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The bloom dynamics and trophic ecology of salps and doliolids in Storm Bay, Tasmania

By

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STATEMENTS AND DECLARATIONS

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Signed

Nurul Huda Ahmad Ishak

Date 08/12/2014

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"Tenang-tenang air laut,

Sampan kolek mudik ke tanjung

Hati terkenang mulut menyebut

Budi baik rasa nak junjung"

I feel that neither English nor Malay can possibly say what I feel.

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ABSTRACT

Zooplankton are important grazers of primary production and play a central role in the transfer of energy from primary producers to higher order consumers. Zooplankton are sensitive to environmental variability, making them useful indicators of climate change; importantly, their physiology is strongly coupled to temperature, they exhibit generally short life cycles and they are excluded from most pressures associated with commercial fishing. However, given the diversity of organisms found in the pelagic environment the responses of different groups of zooplankton to environmental variability are most likely different. In this study I have investigated the bloom dynamics and trophic ecology of dominant thaliaceans in Storm Bay: two species of salp (*Thalia democratica* and *Salpa fusiformis*) and two species of doliolid (*Dolioletta* sp. and *Doliolum* sp.). Storm Bay is a region of dynamic oceanography that is influenced by (i) warm, low nutrient waters from the East Australian Current in the summer, (ii) cooler, nutrient-rich subantarctic waters in the winter, (iii) the Leeuwin (Zeehan) Current flowing along the west coast and (iv) flows from the Derwent Estuary. Key challenges in this study included the fragility of the gelatinous zooplankton, their unpredictable presence in Storm Bay and the absence of doliolids during certain years.

Monthly field trips were undertaken to Storm Bay for three consecutive years (November 2009 to March 2012) to investigate the blooms of salps and

doliolids and the causes of their patchy distribution. Collections of zooplankton were made at five sites and seven environmental parameters were recorded (temperature, salinity, rainfall, diatom stocks, chlorophyll *a* concentration, presence of the heterotrophic dinoflagellate *Noctiluca scintillans* and total phytoplankton abundance).

Relationships between the distribution of thaliaceans and environmental parameters in Storm Bay were examined using the BIOENV (Biology-Environment) procedure of PRIMER, which highlighted that the bloom patterns of salps and doliolids in Storm Bay were not uniform in time or space due to the variability in environmental parameters. The top three drivers of thaliacean distribution and abundance, according to BIOENV, were salinity, temperature and diatom stocks, with a correlation of 0.433. It was clear that each species showed different environmental preferences. Of the doliolids, *Dolioletta* sp. preferred lower temperatures (mean SST 13.42-14.93 °C) and higher salinity (mean SSS 33.91-34.62) than *Doliolum* sp. (mean SST 16.35-16.76 °C; mean SSS 32.95-33.83). The salp *T. democratica* showed a preference for higher temperatures (mean SST 15.85-17.4 °C) and slightly lower salinity (mean SSS 34.34-34.40) than *S. fusiformis* (mean SST 14.64-15.38 °C; mean SSS 34.57-35.11).

The dietary preferences of salps were investigated using two methods of gut content analysis: Scanning Electron Microscopy (SEM) and High Performance Liquid Chromatography (HPLC). Using SEM, I obtained micrographs of 31 different species of plankton, including copepods, in the guts of the four species

of thaliaceans. HPLC confirmed that diatoms, cryptophytes and green algae were the main dietary preferences for salps.

To investigate further where each species of salp fitted within the planktonic food web in Storm Bay, carbon and nitrogen concentrations and stable isotopic profiles were measured on *T. democratica* and *S. fusiformis*. Because of the fragility of salps, an extension of this research project involved comparing three different methods of preparation of salps for elemental analyses (freshly collected and incised salps rinsed with small volume of Milli-Q filtered water, thawed salps incised and rinsed in small volume of Milli-Q filtered water and freshly collected salps incised and rinsed with ammonium formate). The best method was then used for isotopic analysis of salps and seawater. Carbon and nitrogen elemental analyses were found to show the most consistent results if fresh specimens were incised and rinsed with Milli-Q prior to analysis. *T.democratica* had higher carbon and nitrogen values than *S. fusiformis*, and solitary forms of both species had higher carbon and nitrogen contents than the aggregate forms. Comparison with the literature confirmed the relatively low carbon and nitrogen concentrations of these gelatinous organisms when compared to crustacean plankton. The present study does point to the need to consider the life stages separately for any research, e.g. ecosystem modelling, that is attempting to produce realistic carbon budgets for a system.

This study provided further insight into the current understanding of the impacts of environmental variability on important marine zooplankton; specifically on salps and doliolids. Further, this study increased our knowledge of

the dietary preferences of salps and added significant information to the little-known diets of doliolids. It also identified some issues with methods used for preparing gelatinous species for biochemical analyses and provided recommendations for optimal preparation of specimens. These findings will significantly increase our ability to determine how climate-driven oceanographic changes will affect the distribution of these important zooplankton species in Australian waters and in other areas globally.

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CHAPTER 1

INTRODUCTION AND THESIS OUTLINE

The consequences of climate change are being observed worldwide and it is predicted to have significant impacts on humans and both terrestrial and aquatic ecosystems (Honda et al. 2012; Field et al. 2014). The effects of climate change may be more serious for marine species than terrestrial systems (Miller 2004) because marine communities are thought to be more strongly regulated by community interactions than terrestrial communities (Shurin et al. 2006), and range shifts of species are occurring faster in marine than in terrestrial systems (Sorte et al. 2010). We now know via the Intergovernmental Panel on Climate Change (IPCC) reports that 90% of excess heat goes into the ocean. As a store of heat on planet Earth, the oceans' capacity to absorb heat is about 1000 times more than that of the atmosphere (Mason, 1999). The ocean also plays a major role in the Earth's carbon cycle via the Biological Pump, where carbon sinks out of the euphotic zone to the oceans' interior (Post et al. 1990). Understanding the susceptibility of marine biota to changes in climatic and environmental phenomena is of fundamental importance to studies on marine ecosystems (Wassmann et al. 2011).

In Australia, there are increasing trends in extreme weather-related events, including heat waves, prolonged high temperatures, severe tropical cyclones and many more. According to the report on the State of The Climate 2014 (Bureau of

Meteorology 2014a), sea-surface temperatures (SST) around parts of Australia have been mostly well-above average since 2010, with persistent regions of very warm to highest-on-record temperatures to the south and west of the continent throughout much of 2013. Moreover, the Australian regions have warmed by 0.9 °C since 1900, whilst the global mean atmospheric temperature has risen by 0.85 °C from 1880 to 2012. Additionally, the rate of warming has increased from 0.08 °C decade^{-1} for the period 1910-2008 to 0.11 °C decade^{-1} for the period 1950-2008. With the amount of heat stored in the global oceans increasing, Australian temperatures are projected to continue to increase, with more extremely hot days and fewer extremely cool days (Bureau of Meteorolgy 2014a,b). Moreover, predictions of spatial change in water temperatures suggest that, by the 2030s, sea surface temperatures (SSTs) are estimated to be ~1°C warmer (relative to 1980-1999). Finally, climate models predict that water temperature will rise within the range from 1.5 to 3.0°C by the 2070s, with the greatest warming in the Southern Hemisphere occurring around south eastern Australia (Lough 2009).

This warming will have a profound effect on ocean ecosystems, leading to biological changes, especially at the plankton level (Boyce et al. 2010). For example, rising seawater temperature has the potential to create fundamental changes to zooplankton distribution, abundance, physiology and phenology, as well as changes in community structure and function (Richardson et al. 2009). One effect of warming ocean waters in marine ecosystems is the shift in the ranges of species. Such changes may affect the amount of energy transferred to

higher tropic levels and this could lead to more unstable ecosystems (Sorte et al. 2010).

At present our understanding of the impacts of sea temperature increases is poor, as there are few long-term data series for biological organisms in the marine environment (Richardson and Poloczanska 2008), particularly in the Southern Hemisphere. This is problematic because marine organisms may show a more rapid response to temperature rises than terrestrial species (Richardson and Poloczanska 2008), especially at the lower end of the food chain where many species exhibit short life cycles on the scale of days to weeks (Richardson et al. 2009). The value of long term data sets is illustrated by the Southern Ocean Continuous Plankton Recorder program that has been running since the early 1990s (Hosie et al. 2003). This study highlighted changes in the Southern Ocean that included variability in zooplankton abundance, species composition and community patterns. Zooplankton are a critical step in the transfer of energy from primary producers to higher-order consumers and play a useful role as indicators of climate change, as their physiology is strongly coupled to temperature, they exhibit short life cycles and they are generally safe from the pressures associated with commercial fishing (Richardson 2008).

A group of gelatinous zooplankton, collectively known as thaliaceans, which includes salps, doliolids and pyrosomes, are periodically important grazers in temperate marine ecosystems and sometimes form 'blooms' that are capable of depleting phytoplankton production (Boero et al. 2013). These thaliaceans tend to favour warmer, low productivity waters and their feeding mode

(continuous filtration) means that they are capable of indiscriminately clearing large volumes of seawater (Lucas and Dawson, 2014). Many crustacean plankton are also filter feeders but their feeding modes affect the size of particles captured, and thus restrict the overall size range of prey that they can consume (Brum et al. 2014). Gelatinous species have a fundamentally different life cycle to crustacean zooplankton. They can reproduce rapidly and devour a very wide range of particle sizes. Thaliaceans are a natural component of tropical, subtropical, temperate and some of the polar marine ecosystems but their appearance in the water column appears to be less predictable than that of other zooplankton, especially crustacea (Boero et. al, 2008). Blooms may appear suddenly, they may last for a short time, may occur over vast scales, suddenly disappear and are not recurrent on a regular basis (Cole 1952; Kawahara et al. 2006; Paffenhöfer 2013). As waters warm and the composition of the pelagic community changes zooplankton communities might shift from being crustacean-dominated to gelatinous-dominated, and this has important implications for energy transfer up the food chain.

1.1 Zooplankton as indicators of environmental change

The fact that thaliaceans may be strongly affected by climate change is undeniable. Studies of long-term datasets of salps have shown that these creatures are very useful for indicating climate change impacts. For example, regional warming near the Antarctic Peninsula resulted in the highest salp

densities occurring during three summers within the 1984–1996 period. Exceedingly high salp densities were correlated with high mean air temperatures recorded in 1989-1990, 1992-1993 and 1993-1994 (Loeb et al. 1997). Furthermore, from two 24-year time series (1967-1990) in the coastal waters of the Western Mediterranean, it was shown that blooms of *Salpa fusiformis* and *Thalia democratica* occurred when both sea temperature and stability of the water column increased rapidly (Menard et al. 1994). Meanwhile, the doliolids *Doliolum nationalis* and *Dolioletta gegenbauri* (same study sites and period of time as the Mediterranean studies; Menard et al. 1997) showed preference for low-salinity events, and their abundance correlated negatively with high wind velocities (Menard et al. 1997). Given the sparse but promising literature, I focused on salps and doliolids to examine whether Storm Bay would be a suitable indicator site to monitor climate-induced changes in productivity in south east Tasmania.

1.2 Zooplankton as indicators of ecological change

Marine zooplankton are *excellent biological indicators of ecological change* (Hays et al. 2005). Zooplankton are poikilotherms, so their physiological processes such as digestion and reproduction are sensitive to changes in temperature. They can be used to evaluate and monitor the whole community structure of marine ecosystems (Beaugrand et al. 2003), and their sensitivity to environmental stressors such as temperature and stratification can be used to reveal the impact

of climate change on the ocean. Many species are relatively easy to identify and quantify and most have short life spans (generally < 1 year; Paerl et al. 2007). Consequently, phenological variability (changes in life history parameters) of marine zooplankton under changing environmental conditions is evident in changes in population size, reproductive and developmental status and, for some species, the timing and duration of seasonal dormancy (Ji et al. 2010).

Some species have already been observed to shift their seasonal cycles and distributions in response to climate change (Parmesan 2006). For example, the freshwater cladoceran *Simocephalus vetulus* has responded to increasing water temperature by both reproducing at a younger age and producing more offspring per female (Van Doorslaer et al. 2007). The marine copepod *Neocalanus plumchrus* reached its peak abundance several weeks earlier in warm years and at warmer locations in a study of the subarctic Northeast Pacific (Mackas et al. 2007). Latitudinal shifts in the centres of abundance of calanoid copepods in the genera *Neocalanus* (*Neocalanus plumchrus/flemingeri*) and *Calanus* (*Calanus hyperboreus, Calanus glacialis, Calanus finmarchicus,* and *Calanus marshallae*) were observed, along with changes in the life cycle timing of *N. plumchrus* (several weeks earlier in warm years and at warmer locations). By examining changes or patterns in their abundance and distribution, future phenological changes can be predicted.

The most extensive studies of changes in marine plankton phenology resulting from environmental change come from the North Sea, where Continuous Plankton Recorders have been deployed extensively over several

decades. The paper that first synthesized seventy years of plankton and intertidal observations from 1920 to 1990 (Southward et al. 1995) highlighted changes in the distribution and abundance of zooplankton in the Western English Channel. Beaugrand et al. (2002) reported evidence of biogeographical shifts of crustaceans in the eastern North Atlantic Ocean and European shelf seas based on data collected over 40 years (1960-1999). It is believed that the changes were influenced by both the increasing trend in Northern Hemisphere temperatures and the climatic phenomenon of the North Atlantic Oscillation (NAO). The NAO is one of the most prominent and recurrent patterns of climate variability from the eastern seaboard of the United States to Siberia and from the Arctic to the subtropical Atlantic that drives winter climate over the Northern Hemisphere. Its variations are important to the environment (Hurrell et al. 2003) because there is potential for more violent swings of NAO as temperatures get warmer (Woods Hole Oceanographic Institution 2009).

1.3 Zooplankton and the Tasmanian marine ecosystem

The first quantitative sets of zooplankton observations from eastern Australia were collected during the *Warreen* voyages of 1938-1939 and 1940-1942 (Baird et al. 2011). Though the original purpose of the *Warreen* voyages was to survey pelagic fish stocks, the voyages also collected 1742 plankton samples and greatly improved the understanding of the taxonomy (Thompson 1948; Zeidler 1998)

and ecology (Sheard 1953; Wood 1954) of eastern Australian zooplankton (Baird et al. 2011). The zooplankton dataset from the *Warreen* voyages was also significant, due to the large number of stations sampled (444) and its focus on gelatinous zooplankton, which are now known to be affected by anthropogenic factors such as eutrophication and fishing (Hay 2006; Richardson et al. 2009; Baird et al. 2011).

The lack of knowledge of Tasmanian zooplankton was addressed by Taw (1975), who undertook systematic sampling along the east coast of Tasmania between 1971 and 1973. Prior to Taw's study, a number of national and international studies had identified zooplankton species, whose distribution included eastern Tasmania, as indicators of coastal, oceanic, subtropical and subantarctic water masses (Vervoot 1957; Brodskii 1967; Jillett 1968), however, local confirmation of these distribution patterns was needed. To capture an extensive range of zooplankton, Taw (1975) sampled inshore, oceanic and estuarine sites and used plankton nets with mesh sizes of between 200 µm and 300 µm. This dataset provided a basis for comparisons with recent data (e.g. Johnson et al. 2011), revealing significant changes in eastern Tasmania zooplankton distributions over the past 30 years.

The association of phytoplankton species with the seasonality of water masses in Sydney coastal waters was detailed by Hallegraeff and Reid (1986), where dinoflagellate genera such as *Gyrodinium* and *Gonyaulax* were attributed to warm waters. Later studies investigated the distribution of other commercially valuable species such as Southern Bluefin Tuna (Young et al. 1996) and Yellowfin

Tuna (Young et al. 2001), revealing that upwelling of nutrients can create chlorophyll hotspots, leading to zooplankton and fish aggregations. Harris et al. (1991) highlighted the importance of physical and chemical drivers in commercial fisheries, and provided critical information for future successful management under changing hydrological conditions. Whilst some fundamental approaches and basic information on plankton and the Tasmanian marine ecosystem were provided in early studies, this is the first attempt to conduct intensive research on distribution patterns of thaliaceans and their responses to environmental variability in Tasmanian waters.

1.4 Thesis outline

In this study I have examined the abundance and distribution of salps and doliolids, and their responses to environmental conditions, in Storm Bay, at the southern end of Tasmania. Storm Bay is located to the south of the Derwent Estuary, the most densely settled part of Tasmania, making the bay a site of substantial ecological and economic interest. The currents along the coastal boundary around Tasmania demonstrate a distinct seasonal cycle (Harris et al. 1987). Harris (1991) documented the interaction between warmer EAC water from the northeast and cooler waters and identified associated productivity changes in Storm Bay. Ecological studies that focused on zooplankton biomass in Storm Bay were conducted around 30 years ago by Clementson et al. (1989), Taw

and Ritz (1979), Hosie (1982) and Ritz and Hosie (1982). Clementson et al. (1989) showed that climatic events have significant impacts on the food chain. The abundance of the key link in the food chain of Storm Bay, the euphausiid *Nyctiphanes australis*, decreased substantially when the temperature was higher, while salps became more abundant. Being the only euphausiid species found in the study area, *Nyctiphanes australis* forms a significant part of the zooplankton biomass in Storm Bay, especially during summer when it swarms in dense aggregations (Hosie 1982; Hosie and Ritz 1983; O'Brien 1988). Earlier studies in Storm Bay (Harris et al. 1991) hinted at the relationship between weather patterns and blooms of thaliaceans, whereby more frequent westerly winds contributed to blooms of gelatinous species. However, this early work provided no information on individual species and numbers so it is impossible to reach any firm conclusions concerning the frequency of gelatinous blooms.

The objectives (Fig.1.1) of this thesis were to:

1. Determine the conditions favourable to thaliaceans through examining abundance and distribution patterns associated with seasonal environmental changes in Storm Bay, and to explore the environmental variability and investigate the potential for Storm Bay (community) to act as an indicator of productivity for Southern and Eastern Tasmania (Chapter 3).

2. Determine the dietary preferences of salps by examining the food particles in the gut contents and by undertaking gut pigment analysis (Chapter 4).

Information obtained will provide a key to studies of food web structure in the Storm Bay ecosystem, besides supporting the analysis from phytoplankton count data presented in Chapter 3.

3. Analyse the trophic linkages of thaliaceans by focusing on elemental analysis and stablie isotopic signatures of the salps in Storm Bay (Chapter 5).

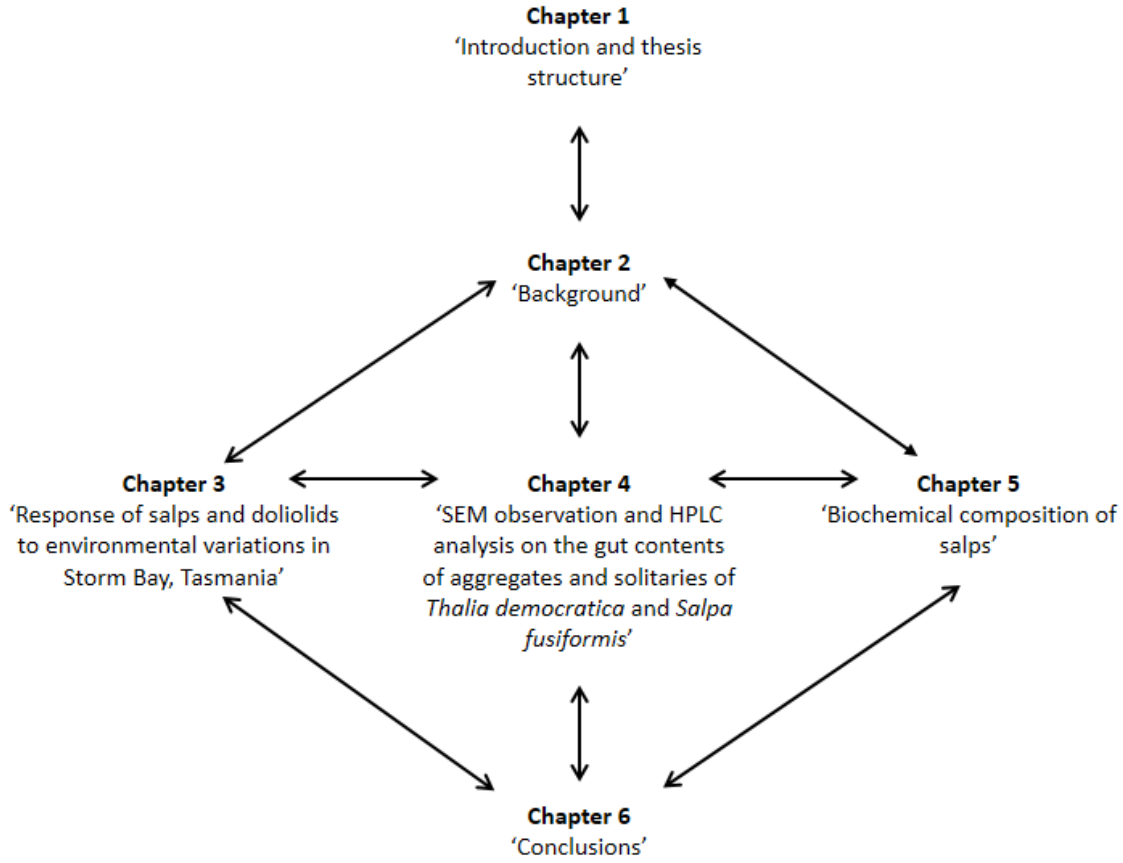

Fig. 1.1. Thesis outline: The bloom dynamics and trophic ecology of salps and doliolids in Storm Bay, Tasmania.

CHAPTER 2

BACKGROUND

2.1 Study Area: Storm Bay, Southeast Tasmania

2.1.1 Large-scale oceanography

The Tasmanian coastline has a complex oceanography that is characterized by large fluctuations in temperature, salinity and nutrients, which are variable on both temporal and spatial scales. One early study by Newell et al. (1961) of salinity and temperature in waters around Tasmania showed a temperature difference of at least 4 °C across the State from the south-west to north-east in summer. This is due to the fact that south-eastern Tasmanian waters (Fig. 2.1) are influenced by waters with subtropical origins from the east while western and southern waters are influenced by cooler waters from the subantarctic and the Leeuwin (=Zeehan) Current flowing from the west (Harris et al. 1987; Clementson et al. 1989).

Generally, the variability in surface ocean properties is different between the east and west coasts of Tasmania. On the east coast there is a sharp division between the East Australian Current (EAC) (which forms the western boundary of the South Pacific Ocean's subtropical gyre) from the northeast and the Leeuwin Current from the northwest that centres on the Tasman Peninsula off southeast Tasmania (Baines et al. 1983; Ridgway, 2007). Western boundary

currents are narrow and fast-flowing surface currents located on the western sides of subtropical gyres (Wu et al. 2012). Cresswell (2000) and Ridgway (2007) confirmed that the seasonal flow around Tasmania occured as distinct summer and winter states. The EAC flows from tropical northern Australia down along the eastern seaboard of Australia and is characterized by warm, saline, nutrient-poor water (Longhurst 2007). There is a separation point at ~31°S, where the majority of the flow heads east into the Tasman Sea and the remainder flows south along the Tasmanian coastline. In the summertime (January-March) there is increased poleward advection of water from the EAC that follows the shelf break around southern Tasmania. West of the Tasman Peninsula (Fig. 2.1), the northward current draws up cool, fresh subantarctic surface water from the south in winter. During winter, off the west coast, the Leeuwin Current projects warm and relatively saline waters down the western Tasmania coast and around the southern tip of Tasmania.

There is a lack of long-term marine data sets in Australian waters (Webster and Bourne 2012) and, in particular, a need for a long-term monitoring program targeting the zooplankton of the EAC has been identified (Suthers and Rissik 2009; Poloczanska et al. 2012). It is predicted that the EAC will show the largest warming of any major current in the Southern Hemisphere this century, with an increase of ~1°C by the 2030s and 1.5-3.0°C by the 2070s, with greatest warming east/northeast of Tasmania (Lough 2009). The EAC has increased in strength and therefore influence over the past 60 years, and this increase is expected to continue in the future (Ridgeway and Hill 2009). The rate of warming in

conjunction with the increasing strength of the EAC combine to make the EAC a global 'hotspot' for climate change, increasing the importance of long-term data needs for this region.

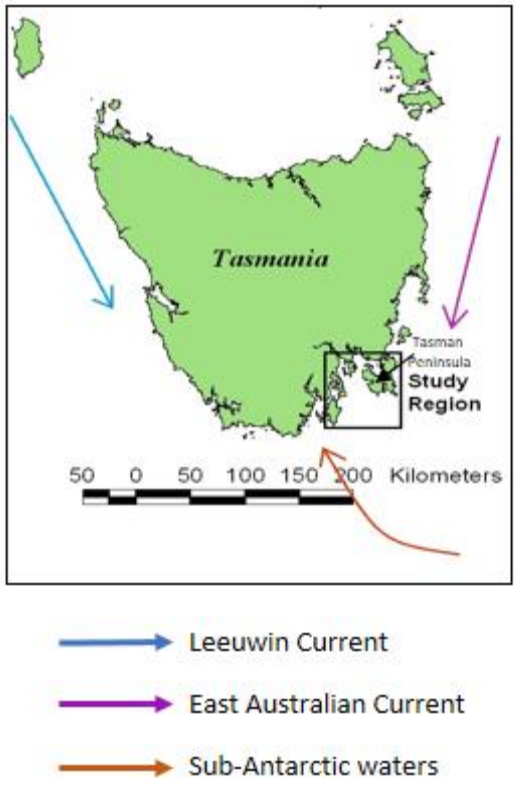

Fig. 2.1. Currents around Tasmania. Box highlights the study region in southeastern Tasmania.

2.1.2 ENSO as the dominant driver of climate variability

The El Niño –Southern Oscillation (ENSO), comprising positive (El Niño) and negative (La Niña) phases/events, is a coupled ocean-atmosphere phenomenon whose effects are experienced globally and is the major source of natural interannual climate variability in Australia (Holbrook et al. 2011). The Southern Oscillation refers to a major see-saw of air pressure and rainfall patterns

between the Australian/Indonesian region and the eastern Pacific Ocean and is experienced as substantial change in the sea temperatures of the upper ocean. Generally, the western Pacific is up to 10 °C warmer than the central and eastern Pacific, a result of the cool water that flows north in the Humboldt Current along the west coast of South America (from deep cold water that upwells along the Peruvian Coast), then flows westward along the equator under the influence of the equatorial Trade Winds, where it is gradually heated. Strong coupling of the tropical upper ocean with the atmosphere influences many aspects of ENSO climate variability (Holbrook et al. 2011).

