tidal exchange in some cases. In contrast, the Eastern continuous-flowing Drains consistently displayed 'fresher' salinities with most having annual salinity means of less than 2 ppt.

When the Continuous-flow Eastern Drains were analysed against groundwater ponds and springs, which had been earlier inferred to be groundwater-fed, no significant differences ($P > 0.05$) in salinities were observed. These salinity results suggest that Eastern drains are likely to be predominantly groundwater-fed but, in addition, are likely to be influenced from time to time by surface water runoff (especially in winter months due to rainfall).

The difference in salinity conditions between drain types (i.e. with Flow and Region combined) is likely to influence the assemblages of the resident biota such as fish, macroinvertebrates, plants and algae that reside in them. The consistent low salinities in the Continuous-flow Eastern Drains suggest a mechanism explaining how a freshwater biota (such as insects, oligochaetes) dominated whereas the biota of Seasonal-flow Western drains displayed more estuarine to marine salinity regimes at various times and so their species compositions (crustaceans, fish, polychaete worms) reflected these fluctuations in salinities (Chapter 4).

Mean temperatures across all location types showed a typical annually-variable (i.e. strongly seasonal) pattern with Marine and Drain sites having greater annual ranges of 9-25°C and 8-27°C, respectively. Ponds and Springs also varied but the variability around their means was less pronounced (approximately 10-22°C); this effect was suggestive of groundwater origins. Mean temperatures in Eastern Drains (16-19°C) sites were cooler but with a smaller range than those in Western Drains (13-24°C) further suggesting that Continuous-flow Drains in that region are supported and influenced by groundwater inputs.

Nitrogen concentrations varied between location types with the groundwater-fed Ponds having the highest mean values of TIN and nitrate. Significant differences (P <0.001) were observed when drains were analysed by region. Continuous-flow Eastern Drains displayed consistently higher concentrations of TIN whereas Continuous-flow Western Drains were significantly lower (about half).

Percentage contributions from nitrate to TIN were considerably higher (70-80%) in Ponds, Springs and Eastern Drain, than Western Drains (only 17%). Greater than 99% of NOx was made up of nitrate. These patterns suggest that quite different sources of nitrogen may be involved here, e.g. from dairying land use in the east versus other forms of agriculture to the west.

Ponds and Beach Springs showed similar values of ^{2}H. When drains were separated by flow regime and region combined, Continuous-flow Eastern Drains once again more closely reflected those values observed in known groundwater-fed Ponds and Beach Springs (i.e. more enriched values), whereas Western Drains generally were considerably lower in ^{2}H (i.e. relatively depleted).

A similar pattern was observed in location type and region for ^{18}O where Western Drains again showed smaller ^{18}O values than Eastern Drains, Ponds and Beach Springs. This again supports the evidence that Continuous-flow Eastern Drains are much more likely to be groundwater-fed than those in the west were.

8.1.2 Altered groundwater discharges patterns and the impacts on biotic assemblages

Many intermittent estuaries exist across the state border in the south-western region of Victoria. These intermittent estuaries are creeks and rivers that are subjected to floods, marine inputs and mouth closures at different times of the year. These systems biologically have many estuarine representatives but also have freshwater and marine representatives at different times of the year, and under different inflow/outflow scenarios.

The physical and chemical effect that continuous-flow, groundwater-fed coastal waterbodies within the South East Region have on influencing biotic assemblages is significant. That is, many of these drains, particularly the majority of drains east of Port Macdonnell, remain fresh all year round and lack any true estuarine component all the way to the sea. This physical and chemical monotone has a major effect on the biotic assemblages that reside there.

Investigations of instream macrofauna assemblages (Chapter 4) revealed that these continuous-flow, groundwater-fed coastal waterbodies have shifted from what should have been seasonally intermittent and estuarine in nature and thus dominated by biotic assemblages containing crustaceans, molluscs, annelids, to being more consistent and typical of freshwater systems that are dominated by insects and freshwater species of the above groups.

8.1.3 Aquatic macrofauna communities of coastal south east waterbodies

The shift of coastal estuarine creeks to freshwater drains noted above had profound effects on the biological assemblages that reside there. The biotic assemblages observed resembled more often those animals and plants that could be found living in the lowland freshwater streams further inland. For example, seagrasses were not observed in these drains but many freshwater higher plants and green algae were seen (*P*ers. obs.).

The study identified approximately 140 morphospecies and 60 families of animals from eight orders across five major phyla. As expected, the macrofauna observed was dominated by freshwater representatives. Insects in particular were the most speciose (73% of all species sampled) and most numerically dominant (69% of all individuals collected). Patterns of species richness and total morphospecies abundance varied between years (but with 2005 being the most abundant and diverse), and differed between flow regimes and regional groupings across years. Seasonal-flowing and all Western drains generally had statistically larger means (P <0.05) of macrofaunal abundance and diversity that Continuous-flowing or all Eastern Drains.

8.1.4 Beach Spring Meiofauna: tiny animals in turbulent sands

Macrofauna in these beaches were sparse and did not appear to reflect groundwater influences (Pers. obs.). In contrast, discharging flows from the beach springs are impressive and serve to change not only the water quality but also the consistency of the substrate. Away from springs, intertidal beach sediments are reasonably consolidated. Quite near to the springs the sand becomes much looser and a sand-like slurry develops from the constant motion due to the discharge within the springs.

Thus the beach springs are subject to constant turbulence from discharging ground water and marine influences such as waves and tides on a daily basis. Therefore the microscopic animals that reside between sand grains (i.e. meiofauna) in these springs need to adapt to changing salinities and be able to complete their life histories in these arguably inhospitable habitats.

Almost 50 species from 9 phyla were collected during the meiofaunal study (Chapter 5). Nematodes were the most dominant group of organisms (84% of all numbers collected); they were also the most speciose. More species were observed away from the springs but the springs did have species that were uniquely sampled there. These results suggest that the groundwater itself and/or physical characteristics created by the combination of discharging groundwater and marine conditions may influence meiofaunal community composition on these sandy beaches.

The higher taxonomic groups observed in this study were comparable to other Australian sandy-beach meiofaunal studies (e.g. Dye & Barros 2005; McLachlan 1985). This study also found slightly more nematode representatives than were reported in the studies reviewed by Nicholas and Trueman (2005).

This study is probably the first anywhere to investigate the meiofauna of associated with groundwater beach springs on an exposed, high-energy open beach. As a result, it is possible that two specimens observed here have not been described previously (J. Gwyther, pers comm.).

8.1.5 Tracing nitrogen isotopes in beach fauna

No distinctive fauna was found that could be used to either trace food webs or test for groundwater dependence via transplant experiments. Bivalves (*Paphies elongata*) that were captured in an area under direct influence of discharging beach springs were likely to be from the same local population as those captured in an area well removed from groundwater discharge. The two groups had similar morphometrics (flesh weight versus shell length), flesh $\delta^{15}N$ and %N contents (Chapter 6). A difference in $\delta^{15}N$ accumulation as a result of groundwater influences was not evident in the tissue mass of *P. elongata* sampled in this study. These filter-feeding bivalves did not appear to be strongly influenced nutritionally by organic matter derived from groundwater sources. These results argue against any general groundwater dependence of this taxon, and groundwater discharge did not influence the food chain of which they are part (i.e. surf diatoms and possibly zooplankton to filter-feeding bivalves). Nearshore marine consequences of groundwater outflows would appear to be limited.

8.1.6 Biomass and productivity in coastal drains

The comparative assessment of biomass and productivity (Chapter 7) of flora growing in Seasonal- versus Continuous-flow Drains found that standing stock biomass ranged on average from 0 to >263 $gDW.m^{-2}$ (Green Point and Piccaninnie Ponds Outlet, respectively) and that Continuous-flow Drains had greater standing-stock biomass than Seasonal-flow Drains. Mean daily productivity of periphyton growing in the drains ranged from <1 to 18 $gDW.m^{-2}.d^{-1}$ (Deep Creek and Eight Mile Creek, respectively). Mean periphyton productivity in Seasonal-flow Drains was about double that of Continuous-flow Drains.

Continuous- and seasonal-flow drains had approximately similar mean $\delta^{15}N$ values in flora but their contents ranged, on average, between 4 and >9 ‰ $\delta^{15}N$. Such differences could have flow-on effects to grazing and detritivorous animals living in these drains (Chapter 4).