Australia's climate is strongly influenced by the ENSO cycle as it has significant effects on the intensity of the southward flowing Leeuwin Current, and Australia's west coast waters (Holbrook et al. 2009). The Southern Oscillation Index (SOI) gives an indication of the development of La Niña or El Niño events in the Pacific Ocean and is calculated via the standardized anomaly of the Mean Sea Level pressure difference between Tahiti and Darwin (McLean, 2009). El Niño arises when SST in the central to eastern Pacific Ocean are significantly warmer than normal; these phases are defined when the SOI falls below negative 8 for a sustained period of several months (Bureau of Meterology 2012). The colder, nutrient-rich waters that normally upwell along north the western flank of South America gradually weaken or disappear and so the central and eastern Pacific become almost as warm as the western part. El Niño events often lead to increased dry conditions across the Australian continent, (though this can be highly variable), warmer than normal day time temperatures in

winter/spring, and reduced cloudiness and rainfall. In southeast Australia the warm EAC does not penetrate as far south, resulting in cooler surface waters and stronger westerly winds. The net effect is for more productive surface waters.

La Niña, defined as positive SOI > 8, is the other of two phases that dominate global inter-annual climate variability. La Niña will generally arise as a result of increased surface winds over the central Pacific Ocean and colder SST in the eastern equatorial Pacific. La Niña events arise when the eastern Pacific is much cooler than normal and the westward equatorial winds associated with the atmospheric Walker Circulation (large-scale east–west atmospheric overturning circulation over the eastern Pacific that is closely tied to the El Niño–Southern Oscillation) (Gill 1980; Tokinaga et al. 2012) is stronger than average (Bureau of Meterology 2012). These changes can bring widespread rain. La Niña conditions are typified by relatively calm weather and temperatures are generally cooler than average. In Tasmania, La Niña conditions are characterised by warm, low productivity surface waters and higher rainfall.

In the last two decades, there were two major events of La Niña recorded in Australia. The 1998–2001 La Niña affected three consecutive years from autumn 1998 to autumn 2001, while the 2010–2012 La Niña events consisted of two peaks in summer; the 2010–2011 peak was one of the strongest on record, comparable to the events of 1917–1918, 1955–1956 and 1975–1976. The 2010-2012 La Niña events were associated with record rainfall over much of Australia and some of the biggest floods (Bureau of Meteorology 2012). In the Tasman Sea, while El Niño/La Niña are natural phenomena, they are predicted to

intensify (i.e. become stronger, more frequent) under climate change (Oliver and Holbrook 2014).

2.2 Thaliaceans

Thaliaceans are gelatinous holoplanktonic tunicates whose population dynamics, life history and feeding biology contrast to copepods and other planktonic crustaceans (Madin and Deibel 1998). The class Thaliacea is comprised of around 72 described species in three orders: the Pyrosomatida (pyrosomes), Salpida (salps) and Doliolida (doliolids). These organisms generally have much shorter generation times than other zooplankton and exhibit complex life cycles that alternate sexual (solitary) and asexual (aggregate) generations (Madin 1974). Some species can reproduce rapidly, resulting in population increases of up to 2.5 times per day (Heron 1972). Thaliaceans play an important energetic role in the ocean as their faecal pellets and dead bodies transport organic matter from the photic to the benthic zone. This is due to their large faeces sinking faster than those of other zooplankton (Madin 1982; Henschke et al. 2013). Bruland and Silver (1981) had shown that salp faeces sink faster than those of copepods; Patonai et al. (2011) showed that doliolid pellets sink far slower (in relation to their volume) than copepods' and other taxa's pellets. This is because doliolids pellets are fluffy and not compact, while copepod and salp pellets are compact.

Thaliaceans are widespread and they can be found almost everywhere in the world's ocean. Some species are known to occur periodically in large blooms and high densities that cover large areas (Madin et al. 2006; Deibel and Paffenhöfer 2009). For example, large swarms of thaliaceans have been recorded in the California Current, the North Sea, the Gulf of Guinea, Red Sea, and the north east and southeastern coasts of the USA (Le Borgne 1983; Paffenhöfer and Lee 1987; Michael and Silver 1988; Pond and Sargent 1998; Reid et al. 2003; Madin et al. 2006; Marimuthu et al. 2013). *Pyrosoma atlanticum*, which has not been well studied ecologically, has been found in a large swarms in the Atlantic Ocean (Drits et al. 1992), while giant colonies of *Pyrosoma spinosum* have been found in south east Australia and New Zealand (Griffin 1970).

2.2.1 Salps

Salps are filter-feeding thaliaceans that feed continuously (Fiala-Medioni 1978). They have a mucous filter that is secreted by the complex endostyle structure (a ciliated groove visible as a horizontal line on the ventral midline of the pharynx; Godeaux 1989). The fine filter mesh size retains particles down to the size of bacteria (Alldredge and Madin 1982) and up to 1 mm (Kremer and Madin 1992). The structure and operation of the fine mesh 'mucous' feeding filters have been described by Bone et al. (2003). In salps, water is forced through the sieve by rhythmic contraction of muscles in the body wall, and the filtered water acts as a jet that propels the animal, meaning that salps must swim while eating (Denny 2008).

Life stages

Salps have a complex life cycle with alternating aggregate and solitary generations (Fig. 2.2) (Alldredge and Madin 1982). In the aggregate stage of the life cycle, salps (Fig. 2.3) are generally found in chains (Madin et al. 1996), thus limiting the number of predators that can feed on them (Kremer 2002). The aggregate individuals (the parents, also known as blastozooids) reproduce sexually; a solitary embryo first develops inside each blastozooid, and will be eventually released when it is fairly large relative to the blastozooids. On release, embryos grow to be mature solitaries (oozoids) (Fig. 2.4), which produce 'stolons' (buds of young aggregates) asexually (Fig. 2.4). The chain that is formed from a stolon will asexually bud off blocks of between 20 to 80 genetically identical embryos (Fig. 2.5) (Heron 1972).

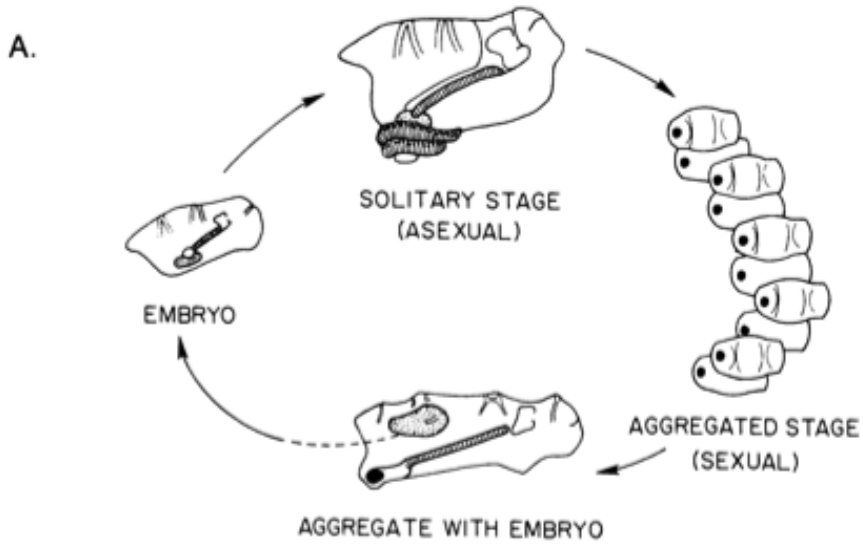

Fig. 2.2. The life cycle of salps (from Alledredge and Madin 1982)

Fig. 2.3. Chain of aggregates of *T. democratica* from Storm Bay.

Fig. 2.4. *Left hand panel*. Solitary stage of *T. democratica*, with muscle bands dyed in Toluidine Blue. *Right hand panel*. Mature solitary (oozoid) that is about to reproduce 'stolons' (buds of young aggregates).

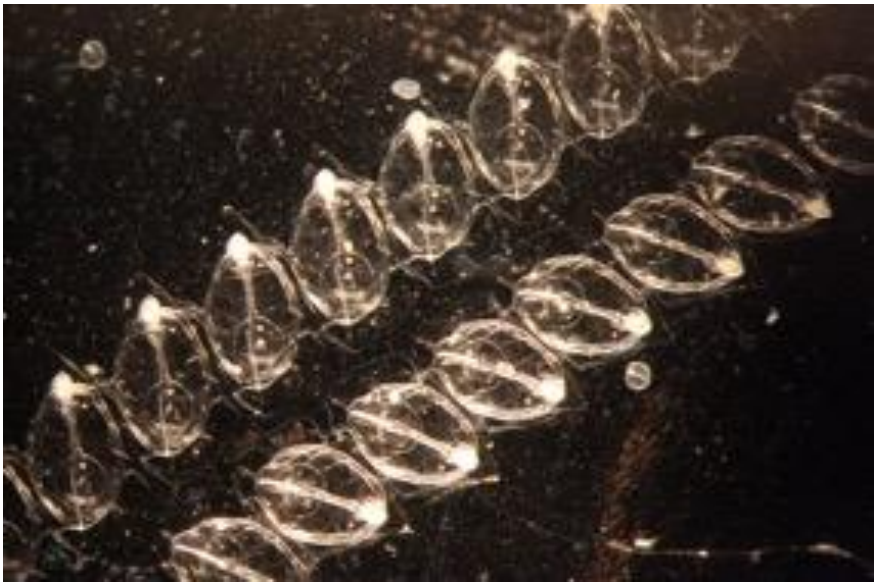

Fig. 2.5. Buds of young aggregates of *T. democratica*, from Storm Bay.

Distribution

Salps swarms have been recorded from all over the world (Kremer 2002; Paffenhöfer 2013). Their large swarms have been found in all oceans including the Arctic Ocean, Southern Ocean, Indian Ocean, Pacific Ocean, Atlantic Ocean and subtropical Inland Sea of Japan, Kuroshio (Sewell 1926; Menon 1931; Nair 1949; Sewell 1953; Casereto and Nemoto 1986; Matsueda et al. 1986; Chiba et al. 1999 Tsuda and Nemoto 1992). The average density of salps swarms makes them the third most common planktonic animal in the S.E. Australian area (Thompson 1948). Several studies previously carried out in Storm Bay recorded the presence of *S. fusiformis*, *Salpa maxima*, *T. democratica* and other *Thalia* spp. (Tranter 1962; Clementson et al. 1989; Harris et al. 1991). *T. democratica* swarm abundance in the Tasman Sea was found to be 20 times greater than the maximum abundance previously sampled during 1939-1941 (Henschke 2009).

General ecology

Salps sometimes occur in high densities and bloom over large areas, but their occurrence and their relationship to environmental conditions, such as survivability under natural temperature and food regimes, are not well understood. The events that trigger salp blooms are unclear (Kremer 2002). However, there is evidence that low chlorophyll concentrations and an abundance of small food particles do appear to increase salp blooms along the Antarctic Peninsula (Moline et al. 2004). Strong negative correlations between chlorophyll *a* and salp concentrations were reported at several sites in the Hauraki Gulf in New Zealand (Zeldis et al. 1995), and from the Southern Ocean (Kawaguchi et al. 2004). However, sometimes salps may be a major competitor of krill and its phytoplankton food stock (Siegel and Loeb 1995). For instance, *Salpa thompsoni's* high filtration capacity and its rapid population growth have made it capable of outcompeting Antarctic krill (*Euphausia superba*) for resources in the Elephant Island area near the Antarctic Peninsula.

Although the size of salp chains limits the number of predators that can feed on them, there are some common predators, such as pelagic fish and anchovies. Mianzan et al. (2001) found a dense aggregation of the salp *Iasis zonaria* in the stomachs of the anchovy *Engraulis anchoita*. Other predators on salps include medusae, siphonophores, heteropods and amphipods (Madin et al. 1996). However, salps are not a preferred prey for higher trophic level organisms such as planktivorous penguins and seabirds (Moline et al. 2004; Cox 2010). They

are considered to be nutritionally poor and are believed to have low protein, lipid and carbohydrate contents (Madin et al. 1981; Verity and Smetacek 1996; Dubischar et al. 2006)

2.2.2 Doliolids

Doliolids are relatively small, transparent, barrel-shaped gelatinous thaliaceans that swim by the rapid contraction of the muscle bands encircling the body (Bone and Ryan 1974). They can be found living primarily in neritic and shelf break waters. Doliolids also use a mucous net to filter particles, but the flow of water through the net is primarily driven by cilia alone (Alldredge and Madin 1982; Denny 2008). They cannot filter the water column as fast as salps because the use of cilia instead of muscle bands to pump water through their mucous feeding net is not as effiecient a mechanism. However, the use of cilia in feeding means that doliolids have separated filtering and swimming, so they are able to feed while remaining stationary. Doliolids exhibit greater fecundity (both sexual and asexual) and longer generation times than salps (Deibel and Lowen 2012).

Doliolids feed primarily on diatoms, flagellates and other phytoplankton species, but also capture particles as small as 0.7 mm in diameter (Denny 2008). Since the life cycle of doliolids consists of several stages, including eggs, larvae, oozoids and early nurses, they are easily exposed to a range of potential predators, although information on predators of doliolids is scarcer than for salps (Paffenhöfer 2013).

Life stages

Although the life cycle of doliolids (Fig. 2.6) is similar in pattern to salps, with alternation of sexual (gonozooid; Fig. 2.7) and asexual (oozooid) stages, it is further complicated by polymorphism in the asexual generations by the oozooids, i.e., asexual phorozooids and trophozooids (Alldredge and Madin 1982; Deibel and Lowen 2012). Like salps, hundreds to a few thousand buds are produced from a rudimentary ventral stolon by the mature asexual stage called the 'oozoid' (or 'Old Nurse') (Braconnot 1970) (Fig. 2.8).

The nutrition for the entire colony is thought to be provided by the trophozoids, which includes all stages from the developing buds to the muscular oozoid. The phorozooids are attached to a 'thread-like tail' form (cadaphore) by a stalk containing from 80 to 100 buds that eventually develop into the gonozooid stage and later reproduce sexually. The phorozooids continue to grow in a free-living state soon after release from the stolon. The gonozooids are released by the phorozooids upon reaching a threshold size. Within several days, it will develop an ovary and testes. After 10-14 days eggs and sperms are released intermittently, resulting in the development of larvae and then oozooids (Deibel and Lowen 2012; Paffenhöfer and Köster 2011).

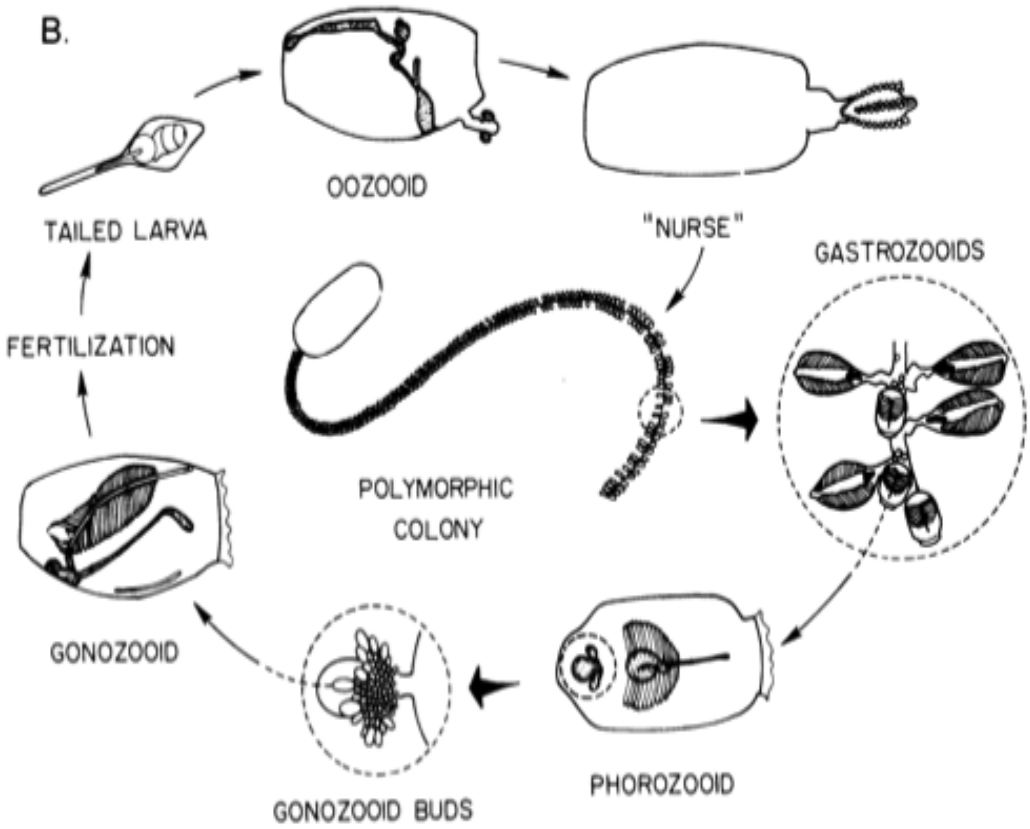

Fig. 2.6. Life cycle of doliolids (from Alldredge and Madin 1982).

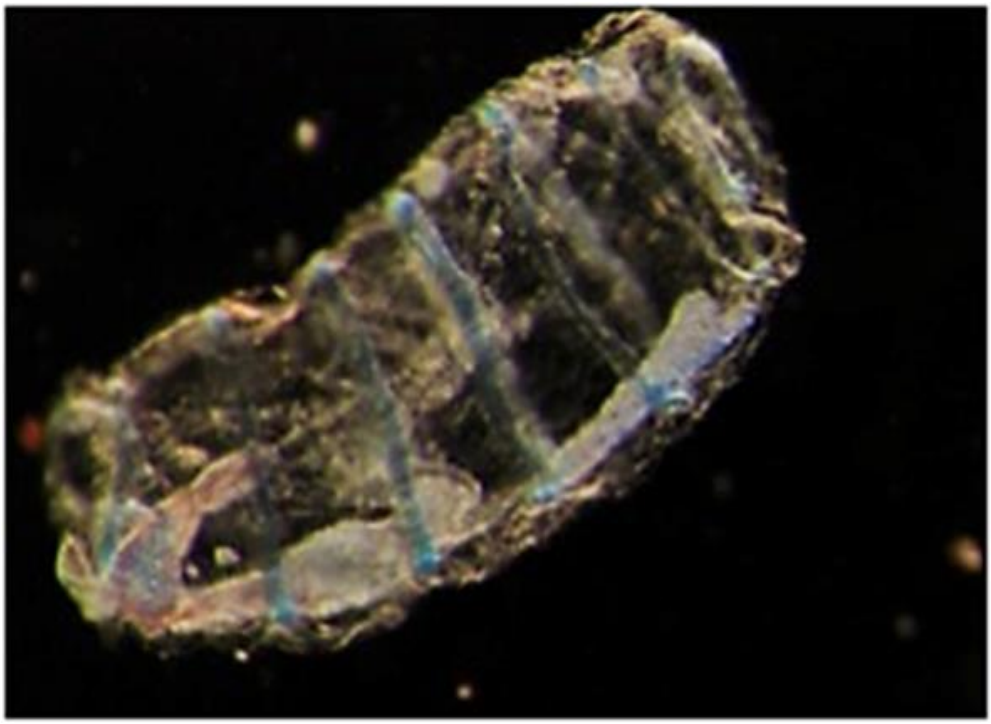

Fig. 2.7. Gonozoid of *Doliolum* sp. from Storm Bay. Muscle bands have been stained with Toluidine Blue

Fig. 2.8. Old nurse of *Doliletta* sp. from Storm Bay. Muscle bands have been stained with Toluidine Blue.

Distribution

Frequently observed species of doliolids in south-eastern Australian include *Dolioletta gegenbauri* and *Doliolum denticulatum*. *Doliolum denticulatum* is by far the most common species found it the region of the Great Barrier Reef and in the south east Australian waters (Thompson 1948). *Doliolum denticulatum* was four times more abundant than *Dolioletta gegenbauri* in south-east Australian waters (Thompson 1948). In Tasmania, *Dolioletta gegenbauri* has been found in small numbers off the east coast by Thompson (1948), and unidentified oozoids of doliolids were identified in Taw's samples from the east coast (Taw, 1975).

General ecology

Not a lot is known about the contribution of doliolids to marine foodwebs. Similar to salps, doliolids can occur irregularly at high concentrations (Deibel and Paffenhöfer 2009). Prolonged blooms of doliolids depend on a high rate of gonozooid production by phorozooids (first two-thirds of their life-time, i.e. up to 12 days). After that, depending on their feeding rate, gonozooids can persist for up to 2.5 weeks. Basically, phorozooids and gonozooids in sequence can maintain high concentrations of *Dolioletta gegenbauri* for weeks (Paffenhöfer 2013). While the main reasons for their sudden abundance were their reproductive strategies, it has been estimated that predation and food availability could affect doliolid blooms (Deibel and Lowen, 2012). There are only a few publications on predators of doliolids (Harbison 1998). Copepods of the genus *Sapphirina* were considered to be an active predator of doliolids (Takashi et al. 2012), along with two species of pelagic fishes from New Zealand (*Caprodon longimanus* and *Caesioperca lepidoptera*) that feed almost exclusively on doliolids (Kingsford and MacDiarmid 1988).

In contrast to salps, doliolids, especially *Dolioletta gegenbauri,* are found in waters containing a wide range of phytoplankton concentrations (Deibel 1998). However, according to Deibel and Lowen (2012) low food supply can limit the development of doliolid blooms. Another factor that may contribute to the limitation of doliolid blooms is predation (Paffenhöfer 2013). During the long period of phorozooid development on the nurse's cadophore, predation could be

a significant link preventing a continuous supply of phorozooids and, later, gonozooids, and consequently restricting the development of blooms.

Although salps and doliolids are in the Class Thaliacea, there are differences that separate them at the Order level. Both groups share some similar characteristics, for example their limited mobility, but at the same time they are different in terms of their propulsion technique. Other differences in characteristics include their anatomy, feeding and reproduction (Table 2.1).

Table 2.1. Similarities and differences between salps and doliolids.

Characteristic	Salps	Doliolids
Anatomy	The internal organs are compacted along the posterior ventral surface of the zooid and the pharyngeal gill slits are reduced to a single, central gill bar.	The internal organs are compacted along the posterior ventral surface of the zooid and the pharyngeal gill slits are expanded to reach across the entire pharyngeal cavity.
Feeding	The feeding current is driven primarily by continuous, rhythmic contractions of the circumferential muscles, and assisted by the cilia of the pharyngeal gill bar.	The feeding current is driven primarily by cilia of the many ostia perforating the pharyngeal gill.
	Rely on muscular pumping alone, meaning that they pulse continuously throughout their lives.	Pump water by a combination of ciliary and muscular mechanisms.
Reproduction	Lower lifetime fecundity, and shorter generation times.	Greater fecundity and longer generation times.

CHAPTER 3

RESPONSE OF SALPS AND DOLIOLIDS TO ENVIRONMENTAL VARIATION IN STORM BAY, TASMANIA

3.1 Highlights

- Studies on salps and doliolids were carried out during 3 consecutive summers (2009-2010, 2010-2011 and 2011-2012) in Storm Bay, Tasmania.
- Salps were absent in the years influenced by La Niña conditions, i.e. high rainfall and lower SST.
- The two doliolids were positively correlated with different phytoplankton assemblages: *Doliolum* sp. with diatoms and dinoflagellates and *Dolioletta* sp. with flagellates.
- Doliolids were frequently dominant in lower salinity and nutrient-rich shelf and coastal waters, while salps mostly showed higher abundances in warm oceanic waters.
- Spatial distribution patterns of doliolids and salps were distinct and were closely associated with hydrographic characteristics.

3.2 Introduction

Swarms or patches of thaliaceans are believed to be ephemeral as they often occur at unpredictable locations and times. The irregularities of occurrences have restricted our ability to gain knowledge of the trigger of thaliacean blooms (Boero et. al. 2008). A better understanding of the environmental drivers of thaliacean blooms is needed to address current uncertainties in the causes of their formation.

Tasmanian waters have a complex oceanography that is characterized by large fluctuations in temperature, salinity and nutrients that are variable on both temporal and spatial scales. This is due to the fact that south-eastern Tasmanian waters are influenced by waters with subtropical origins from the east while western and southern waters have a south-westerly and sub-antarctic influence (Harris et al. 1987; Clementson et al. 1989).

The EAC has extended its southward penetration over the past 60 years (Ridgeway and Hill 2009). With mean positive trends of 2.28 °C century^{-1} and 0.34 psu century^{-1} over the 1944-2002 period, there has been an enhancement in temperature and salinity in the region. SST has increased in winter over the last 10 years, and in summer both temperature and salinity are greater than in winter. Oke and England (2004) observed changes in the latitude of subpolar westerly winds, whereby there was a poleward shift of the westerly wind belt by about 5.4°S. Their studies agreed well with reports of patterns in the zonal winds of Hobart by Harris et al. (1988). The southward movement of westerly wind belt

would have displaced the EAC southward and this would serve to block the northward penetration of subantarctic water.

Harris et al. (1991) documented the interaction between warmer EAC-derived waters and cooler temperature waters and identified associated productivity changes in Storm Bay. These included changes in both the magnitude of productivity and in the composition of primary producers and consumers. Clear signals in the nutrient status of waters indicated the influence (timing and duration) of the EAC, while the magnitude and composition of primary producers (predominantly single-celled algae) highlighted the productivity of the region and the influence of westerly winds. This research clearly demonstrated the potential for Storm Bay to act as an indicator of productivity for southern and eastern Tasmania. In this study I investigated the spatial and temporal patterns in abundance and distribution of thaliaceans in Storm Bay. Environmental drivers that promote blooms of these organisms were investigated based on observation and multivariate analyses.

3.3 Methods

3.3.1 Study area

Five locations were chosen in Storm Bay. Sites 1, 5 and 6 represented coastal waters, while sites 2 and 3 were further south and more oceanic in nature (Fig. 3.1). Site 4 was further offshore and was abandoneded as a sampling site after the first trip. Monthly field trips were carried out beginning in November 2009 and continuing throughout 2012 (Table 3.1-3.3).

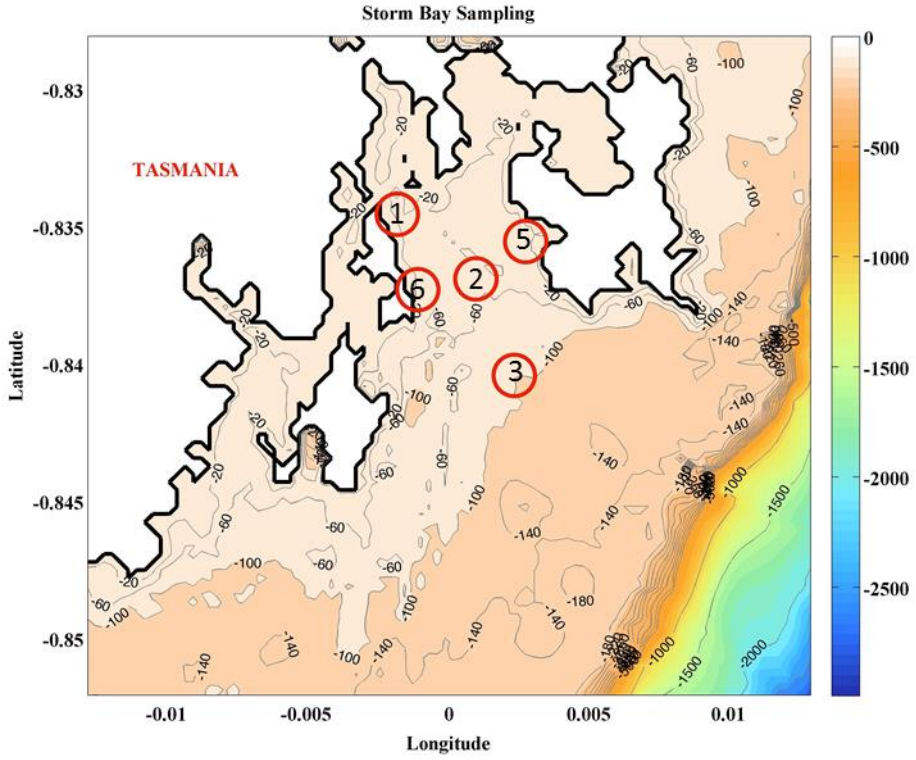

Fig. 3.1. Map of study area, Storm Bay. Thaliaceans were collected from 5 sites in Storm Bay. Colourbar shows bathymetry.

Table 3.1: Details on field trip in the summer of 2009/2010

Summer/ Trip	Date/Time of each sample were taken	Station positions	Code used in BEST analyses diagram	Depth (m)
Summer 2009/2010 Trip 1	9 Nov 2009	Site 1	SB1	16
		Site 2	SB2	50
		Site 3	SB3	90
		Site 5	SB5	30
		Site 6	SB6	30
Summer 2009/2010 Trip 2	14 Dec 2009	Site 1	2SB1	15
		Site 2	2SB2	50
		Site 3	2SB3	90
		Site 5	2SB5	30
		Site 6	2SB6	30
Summer 2009/2010 Trip 3	21 Jan 2010	Site 1	3SB1	16
		Site 2	3SB2	45
		Site 3	3SB3	90
		Site 5	3SB5	35
		Site 6	3SB6	40
Summer 2009/2010 Trip 4	25 Feb 2010	Site 1	4SB1	18
		Site 2	4SB2	45
		Site 3	4SB3	80
		Site 5	4SB5	35
		Site 6	4SB6	35

Table 3.2: Details on field trip in the summer 0f 2010/2011

Summer/ Trip	Date/Time of each sample were taken	Station positions	Code used in BEST analyses diagram	Depth (m)
Summer 2010/2011 Trip 14	24 Nov 2010	Site 1	14SB1	18
		Site 2	14SB2	45
		Site 3	14SB3	90
		Site 5	14SB5	30
		Site 6	14SB6	40
Summer 2010/2011 Trip 15	14 Dec 2010	Site 1	15SB1	16
		Site 2	15SB2	45
		Site 3	15SB3	90
		Site 5	15SB5	35
		Site 6	15SB6	35
Summer 2010/2011 Trip 16	19 Jan 2011	Site 1	16SB1	14
		Site 2	16SB2	40
		Site 3	16SB3	90
		Site 5	16SB5	35
		Site 6	16SB6	35
Summer 2010/2011 Trip 17	21 Mar 2011	Site 1	17SB1	18
		Site 2	17SB2	45
		Site 3	17SB3	80
		Site 5	17SB5	35
		Site 6	17SB6	35

Table 3.3: Details on field trip in the summer 0f 2011/2012

Summer/ Trip	Date/Time of each sample were taken	Station positions	Code used in BEST analyses diagram	Depth (m)
Summer 2011/2012 Trip 25	25 Nov 2011	Site 1	25SB1	18
		Site 2	25SB2	45
		Site 3	25SB3	90
		Site 5	25SB5	30
		Site 6	25SB6	40
Summer 2011/2012 Trip 26	15 Dec 2011	Site 1	26SB1	16
		Site 2	26SB2	45
		Site 3	26SB3	85
		Site 5	26SB5	35
		Site 6	26SB6	35
Summer 2011/2012 Trip 27	19 Jan 2012	Site 1	27SB1	18
		Site 2	27SB2	15
		Site 3	27SB3	30
		Site 5	27SB5	30
		Site 6	27SB6	35
Summer 2011/2012 Trip 28	21 Feb 2012	Site 1	28SB1	15
		Site 2	28SB2	40
		Site 3	28SB3	35
		Site 5	28SB5	40
		Site 6	28SB6	35

3.3.2 Field sampling

Zooplankton samples were obtained with a 0.75 m diameter bongo net with 100 µm mesh size, which was hauled vertically through the water from 2 m above the bottom to the surface, from a stationary vessel. A flow meter (General Oceanic) was fitted in the mouth of one of the nets, and enabled the volume of filtered seawater to be recorded, leading to the calculation of abundance on a per cubic metre basis.

At each sampling site a Seabird SBE 19 plus CTD with fluorescence/turbidity (WETLabs), dissolved oxygen (Seabird SBE 43) and PAR Biospherical Instruments) sensors was lowered to 2-5 m above the seabed. A YSI Sonde measuring salinity, temperature and chlorophyll *a* was also deployed at each site to act as a backup in case the Seabird failed. The data were stored in the internal memory of the instruments and these were downloaded in the laboratory using SBED data-processing Win 32 for the Seabird and EcoWatch version 3.18.00 for the YSI Sonde. Sea surface temperature maps were also sourced from http://oceancurrent.imos.org.au/ a site maintained by the Australian Intergrated Marine Observing System (IMOS). Rainfall data were obtained from http://www.bom.gov.au. The rainfall data have been summarized from the records of Cape Bruny Lighthouse, Hobart (Ellerslie Rd), Maria Island and Tasman Island. All these stations were located adjacent to Storm Bay sites. The data were collected from the Bureau of Meteorology website.

At each site an 8 L Niskin bottle was used to collect water samples from 1 m and 10 m below the surface, and at ~2 m above the seabed. For the deeper site 3, samples were also taken at 50 m below the surface. All samples were analyzed for chlorophyll *a* concentration. Additional samples were collected for phytoplankton identification and enumeration by the use of a 0 -12 m tube that was deployed from the surface and stoppered with a rubber bung. Samples for phytoplankton were preserved in acid Lugol's solution, and chlorophyll *a* samples were kept cold and dark until processing in the laboratory.

3.3.3 Analytical methods

Samples for phytoplankton analysis were transferred to 1 L measuring cylinders (volume recorded) and allowed to settle for at least 24 hours. After this time approximately 900 mL was siphoned off and the remaining sample was transferred to a 100 mL measuring cylinder and again allowed to settle for at least 24 hours. After this time approximately 90 mL was siphoned off, the final volume recorded and thoroughly mixed before a 1 mL aliquot was taken and placed in a Sedgwick Rafter counting chamber and examined at 400 to 1000 x using a Leica Fluorescence microscope. Immersion oil was added when necessary to count the small cells. A Leica camera attached to the phototube enabled a photographic reference collection to be developed. For chlorophyll *a* analysis, the GFF filters with filtered samples were cut into small pieces and were placed in 15 mL centrifuge vials (with 10 mL of 90% acetone). The samples were sonicated for no longer than 20 s. The vials were wrapped in aluminium foil to

exclude light and were left to extract the chlorophyll *a* at 4°C for 16 to 24 hours. Samples were centrifuged for 15 min at 4500 rpm, with the chamber chilled to 4 °C. Samples were analysed using a GBC Cintra 10e spectrophotometer.

Zooplankton samples were preserved in 5% formaldehyde-seawater buffered with Borax, and all gelatinous organisms were identified and counted using a Leica M165C stereomicroscope. When necessary, zooplankton samples were split with a Folsam plankton splitter to reduce the numbers of organisms counted to between 400 and 1,200. The estimated density of thaliaceans m^{-3} was calculated. Toluidine Blue was used to stain muscle fibres and aid in identification.

3.3.4 Statistical analyses

The distributions of thaliaceans in relation to sampling sites and environmental variables were analyzed by using PRIMER 6 (Plymouth Routine in Marine Environmental Research; Clarke and Gorley 2006). Bray-Curtis dissimilarity index was applied to quantify the compositional dissimilarity between different sites, based on counts of thaliaceans at each site. Multi-dimensional scaling (MDS) (Clarke, 1993) of the Bray-Curtis similarity matrix was used to visualise the relationships between sampling sites. Relationships between thaliaceans and environmental variables were tested by BEST analysis, one of the multivariate routines available in PRIMER (Clarke, 1993). BEST analysis determines which suite of environmental variables best explained the patterns seen in the distributions of the thaliaceans over the three summers while principle

components analysis (PCA) was used to examine the relationships between the seven environmental variables and the sampling sites where thaliaceans were recorded.

3.4 Results

3.4.1 Physical parameters in Storm Bay

In the summer of 2009-2010, warm saline water that is derived from the EAC penetrated further south along the north-east coast of Tasmania (Fig. 3.2), and Storm Bay showed warmer than average SST, with mean SST measuring between 13.67 and 18.73°C (Fig. 3.3-3.8). In the summer of 2010-2011, mean SST ranged from 13.04 to 16.76°C and during the summer of 2011/2012 it ranged from 14.64 to 17.86 °C. In early summer 2009-2010, mean sea surface salinity (SSS) measured between 33.89 and 34.89 (Fig. 3.9 – 3.14). In the following summer, when there was a La Niña event, mean salinity ranged dropped to a low of 32.95, but also reached as high as 35.02. Finally in the summer of 2011-2012, the range of mean salinity was the highest, recorded at 33.99-35.11.

The mean value of rainfall around Storm Bay was the highest when there were La Niña conditions in summer 2010/2011 (November 2009, at 81.85 mm) followed by summer 2009/2010 (in November 2010, at 80.25 mm) and lastly summer 2011/2012 (in February 2012, at 21.05 mm) (Fig. 3.15).

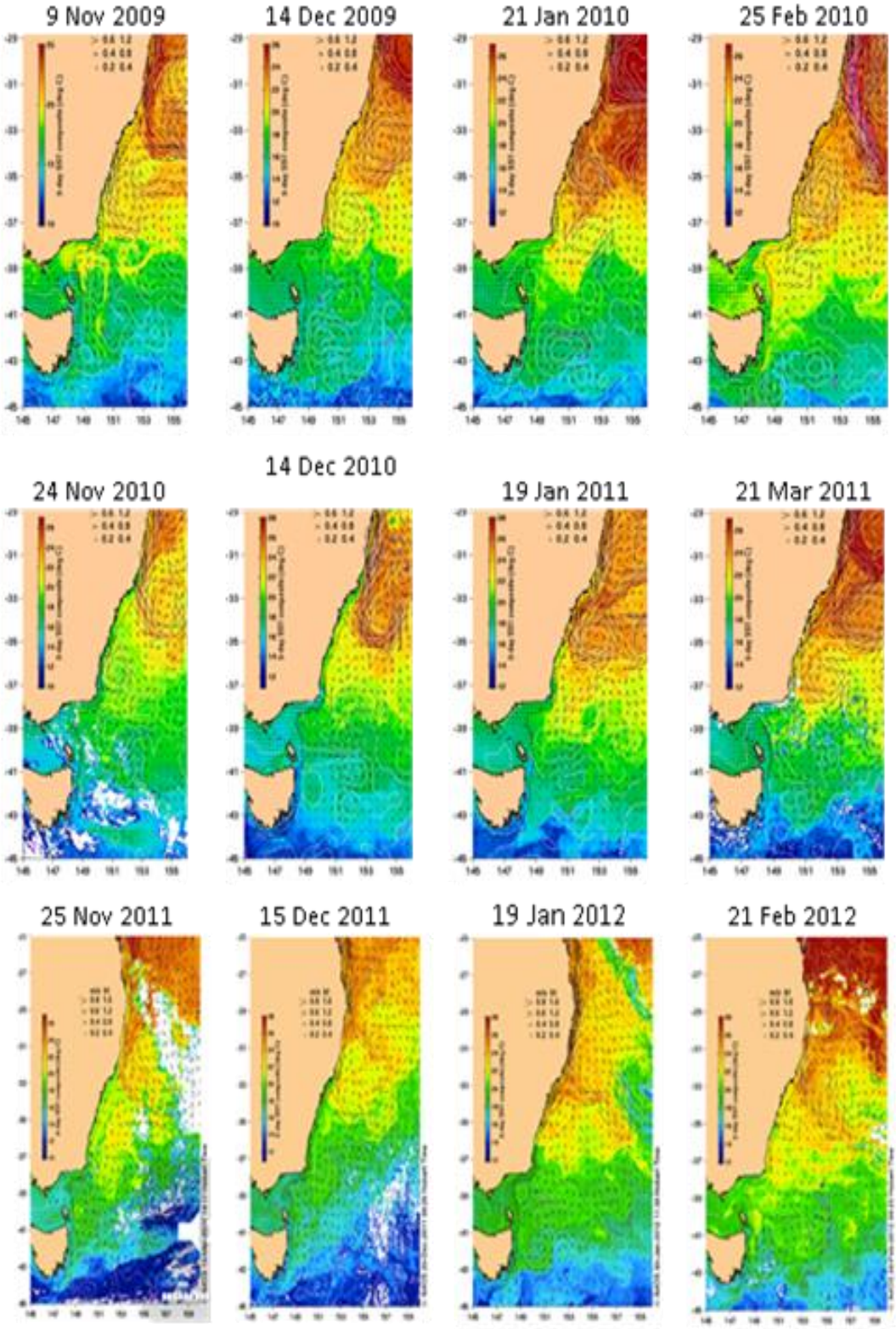

Fig. 3.2. Details the monthly sea surface temperatures corresponding to the survey in each year along the east coast of Tasmania. SST and ocean currents for summer

2009-2010, summer 2010-2011 and summer 2011-2012 showing the EAC flowing south along mainland Australia towards Tasmania. Current speed (ms^{-1}) denoted by > (Images provided by David Griffin, CSIRO Marine and Atmospheric Research).

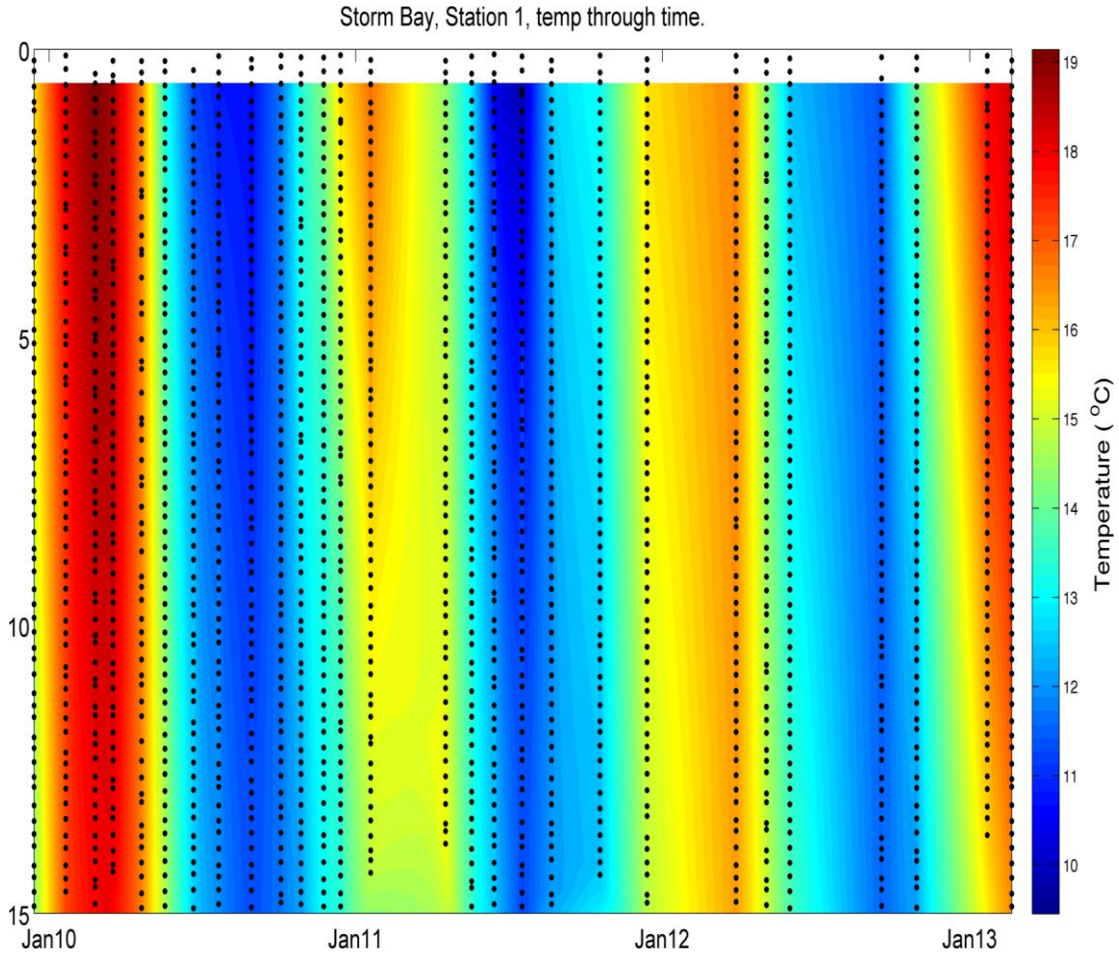

Fig. 3.3. Vertical temperature profiles at site 1, from January 2010 to January 2013.

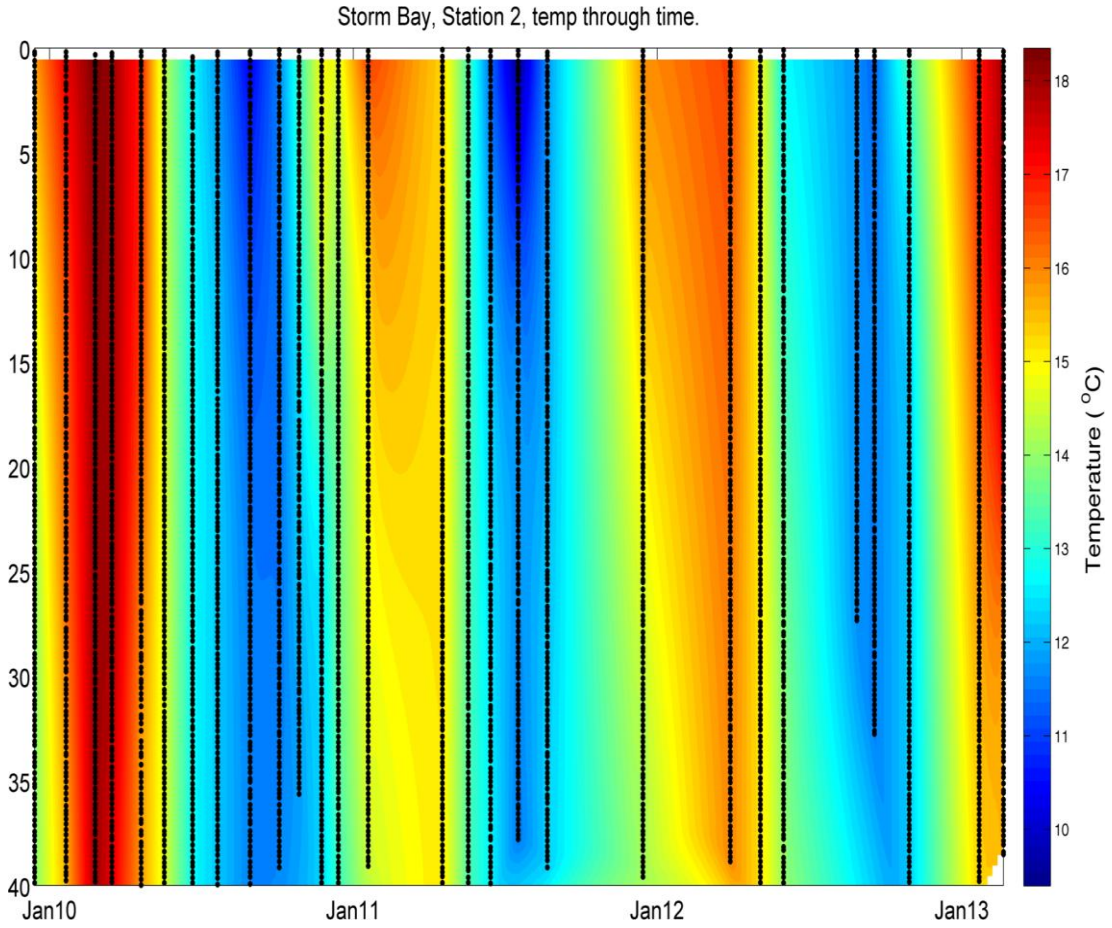

Fig. 3.4. Vertical temperature profiles at site 2, from January 2010 to January 2013.

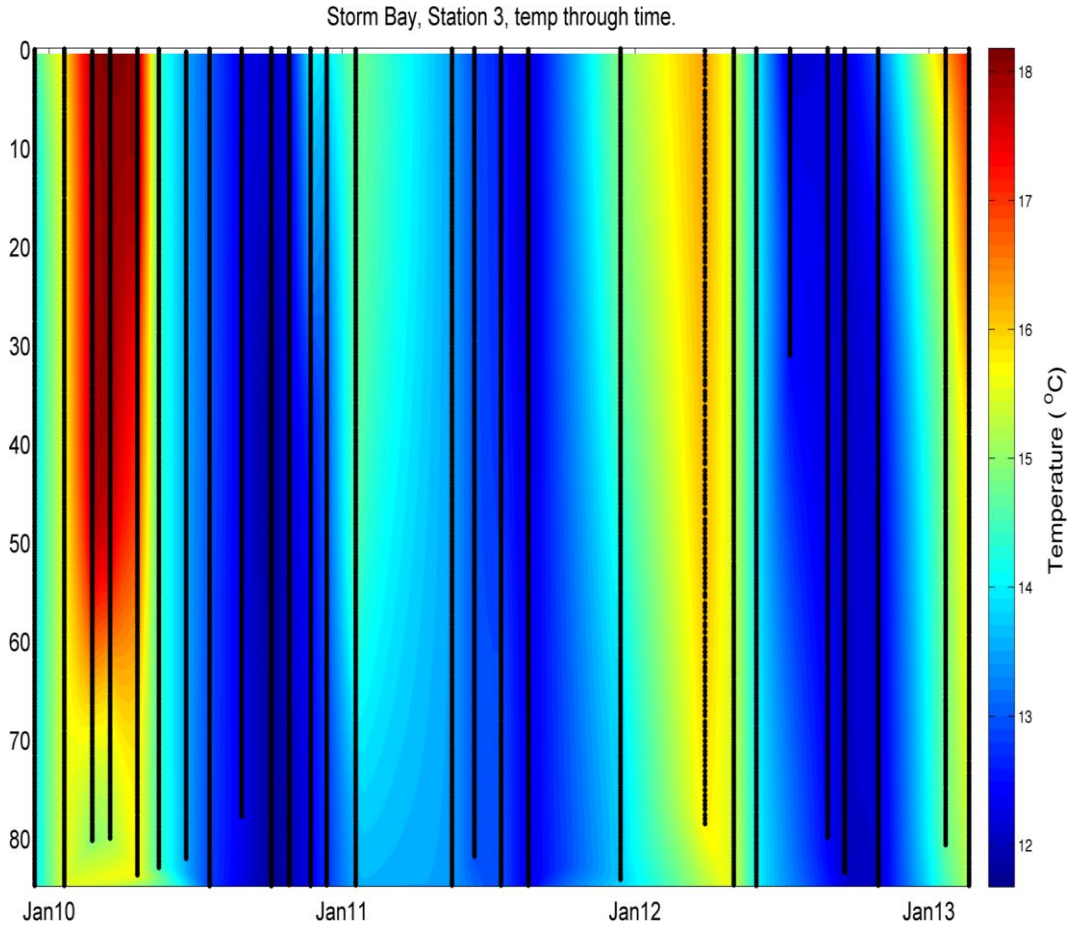

Fig. 3.5. Vertical temperature profiles at site 3, from January 2010 to January 2013.

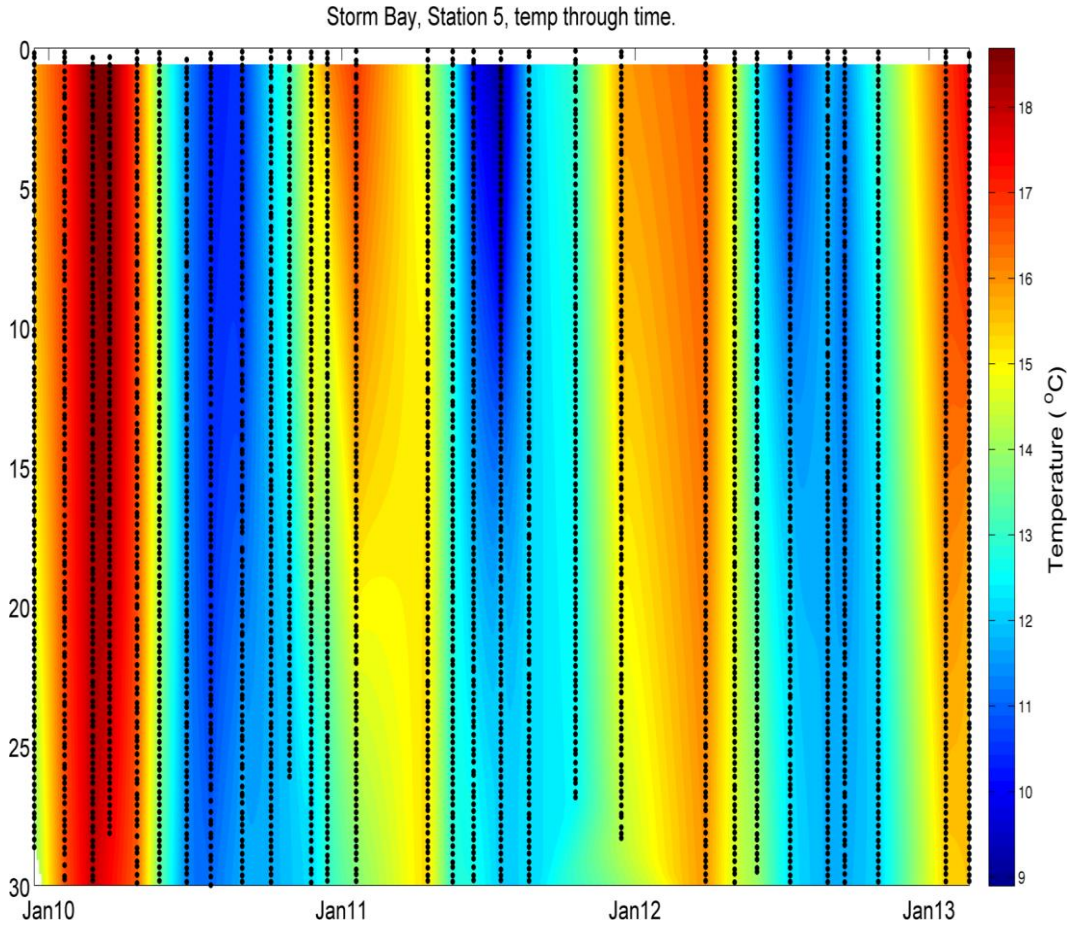

Fig. 3.6. Vertical temperature profiles at site 5, from January 2010 to January 2013.