8.2 IMPLICATIONS OF THESE FINDINGS FOR REGIONAL MARINE ECOLOGY

A benefit of undertaking complex studies with this significant coastal region is that, for most of the sites sampled in this study, human traffic to many of these water bodies is limited. They are fundamentally removed from built up human areas and remnant vegetation areas remain. In no case of the ecosystems, habitats or organisms studied during this project was a dependence upon groundwater as a resource found. At best, any 'dependencies' as such are more likely to be 'influences' (e.g. water quality) and/or opportunistic refuges and permanent habitats (e.g. flora and fauna) in what is a semi-arid landscape. This study was not able to identify any key indicator species to assess dependence on groundwater but the possibility of using insect diversity may warranted further consideration and application.

This lack of a distinctive groundwater-associated fauna and flora made it unproductive to carry out my planned experimental transplants (see Section 4.4.1); essentially there was no different assemblage to transplant. This means that I have not been successful in determining the exact dependence upon groundwater of relevant species and their assemblages. Whilst disappointing, this does lead to another conclusion: that, although groundwater is a longstanding coastal feature of the SE region of SA, it currently has no suite of species absolutely relying upon it.

Some biotic assemblages were judged to be partially dependent upon groundwater in that they were more closely associated with flows or more common where groundwater influenced the environment (e.g. driving and supporting dominance of insect diversity in low salinity freshwater creeks and drains).

It is unlikely that the continuous flow of groundwater to coastal creeks has influenced the evolution of plants and animals but merely they are now supported through the supplement of surface-water runoff that may not have been there under previously ‘natural’ conditions in summer and autumn.

Groundwater that discharges through small but distinct habitats (e.g. groundwater discharging via springs on sandy beaches) is also likely to influence island-like assemblages that are unique and contrasting for adjacent surrounds (e.g. meiofaunal communities).

What is apparent is that groundwater changes the physical conditions of habitats it discharges into, and in an abrupt manner. That is, for the continuous-flow coastal drains and creeks sampled here, no true estuarine conditions exist. These systems go from fresh to marine salinities within metres. This is similar for groundwater springs on sandy beaches. In these beach spring systems any changes (in a low energy environment) is likely to be measured in millimetres or centimetres. The reality for the beach springs investigated here is that the animals within the springs are likely to be subjected to daily changes in conditions from fresh to marine. Depending on tides and waves, these changes may ever occur over seconds.

Thus any animals that reside in these habitats must be either resilient to chemical (i.e. salinity) and physical (i.e. water turbulence, moving sediments) disturbance or have the ability to quickly move within the sediment structure of the springs for survival.

Climate change and rising sea levels may have an impact on these environmental characteristics and thus biotic assemblages in future decades. The fish kill that was observed due to large swells combination with high tides pushing marine salinity water a distance upstream in what is now a freshwater system may be an example of things to come (see fish kill Discussion in Chapter 4). This situation could also occur from the inland side if excessive drawdown of the regional groundwater resulted in a reduction in the geohydrological head localised groundwater (i.e. ground outflow may reduce, thus facilitating circumstances that return these creeks and drains to marine and/or estuarine conditions. In either case, it is likely that the critical factor will be the speed at which any change may happen. It is possible that the slower and gradual change to the chemical and physical characteristics of these systems, the more likely and successfully species adaptation (e.g. allow upstream migration to fresher water sources) and/or changes (e.g. for partially-dependent aquatic organisms such as insects and estuarine fishes) might occur.

Piccaninnie or Ewens Ponds may become key components in establishing estuarine transitional zones under conditions of rising sea level. Both ponds extend deep (Scholz 1990) into the aquifer and so are likely to still discharge even if a modest reduction in the hydrological groundwater head is observed). Rising sea level may push upstream in the outlet creek creating a genuine fresh/marine water mixing area resulting in estuarine conditions. In large events, that may result in seawater reaching the pond but that may be mitigated (to a degree) by the rapid turnover rate of these systems (Scholz 1990).

Groundwater that eventually gets to the nearshore environments, either naturally (e.g. via aquifers, beach springs) or artificially (e.g. human action) resulting in runoff of used irrigation water, drainage construction and operation, can change the nature of the receiving waterbody. In the South East Region of South Australia, this is most clearly seen in the absence of any true estuarine habitats Groundwater accession to the coastline is a notable feature of the South East region of South Australia and so it was worth studying possible or perceived effects. The findings presented here are also likely to be relevant to other South Australian places with semi-arid climes, karstic geology and aquifers located near the ground surface, e.g. the lower Eyre Peninsula, and far west coast).

8.2.1 Management implications

There was only very limited background data associated with this region at the commencement of this project (2003). Thus it was very important to collect relevant and well-replicated environmental data to permit future statistical testing and allow for design of direct experimentation. The results of the present study can provide regional natural resource managers with critical biological and water-quality data information that may guide future monitoring. In particularly, this study provides valuable observations and understanding that fills many knowledge gaps associated with groundwater as a coastal resource and player in nearshore ecosystem interactions, particularly in this region of south east South Australia.

The results of this study could, at a minimum, be used by natural resource managers to shape biodiversity conservation programs. Identification of any uniqueness in the results here may be used to lever support and win over external funding opportunities for habitat protection programs, or for additional collaborative research activities.

A focus of the study was to address the SENRMB understanding of perceived local dependencies on groundwater that nearshore coastal ecosystems may have. Any observed relationships could then be used by SENRMB to, in part, inform future water-allocation planning. At this time of its completion, limited formal management recommendations could be made. Issues to address further information gaps and research improvements have been made previously in the thesis.

Leveraging of the preliminary findings of this study, some outcomes of a subsequent study (an ARC-Linkage project [see Fairweather *et al.* 2011]) identified a number of management recommendations to assist SENRMB decision making in with regard to future reviews of water allocation policy.

Specifically, these included monitoring and research focussed around (Fairweather *et al.* 2011):

- Flow regimes and water quality (salinity, dissolved oxygen, nutrients) of drains and beach springs
- Effects on fish populations of discharging groundwater including any links to fisheries production and any effects further offshore
- Understanding nitrogen cycle in relation to the various sources of nitrogen in the catchment
- Freshwater character of drains and coastal creeks including any effects of manipulating or diverting water flow
- Changes in the creeks draining Picanninie Ponds and/or Ewens Ponds

This study provided some important background information and baseline data that could be used by regional natural resource managers to guide future investigations and monitoring programs. Monitoring programs could be delivered by qualified academics with strict research and experimental focus, to those of community-based programs with participation, awareness and applied outcomes.

8.2.2 Future Studies

The high productivity of periphyton and other plants seen in the drains and coastal creeks is probably benefiting from both the continuously-flowing conditions and the abundance of nutrients coming down the drains. This primary production in turn provides considerable loads of detritus as dead organic matter for fuelling the food webs of the region (that we are only now coming to understand; Duong 2009; Fairweather *et al.* 2011). It would be very interesting to trace these pathways up into the larger animals that are either fished (e.g. lobster, finfish, squid, giant crab) or otherwise enjoyed by ecotourists (e.g. shorebirds, marine mammals), to ascertain if groundwater is indirectly supporting these human activities.

8.3 OVERALL CONCLUSIONS

Concluding statements are given here in response to the questions posed in Chapter 1 flow chart (Figure. 1.2):

8.3.1 Are coastal waterbodies such as drains, ponds & springs and nearshore marine environments in SE SA influence by groundwater discharge?

This study (Chapter 3) found that coastal waterbodies such as ponds, beach springs and Eastern drains are influenced or driven by groundwater discharge and that a strong influence on Western drains was not apparent. The study did not find an ecological influence by groundwater on nearshore coastal ecosystems.

8.3.2 What are the temperature, salinity, nutrient and isotopic characteristics of these coastal waterbodies?

Ponds, springs and Continuous-flow Eastern Drains were considered to be 'fresh', whereas seasonal-flow Western Drains were brackish to marine in nature (Chapter 3). Mean temperature in drains varied between sites but Eastern drains were slightly cooler and more stable than seasonal drains. Ponds and springs were coolest and had less annual temperature variation than drains. Total nitrogen concentrations varied between location types. Ponds had the largest concentrations of TIN and nitrate than other groups. Continuous-flow Eastern Drains had higher TIN concentrations than Continuous-flow Western Drains. The percent contributions from nitrate to TIN were considerably higher in Ponds, Springs and Eastern Drains, than Western Drains. Ponds, Beach Springs and Continuous-flow Eastern Drains showed similar δ^2H and $\delta^{18}O$ values between them, suggesting isotopic enrichment. All this evidence suggests that Continuous-flow Eastern Drains are most likely to be groundwater-fed.

8.3.3 Do groundwater-fed drains maintain continuous or seasonal connection to nearshore marine environments?

Typically (Chapter 3), groundwater-fed drains maintain continuous flow to nearshore marine environments but discharge rates may vary within sites and any year.