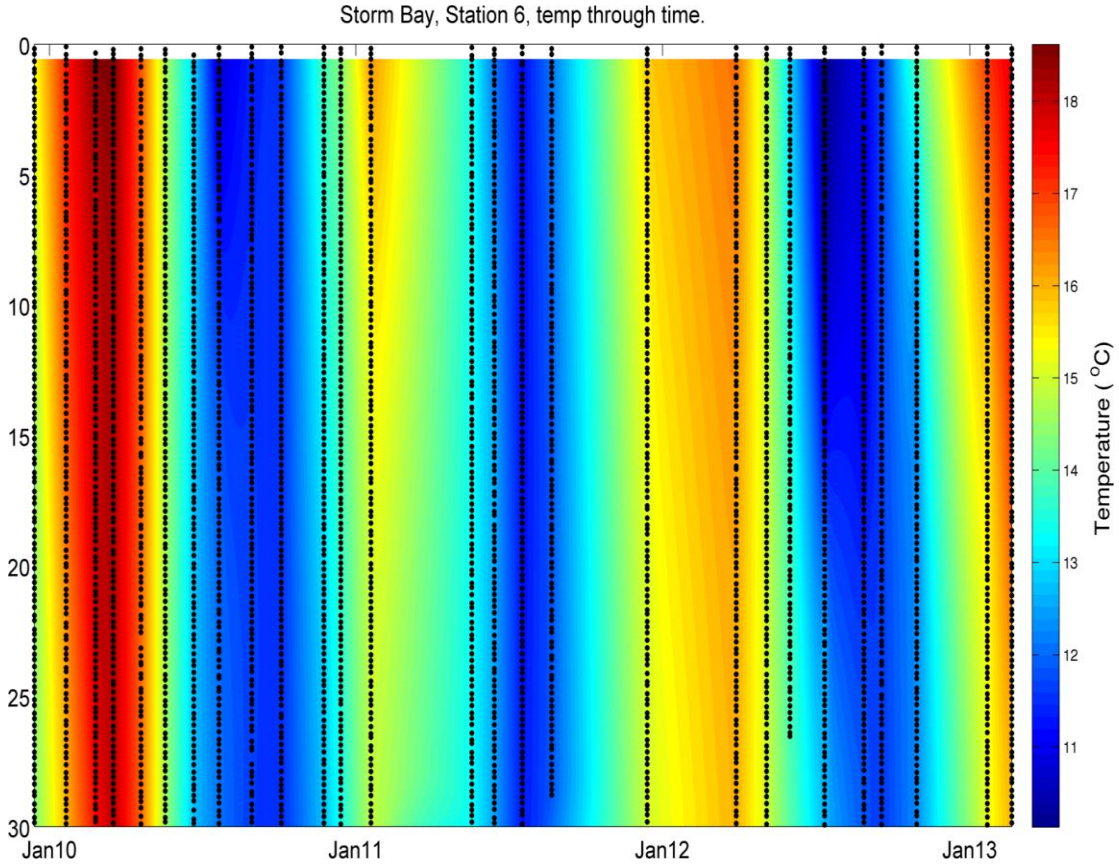

Fig. 3.7. Vertical temperature profiles at site 6, from January 2010 to January 2013.

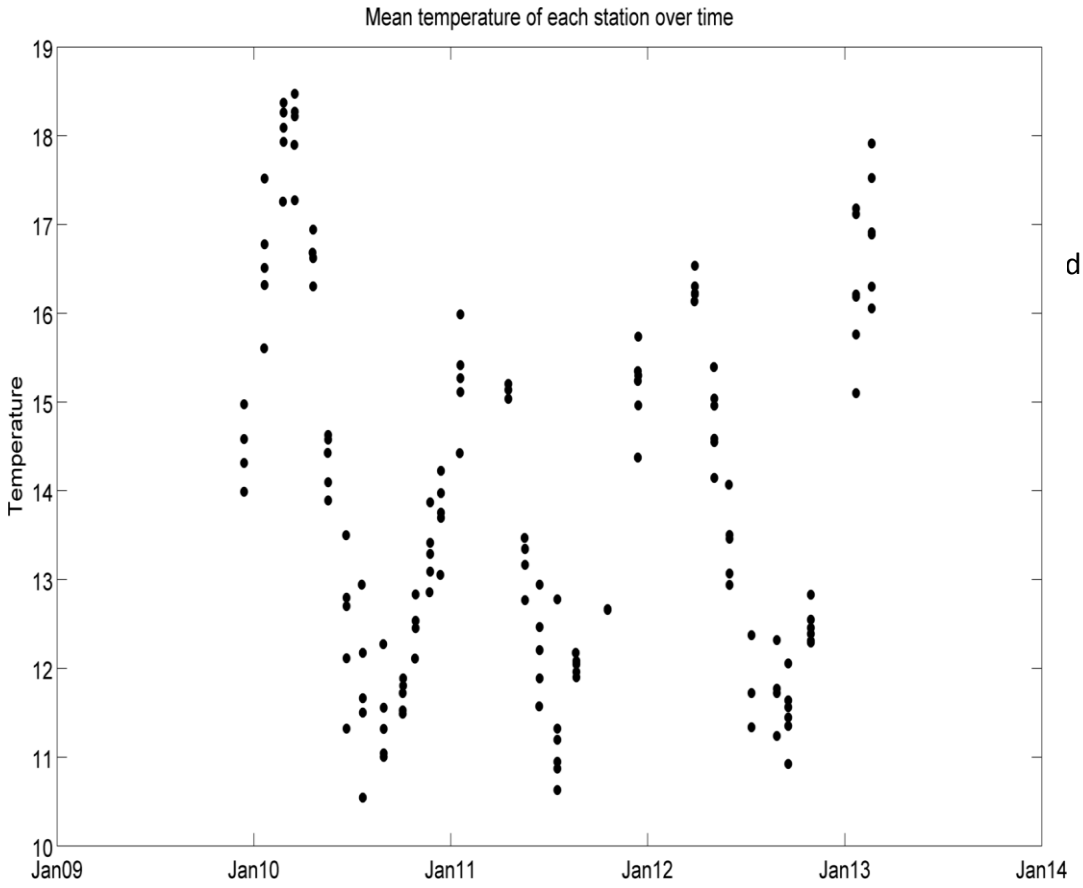

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Fig. 3.8. Mean temperature across Storm Bay over the sampling period.

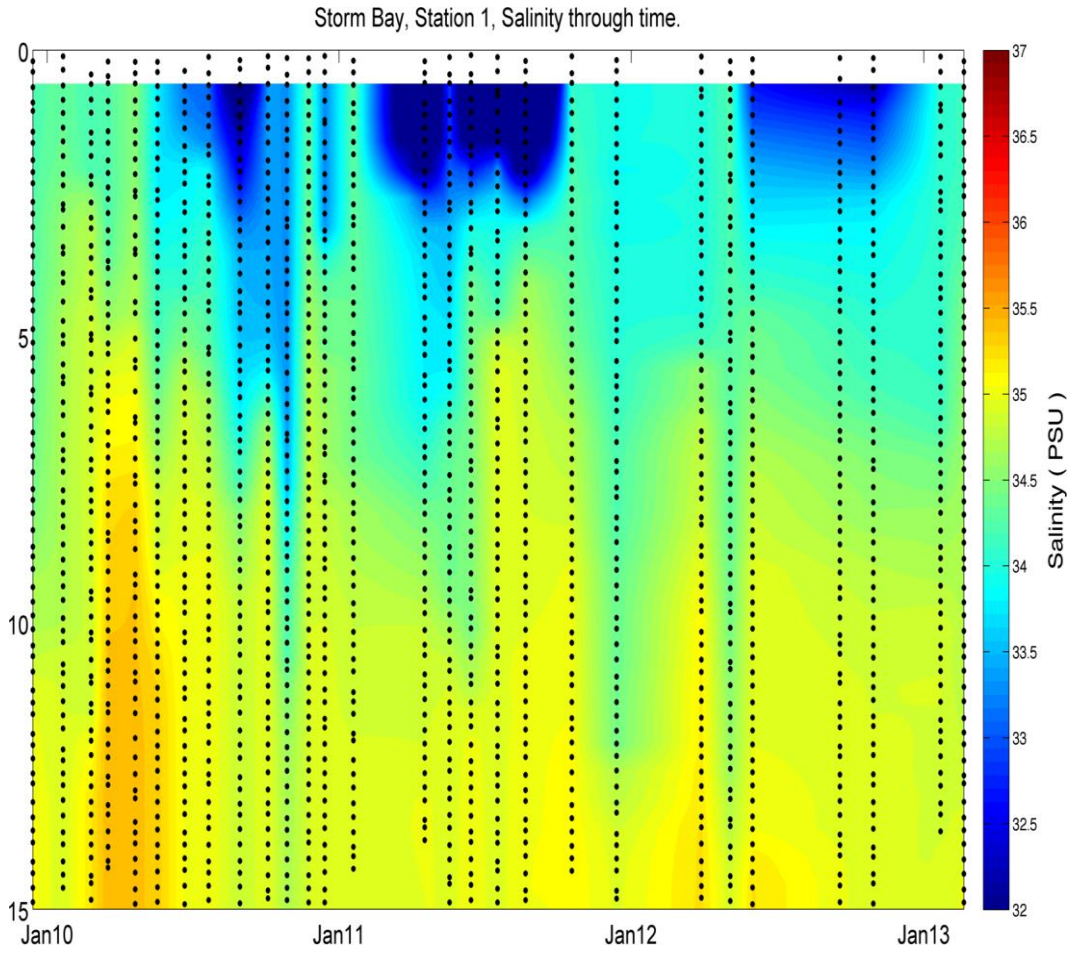


Fig. 3.9. Vertical salinity profiles at site 1, from January 2010 to January 2013.

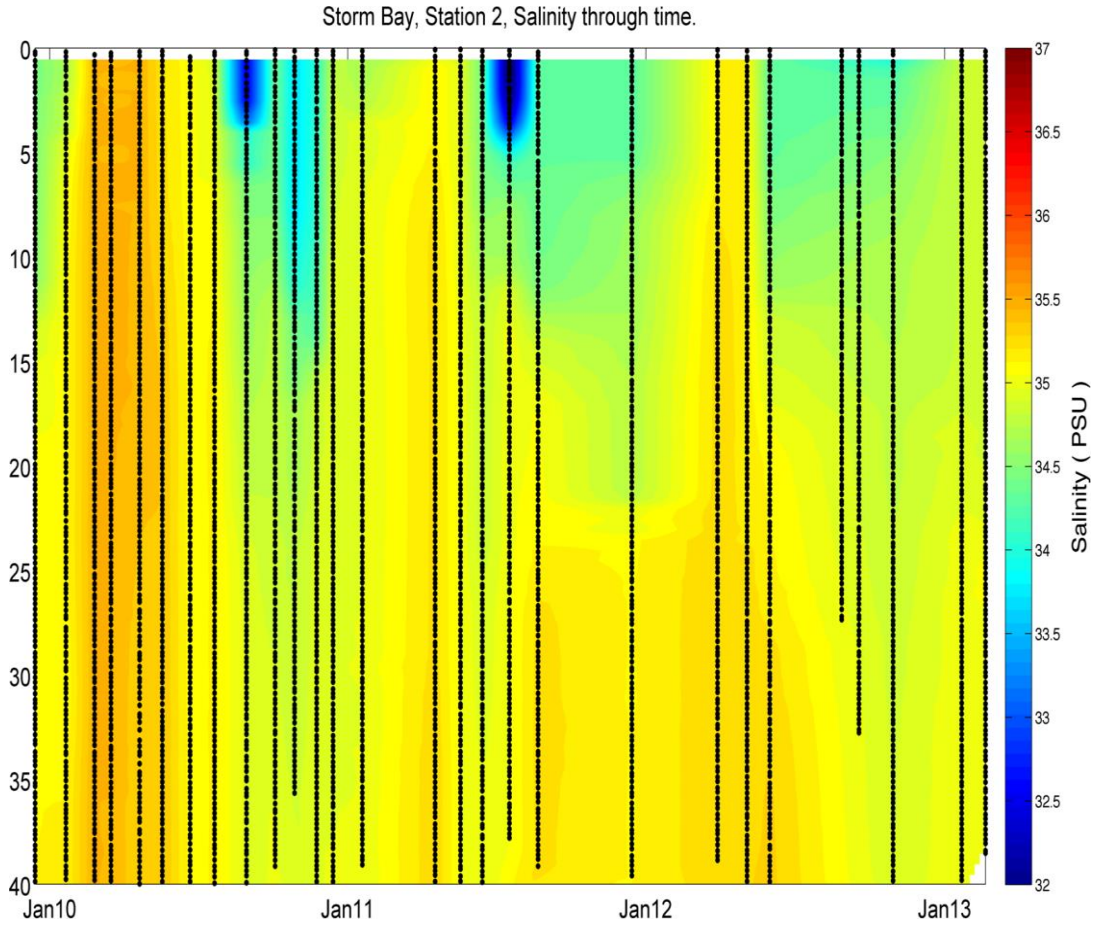


Fig. 3.10. Vertical salinity profiles at site 2, from January 2010 to January 2013.

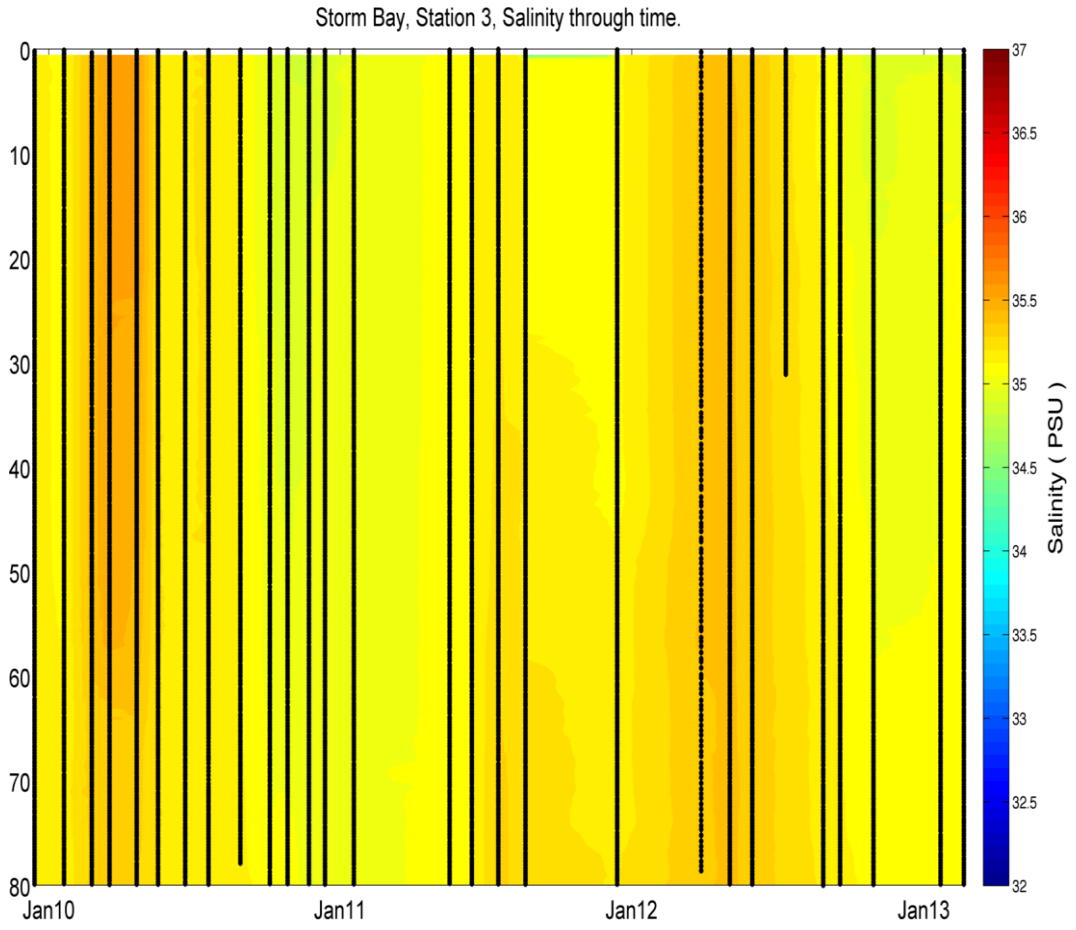


Fig. 3.11. Vertical salinity profiles at site 3, from January 2010 to January 2013.

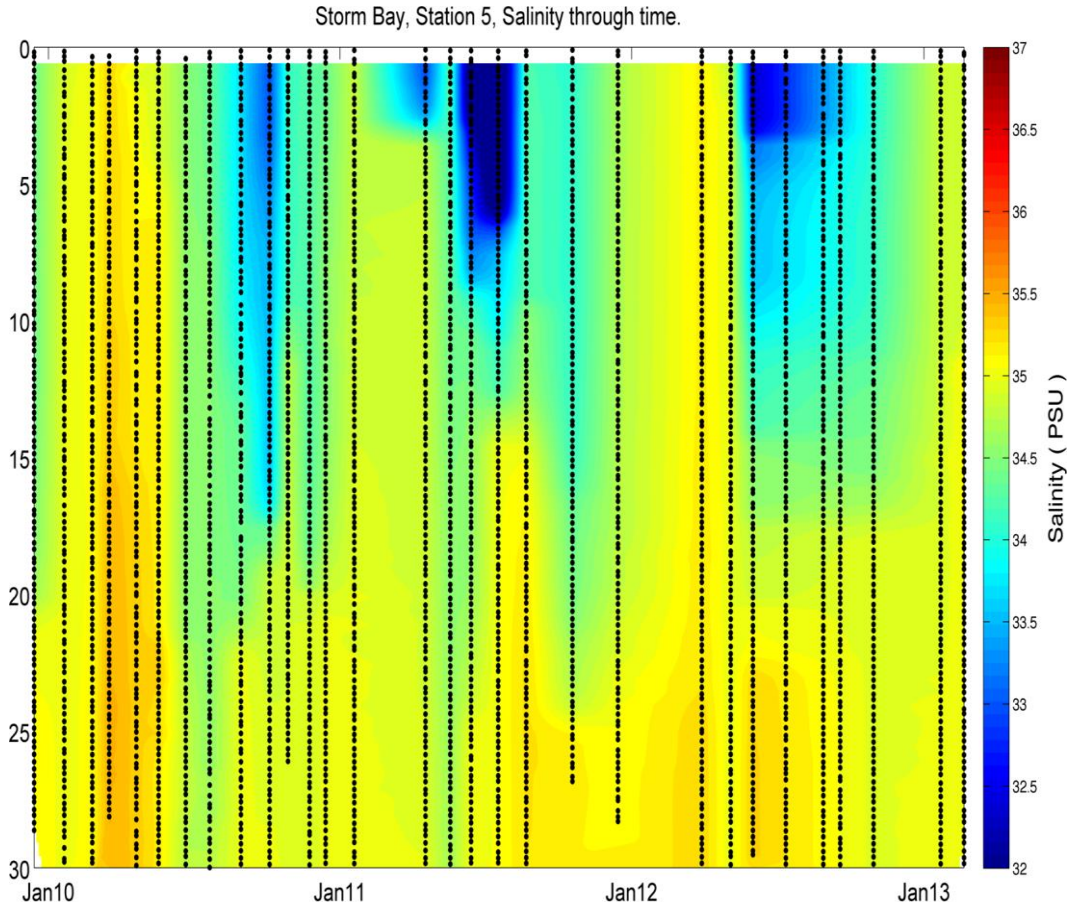


Fig. 3.12. Vertical salinity profiles at site 5, from January 2010 to January 2013.

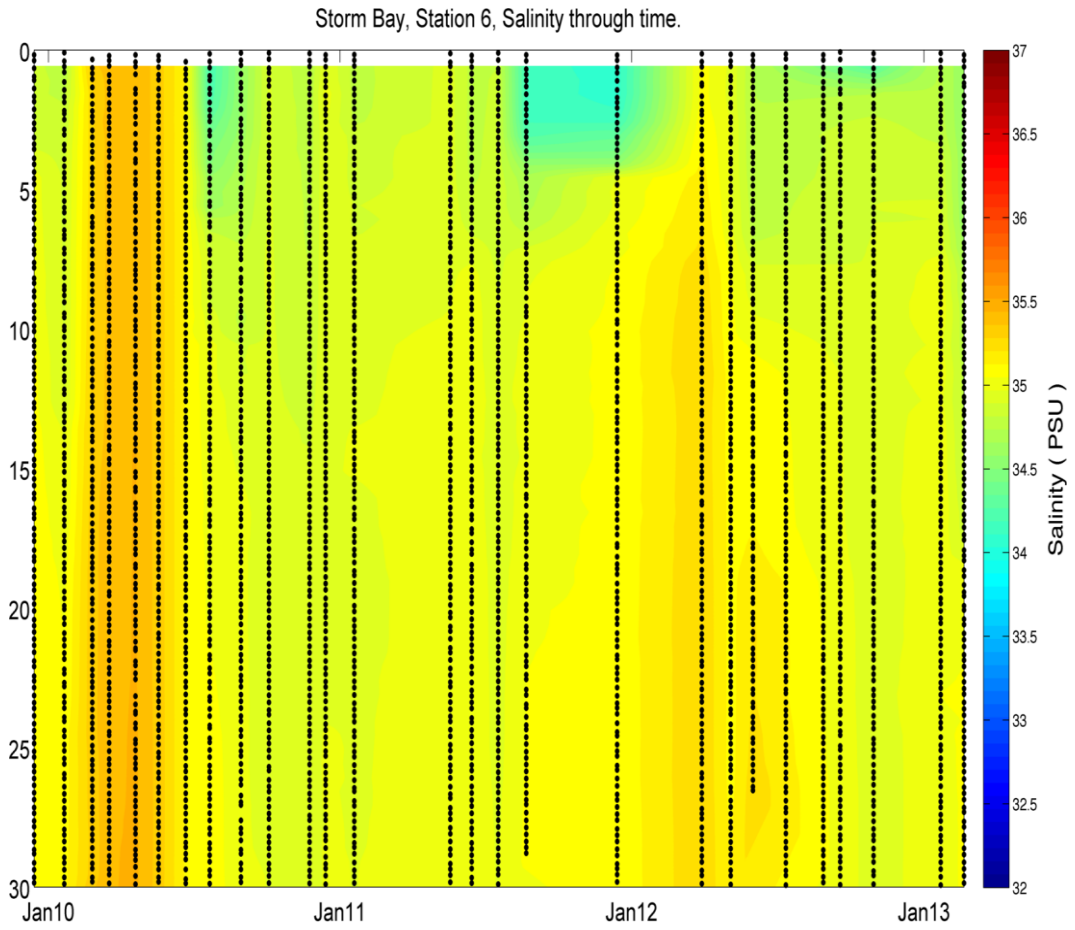


Fig. 3.13. Vertical salinity profiles at site 6, from January 2010 to January 2013.

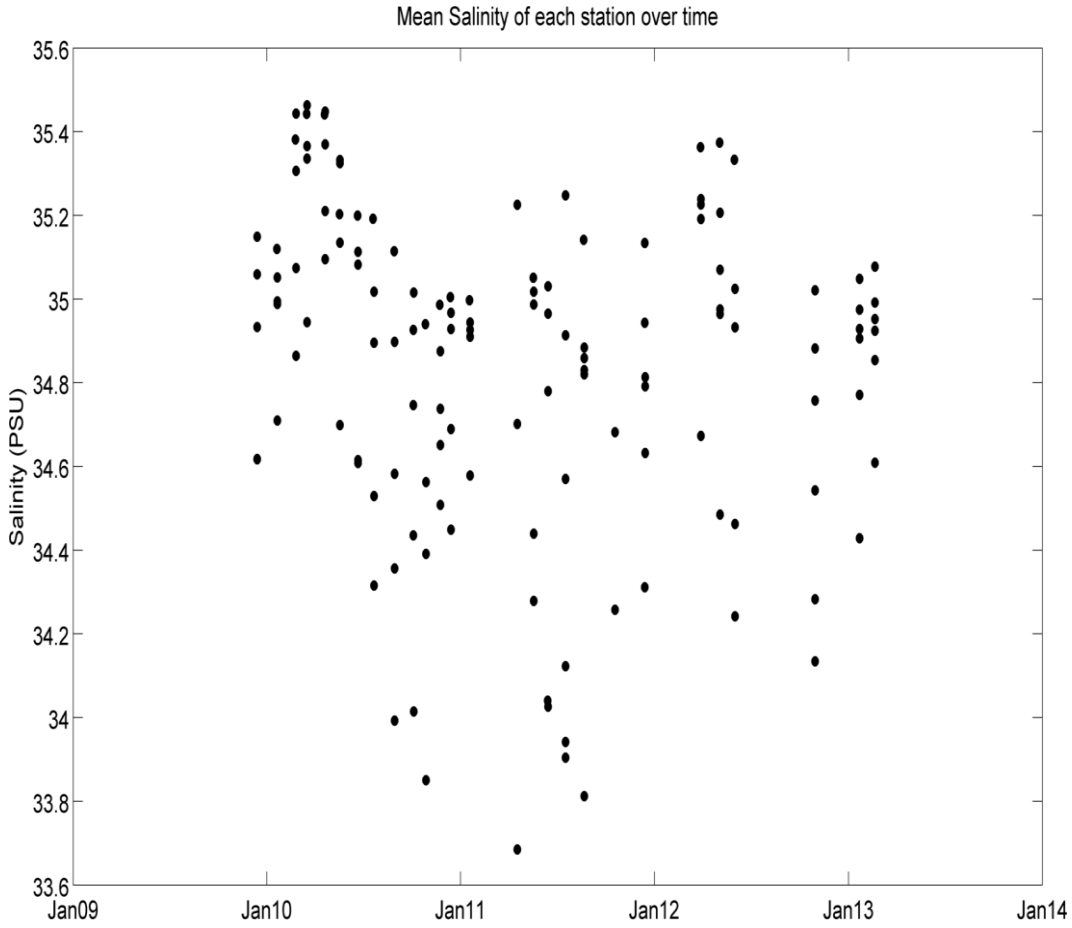


Fig. 3.14. Mean temperature across Storm Bay over the sampling period.

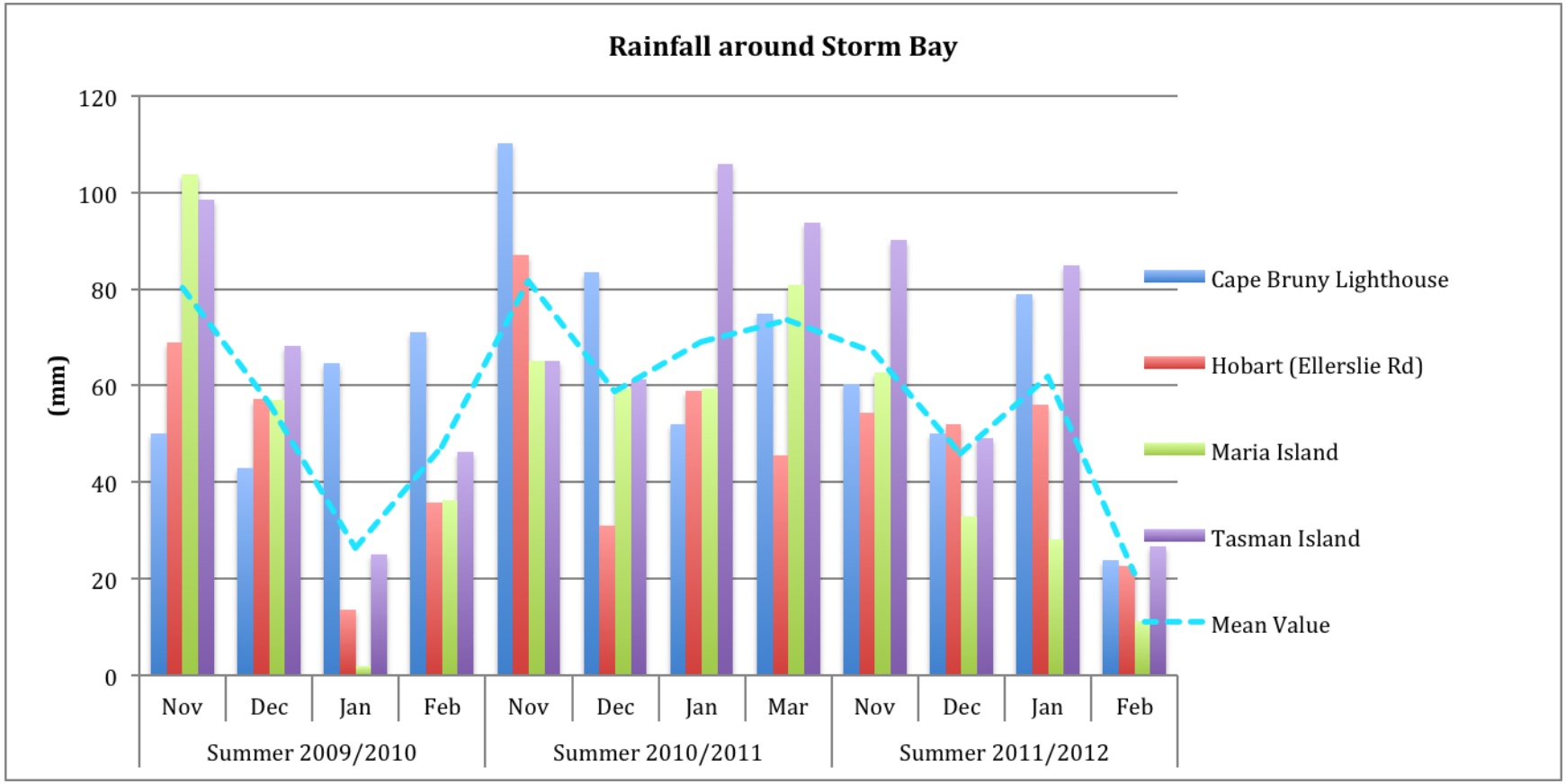


Fig. 3.15. Rainfall around Storm Bay during 3 summers. Cape Bruny Lighthouse, Hobart (Ellerslie Rd), Maria Island and Tasman Island were located to the south, west, north and northeast of Storm Bay, respectively.

3.4.2 Abundance and distribution of thaliaceans in Storm Bay

In early summer 2009-2010, doliolids were abundant and were observed at all five sites in Storm Bay. *Dolioletta* sp. was abundant at sites 2, 3 and 5 in November. As the season progressed, the SST increased and as a result, in January 2010, there were salp blooms (*T. democratica*) at sites 2 and 3 (Fig. 3.16). All thaliaceans had disappeared from Storm Bay by February 2010.

In the 2010-2011 summer the La Niña conditions that influenced much of Australian weather, resulting in very wet conditions continent-wide (Giles 2012; Trenberth 2012), was reflected in lower SST in Storm Bay (Fig. 3.9-3.14). Coincidentally, salps were exceedingly rare (small numbers of *S. fusiformis* were observed at site 3 in December 2010), although *Doliolum* sp. was common in early autumn (Fig. 3.17).

In the summer of 2011-2012, little warm water from the EAC penetrated south to Tasmania (Fig. 3.2). There were only small numbers of salps and doliolids at sites 2 and 3 (Fig. 3.18). The SST increased towards February and there was an outbreak of salps in January. Although there was also a bloom of salps in the summer of 2009/2010, this time a different pattern was evident. The event started with a bloom dominated by *Salpa fusiformis* at Site 2 in January, followed by an increased number of *Thalia democratica* that were more abundant than *S. fusiformis* by February (Figure 3.18). The highest abundance recorded for each salp and doliolid species in Storm Bay across the sampling period is depicted in Table 3.4, highlighting the key environmental drivers.

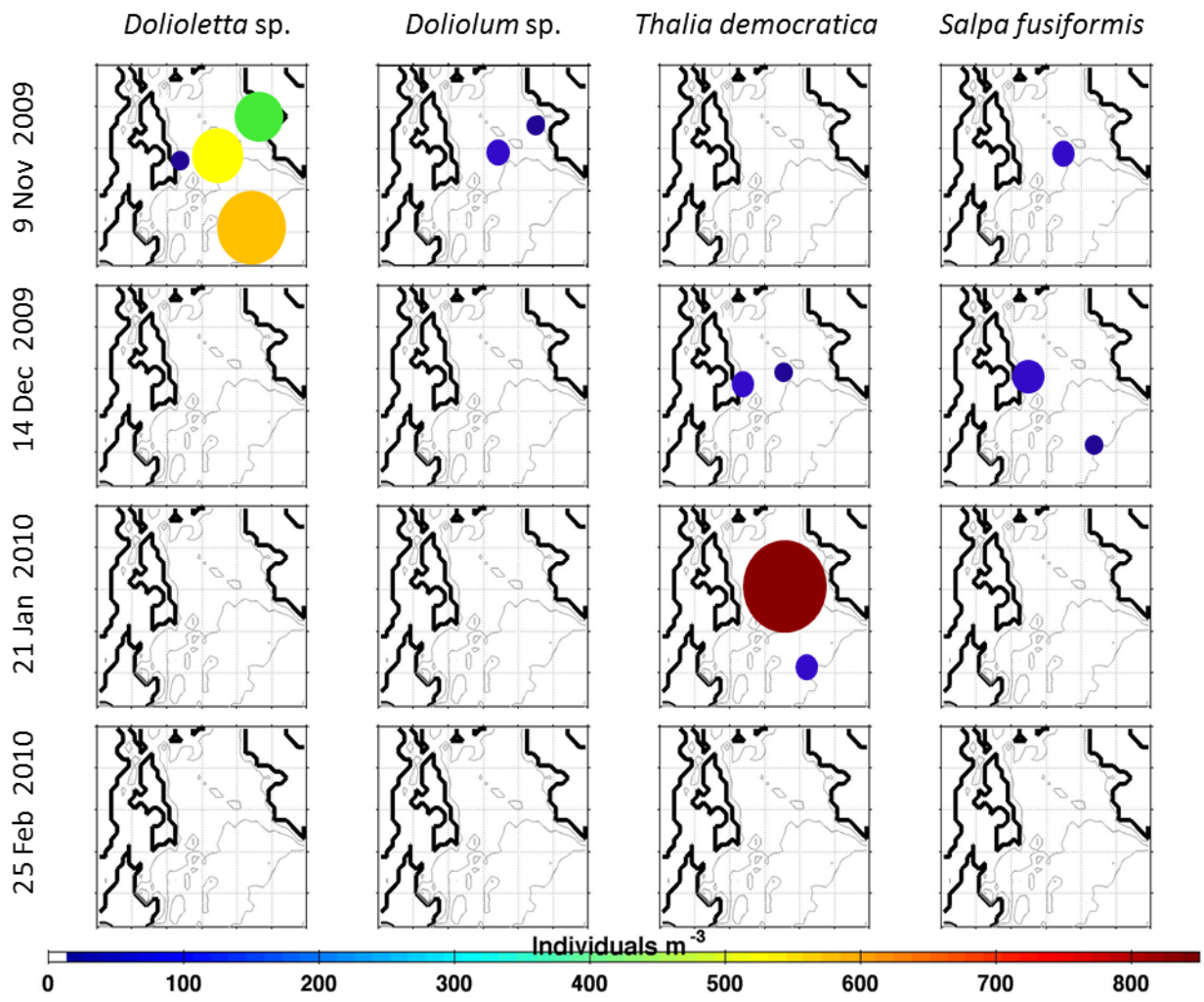


Fig. 3.16. The abundance and distribution of thaliaceans during the summer of 2009-2010. Colour bar and circle size shows abundance of each species at each sampling site. There was a bloom of *T. democratica* at site 2 in January 2010.

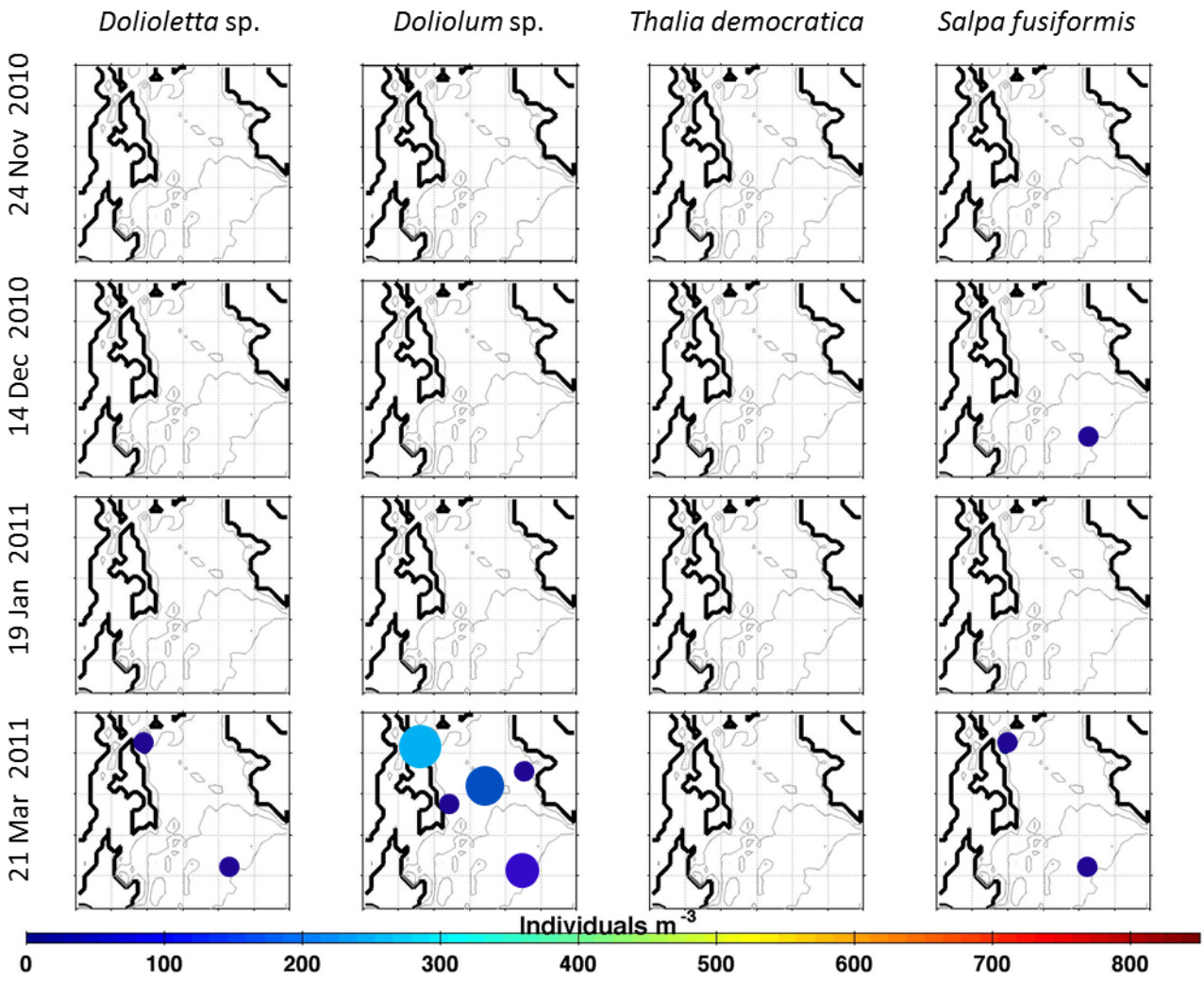


Fig. 3.17. The abundance and distribution of thaliaceans during the summer of 2010-2011. Colour bar and circle size show abundance of each species at each sampling site. *Doliolum* sp. were common in March 2011, distributed over all the sites.

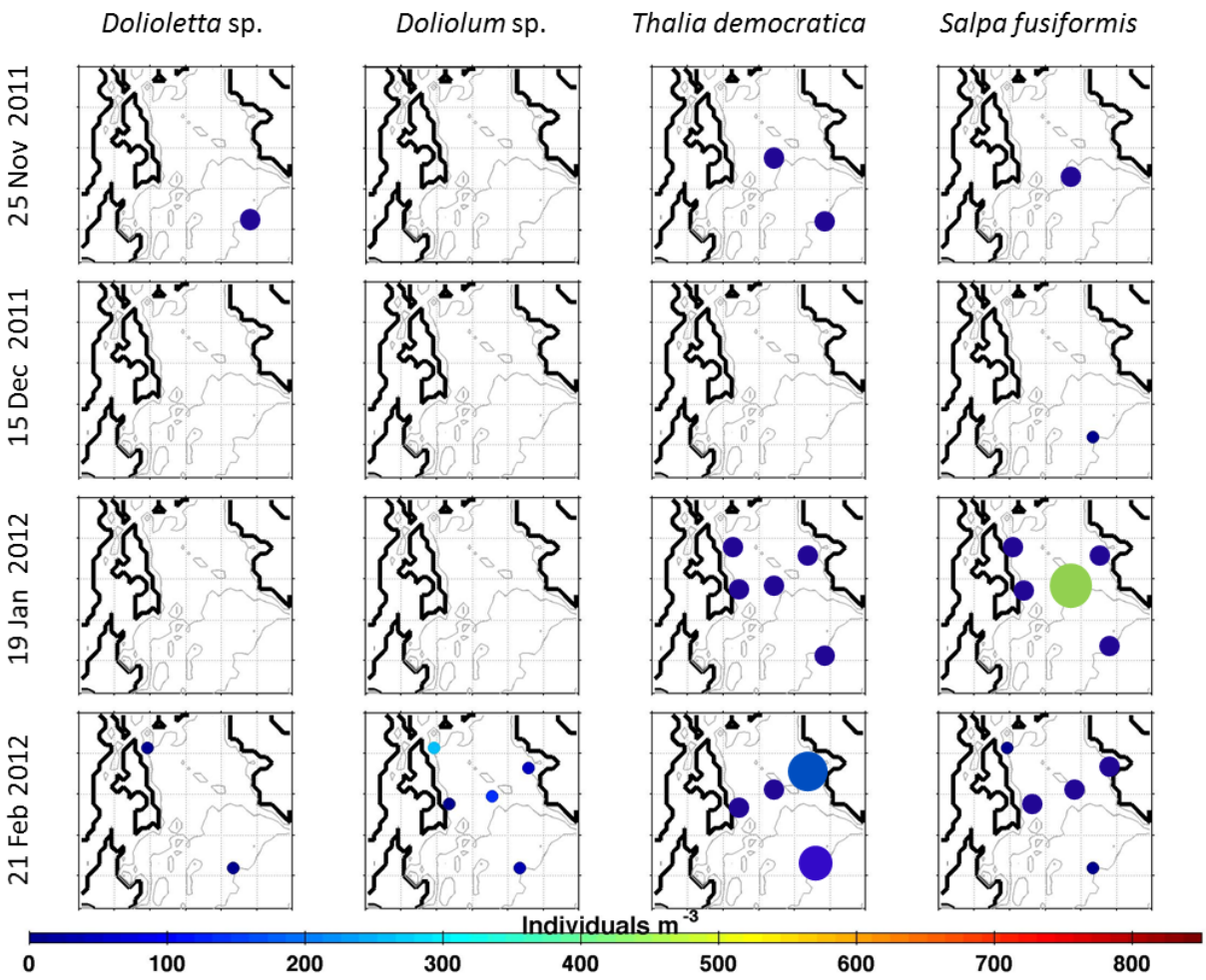


Fig. 3.18. The abundance and distribution of thaliaceans during the summer of 2011-2012. Colour bar and circle size show abundance of each species at each sampling site. Both *T. democratica* and *S. fusiformis* occurred in high densities in January and February 2012.

Table 3.4. The highest abundances recorded for each species in Storm Bay showing the month, year when each was dominant.

Species	Dominant month	Site	Abundance (number m^{-3})	Mean SST (°C)	Mean SSS	Rainfall (mm)
Dolioletta sp.	Nov 2009	1	10	14.92	33.91	80.25
		2	542	14.84	34.62	
		3	564	13.42	34.33	
		5	477	14.63	34.52	
		6	65	14.93	34.41	
Doliolum sp.	Mar 2011	1	262	16.76	33.06	73.70
		2	138	16.75	33.58	
		3	34	16.38	33.77	
		5	40	16.35	33.83	
		6	10	16.48	32.95	
T. democratica	Jan 2010	2	924	17.40	34.40	26.28
		3	389	15.85	34.34	
S. fusiformis	Jan 2012	2	354	15.25	34.57	61.93
		3	47	14.64	35.11	
		5	82	15.38	34.94	
		6	28	14.98	34.82	

3.4.3 Primary production

3.4.3.1 Chlorophyll *a*

Chlorophyll *a* concentrations were highest in summer of 2009/10, with a second bloom evident in the bay at sites 2 and 3 during the summer of 2011/12 (Fig. 3.19-3.23). Mean chlorophyll *a* concentrations at 10 m at each site for the three summers are shown in Table 3.5. The highest mean concentration of chlorophyll *a* was in summer 2010/2011, when it reached 5.65 mg m^{-3} across the bay. The

concentrations in the summer 2009/2010 and summer 2011/2012 were similar, with 2.41 mg m^{-3} and 2.57 mg m^{-3}, respectively.

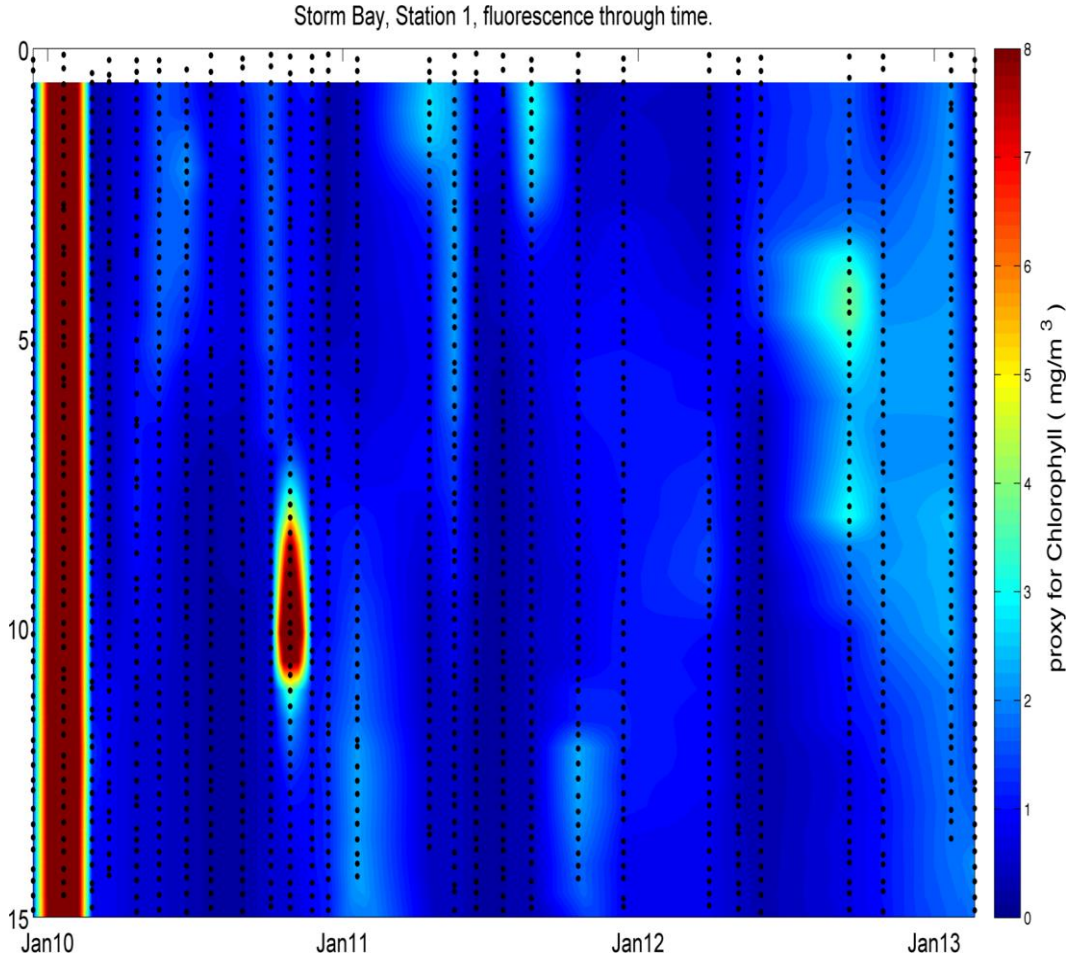


Fig 3.19. Vertical chlorophyll *a* profiles at site 1, from January 2010 to January 2013**.**

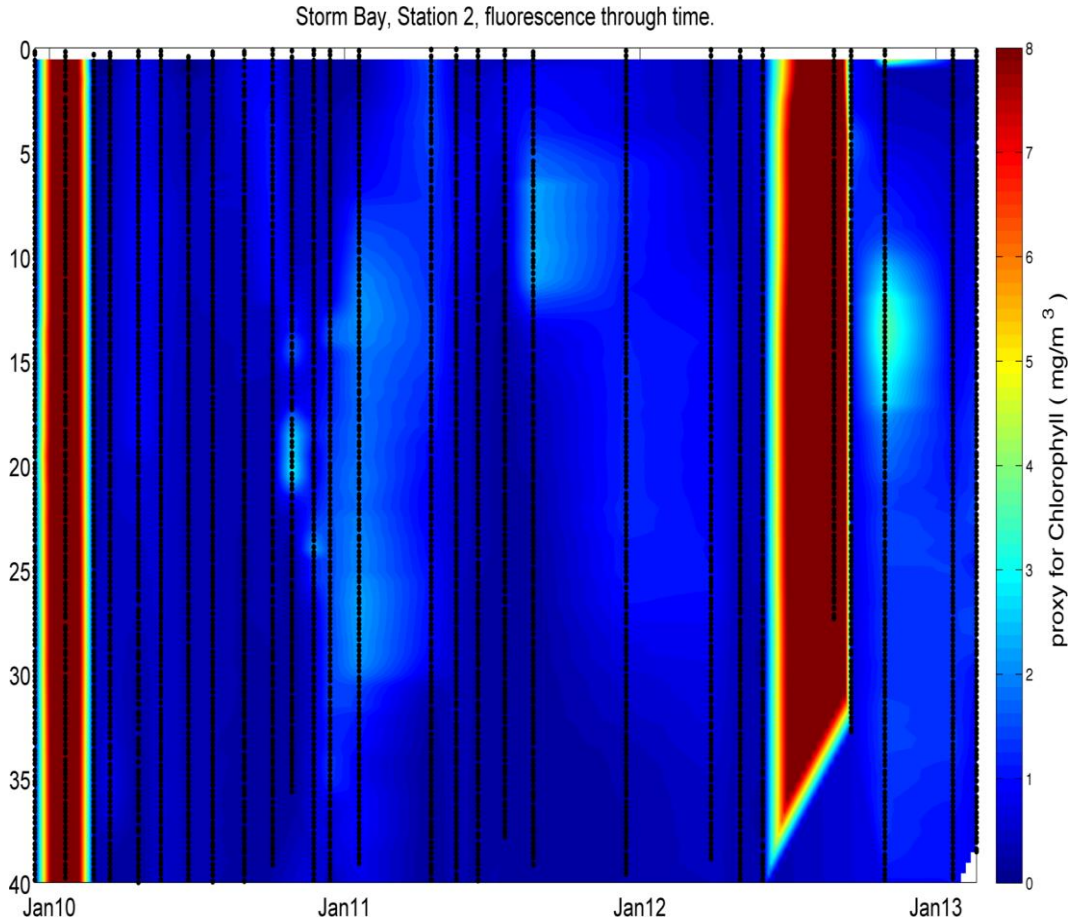


Fig 3.20. Vertical chlorophyll *a* profiles at site 2, from January 2010 to January 2013.