8.3.4 What are the differences in the macrobiota that reside in seasonal versus continuous groundwater-influenced drains?

Patterns of species richness and total morphospecies abundance varied between years, flow, and region (Chapter 4). Seasonal-flow and Western Drains generally had greater individual abundances and species richnesses than Continuous-flow and Eastern Drains. Insects dominated Continuous-flow Drains as per purely freshwater habitats. Continuous groundwater flow can also influence physical habitat conditions that can influence macrofauna abundance (Zipperle & Reise 2005, Miller *et al.* 2009, Dale & Miller 2007, 2008).

8.3.5 Does primary productivity and standing stock biomass differ between seasonal and continuous groundwater-influenced drains?

Standing stock biomass of flora was greater in groundwater-fed continuous flow drain than season-flow drains (Chapter 7). Productivity was greater in seasonal-flow drains than continuous flow drains.

8.3.6 Are different meiofaunal communities observed in groundwater-fed beach springs compared to intertidal marine sands?

Meiofaunal abundance and species richness was less in the springs than in nearby marine intertidal sediment (Chapter 5). Different assemblages were observed between the groups.

8.3.7 Are there differences in nitrogen isotope ratios in the flesh of intertidal bivalves found in areas influenced by groundwater-fed beach springs compared to intertidal marine sands?

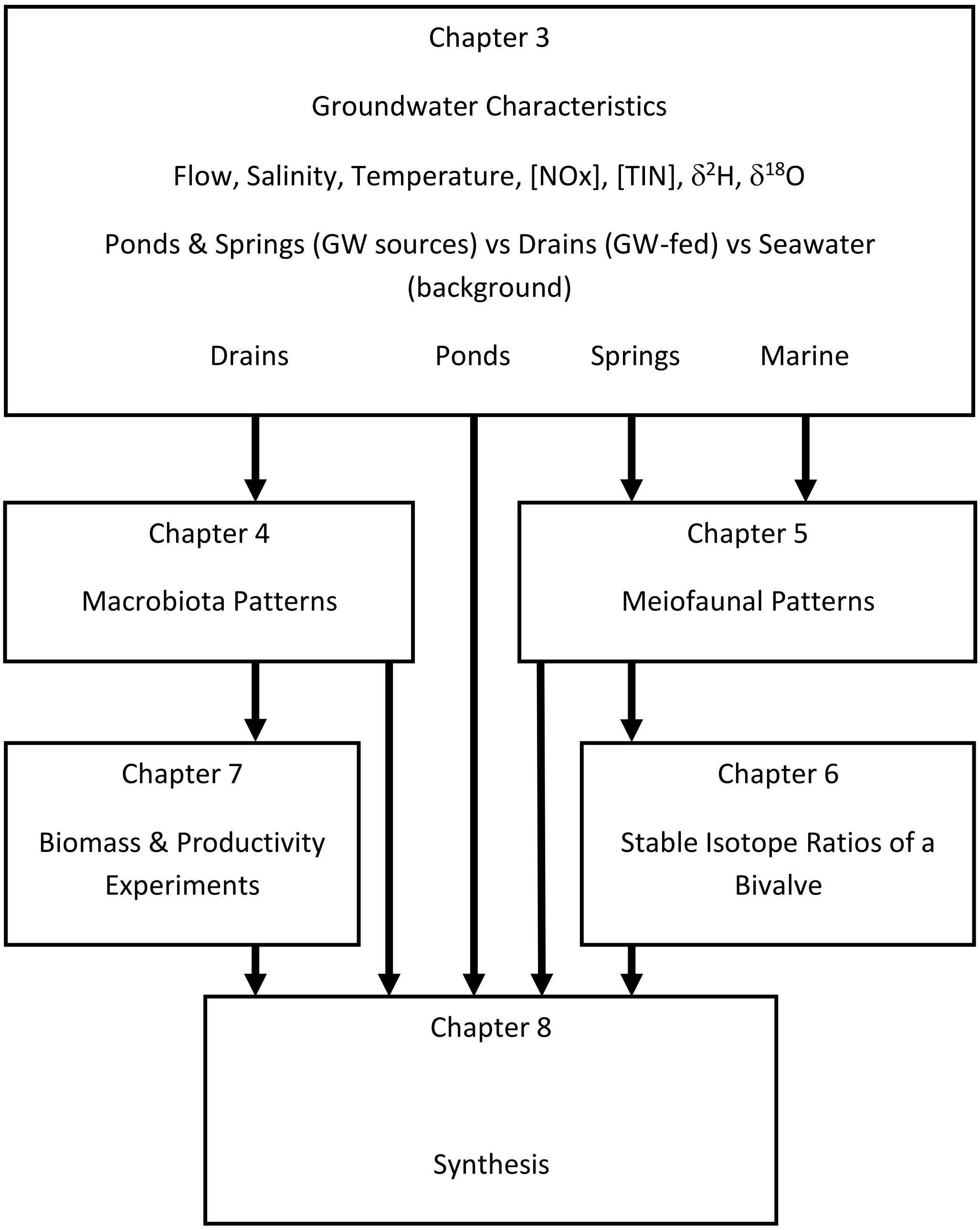
No difference in nitrogen isotope ratios in the flesh of intertidal bivalves found in areas influenced by groundwater-fed beach springs compared to intertidal marine areas not near groundwater beach springs was observed (Chapter 6).

In closing, one aim of this study was to deviate from the dominant physical and chemical foci of the majority of previous research in to groundwater discharge within (or in the case of this study, to) nearshore marine ecosystems (or submarine groundwater discharge, as it is also known) and to address the effects of the phenomena form an ecological focus. I also hope that an additional outcome of this research project has been to point the way, in a methodological sense, for further research elsewhere upon the ecological effects of groundwater to nearshore and coastal marine ecosystems.

As stated in Chapter 1, Johannes (1980) is probably one of the earliest and most regularly cited papers in the submarine groundwater discharge realm. Johannes (1980) posed the question “what effects does SGD have on the ecological components of nearshore marine environments?” Whilst many authors have acknowledged or restated this question in one form or another, the ecological component has never truly been addressed. Although some of the main objectives and early direction this project hoped to find and achieve were not completely realised, I do hope that it has, in some small way, remained true to the ecological and Australian focus it set out to achieve. Specifically, that being we now know more about the ecological impacts of groundwater discharges to nearshore marine ecosystems in south east South Australia.

FIGURES

Figure 8.1: Factors potentially influencing groundwater effects in the coastal and nearshore ecosystems studied in this thesis (including the chapter number where each issue is primarily addressed).



REFERENCES

Akroyd JAM, Walshe KAR, Millar RB (2002) Abundance, distribution, and size structure of toheroa (*Paphies ventricosa*) at Ripiro Beach, Dargaville, Northland, New Zealand. New Zealand Journal of Marine and Freshwater Research. 36: 547-553

Allan J (1959) Australian Shells. Georgian House, Melbourne.

Anderson, M.J. (2004). PERMDISP: a FORTRAN computer program for permutational analysis of multivariate dispersions (for any two-factor ANOVA design) using permutation tests. Department of Statistics, University of Auckland, New Zealand.

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth UK

ANZECC (Australian and New Zealand Environment and Conservation Council) (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Volume 1 - The Guidelines, Australian and New Zealand Environment and Conservation Council and the Agriculture and Resource Management Council of Australia and New Zealand.

Ardisson P-L, Bourget E (1997) A study of the relationship between freshwater runoff and benthos abundance: a scale oriented approach. Estuarine Coastal & Shelf Science 45:535-545

Arundel HP (2003) Invertebrate larval dynamics in seasonally closed estuaries. Thesis (*P*h.D.)--Deakin University, Victoria, 335pp.

Ataie-Ashtiani B, Volker RE, Lockington DA (2001) Tidal effects on groundwater dynamics in unconfined aquifers. Hydrological Processes 15:655-669

Barton J. & Sherwood J. (2004) Estuary opening management in Western Victoria: An information analysis. Parks Victoria Technical Series No. 15. Parks Victoria, Melbourne.

Barton, JL, Pope, AJ, Quinn, GP and Sherwood, JE (2008) Identifying threats to the ecological condition of Victorian estuaries. Department of Sustainability and Environment Technical Report.

Bayari CS, Kurttas T (2002) Coastal and submarine karstic discharges in the Gokova Bay, SW Turkey. Quarterly Journal of Engineering Geology & Hydrogeology 35:381-390

Becker A (2007) Fish use of flooded margins of an intermittently open estuary. Thesis (*P*h.D.)--Deakin University, Victoria, 2000 180pp.