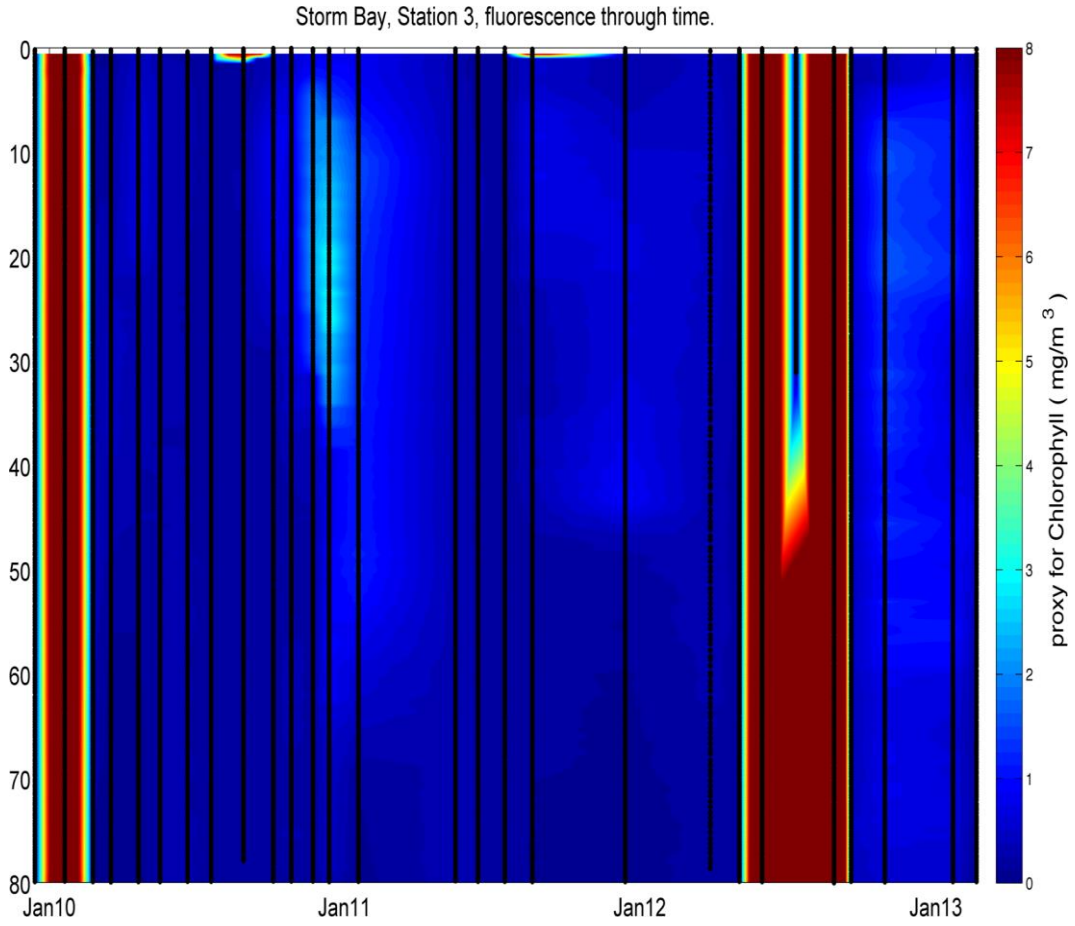


Fig 3.21. Vertical chlorophyll *a* profiles at site 3, from January 2010 to January 2013.

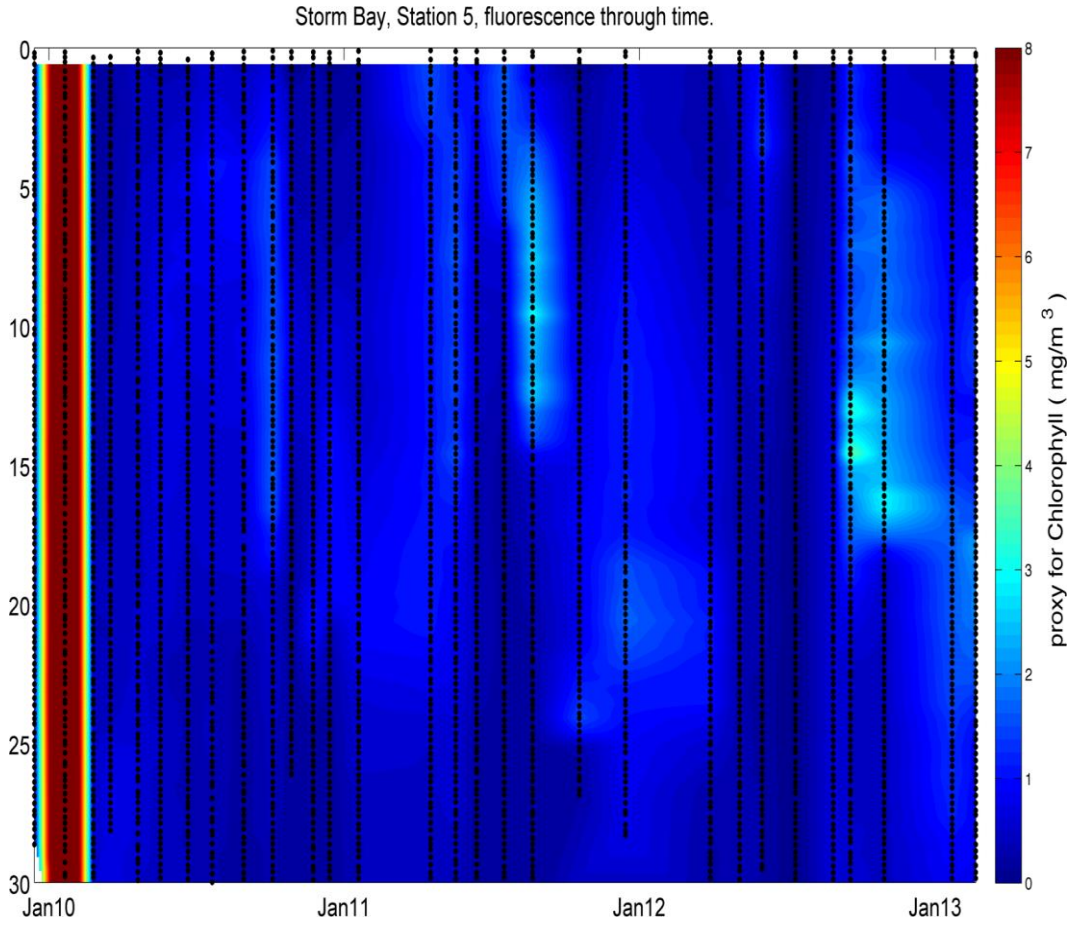


Fig 3.22. Vertical chlorophyll *a* profiles at site 5, from January 2010 to January 2013.

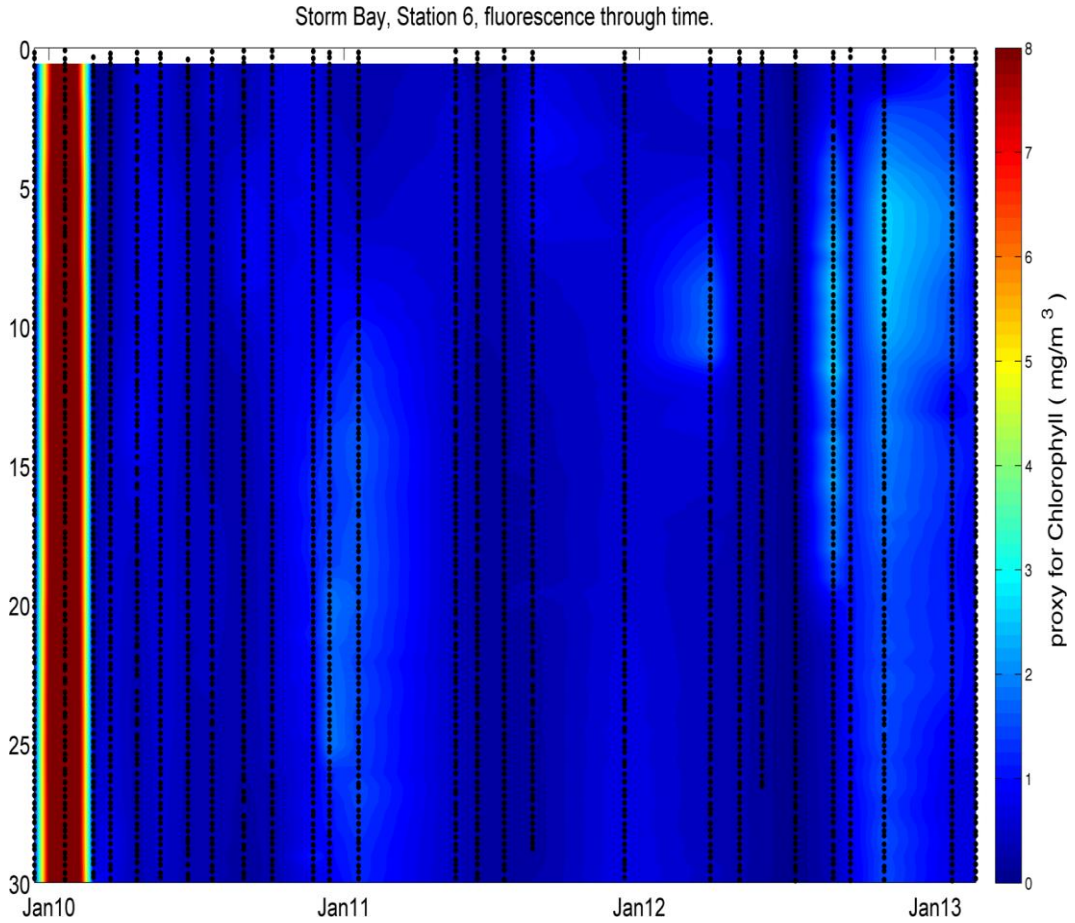


Fig 3.23. Vertical chlorophyll *a* profiles at site 6, from January 2010 to January 2013.

Table 3.5. Chlorophyll *a* concentrations (10m depth) across each site for the three summers and the total mean concentration across Storm Bay.

Summer	**Site**	**Chlorophyll *a*, mg m^{-3}**	**Total mean chlorophyll *a*, mg m^{-3}**
2009-10	SB1	4.12	2.41
	SB2	1.47	
	SB3	2.08	
	SB5	2.48	
	SB6	1.88	
2010-11	SB1	5.02	5.65
	SB2	4.14	
	SB3	11.87	
	SB5	2.87	
	SB6	4.28	
2011-12	SB1	3.59	2.57
	SB2	2.11	
	SB3	3.09	
	SB5	2.33	
	SB6	1.74	

3.4.3.2 Total phytoplankton

Figs. 3.10 -3.12 show the abundances of phytoplankton stocks (flagellates, dinoflagellates and diatoms) over the three summers. Highest phytoplankton stocks occurred in 2009/10, then they were almost an order of magnitude lower during 2010/2011 and then relatively high again in 2011/2012. In summer 2009/2010, phytoplankton were visibly higher in coastal waters (sites 1, 5 and 6), abundances ranging from 1.4 X 10^5 to 1.2 X 10^6 cell L^{-1}, an order of magnitude

higher than at sites 2 and 3 (range: 8.8 X 10^4-8.0 X 10^5 cell L^{-1}). During 2010/11, phytoplankton stocks were generally highest at site 3 and ranged from 4.3 X 10^4 to 3.3 X 10^5 cell L^{-1}. Phytoplankton were distributed fairly evenly during summer 2011/2012, with the range of abundances from 1.3 X 10^6 to 1.6 X 10^6 cell L^{-1}.

Fig. 3.27 shows diatom species and their abundance per sample during the three summers. Twenty-nine species of diatoms were observed throughout the study, the lowest number of species was seen in the summer of 2009/2010 (the year when overall total phytoplankton stocks were highest). However, each individual species was in greater abundance in 2009/10 compared to the other two summers and the numbers of species gradually increased by the end of the season. In the summer of 2010/2011, although the number of diatoms species (35) was slightly higher than the in the previous summer, the abundances of all species were lower. The greatest variety of diatom species in Storm Bay was recorded in summer 2011/2012, at about 44 species.

3.4.3.3 Relationship between phytoplankton and thaliaceans

When *T. democratica* bloomed in January 2010 at site 2, total phytoplankton stocks were 2.7 X 10^5 cell L^{-1}, comprising 2.5 X 10^5 flagellates L^{-1} flagellates and 7.9 X 10^3 diatoms L^{-1}. The highest number of *S. fusiformis* was recorded on January 2012, when phytoplankton stocks reached 1.6 X 10^5 cell L^{-1}, predominantly comprised of 1.6 X 10^5 flagellates L^{-1} and 3 X 10^2 diatoms L^{-1}.

Table 3.3 summarizes the relationships between phytoplankton abundances and taxonomy and each thaliacean species. *Dolioletta* sp. occurred

in high abundance at site 3 when the phytoplankton cell counts were 3.0 X 10^5 L^{-1}, with 1.5 X 10^5 flagellates L^{-1} and 3.5 X 10^3 diatoms L^{-1}. *Doliolum* sp. occurred in large numbers when total phytoplankton cell counts were 6.2 X 10^4 cell L^{-1}, with 5.1 X 10^4 L^{-1} of flagellates, in March 2011. However, unlike other species, *Doliolum* sp. appeared in high numbers when total diatom numbers were high (3.3 X 10^5 cell L^{-1} out of 6.1 X 10^5 cell L^{-1} of total phytoplankton cell counts). While salps were observed in high abundances when phytoplankton stocks were low, *Dolioletta* sp. were found when chlorophyll *a* concentrations were high.

During the three summers *Skeletonema* sp., *Pseudo-nitzschia delicatissima*, *Pseudo-nitzschia seriata*, *Chaetoceros* spp., *Leptocylindricus danicus* and *Rhizosolenia cf. setigera* were species of diatoms that occurred across Storm Bay in large numbers, independent of rainfall or temperature.

The temporal and spatial patterns of the diatoms appeared to affect the thaliacean abundance in Storm Bay. In early summer of 2009/2010, doliolids were abundant and there were many diatom species present. When doliolids and salps were all together at site 2 in November 2009*, Skeletonema* spp., *Pseudo-nitzschia delicatissima*, *Thalassionema* spp., *Leptocylindricus mediterraneus*, *Leptocylindricus minimus*, *Nitzshia closterium, Guinardia flaccida* and *Navicula* sp. were recorded. As the season progressed, the sea surface temperature increased and doliolids gradually decreased. *T. democratica* bloomed in January 2010 but no doliolids were observed. At this time, there

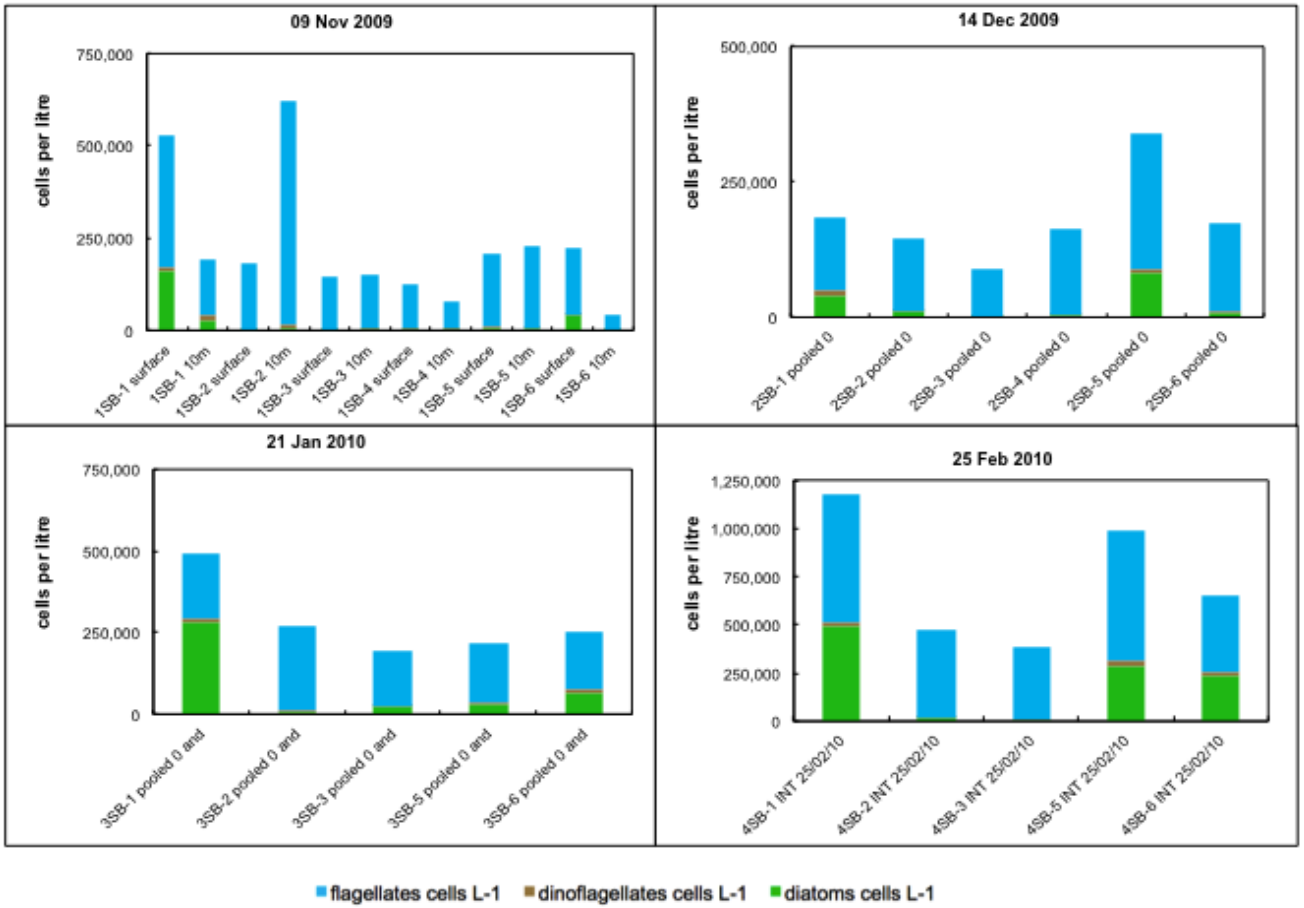


Fig. 3.24. The abundances of phytoplankton stocks (flagellates, dinoflagellates and diatoms) during summer 2009/2010.

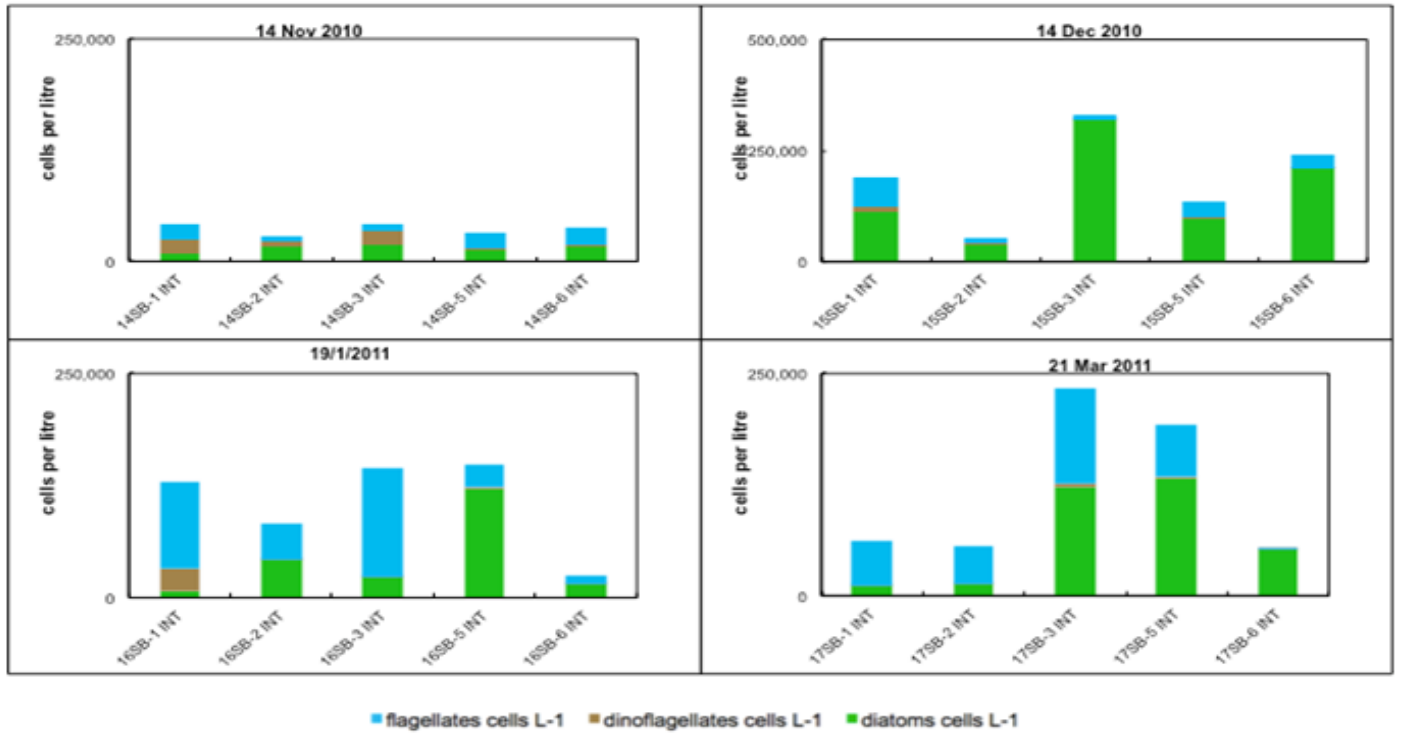


Fig. 3.25. The abundances of phytoplankton stocks (flagellates, dinoflagellates and diatoms) during summer 2010/2011.

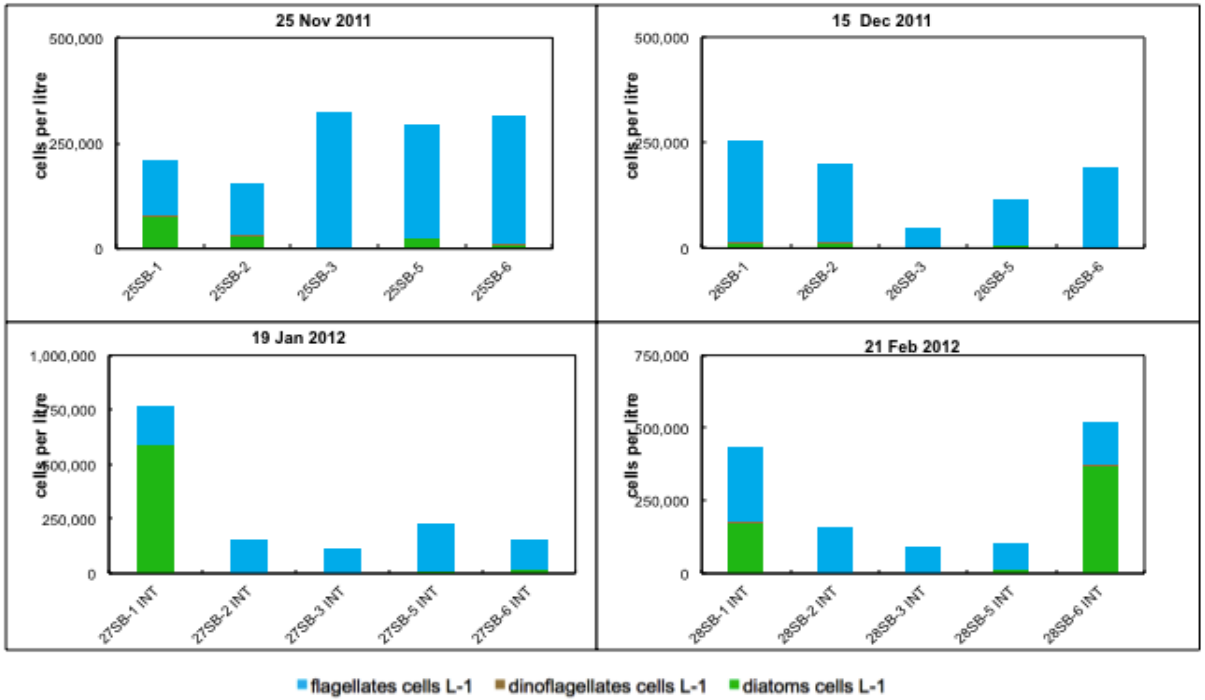


Fig. 3.26. The abundances of phytoplankton stocks (flagellates, dinoflagellates and diatoms) during summer 2011/2012.

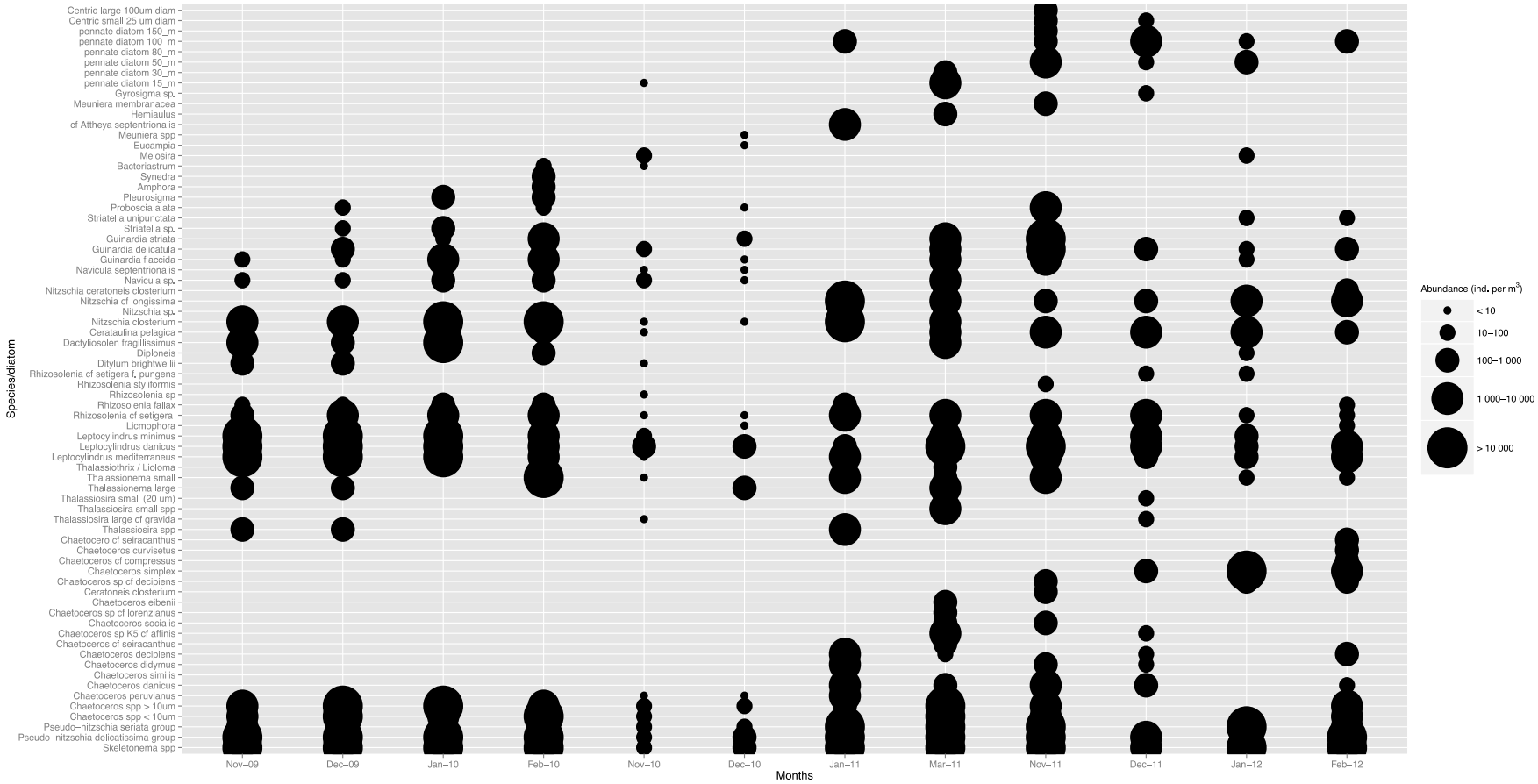


Fig. 3.27. Abundance of diatoms during the 3 summers (2009-1010, 2010-2011, 2011-2012) in Storm Bay. Total numbers of diatom species were the highest in summer of 2011/2012, followed by summer of 2010/2010 and summer of 2009/2010.

were massive numbers of *Pseudo-nitzshia delicatissima* followed by *Leptocylindricus mediterraneus*, *Chaetoceros* spp., *Skeletonema* spp., *Cerataulina pelagica*, *Dactyliosolen fragillissimus*, *Nitzschia closterium, Rhizosolenia cf setigera, Rhizosolenia fallax* and *Pseudo-nitzschia seriata*.