Becker A, Laurenson LJB (2007) Seasonal and diel comparisons of the diets of four dominant fish species within the main channel and flood-zone of a small intermittently open estuary in south-eastern Australia. Marine and Freshwater Research 58: 1086-1095

Becker A, Laurenson LJB (2008) Presence of fish on the shallow flooded margins of a small intermittently open estuary in south-eastern Australia under variable flooding regimes. Estuaries and Coasts 31: 43-52

Becker A, Laurenson LJB, Bishop K (2009) Artificial mouth opening fosters anoxic conditions that kill small estuarine fish. Estuarine, Coastal and Shelf Science. 82: 566-572

Beentjes MP, Carbines GD, Willsman AP (2006) Effects of beach erosion on abundance and distribution of toheroa (*P*aphies ventricosa) at Bluecliffs Beach, Southland, New Zealand. New Zealand Journal of Marine and Freshwater Research 40: 439-453

Belanger TV, Montgomery MT (1992) Seepage meter errors. Limnology & Oceanography 37: 1787-1795

Bokuniewicz H, Buddemeier R, Maxwell B, Smith C (2003) The typological approach to submarine groundwater discharge (SGD). Biogeochemistry 66: 145-158

Blanco AC, Watanabe A, Nadaoka K, Motooka S, Herrera EC, Yamamoto T (2011) Estimation of nearshore groundwater discharge and its potential effects on a fringing coral reef. Marine Pollution Bulletin 62: 770–785

BOM (Bureau of Meteorology) (2005) Climate Averages for South Australian Sites.

http://www.bom.gov.au/climate/averages/tables/ca_sa_names.shtml Modified 11 August 2004. Accessed 11 January 2006

Bratton JF (2010) The three scales of submarine groundwater flow and discharge across passive continental margins. The Journal of Geology 118: 565-575

Burnett WC, Taniguchi M, Oberdorfer J (2001) Measurement and significance of the direct discharge of groundwater into the coastal zone. Journal of Sea Research 46: 109-116

Bussman I, Schlömer S, Schlüter M, Wessels M (2011) Active pockmarks in a large lake (Lake Constance, Germany): Effects on methane distribution and turnover in the sediment. Limnology and Oceanography 56: 379-393

Cable JE, Burnett WC, Chanton JP (1997a) Magnitude and variations of groundwater seepage along a Florida marine shoreline. Biogeochemistry 38: 189-205

Cable JE, Burnett WC, Chanton JP, Corbett DR, Cable PH (1997b) Field evaluation of seepage meters in the coastal marine environment. Estuarine Coastal & Shelf Science 45: 367-375

Carruthers TJB, van Tussenbroek BI, Dennison WC (2005) Influence of submarine springs and wastewater on nutrient dynamics of Caribbean seagrass meadows. Estuarine, Coastal and Shelf Science 64: 191-199

CFS (1999) Map Book Region 5 (*P*art): Lower South East. Government of South Australia. Country Fire Service of South Australia, Naracoorte

Charette MA, Buesseler KO, Andrews JE (2001) Utility of radium isotopes for evaluating the input and transport of groundwater-derived nitrogen to a Cape Cod estuary. Limnology & Oceanography 46: 465-470

Clark ID, Fritz P (1997) Environmental Isotopes in Hydrogeology. Lewis Publishers. Boca Raton.

Clarke KR, Gorley RN (2006) PRIMER v6: User Manual/Tutorial, PRIMER-E, Plymouth

Cole RG, Hull PJ, Healy TR (2000) Assemblage structure, spatial patterns, recruitment, and post-settlement mortality of subtidal bivalve molluscs in a large harbour in north-eastern New Zealand. New Zealand Journal of Marine and Freshwater Research. 34: 317-329

Connell SD, Gillanders GM (eds) (2007) Marine Ecology. Oxford University Press. South Melbourne, Australia. 630pp.

Corbett DR, Chanton J, Burnett W, Dillon K, Rutkowski C, Fourqurean JW (1999) Patterns of groundwater discharge into Florida Bay. Limnology & Oceanography 44: 1045-1055

Corbett DR, Dillon K, Burnett W, Chanton J (2000) Estimating the groundwater contribution into Florida Bay via natural tracers, 222Rn and CH4. Limnology & Oceanography 45: 1546-1557

Cotton BC (1984) South Australian Mollusca Pelecypoda. Government Printers, Adelaide. Pp???

Courtenay GC, Gladstone W, Schreider M (2004) Assessing response of estuarine intertidal assemblages to urbanised catchment discharge. Environmental Monitoring and Assessment. 107: 375-398

Cranfield HJ, Michael KP (2001) Growth rates of five species of surf clams on a southern North Island beach, New Zealand. New Zealand Journal of Marine and Freshwater Research. 35: 909-924

Cronk JK, Siobhan M (2001) Wetland Plants: Biology and ecology. Lewis Publishers, Boca Raton.

CSIRO (2006) Groundwater-Dependent Ecosystems. Australian Journal of Botany [Special Edition] 54(iss. 2): 91-237

Cummings VJ, Thrush SF (2004) Behavioural response of juvenile bivalves to terrestrial sediment deposits: implications for post-disturbance decolonisation. Marine Ecology Progress Series 278: 179-191

Currie DR, Small K (2006) The influence of dry-season conditions on the bottom dwelling fauna of an east Australian sub-tropical estuary. Hydrobiologia 560: 345-361,

Dale R K, Miller D C (2007) Spatial and temporal patterns of salinity and temperature at an intertidal groundwater seep. Estuarine, Coastal and Shelf Science 72: 283-298

Dale R K, Miller D C (2008) Hydrologic interactions of infaunal polychaetes and intertidal groundwater discharge. Marine Ecology Progress Series. 363: 205-215

Danielopol DL, Griebler C, Gunatilaka A, Notenboom J (2003) Present state and future prospects for groundwater ecosystems. Environmental Conservation 30: 104-130

Davenport SR, Bax NJ (2002) A trophic study of a marine ecosystem off south-eastern Australia using stable isotopes of carbon and nitrogen. Canadian Journal of Fisheries and Aquatic Sciences 59: 514-530

de Sieyes NR, Yamahara KM, Paytan A, Boehm AB (2011) Submarine groundwater discharge to a high-energy surf zone at Stinson Beach, California, estimated using radium isotopes. Estuaries and Coasts 34: 256–268

Dillon PJ (1988) An evaluation of the sources of nitrate in groundwater near Mount Gambier, South Australia. CSIRO Water Resources Series No. 1.

Dole-Olivier MJ, Galassi DMP, Marmonier P, Creuze des Chat Chatelliers (2000) The biology and ecology of lotic microcrustaceans. Freshwater Biology 44: 63-91

Dollar SJ, Atkinson MJ (1992) Effects of nutrient subsidies from groundwater to nearshore marine ecosystems of the island of Hawaii. Estuarine Coastal & Shelf Science 35: 409-424

Dye AH (2006a) Is geomorphic zonation a useful predictor of patterns of benthic infauna in intermittent estuaries in NSW, Australia. Estuaries and Coasts 29: 455-464

Dye AH (2006b) Influence of isolation from the sea on spatial patterns of macroinfauna in intermittently closed/open coastal lakes in New South Wales. Austral Ecology 31: 013-924

Dye AH, Barros F (2005a) Spatial patterns of macrofaunal assemblages in intermittently closed/open coastal lakes in New South Wales, Australia. Estuarine, Coastal and Shelf Science 64: 357-371

Dye AH, Barros F (2005b) Spatial patterns in meiobenthic assemblages in intermittently open/closed coastal lakes in New South Wales, Australia. Estuarine Coastal and Shelf Science 62: 575-59.

Eamus D, Froend R (2006) Groundwater-dependent ecosystems: the where, what and why of GDE's. Australian Journal of Botany 54: 91-96

Eamus D, Froend R, Loomes R, Hose G, Murray B (2006a) A functional methodology for determining the groundwater regime needed to maintain the health of groundwater-dependent vegetation. Australian Journal of Botany 54: 97-114

Eamus D, Hatton T, Cook P, Colvin C (2006b) Ecohydrology: Vegetation function, water and resource management, CSIRO Publishing, Collingwood 348 pp.

Edgar GJ (2000) Australian Marine Life: the Plants and Animals of Temperate Waters. Reed New Holland, Sydney, Australia. 544pp.

Edgar GJ (2001) Australian Marine Habitats in Temperate Waters. Reed New Holland, Sydney, Australia. 224pp.

Edgar GJ, Barrett NS (2000) Effects of catchment activities on macrofaunal assemblages in Tasmanian estuaries. Estuarine, Coastal and Shelf Science 50: 639-654

Edgar GJ, Barrett NS, Last PR (1999) The distribution of macroinvertebrates and fishes in Tasmanian estuaries. Journal of Biogeography 26: 1169-1189

Ellins KK (1992) Stable isotopic study of the groundwater of the Martha Brae River Basin, Jamaica. Water Resources Research 28: 1597-1604

Fairweather PG, Henry RJ, Langley, RA, Duong SHL (2011) Assessing dependence on groundwater of nearshore coastal habitats in south east South Australia. Flinders University

Fetter CW (2001) Applied Hydrology. Prentice-Hall Publishing. Upper Saddle River, New Jersey, USA.

Fleury P, Bakalowicz M, de Marsily G (2007) Submarine springs and coastal karst aquifers: A review. Journal of Hydrology 339: 79-92

Fry B (1999) Using stable isotopes to monitor watershed influences on aquatic trophodynamics. Canadian Journal of Fisheries and Aquatic Sciences 55: 2167-2171

Garrison GH, Glenn CR, McMurtry GM (2003) Measurement of submarine groundwater discharge in Kahana Bay, O'ahu, Hawai'i. Limnology & Oceanography 48: 920-928

Gobler CJ, Boneillo GE (2003) Impacts of anthropogenically influenced groundwater seepage on water chemistry and phytoplankton dynamics within a coastal marine system. Marine Ecology-Progress Series 255: 101-114

Gouramanis C, Wilkins D, De Deckker P (2010) 6000 years of environmental changes recorded in Blue Lake, South Australia, based on ostracod ecology and valve chemistry. Palaeogeography, Palaeoclimatology, Palaeoecology 297:223–237

Grant CM, Creese RG (1995) The reproductive cycle of the tuatua – Paphies subtriangulata (Wood, 1828) in New Zealand. Journal of Shellfish Research 14: 287-292

Grant CM, Hooker SH, Babcock RC, Creese RG (1998) Synchronous spawning and recruitment and reproductive incompatibility of two bivalve species: Paphies subtriangulata and Paphies australis. Veliger 41: 148-156

Green EP, Short FT (2003) World Atlas of Seagrasses, University of California Press, Berkeley

Haddon M, Willis TJ, Wear RG, Anderlini VC (1996) Biomass and distribution of five species of surf clam off an exposed west coast North Island Beach, New Zealand. Journal of Shellfish Research 15: 331-339.

Hastie BF, Smith SDA (2006) Benthic macrofaunal communities in intermittent estuaries during a drought: comparisons with permanently open estuaries. Journal of Experimental Marine Biology and Ecology 330: 356-367

Hatton T, Evans R (1998) Dependence of Ecosystems on Groundwater and its Significance to Australia, Land & Water Resources Research & Development Corporation, Canberra

Hayashi M, Rosenberry D O (2002) Effects of Ground Water Exchange on the Hydrology and Ecology of Surface Water. Ground Water 40: 309-316

Hays R L, Ullman W J (2007) Dissolved Nutrient Fluxes through a Sandy Estuarine Beachface (Cape Henlopen, Delaware, USA.): Contributions from Fresh Groundwater Discharge, Seawater Recycling, and Diagenesis. Estuaries and Coasts 30: 710-724

Healy JM, Lamprell K (1998a) Subclass Heterodonta in Mollusca: The Southern Synthesis (eds) Beesley PL, Ross GJB, & Wells A. CSIRO Australia Publishing, Melbourne. p301-396

Healy JM, Lamprell K (1998b) Superfamily Mactroidea in Mollusca: The Southern Synthesis (eds) Beesley PL, Ross GJB, & Wells A. CSIRO Australia Publishing, Melbourne. p336-340

Herczeg AL, Leaney FW, Dighton JC, Lamontagne S, Schiff SL, Telfer AL, English MC (2003) A modern isotope record of changes in water and carbon budgets in a groundwater-fed lake: Blue Lake, South Australia. Limnology and Oceanography 48: 2093-2105

Herczeg AL, Leaney FW, Stadter MF, Allan GL, Fifield LK (1997) Chemical and isotopic indicators of point source recharge to a karst aquifer, South Australia. Journal of Hydrology 192 271-299

Herrera-Silveira JA, Medina-Gomez I, Colli R (2002) Trophic status based on nutrient concentration scales and primary producers community of tropical coastal lagoons influenced by groundwater discharges. Hydrobiologia 475: 91-98

Hewitt JE, Norkko J (2007) Incorporating temporal variability into studies: an example using suspension feeding bivalves and elevated suspended sediment concentrations. Journal of Experimental Marine Biology and Ecology 341: 131-141

Higgins RP, Thiel H (1988) Introduction to the Study of Meiofauna. Smithsonian Instituition Press. Washington.

Hirst A (2004) Broad-scale environmental gradients among estuarine benthic macrofaunal assemblages of south eastern Australia: implications for monitoring estuaries. Marine and Freshwater Research 55: 79-92

Hirst A, Alpine J, Crawford C (2006) Benthic macroinvertebrate communities of high conservation value in Thirsty and Little Thirsty Lagoons, Cape Barren Island. Papers and proceedings of the Royal Society of Tasmania. 140: 2006 1-7

Hirst AJ, Kilpatrick R (2007) Spatial and temporal variation in the structure of estuarine macroinvertebrate assemblages: implications for assessing the health of estuaries. Marine and Freshwater Research 58: 866-879

Hoefel FG, Evans RL (2001) Impact of low salinity porewater on seafloor electromagnetic data: A means of detecting submarine groundwater discharge? Estuarine Coastal & Shelf Science 52: 179-189

Hooker SH, Creese RG (1995) The reproductive biology of pipi, Paphies australis (Gmelin, 1790) (Bivalvia: Mesodesmatidae). I. Temporal patterns of the reproductive cycle. Journal of Shellfish Research 14: 7-15

Hooker SH, Creese RG (1995) The reproductive biology of pipi, Paphies australis (Gmelin, 1790) (Bivalvia: Mesodesmatidae). II. Spatial patterns of the reproductive cycle. Journal of Shellfish Research 14: 17-24

Hooker SH (1997) Larval and post-larval development of the New Zealand pipi, Paphies australis (Bivalvia : Mesodesmatidae). Bulletin of Marine Science 61: 225-240.

Hulings NC, Gray JS (1971) A Manual for the Study of Meiofauna. Smithsonian Institution Press. Washington.

Hull PJ, Cole RG, Creese RG, Healy TR (1998) An experimental investigation of the burrowing behaviour of Paphies australis (Bivalvia:Mesodesmatidae). Marine and Freshwater Behaviour and Physiology 31: 167-183

James RJ & Fairweather PG (1995) Comparison of rapid methods for sampling the pipi, Donax deltoides (Bivalvia: Donacidae), on sandy ocean beaches. Marine & Freshwater Research 46: 1093-1099

James RJ, Fairweather PG (1996) Spatial variation of intertidal macrofauna on a sandy ocean beach in Australia. Estuarine, Coastal & Shelf Science 43: 77-103

Johannes RE (1980) The ecological significance of the submarine discharge of groundwater. Marine Ecology-Progress Series 3: 365-373

Johannes RE, Hearn CJ (1985) The effects of submarine groundwater discharge on nutrient and salinity regimes in a coastal lagoon of Perth, Western Australia. Estuarine Coastal & Shelf Science 21: 789-800

Johannes RE, Rimmer DW (1984) Some distinguishing characteristics of nesting beaches of the green turtle Chelonia mydas on North West Cape Peninsula, Western Australia. Marine Biology 83: 149-154

Kamermans P, Hemminga MA, Tack JF, Mateo MA, Marba N, Mtolera M, Stapel J, Verheyden A, Van Daele T (2002) Groundwater effects on diversity and abundance of lagoonal seagrasses in Kenya and on Zanzibar Island (East Africa). Marine Ecology-Progress Series 231: 75-83

Katz BG (2004) Sources of nitrate contamination and age of water in large karstic springs of Florida. Environmental Geology 46: 689–706

Kefford BJ, Palmer CG, Nugegoda D (2005) Relative salinity tolerance of freshwater macroinvertebrates from the south-east Eastern Cape, South Africa compared with Barwon Catchment, Victoria, Australia. Marine and Freshwater Research 56: 163-171

Kefford BJ, Palmer CG, Nugegoda D (2007) Salinity tolerance of riverine microinvertebrates from the southern Murray-Darling Basin. Marine and Freshwater Research 58: 1019-1031

Kefford BJ, Papas PJ, Nugegoda D (2003) Relative salinity tolerance of macroinvertebrates from Barwon River, Victoria, Australia. Marine and Freshwater Research 54: 755-765

Keser M, Swenarton JT, Vozarik JM, Foertch JF (2003) Decline in eelgrass (Zostera marina L.) in Long Island Sound near Millstone Point, Connecticut (USA) unrelated to thermal input. Journal of Sea Research 49: 11-26

Kingsford M, Battershill C (eds) (1998) Studying Temperate Marine Environments: A handbook for ecologists. Canterbury University Press, Christchurch, NZ. 355pp.