Table 3.6. Summary of thaliacean preferences and responses to different types of phytoplankton assemblages in Storm Bay over the three summers.

Thaliacean	**Phytoplankton preferences**	**Responses**
Dolioletta sp.	Flagellates, cryptophytes and prymnesiophytes	Disappear when diatom biomass >25%
Doliolum sp.	Diatom biomass up to 80%; Occur if flagellate biomass is low as long as *Phaeocystis* is present	Tolerate well the presence of diatoms with total biomass from 40-98%
T. democratica	90 - 95% flagellates	Bloom when the proportion of diatoms and dinoflagellates have a similar percentage to flagellates
S. fusiformis	High proportions of flagellates	Favour the condition of high percentage of flagellates over diatoms

In the wet summer of 2010/2011, salps were exceedingly rare though doliolids were common in early autumn. When *Dolioletta* sp. appeared in December 2010, few diatoms were observed in Storm Bay and only two species

(*Leptocylindricus danicus* and *Thalassionema* spp) appeared at the same sites where *Dolioletta* sp. was collected. As mentioned previously, *Doliolum* sp. was abundant at all sites on March 2011. At the same time there were 5 dominant diatom species present (*Skeletonema* spp., *Pseudo-nitzschia delicatissima*, *Pseudo-nitzschia seriata*, *Leptocylindrus danicus*, *Chaetoceros* spp. and *Rhizosolenia cf setigera*), as well as several other species that were present in large numbers: *Guinardia striata*, *Thalassiosira* small, *Guinardia delicatula*, *Navicula septentrionalis* and *Navicula* sp.

In early summer of 2011/2012, when SST were lower than in the previous two summers, there was only a small number of salps and *Dolioletta* sp. and four species of diatoms were quite abundant at this time. They were *Guinardia striata, Guinardia delicatula, Rhizosolenia cf setigera,* and *Cerataulina pelagica.* When *Chaetoceros simplex*, *Nitzschia cf longissima, Cerataulina pelagica* were highly abundant, there was an outbreak of salps in January. The bloom was dominated by *S. fusiformis* at site 2 in January, followed by quite high abundance of *T. democratica* in February; during that month *T. democratica* was more abundant than *S. fusiformis*.

3.4.4 Attribution of environmental impact

Principle components analysis was used to examine the relationships between the seven environmental variables and the sampling sites where thaliaceans were recorded (Fig. 3.28).). BEST analysis determines which suite of

environmental variables best explained the patterns seen in the distributions of the thaliaceans over the three summers.

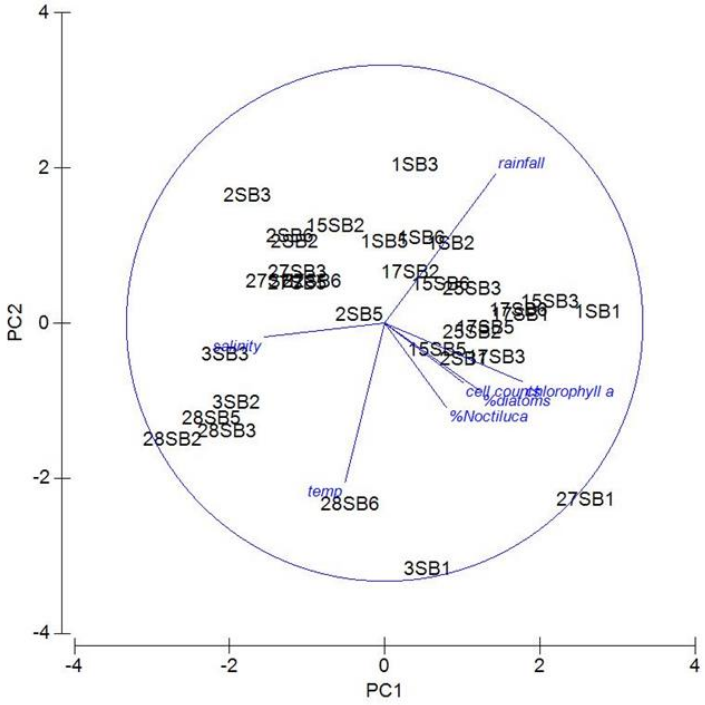


Fig. 3.28. Principle components analysis shows the relationship between the sampling sites and the environmental variables.

According to BEST analysis, of the seven environmental variables examined, the top three drivers of thaliacean abundance in Storm Bay were salinity, diatom concentrations and temperature, with a correlation of 0.433. To examine these relationshops further, multi-dimensional scaling (MDS) plots were used to show the relationships between the sampling sites and the three key environmental drivers. Salinity (Fig. 3.29) played a major role in driving thaliacean abundance during three summers in Storm Bay. Site 1 (shown as SB1 on Fig. 3.29) was where all *Dolioletta* sp., *Doliolum* sp., *T. democratica* and *S. fusiformis* occurred across the sites in summer of 2009/2010, while points 27SB and 28SB highlight the sites

in Storm Bay during the summer of 2011/2012 where *T.democratica* and *S.fusiformis* occurred in large numbers.

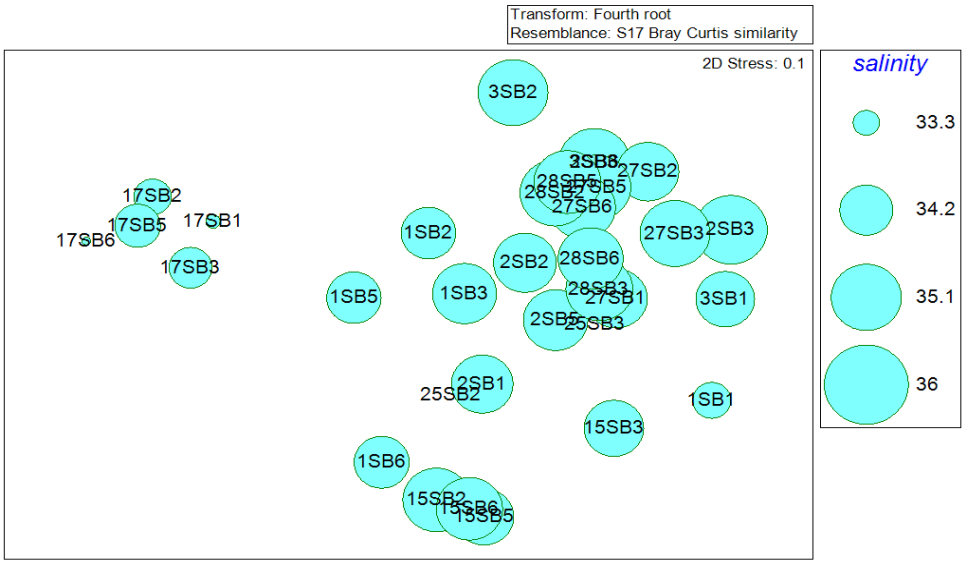


Fig. 3.29. The relationship between salinity and thaliaceans during three summers. Overall salinity influenced all sites in Storm Bay in consecutive summers.

Diatom stocks (Fig. 3.30) significantly influenced doliolids in the summer of 2010/2011; the sizes of the circles are shown according to the value of the percentages of diatom stocks. Point 15SB represents moderate abundances of *Dolioletta* sp., while all sites within point 17SB showed the largest numbers of *Doliolum* sp. Temperatures (Fig. 3.31) affected the abundance and distribution of thaliaceans during the three summers across all sites. The four remaining variables, rainfall, chlorophyll *a* concentration, total phytoplankton and *Noctiluca scintillans* concentrations, did not add significantly to the BEST correlation and are not shown here.

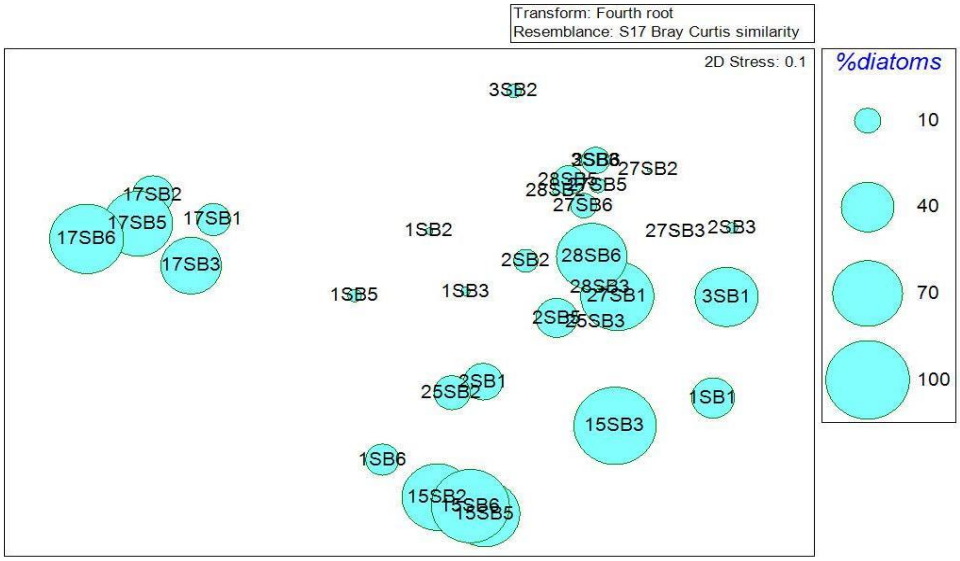


Fig. 3.30. The relationship between diatom concentration and thaliaceans during three summers. In particular, large numbers of diatoms occurred at sites 15SB and 17SB, which were sampled during the summer of 2010/2011, and coincided with large numbers of doliolids.

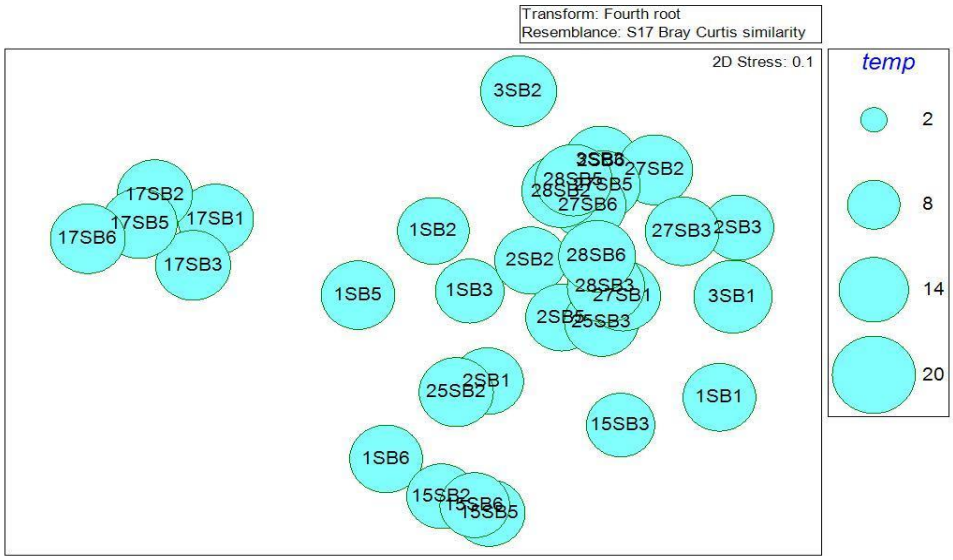


Fig. 3.31. The relationships between temperature and thaliaceans during three summers.

3.5 Discussion

There were three distinct patterns of thaliacean abundance throughout the three summers in Storm Bay. In the first summer season (2009/2010) there were small numbers of doliolids across the sites, from November until December. Later, when the waters were getting warmer, *T. democratica* bloomed at site 2. The next summer (2010-2011) was known as a 'wet summer' due to La Niña conditions that affected the whole of Tasmania and contributed to changes in rainfall patterns and lower temperatures (Bureau of Meterology 2012). Doliolids were common in early summer and salps were extremely rare all year. In the summer of 2011-2012 environmental conditions were different again; little warm water from the EAC penetrated as far south as Tasmania, with most of the flow leaving the separation point to head east into the Tasman Sea. There were no salps and doliolids in samples collected in December, though *S. fusiformis* were abundant in samples collected in January, particularly at site 2. Also, *T.democratica* abundance was dense in samples from coastal waters (sites 5 and 6). The abundance of *S. fusiformis* declined in February whereas *T.democratica* abundance remained high at coastal sites (J. Beard, personal observation 2012).

T. democratica always occurred in coastal waters at sites 5 and 6, in contrast to *S. fusiformis* that was always dominant at the more marine site, (site 3). This observation suggests that *T. democratica* was more tolerant of lower salinities than *S. fusiformis*.

Doliolids were more abundant in low temperature conditions and following rainfall events; this also suggests that they were influenced by low salinity

events. In summer 2010-2011, *Doliolum* sp. was common in coastal waters (site 1), whereas *Dolioletta* sp. appeared at sites other than site 1. It is known that *Doliolum* sp. has more a coastal distribution and develops intense swarms in zones influenced by freshwater inputs such as estuaries (Berner and Reid 1961).

The abundance and occurrence of salps and doliolids were not uniform in time or space. This is due to their distinct features in terms of morphology, feeding behaviour, reproduction, predators, as well as their life history traits. Iguchi and Kidokoro (2006) observed a high biomass of the thaliaceans *Tethys vagina* in the Japan Sea that was related to an area with high chlorophyll *a* concentration. According to Drits et al. (1992) and Menard et al. (1994), thaliaceans are found in dense populations during the phytoplankton spring bloom. Based on these observations, it was hypothesized that phytoplankton stocks can be considered as an important factor to determine the effects of environmental change on thaliaceans. However, in this study, we found that phytoplankton stocks were the factor that least contributed to thaliacean abundance.

Tew and Low (2005) proposed that the distribution patterns of *Doliolum denticulatum, T. democratica* and *Thalia orientalis* in Taiwanese coastal waters were related to reproduction, food availability, and hydrography, while Zhang et al. (2003) suggested that the abundance of thaliaceans in the eastern Taiwan Strait increased with increasing temperatures and phytoplankton concentrations. Similarly, Henschke et al. (2011) concluded that temperatures and phytoplankton stocks were both equally important in promoting blooms of *T.*

democratica. Moreover, seawater temperature is known to have a profound effect on salp distributions (Brandon et al. 2004), as their ability to reproduce rapidly by asexual reproduction can grow the population quickly.

The abundance and distribution of thaliaceans in Storm Bay throughout the three summers demonstrate several factors contributing to bloom development of salps and doliolids. Based on observations throughout three summers, a small number of environmental drivers has been identified. Although little is known on thaliacean abundance versus hydrographic variables over short periods of time except of a paper by Nakamura(1998), we could interprate that there might be unknown continuously ongoing circulation and hydrographic processes during the 4 week intervals between samplings that led to each of field data every 4 weeks in this study. In essence, the observed thaliacean abundance and distribution on a certain day would be the result of longer term primary productivity, and not exclusively of the food abundances found that particular day; as well as longer term circulation in the Bay.

Salinity a stronger influence than temperature

Although temperature was a significant driver of thaliacean distribution, as shown by BEST analsyis, this study suggested that salinity had a greater influence than temperature. *Dolioletta* sp. reached highest abundance in early summer 2009/2010 when the mean salinities were between 33.91 and 34.62, while *Doliolum* sp. were recorded in large number in late summer 2010/2011 when the mean salinity ranged from 32.95 to 33.83. In contrast, salps showed a preference

towards higher salinities than shown by the doliolids. Salinity was 34.34-34.40 when *T. democratica* bloomed in January 2010. Also *S. fusiformis* were found in large numbers when the salinities exceeded 34.57-35.11. Such salinity preferences are probably related to seasonal differences. Doliolids started to appear either during early summer (November) or late summer (March). In contrast, salps were found in the middle of summer (January).

Meanwhile salps showed preference for higher temperatures. The first salp bloom was observed during summer 2009-2010 (December and January). Though both species of salps, *T. democratica* and *S. fusiformis*, occurred during the same period, *T. democratica* was clearly dominant. *T.democratica* started to appear slowly at sites 2, 3 and 5 in November 2009 and at sites 1, 2, 5 and 6 in December 2009 when the temperature was between 13.42 and 15.31°C. The abundance of salps in November was, however, low and far less than doliolids at that time. Later there was a significant bloom of *T. democratica* in January 2010 at sites 2 and 3 when the temperature range was 15.85-17.4°C.

I conclude that the optimum temperature for *Dolioletta* sp. in this study ranged between 13.42 and 14.93 °C, while for *Doliolum* sp. the temperature range was very narrow (16.35-16.76 °C). Thompson (1948) suggested that *Doliolum denticulatum* has characteristics of a tropical and sub-tropical species as their numbers increased with warmer conditions. *Dolioletta gegenbauri*, however, showed a different preference for colder waters. Although summer starts in December, the SST at sites 2 and 3 in 2011/2012 reached its peak in March 2013 (coincident with the highest abundance of *Doliolum* sp.

Diatoms are a stronger influence than total phytoplankton concentrations

Total phytoplankton in this study encompassed diatoms, flagellates and dinoflagellates. *Doliolum* sp. were observed in largest abundance when the percentage of diatoms present in the phytoplankton exceeded 70%, while other thaliaceans appeared to be negatively influenced by large numbers of diatoms. In summer 2009/2010 I recorded the lowest number of diatom species of all the three summers. However, despite this lower diatom diversity, each thaliacean present occurred in abundance. In November 2009 when *Dolioletta* sp. occurred in high densities at sites 2, 5 and 6 (range: 477-564 m^{-3}) diatom abundance was extremely low (range: 335-41,475 L^{-1}), but in March 2011, when *Doliolum* sp. appeared in large numbers (138-262 m^{-3}), the number of diatoms increased to 13,320- 132,400 L^{-1}. Summer 2011/2012 recorded the highest diversity of diatom species in Storm Bay although their abundance was not high. *T. democratica* and *S. fusiformis* co-existed during this time.

Interestingly, salps and doliolids indicated preference towards different types of diatoms. When less dominant diatom species were prevalent in Storm Bay, such as *Guinardia flaccida, Guinardia striata, Guinardia delicatula,* and *Thalassionema* spp., doliolids were present. In contrast, salps blooms occurred when *Pseudo-nitzshia delicatissima, Pseudo-nitzschia seriata*, *Cerataulina pelagica*, *Rhizosolenia cf setigera, Rhizosolenia fallax*, *Leptocylindricus mediterraneus*, *Nitzschia closterium* and *Nitzschia cf longissima* were abundant.

Thaliaceans are known to occur regularly in nutrient-rich upwelled water, which is ideal for phytoplankton productivity (Deibel and Paffenhöfer 2009).

Furthermore, in the northern part of the Levantine Sea, the Japan Sea and the northern Arabian Sea, the abundance of most salps (*Thetys vagina, T. democratica, S. fusiformis, Thalia orientalis*) and doliolids (*Doliolum nationalis, Doliolum denticulatum, Doliolinetta intermedia, Doliolina muelleri, Doliolina krohni, Dolioletta gegenbauri*) were directly proportional to concentrations of chlorophyll *a* (Naqvi et al. 2002; Iguchi and Kidokoro 2006; Weikert and Godeaux 2008). In Storm Bay, chlorophyll *a* concentrations were used to highlight the appearance of the spring phytoplankton bloom. When *Dolioletta* sp. appeared in early summer 2009/2010, the chlorophyll *a* concentration value was between 0.33 and 0.61mg m^{-3}, while the chlorophyll *a* concentration was higher (0.5-1.0 m^{-3}) when *Doliolum* sp. were found in March 2011. Meanwhile in January 2010 *T. democratica* bloomed in January 2010, when the chlorophyll *a* concentration was only 0.34-0.42 m^{-3}. Two years later in January 2012, at the same site, the chlorophyll a concentration was 0.25-0.48 m^{-3} when a different species, *S. fusiformis*, bloomed.

During the wet summer of 2010/2011 the heterotrophic dinoflagellate *Noctiluca scintillans* increased substantially across Storm Bay. From November 2010 to January 2011, *N. scintillans* exceeded 50% of total phytoplankton biomass and salps and doliolids were exceedingly rare. According to Deibel and Lowen (2012), limitation and proliferation of phytoplankton could both affect salp and doliolid abundance, whether by promoting their numbers or causing their fairly sudden decreases. In this case, the presence of *N. scintillans* was coincidence with the decreased number of salps and doliolids in Storm Bay.

Being filter feeders, thaliaceans employ a filtering net made of mucus secreted by the endostyle. They naturally feed on particles primarily in proportion to their availability in the water column; i.e. they are believed to be indiscrimate feeders). The mechanical capacity of the filters to retain and ingest particles is also important.

Some species of thaliaceans are adapted to feeding on ambient concentrations of particulate material. However, their unregulated filtration rate does not work well under higher concentrations of particulate material. When a high particle concentrations are encountered, a bolus is formed that blocks the esophagus, dramatically decreasing the amount of material that enters the stomach. This bolus is formed at particle concentrations that are greater than 5.0 ppm in the salp *Pegea confoederata* (Harbison et al. 1986). Sometimes these blockages can be fatal (Alldredge and Madin 1982), and this may also occur in doliolids.

Under favourable conditions, when there is an adequate food supply but volume is not high enough to cause clogging, salp biomass can increase exponentially since they have some of the highest growth rates and filtration rates amongst thaliaceans (Alldredge and Madin 1982; Dubischar and Bathmann 1997; Perissonoto and Pakhomov 1997). In Storm Bay, *Dolioletta* sp., *Doliolum* sp., *T. democratica* and *S. fusiformis* occured in high abundance at all sites from November 2009 to January 2010 when the phytoplankton stocks ranged from 2.0 X 10^5– 6.0 X 10^5 cell L^{-1}. However, when the concentrations reached higher than 6.0 X 10^5- 1.3 X 10^6 cells L^{-1} there were no thaliaceans observed at all.

Flagellates comprised a high proportion of phytoplankton stocks when thaliaceans were present. The most common types of flagellates recorded were flagellates 3-10 µm 'round', flagellates 3-10 µm 'fusiform', prymnesiophytes, prasinophytes and cryptophytes 5 -1 0 µm. *Dolioletta* sp. occurred in high abundance when percentages of total flagellates were greater than diatoms and dinoflagellates. *Doliolum* sp. were recorded in large numbers across all the sites in March 2011 when diatoms and dinoflagellates were more abundant than flagellates. At this time, when only *Doliolum* sp. appeared in Storm Bay, *Phaeocystis* (flagellates) was also recorded across the sites. When *T. democratica* bloomed in January 2010, diatoms and flagellates recorded at sites 2 and 3 were of almost equal percentage. The only times when the percentages of diatoms were almost the same as flagellates were during the blooms of *T. democratica.*

There is a recurring abundance of both salp species at all 5 stations from January to February 2012 which is unique in this oceanographic data set (Fig. 3.17). A look at Fig.3.26 reveals dominance of flagellates on 15 Dec 2011, and their dominance on 4 of the 5 stations on 19 January 2012. Despite their dominance the relatively low abundance of those flagellates could have contributed to those salps' persistence (no clogging of their guts) which would have carried into February 2012.

3.6 Conclusions

Due to scarcity of long-term data of thaliaceans in Australian waters, very little is known about their distribution let alone the features that trigger their blooms.

Realizing the importance of this often overlooked gelatinous creatures, a study on thaliaceans in three consecutive summers (2009-2012) was carried out in Storm Bay, Tasmania, to examine the factors that determine their distribution and abundance.

The most significant drivers in structuring thaliaceans assemblages in Storm Bay were: salinity, followed by the presence of diatoms, and seawater temperatures. Other factors, including local rainfall events, chlorophyll *a* concentration, concentration of *Noctiluca scintillans* and abundance of phytoplankton stocks, were not shown to be statistically significant but probably had localised influence.

Doliolids preferred lower salinity conditions than salps. *Dolioletta* sp. was present when the phytoplankton concentration was high, and it preferred the lowest temperatures of all four species. Most of the thaliaceans in Storm Bay preferred groups of flagellates with size ranges between 3 and 10 µm (based on those findings of SEM observations in chapter 4 and through identification and counting of phytoplankton in laboratory) and they disappeared when diatom biomass was greater than 25%. Meanwhile *Doliolum* sp. occurred in high abundance when salinity was the lowest of all three summers. Unlike other thaliaceans, they could tolerate a high percentage of diatom biomass and also cope with high *Noctiluca scintillans* concentrations. *T. democratica* had a higher optimal temperature than *S. fusiformis*, but they could also tolerate a wider range of temperature than *S. fusiformis*. The food preferences of the two species of salps appeared similar. In fact when both species occurred together at one

time (e.g. January and February 2012), there were record numbers of unicellular phytoplankton at all sites.