Kohout FA, Kolipinski MC (1967) Biological zonation related to groundwater discharge along the shore of Biscayne Bay, Miami, Florida. In: Lauff GH (ed) Estuaries. American Association for the Advancement of Science, Washington DC, p 488-499

Kotwicki L, Grzelak K, Czub M, Dellwig O, Gentz T, Szymczycha B, Böttcher ME (2014) Submarine groundwater discharge to the Baltic coastal zone: Impacts on the meiofaunal community. Journal of Marine Systems 129: 118–126

Krebs CJ (2001) Ecology: the experimental analysis of distribution and abundance. 5th Ed. Addison-Wesley, San Francisco. 695pp.

Lamontagne S, Le Gal La Salle C, Hancock GJ, Webster IT, Simmons CT, Love AJ, James-Smith J, Smith AJ, Kämpf J, Fallowfield HJ (2008) Radium and radon isotopes in regional groundwater, intertidal groundwater, and seawater in the Adelaide Coastal Waters Study area: Implications for the evaluation of submarine groundwater discharge. Marine Chemistry 109: 318-336

Lamprell K, Healy J (1998) Bivalves of Australia (Vol 2). Backhuys Publishers, Leiden.

Lamprell K, Whitehead T (1992) Bivalves of Australia (Vol 1). Crawford House Press, Bathurst.

Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnology & Oceanography 42: 1119-1131

Laws EA, Ziemann D, Schulman D (1999) Coastal water quality in Hawaii: the importance of buffer zones and dilution. Marine Environmental Research 48: 1-21

Leach BF, Davidson JM, Robertshawe M, Leach PC (2001) Identification, nutritional yield and economic role of tuatua shellfish, Paphies spp., in New Zealand Archaeological Sites. People and Culture in Oceania 17: 1-26

Leaney FW, Herczeg AL (1995) Regional recharge to a karst aquifer estimated from chemical and isotopic composition of diffuse and localised recharge, South Australia. Journal of Hydrology 164:363-387

Lee DR (1977) A device for measuring seepage flux in lakes and estuaries. Limnology & Oceanography 22: 140-147

Lercari D, Defeo O (1999) Effects of freshwater discharge in sandy beach populations: The mole crab Emerita brasiliensis in Uruguay. Estuarine Coastal & Shelf Science 49: 457-468

Lester RE, Wright W, Jones-Lennon M (2007) Does adding wood to agricultural streams enhance biodiversity? An experimental approach. Marine and Freshwater Research. 58: 687-698

Lewis RK (1981) Seasonal upwelling along the South-eastern coastline of South Australia. Australian Journal of Marine and Freshwater Research 32: 843-54.

Li L, Barry DA, Stagnitti F, Parlange JY (1999) Submarine groundwater discharge and associated chemical input to a coastal sea. Water Resources Research 35: 3253-3259

Lind PR (2004) Ecological response to environmental flows in two lowland rivers. Thesis (*P*h.D.)--Deakin University, Victoria, 213pp. http://tux.lib.deakin.edu.au/adt-VDU/public/adt-VDU20050704.095818/

Lind PR, Robson BJ, Mitchell BD (2006) The influence of reduced flow during drought on patterns of variation in macroinvertebrate assemblages across a spatial hierarchy in two lowland rivers. Freshwater Biology 51: 2282-2295

Lind PR, Robson BJ, Mitchell BD (2007) Multiple lines of evidence for the beneficial effects of environmental flows in two lowland rivers in Victoria, Australia. River Research and Applications 23: 933-946

Lind PR, Robson BJ, Mitchell BD, Matthews TG (2009) Can sand slugs in rivers deliver conservation benefits? The biodiversity value of tributary junction plug wetlands in Glenelg River, Australia. Marine and Freshwater Research 60: 426-434

Love AJ, Herczeg AL, Armstrong D, Stadter F, Mazor F (1993) Groundwater flow regime within the Gambier Embayment of the Otway Basin, Australia: evidence from hydraulics and hydrochemistry Journal of Hydrology 143:297-338

Loveless AM, Oldham CE, Hancock GJ (2008) Radium isotopes reveal seasonal groundwater inputs to Cockburn Sound, a marine embayment in Western Australia Journal of Hydrology 351: 203– 217

MacKay H (2006) Protection and management of groundwater-dependent ecosystems: emerging challenges and potential approaches for policy and management. Australian Journal of Botany 54: 231-237

Marsden ID (1999a) Reproductive cycles of the surf beach clam Paphies donacina (Spengler, 1793) from New Zealand. Journal of Shellfish Research 18: 539-546

Marsden ID (1999b) Respiration and feeding of the surf clam Paphies donacina from New Zealand. Hydrobiologia 405: 179-188

Marsden ID (2000) Variability in low tide populations of tuatua, Paphies donacina, in Pegasus Bay, Canterbury, New Zealand. New Zealand Journal of Marine and Freshwater Research 34: 359-370

Marsden ID (2002) Recruitment in the swash zone – temporal variations in juvenile recruitment of an exposed sand beach surf clam. Hydrobiologia 477: 47-57

Marshall JC, Steward AL, Harch BD (2006) Taxonomic resolution and quantification of freshwater macroinvertebrate samples from an Australian dryland river: the benefits and costs of using species abundance data. Hydrobiologia 572: 171-194

Marshall NA, Bailey PCE (2004) Impact of secondary salinisation on freshwater ecosystems: effects of contrasting, experimental, short-term releases of saline wastewater on macroinvertebrates in a lowland stream. Marine and Freshwater Research 55: 509-523

Matthews TG (2000) Population dynamics of the infaunal bivalve, Soletellina alba. Thesis (*P*h.D.)--Deakin University, Victoria, 309pp.

Matthews TG, Fairweather PG (2003) Growth rates of the infaunal bivalve Soletellina alba (Lamarck, 1818) (Bivalvia: Psammobiidae) in an intermittent estuary of southern Australia. Estuarine, Coastal and Shelf Science. 58: 873-885

Matthews TG, Fairweather PG (2004) Effect of lowered salinity on the survival, condition and reburial of Soletellina alba (Lamarck, 1818) (Bivalvia: Psammobiidae). AustralEcology. 29: 250-257

Matthews TG, Fairweather PG (2006) Recruitment of the infaunal bivalve Soletellina alba (Lamarck, 1818) (Bivalvia: Psammobiidae) in response to different sediment types and water depths within the intermittently open Hopkins River Estuary. Journal of Experimental Biology and Ecology 334: 206-218

Mazor E (1991) Hydrochemical Fieldwork. John Wiley & Sons, New York.

McClelland JW, Valiela I (1998) Linking nitrogen in estuarine produces to land derived sources. Limnology & Oceanography 43:577-585

McClelland JW, Valiela I, Michener RH (1997) Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanisation in coastal watersheds. Limnology & Oceanography 42: 930-937

McKenzie JL, Quinn GP, Matthews TG Barton J, Bellgrove A (2011) Influence of intermittent estuary outflow on coastal sediments of adjacent sandy beaches. Estuarine, Coastal and Shelf Science 92: 59-69

McKinney R A, Lake J L, Charpentier M A, Ryba S (2002) Using mussel isotope ratios to assess anthropogenic nitrogen inputs to freshwater ecosystems. Environmental Monitoring and Assessment 74: 167-192

McLachlan A (1985) The biomass of macro- and inte¡stitial fruna on clean and wrack-covered beaches in Westem Australia. Estuarine, Coastal and Shelf Science 21: 587-599

McLachlan A, Brown AC (2006). Interstitial Ecology. The Ecology of Sandy Shores (Second Edition). Burlington, Academic Press: p.181-196.

McMahon K, Walker DI (1998) Fate of seasonal, terrestrial nutrient inputs to a shallow seagrass dominated embayment. Estuarine Coastal & Shelf Science 46: 15-25

Michael HA, Lubetsky JS, Harvey CF (2003) Characterizing submarine groundwater discharge: a seepage meter study in Waquoit Bay, Massachusetts. Geophysical Research Letters 30: 1297-1297

Michael HA, Mulligan AE, Harvey CF (2005) Seasonal oscillations in water exchange between aquifers and the coastal ocean. Nature 436: 1145-1148

Migné A, Ouisse V, Riera P, Hubas C, Davoult D (2011) Freshwater seepages and ephemeral macroalgae proliferation in an intertidal bay: II. Effect on benthic biomass and metabolism. Estuarine, Coastal and Shelf Science 92: 161-168

Miller CJ, Davis SE, Roelke L, Li H, Driffill MJ (2009) Factors influencing algal biomass in intermittently connected subtropical lagoons. Wetlands 29: 759-771

Miller DC, Ullman WJ (2004) Ecological Consequences of Ground Water Discharge to Delaware Bay, United States. Ground Water (Oceans Issue) 42: 959-970

Millham NP, Howes BL (1994) Nutrient balance of a shallow coastal embayment: 1. Patterns of groundwater discharge. Marine Ecology-Progress Series 112: 155-167

Montlucon D, Sanudo-Wilhelmy SA (2001) Influence of net groundwater discharge on the chemical composition of a coastal environment: Flanders Bay, Long Island, New York. Environmental Science & Technology 35: 480-486

Moore SK, Suthers IM (2005) Can the nitrogen and carbon stable isotopes of the pygmy mussel, Xenostrobus securis, indicate catchment disturbance for estuaries in Northern New South Wales, Australia? Estuaries & Coasts 28: 714-725

Moore WS (2010) The Effect of Submarine Groundwater Discharge on the Ocean. Annual Review Marine Science 2: 59-88

Muller M (ed) (2001) Exploring the Anatomy of a Region: the South East of South Australia. South East Book Promotions, Mt Gambier 173pp.

Murray BR, Hose GC, Eamus D, Licari D (2005) Valuation of groundwater-dependent ecosystems: a functional methodology incorporating ecosystem services. Australian Journal of Botany 54: 221-229

Murray BR, Zeppel MJB, Hose GC, Eamus D (2003) Groundwater-dependent ecosystems in Australia: It's more than just water for rivers. Ecological Management & Restoration 4: 110-113

Naim O (1993) Seasonal response of a fringing reef community to euthrophication (Reunion Island, Western Indian Ocean). Marine Ecology-Progress Series 99: 137-151

Napier GM, Fairweather PG (1999) An approach to biomonitoring wetlands to assess impacts of agricultural drainage. pp. 553-564 in Wetlands for the Future: Contributions from INTECOL's V International Wetlands Conference, (eds) AJ McComb & JA Davis, Gleneagles Press, Adelaide

Nicholas WL (2001) Seasonal variations in nematode assemblages on an Australian temperate ocean beach; the effect ofheavy seas and unusually high tides. Hydrobiologia 464: 17 -26

Nicholas WL (2002) Ultrastructure of the head of Okranema eileenae Greenslade and Nicholas, 1991 (Thoracostomopsidae: Nematoda). Journal of Nematology 34: 50-61

Nicholas WL (2004a) Crustorhabditis chitwoodi sp nov (Nematoda : Rhabditidae): an intertidal species from the coast ofNew South Wales, Australia, with observations on its ecology and life history. New Zealand Journal of Marine and Freshwater Research 38: 803-808

Nicholas WL (2004b) Oxyonchus longisetosus n. sp., Oxyonchus evelynae n. sp. And Oxyonchus culcitatus weiser, 1959 (Thoracostomopsidae: Enoplida: Nematoda) from Australian ocean beaches, together with observations on the variability of taxonomic characters and a key to species of the genus Oxyonchus. Hydrobiologia 511: 47-64.

Nicholas WL, Hodda M (1999) The free-living nematodes of a temperate, high energy, sandy beach: faunal composition and variation over space and time. Hydrobiologia 394: 113-127

Nicholas WL, Trueman JWH (2005) Biodiversity of marine nematodes in Australian sandy beaches from tropical and temperate regions. Biodiversity and Conservation 14: 823-839

Nicholson G, Jenkins GP, Sherwood J, Longmore A (2008) Physical environmental conditions, spawning and early life stages of an estuarine fish: climate change implications for recruitment in intermittently open estuaries. Marine and Freshwater Research 59: 735-749

Norkko J, Hewitt JE, Thrush SF (2006a) Effects of increased sedimentation on the physiology of two estuarine soft-sediment bivalves, Austrovenus stutchburyi and Paphies australis. Journal of Experimental Marine Biology and Ecology 333: 12-26

Norkko J, Pilditch CA, Thrush SF, Wells RMG (2005), Effects of food availability and hypoxia on bivalves: the value of using multiple parameters to measure bivalve condition in environmental studies. Marine Ecology Progress Series 298: 205-218

Norkko J, Thrush SF, Wells RMG (2006b) Indicators of short-term growth in bivalves: detecting environmental change across ecological scales. Journal of Experimental Marine Biology and Ecology 337: 38-48

Norman GR & Streiner DL (1998) Biostatistics: The bare essentials, BC Decker Inc, Canada

Nowicki BL, Requintina E, Van Keuren D, Portnoy J (1999) The role of sediment denitrification in reducing groundwater-derived nitrate inputs to Nauset Marsh estuary, Cape Cod, Massachusetts. Estuaries 22: 245-259

Ortega-Guerrero, A (2003) Origin and geochemical evolution of groundwater in a closed-basin clayey aquitard, Northern Mexico. Journal of Hydrology 284:26–44

Ouisse V, Riera P, Migné A, Leroux C, Davoult D (2011) Freshwater seepages and ephemeral macroalgae proliferation in an intertidal bay: I. Effect on benthic community structure and food web. Estuarine, Coastal and Shelf Science 91: 272-281

Page HM (1995) Variation in the natural abundance of 15N in the halophyte, Salicornia virginica, associated with groundwater subsidies of nitrogen in a southern California salt-marsh. Oecologia 104: 181-188

Parreira SML (2000) Spatial and temporal variation of infauna in an Australian estuary. Masters Thesis. Deakin University, Warrnambool.

Pavlidou A, Papadopoulos VP, Hatzianestis I, Simboura N, Patiris D, Tsabaris C (2014) Chemical inputs from a karstic submarine groundwater discharge (SGD) into an oligotrophic Mediterranean coastal area. Science of the Total Environment Volume 488: 1-13

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18: 293-320

Peterson CH, Wells FE (1998) Molluscs in Marine and Estuarine Sediments in Mollusca: The Southern Synthesis (eds) Beesley PL, Ross GJB, & Wells A. CSIRO Australia Publishing, Melbourne. p36-46,

Platt HM, Warwick RH (1983) Freeliving Marine Nematodes: Part I. British Enoplids: Pictorial key to world genera and notes for the identification of British species. Cambridge University Press, Cambridge.

Platt HM, Warwick RH (1988) Freeliving Marine Nematodes: Part II. British Chromadorids: Pictorial key to world genera and notes for the identification of British species. EJ Brill & W Backhuys, Leiden.

Portnoy JW, Nowicki BL, Roman CT, Urish DW (1998) The discharge of nitrate-contaminated groundwater from developed shoreline to marsh-fringed estuary. Water Resources Research 34: 3095-3104

Post DM (2002) Using stable isoptopes to estimate trophic position: models, methods and assumptions. Ecology 83: 703-718

Potter IC, Hyndes GA (1999) Characteristics of the ichthyofaunas of southwestern Australian estuaries, including comparisons with holarctic estuaries and estuaries elsewhere in temperate Australia: A review. Australian Journal of Ecology 24: 395–421

Power G, Brown R S, Imhof J G (1999) Groundwater and fish – insights from northern North America. Hydrological Processes 13: pp 401-422.

Quinn GP, Keough MJ (2002) Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge. 537pp.

Quinn GP, Lake PS, Schreiber ESG (1996) Littoral benthos of a Victorian lake and its outlet stream: spatial and temporal variation. Australian Journal of Ecology 21: 292-301.

Ranasinghe R, Pattiaratchi C (1999) Circulation and mixing characteristics of a seasonally open tidal inlet: a field study. Marine & Freshwater Research 50: 281-290

Roberts D (1984) A comparative study of Lasaea australis, Vulsella spongiarum, Pinna bicolor and Donacilla cuneata (Mollusca: Bivalvia) from Princess Royal Harbour, Western Australia. Journal of Molluscan Studies 50: 129-136

Robson BJ (2000) Role of residual biofilm in the recolonization of rocky intermittent streams by bethnic algae. Marine and Freshwater Research 51: 724-732

Robson BJ, Clay CJ (2005) Local and regional macroinvertebrate diversity in the wetlands of cleared agricultural landscape in south-western Victoria, Australia. Aquatic Conservation: Marine and Freshwater Ecosystems 15: 403-414

Robson BJ, Hogan M, Forrester T (2005) Hierarchical patterns of invertebrate assemblage structure in stony upland streams change with time and flow permanence. Freshwater Biology 50: 944-953

Robson BJ, Matthews TG (2004) Drought refuges affect algal recolonization in intermittent streams. River Research and Applications 20: 753-763

Robson BJ, Matthews TG, Lind PR, Thomas NA (2008) Pathways for algal recolonization in seasonally flowing streams. Freshwater Biology 53: 2385-2401

Rogers KM (1999) Effects of sewage contamination on macroalgae and shellfish at Moa Point, New Zealand using stable carbon and nitrogen isotopes. New Zealand Journal of Marine and Freshwater Research 33: 181-188

Rutkowski CM, Burnett WC, Iverson RL, Chanton JP (1999) The effect of groundwater seepage on nutrient delivery and seagrass distribution in the northeastern Gulf of Mexico. Estuaries 22: 1033-1040

Scealy JA, Mika SJ, Boulton AJ (2007) Aquatic macroinvertebrate communities on wood in an Australian lowland river: experimental assessment of the interactions of habitat, substrate complexity and retained organic matter. Marine and Freshwater Research 58: 153-165

Schahinger RB (1987) Structure of coastal upwelling events observed off the south-east coast of South Australia during February 1983 – April 1984. Australian Journal of Marine and Freshwater Research 38: 439-459

Scholz O (1990) Physicochemistry and vegetation of Piccanninie Ponds, a coastal aquifer-fed pond in south-eastern South Australia. Australian Journal of Marine and Freshwater Research 41: 237-246

Schmidt LG, Schultz TE, Correll RL, Gerrit Schrale (1999) Diffuse-source nitrate pollution of groundwater in relation to land management systems in the South East of South Australia. Proceedings International Conference on Diffuse Pollution – Solutions Innovations. Perth Western Australia May 1999. International Association on Water Quality, Australian Water and Wastewater Association.

SENRMB 2010 South East Regional NRM Plan

SECWMB (2001a) Comaum-Caroline Prescribed Wells Area Water Allocation Plan, South East Catchment Water Management Board, Mt Gambier

SECWMB (2001b) Lacepede Kongorong Prescribed Wells Area Water Allocation Plan, South East Catchment Water Management Board, Mt Gambier

SECWMB (2002) Draft South East Catchment Water Management Plan 2003/2007, South East Catchment Water Management Board, Mt Gambier

SECWMB (2003) South East Catchment Water Management Plan 2003/2007. South East Catchment Water Management Board, Mt Gambier. 244pp.

Short FT, Coles RG (eds) (2001) Global Seagrass Research Methods. Elsevier, Amsterdam.

Sigua GC, Tweedale WA (2003) Watershed scale assessment of nitrogen and phosphorus loadings in the Indian River Lagoon basin, Florida. Journal of Environmental Management 67: 363-372

Simmons GMJ (1992) Importance of submarine groundwater discharge (SGWD) and seawater cycling to material flux across sediment/water interfaces in marine environments. Marine Ecology-Progress Series 84: 173-184

Sinclair Knight Mertz (2009) Classification of groundwater-surface water interaction for water dependent ecosystems in the South East, South

Australia. Report to Government of South Australia, Department of Water, Land and Biodiversity Conservation 94pp.

Slomp C P, Van Cappellen P (2004) Nutrient inputs to the coastal ocean through submarine groundwater discharge: controls and potential impact. Journal of Hydrology 295: 64-86

Smith AJ, Nield SP (2003) Groundwater discharge from the superficial aquifer into Cockburn Sound, Western Australia: estimation by inshore water balance. Biogeochemistry 66: 125–144

Somerfield PJ, Warwick RM (1996) Meiofauna in Marine Pollution Monitoring programmes. A laboratory manual. Ministry of Agriculture, Fisheries and Food. Directorate of Fisheries

Spruzen FL, Richardson AMM, Woehler EJ (2008) Spatial variation of intertidal macroinvertebrates and environmental variables in Robbins Passage wetlands, NW Tasmania. Hydrobiologia 598: 325-342

Stieglitz T, Submarine groundwater discharge into the near-shore zone of the Great Barrier Reef, Australia(2005) Marine Pollution Bulletin 51: 51-59

SYSTAT (2004) SYSTAT 11: Statistics 1 SYSTAT Software Inc, Richmond, California.

Taniguchi M (2002) Tidal effects on submarine groundwater discharge into the ocean. Geophysical Research Letters 29: 1561-1561

Taniguchi M, Burnett WC, Cable JE, Turner JV (2002) Investigation of submarine groundwater discharge. Hydrological Processes 16: 2115-2129

Thomson JR (2002) The effects of hydrological disturbances on the densities of macroinvertebrate predators and their prey in a coastal stream. Freshwater Biology 47: 1333-1351

Tobias CR, Harvey JW, Anderson IC (2001a) Quantifying groundwater discharge through fringing wetlands to estuaries: seasonal variability, methods comparison, and implications for wetland-estuary exchange. Limnology & Oceanography 46: 604-615

Tobias CR, Macko SA, Anderson IC, Canuel EA, Harvey JW (2001b) Tracking the fate of a high concentration groundwater nitrate plume through a fringing marsh: a combined groundwater tracer and in situ isotope enrichment study. Limnology & Oceanography 46: 1977-1989

Todd DK (1959) Groundwater Hydrology. John Wiley & Sons, New York

Tomlinson & Boulton 2008

Toth DJ, Katz BG (2006) Mixing of shallow and deep groundwater as indicated by the chemistry and age of karstic springs. Hydrogeology Journal 14: 827–847

Tyler MJ, Twidale CR, Ling JK, Holmes JW (eds) (1983) Natural History of the South East. Royal Society of South Australia, Adelaide 238pp.

Turner JV, Allison GB, Holmes JW (1984) The water balance of a small lake using stable isotopes and tritium. Journal of Hydrology 70: 199-220

Uchiyama Y, Nadaoka K, Rolke P, Adachi K, Yagi H (2000) Submarine groundwater discharge into the sea and associated nutrient transport in a sandy beach. Water Resources Research 36: 1467-1479

Ullman WJ, Chang B, Miller DC, Madsen JA (2003) Groundwater mixing, nutrient diagenesis, and discharges across a sandy beachface, Cape Henlopen, Delaware (USA). Estuarine Coastal & Shelf Science 57:539-552

Umezawa Y, Miyajima T, Kayanne H, Koike I (2002) Significance of groundwater nitrogen discharge into coral reefs at Ishigaki Island, southwest of Japan. Coral Reefs 21:346-356

Underwood AJ (1997) Experiments in Ecology: Their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge, UK, 504pp.

Underwood AJ, Chapman MG (eds) (1995) Coastal Marine Ecology of Temperate Australia. NSW University Press, Kensington, NSW, 341pp.

Underwood AJ, Chapman MG, Connell SD (2000) Observations in ecology: you can’t make progress on processes without understanding the patterns. Journal of Experimental Marine Biology and Ecology 250: 97-115

Valiela I, Bowen JL, Cole ML, Kroeger KD, Lawrence D, Pabich WJ, Tomasky G, Mazzilli S (2001) Following up on a Margalevian concept: Interactions and exchanges among adjacent parcels of coastal landscapes. Scientia Marina 65: 215-229

Vanek VL, Lee DR (1991) Mapping submarine groundwater discharge areas - an example from Laholm Bay, southwest Sweden. Limnology & Oceanography 36: 1250-1262

Warwick RH, Platt HM, Somerfield PJ (1998) Freelivìng Marine Nematodes: Part III. Monhysterids: Pictorial key to world genera and notes for the identification of British species. Field Studies Council Shrewbury.

Wedderburn SD, Walker KF, Zampatti BP. (2007) Habitat separation of Craterocephalus (Atherinidae) species and populations in off-channel areas of the lower River Murray, Australia. Ecology of Freshwater Fish 16: 442–449

Wedderburn SD, Walker KF, Zampatti BP (2008) Salinity may cause fragmentation of hardyhead (Teleostei: Atherinidae) populations in the River Murray, Australia Marine and Freshwater Research 59: 254–258

Wells R (2001) An overview of the geological environmental history of the South East of South Australia and its influence on the pattern of settlement from aboriginal occupation to the present day. In: Muller M (ed) Exploring the Anatomy of a Region: the South East of South Australia. South East Book Promotions, Mt Gambier p4-10

Williams WD (1980) Australian Freshwater Life: The invertebrates of Australian inland waters. MacMillan Company of Australia, South Melbourne, 321pp.

Zipperle A, Reise K (2005) Freshwater springs on intertidal sand flats cause a switch in dominance among polychaete worms. Journal of Sea Research 54: 143-150