Results obtained in this study provide further evidence of the impacts of environmental changes on spatial and temporal patterns of thaliaceans in Tasmania ecosystems. Different weather conditions, increased precipitation, warmer SST and variable salinity lead to variability in both phytoplankton stocks and thaliacean presence and abundance. More frequent periods of increased salinities and SST could result in an increased frequency of salps blooms, while heavy rainfall and increased concentrations of diatoms could contribute to the occurrence of doliolids, ultimately causing fundamental changes to the Storm Bay food web.

CHAPTER 4

SEM OBSERVATION AND HPLC ANALYSIS OF THE GUT CONTENTS OF SOLITARIES AND AGGREGATES OF *Thalia democratica* and *Salpa fusiformis*

4.1 Highlights

- Images of 31 species of phytoplankton obtained from salp' guts were captured with Scanning Electron Microscopy.
- The size range of food particles found in solitary stages of *T. democratica* and *S. fusiformis* were similar: 10 µm – 1400 µm and 8 µm – 1400 µm, respectively.
- A smaller range size of food particles was found in aggregates: 2µm – 250 µm in *T. democratica* and 5µm – 100 µm for *S. fusiformis*.
- The pigments identified suggested that the gut content of the salps contained fucoxanthin, alloxanthin and astaxanthin. This confirmed that the salps had predominately fed on diatoms, cryptophytes and green algae.
- Partially-digested copepods were present only in solitary adults of both species. However, astaxanthin, a main carotenoid in copepods was identified in aggregates of *S. fusiformis.*

4.2 Introduction

Understanding the dietary habits of salps is a key to studies of food web structure in the Storm Bay ecosystem. In this chapter SEM and HPLC analyses were carried out only on salps. There were limited chances for collecting fresh thaliacean samples and they proved to be extremely fragile, so sample numbers were limited. SEM analyses were eventually successful after two initial trials. The first two experiments on preparation for SEM analysis were done using preserved thaliaceans (salps and doliolids) collected in each of the three summers. From the results of these unsuccessful experiments, I concluded that SEM analysis should only be done on freshly collected samples of thaliaceans. It is because, nothing could be done to reinvigorate those specimens from other summers that had been preserved with formaldehyde. Fresh samples for SEM and HPLC were only collected in the summer of 2013/2014. Because doliolids were absent in the samples of summer 2013/2014, only salps (*T. democratica* and *S. fusiformis*) were analyzed for this chapter.

Determining the diet of most marine invertebrates by gut content analysis is rather complicated (Blankenship and Yayanos 2005) and salps are even more complicated. SEM analysis has been used widely in determining the gut contents of invertebrates and fish (Van Dover et al. 1988; Harris et al. 1991; Gallimany et al. 2009; Vannier 2012) as has HPLC (Robinson et al. 1989; Bandaranayake and Des Rocher 1999; Majdi et al. 2011). SEM analyses has been used to determine the morphological condition in the gut contents of a shrimp (*Rimicaris exoculata*) (Van Dover et al. 1988), the gut lining, associated microflora and the nature of

the ingested food of thalassinid prawns, krill (*Euphausia pacifica*) (Bargu et al. 2002), *Euphausia superba* (Meyer et al. 2002), *Euphausia superba* (Nishino and Kawamura 1994), and copepods (Wu et al. 2004). (*Upogebia africana and Callianassa kraussi*) (Harris et al. 1991). SEM studies have also been used to monitor contaminants in marine ecosystems. For example, SEM discovered fiberglass ingestion by *Mytilus galloprovincialis* (Gallimany et al. 2009). Only two studies have reported SEM of gut contents of salps: *Ihlea racovitzai* and *Salpa thompsoni* (Harbou et al. 2011) and *Cyclosalpa bakeri* (a large salp, 10-100 mm in overall length; Madin and Purcell 1992). Harbou et al. (2011) investigated the feeding dynamics of *I. racovitzai* and *S. thompsoni* during different seasons and revealed the presence of *Fragilariopsis* spp., *Pseudo-nitzschia* spp., fragments of *Corethron inerme*, spines of large *Chaetoceros* spp., *Protocystis* spp., the silicoflagellate *Dictyocha,* as well as other algal fragments, faecal pellets and non-definable matter. The study by Madin and Purcell (1992) also found large centric diatoms (*Thalassiosira* spp.) and some other unidentified cells in the gut of *Cyclosalpa bakeri*.

The SEM analysis in the present study was augmented by the use of HPLC, which is capable of detecting signature pigments even where food particles have been physically and chemically altered. Mackas and Bohrer (1976) first used phytoplanktonic pigments as natural markers to study phyto-zooplankton trophic relationships. The chromatographic technique is known for its discriminating power that provides a simultaneous separation of chlorophylls and carotenoid pigments. This technique has become a valuable tool in studies of trophic

relationships between phytoplankton and zooplankton, and can be used to identify dominant food sources selected by herbivores (Quiblier et al. 1994). By identifying and quantifying their biomarker pigments, gut pigment analyses can provide information on feeding selectivity among various microphytic taxa (Majdi et al. 2012). HPLC analyses have been applied to examine the pigments and lipid stores in some tissues, digestive glands and gut contents of the sea cucumber *Holothuria atra*, scallop *Placopecten magellanicus* and the nematodes *Chromadorina bioculata* and *Chromadorina viridis* (Majdi et al. 2011). Previous studies of thaliaceans revealed several pigments in the faeces of *Cyclosalpa bakeri*. These included clorophyll *a* and its derivatives, chlorophyll *c* and phaeophytin *a* and derivatives (Madin and Purcell 1992).

The combination of these two methods enables the analysis of the correlation between the morphological evidence and the synthesized food particles in the salps guts. Furthermore, HPLC analysis has been shown to detect small phytoplankton taxa that are generally underestimated or overlooked using microscopy (Ansotegui et al. 2001). Both techniques are a promising approach to examining food consumed by marine invertebrates. The observation and analysis of food particles in the guts of salps is a fundamental step in understanding the biology and ecology of thaliaceans in the Storm Bay ecosystem.

4.3 Materials and Methods

4.3.1 Scanning Electron Microscopy

4.3.1.1 Sample collection and preparation

Individuals of solitary adults (oozoids) and aggregates (blastozooids) of *T.democratica* and *S. fusiformis* were caught with a Bongo net, fitted with a closed cod-end, at sites 1, 2 and 3 in Storm Bay during summer 2013/2014. Samples were preserved in ethanol within a few hours of collection and prepared using polylysine and also filtering procedures. The guts of the thaliaceans were removed using a pair of very fine forceps. They were then washed 5 times with Milli-Q to make sure no salt was attached. The intestines were then crushed using a scalpel and mounted needle and then pipetted into a centrifuge tube before they were centrifuged for 10-20 minutes for better mixing.

A droplet of polylysine was placed onto marked, EtOH-cleaned coverslips using a syringe (0.2µm), covering the cover slip to within 1-2 mm of the edges. Polylysine was used in this study because it can be used effectively to bind particulates and nanoplankton from intestine samples onto coverslips for SEM. This technique consistently results in better-preserved organisms that are less concealed by detritus than samples dried directly onto glass coverslips (Mazia et al. 1975; Marchant and Thomas 2011).

The intestine samples were then left for 5 minutes to allow the polylysine to hydrostatically attract the specimens to the coverslips. After 5 minutes, the polylysine was discarded into a waste beaker and excess polylysine was washed

off by gently dipping the coverslip into a beaker of distilled water 5 times with no sidewards motion. The excess water was then dried off with filter paper wedges placed gently between forceps and the paper was slid along the margin of the coverslip. Finally, the coverslip was placed, with polylysine side up, in a petri dish lined with filter paper. In a fume hood, a large drop of a gut sample was placed on each coverslip. After that, one drop of OsO_4 (4%) was added to each petri dish and covered. Then the samples were left in a fume hood, covered, for 30 minutes.

Once the holder was loaded with coverslips, it was placed in 30% EtOH/Acetone for 10 minutes. The solvent level was high enough to cover the samples but low enough to allow the holder screw to clear the liquid. Then after 10-20 minutes the holder was transferred to 50% EtOH/acetone for a further 10-20 minutes. This procedure was repeated through the EtOH/acetone series (give % of treatments here), repeating the 100% DRY EtOH stage twice.

4.3.1.2 Stub preparation and mounting

A double-sided adhesive dot was placed centrally on an SEM stub that had been marked with an identification code. Coverslips were quickly removed from the immersion bath and placed centrally onto the sticky dot on the stub, with the sample side up. Once sputter-coated with gold, the sample was then viewed under field emission scanning electron microscope, a JEOL JSM-6701F with a Gatan Alto 2500 cryo chamber.

4.3.2 Salp gut extracts for HPLC

In the laboratory, the guts of several salps were separated from their bodies and the weight of the combined guts was recorded. The guts were ground with a small amount of 100% acetone using a glass mortar and pestle, settled in ice. The ground guts and acetone were transferred to a 10 mL centrifuge tube and sonicated in an ice-water bath for 15 minutes in the dark. The samples were then kept in the dark at 4 °C for approximately 15 hours. After this time 200 µL water was added to the samples, which were sonicated once more in an ice-water bath for 15 minutes. The extracts were transferred to a clean centrifuge tube and centrifuged to separate the gut material. The final extract was filtered through a 0.2 µm membrane filter (Whatman, anatope) prior to analysis by HPLC using a Waters - Alliance high performance liquid chromatography system. This system comprised a 2695XE separation module with column heater and refrigerated autosampler and a 2996 photo-diode array detector. HPLC was carried out using a C8 column and binary gradient system with an elevated column temperature, following a modified version of the Van Heukelem and Thomas (2001) method. Pigments were identified by retention time and absorption spectra from a photo-diode array (PDA) detector and concentrations of pigments were determined from commercial and international standards (Sigma; DHI, Denmark).

4.4 Results

4.4.1 SEM

Although the gut of *S. fusiformis* is slightly larger than that of *T. democratica* in both stages, both species had a similar gut morphology; a slender, elongated V-shape as according to Fredriksson et al. (1988). The guts of both stages, however, were different colours when they were freshly collected; purple-blue for *T. democratica* and brown-green in *S. fusiformis*.

Images of 31 different phytoplankton species from the guts of thaliaceans were captured (Figs. 4.1 - 4.21). The images consisted of *Chrysochromulina* sp., *Pyramimonas* sp., *Navicula* sp., *Pseudo-nitzchia* sp., *Phaeocystis* sp., *Prorocentrum* minimum, *Leptocylindrus mediterraneus, Rhizosolenia* sp., *Cocconeis* sp., *Protoperidinium* sp., *Ceratium* sp., *Emiliania huxleyi*, Malacanosma m*inidiscus, Pyramimonas grossi*, *Cylindrotheca* sp., *Cylindrotheca closterium, Scrippsiella* sp., *Corethron* sp., *Cocconeis* sp. 2, *Thalassionema* sp., *Fragilariopsis* sp., *Thalassiosira* sp., *Cocconeis* sp. 3, *Proboscia* sp., *Nitzschia panduriformis* and 5 unidentified taxa, as well as a large portion of a copepod's body. Diatoms were the major group found, followed by dinoflagellates.

4.4.1.1 Species observed in the guts of solitary *T. democratica*

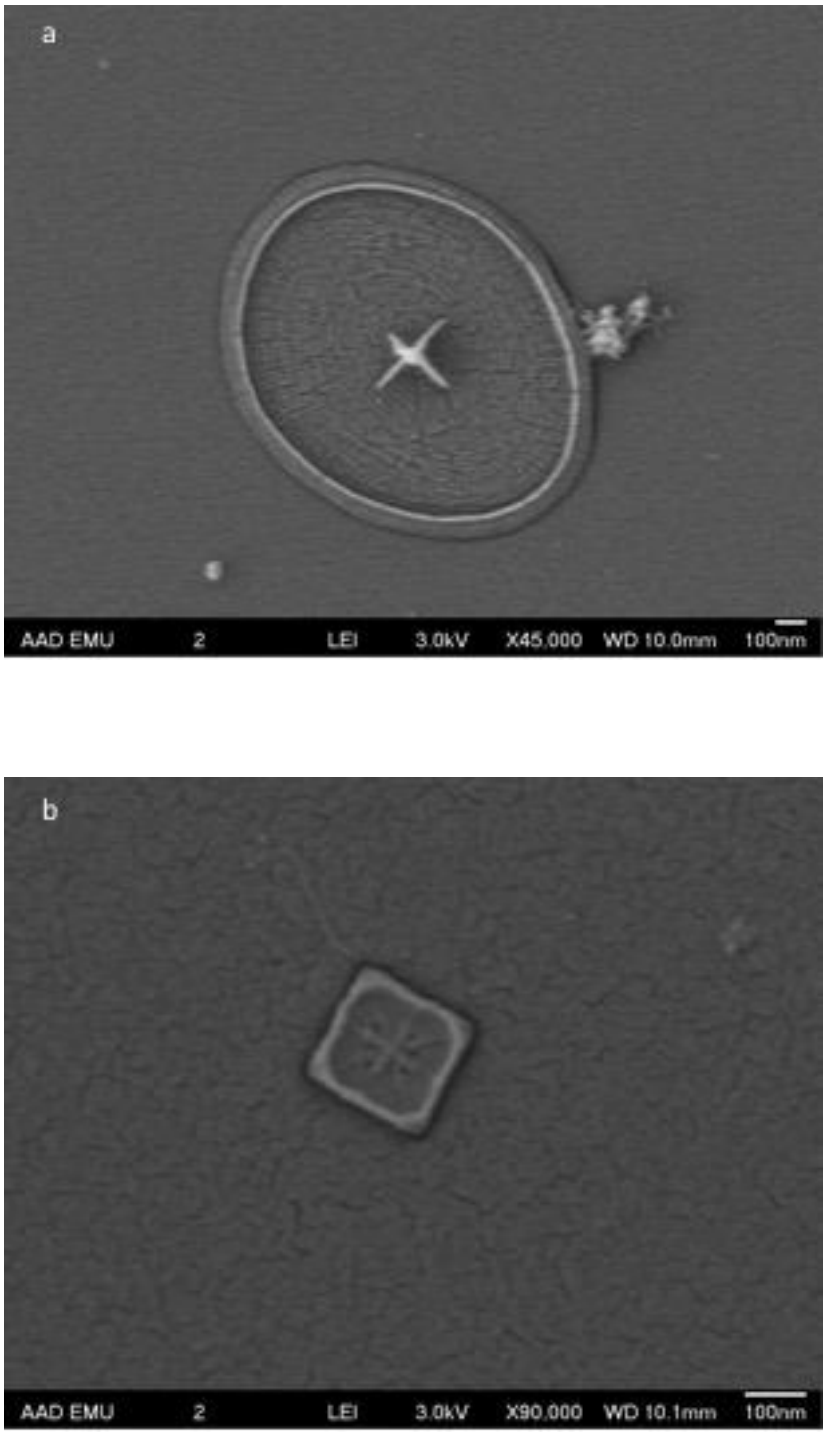


Fig. 4.1. a) *Chrysochromulina* sp. (Prymnesiales), b) Free nanoflagellate scale; box scale from *Pyramimonas* sp. (Prasinophytes, Order Chlorodendales).

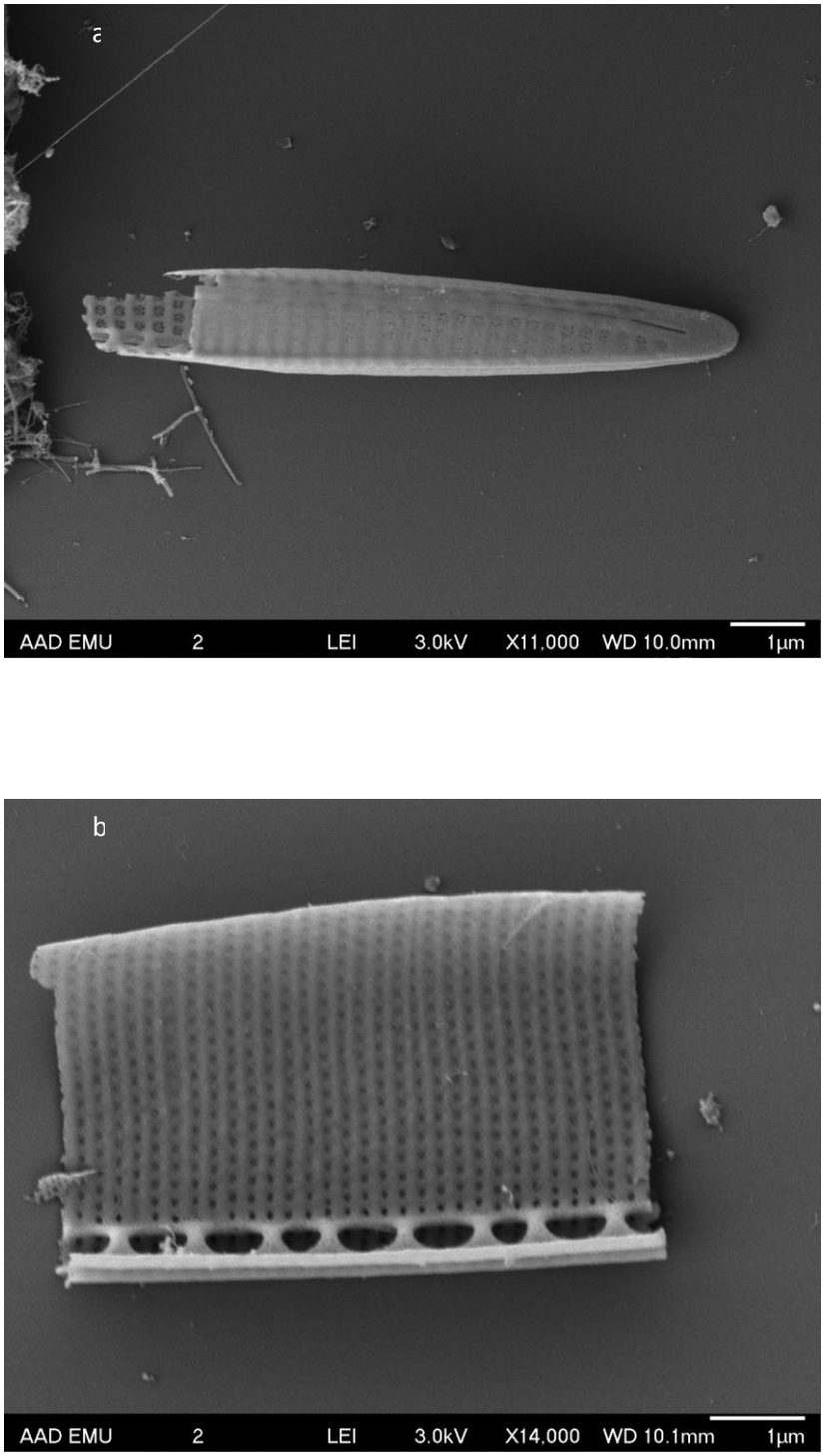


Fig. 4.2. a) *Navicula* sp. (Diatom) b) *Pseudo-nitzchia* sp. (Diatom)

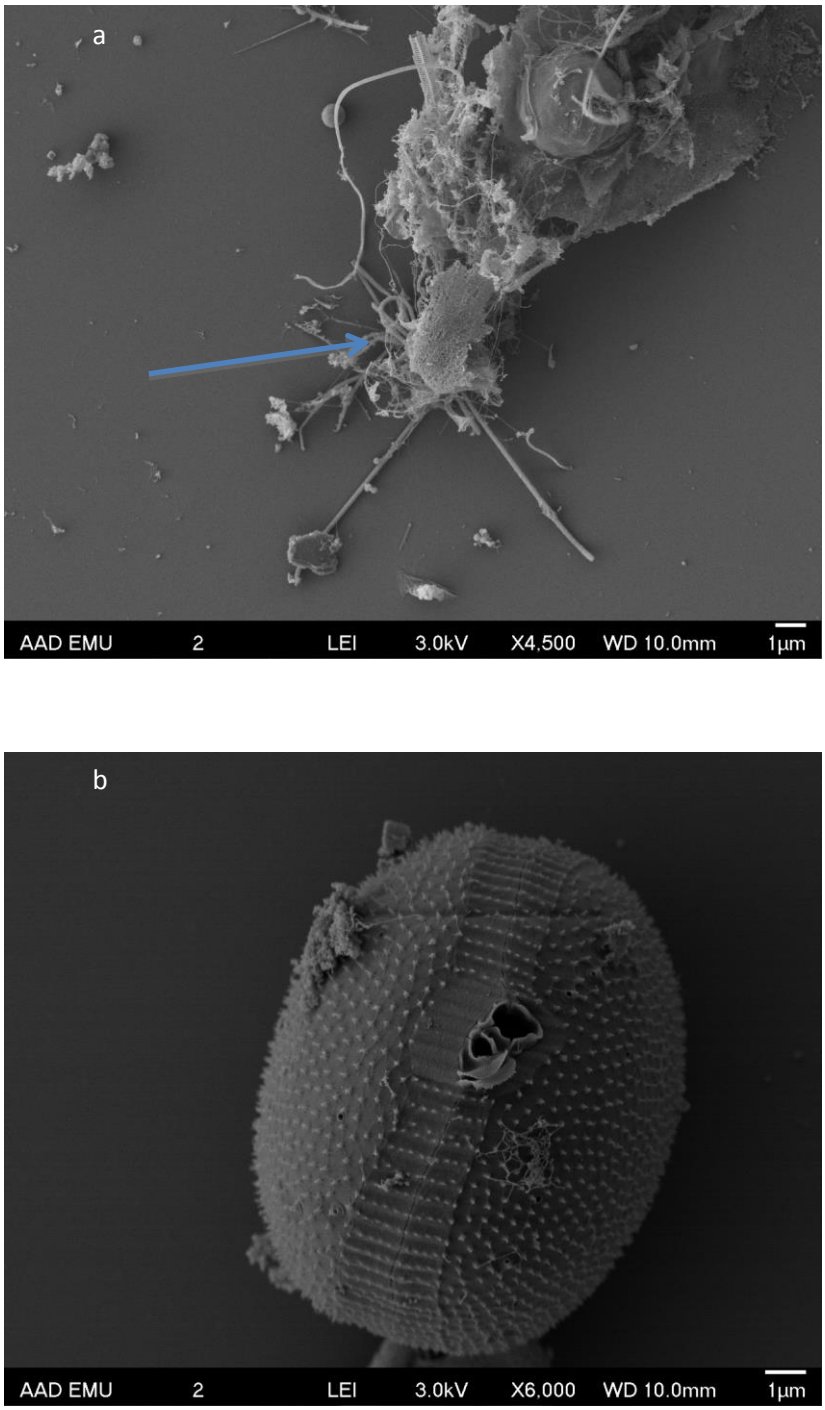


Fig. 4.3. a) *Phaeocystis* sp. (Prymnesiales) b) *Prorocentrum minimum* (Dinoflagellate).

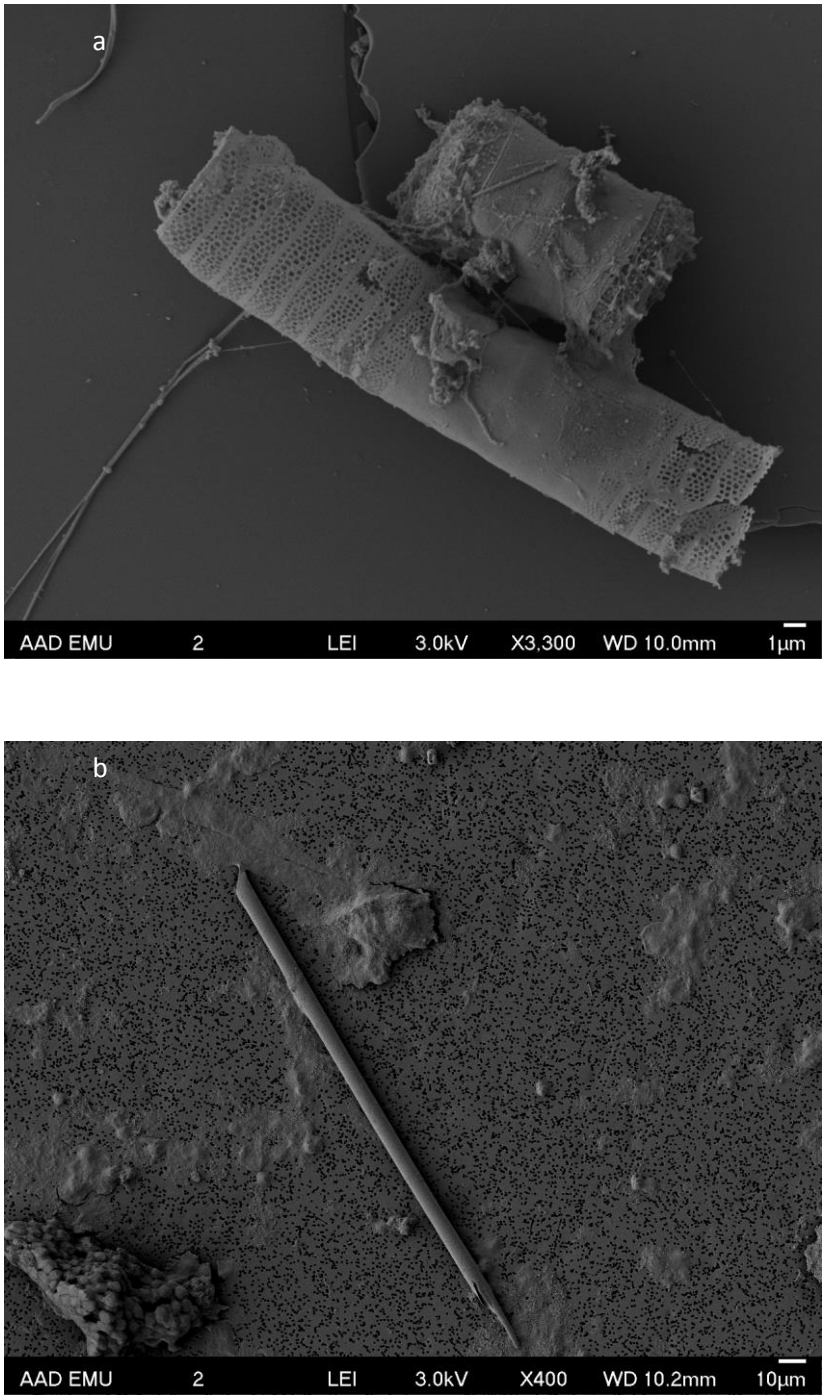


Fig. 4.4. a) *Leptocylindrus mediterraneus* (Diatom) b) *Rhizosolenia* sp. (Diatom).

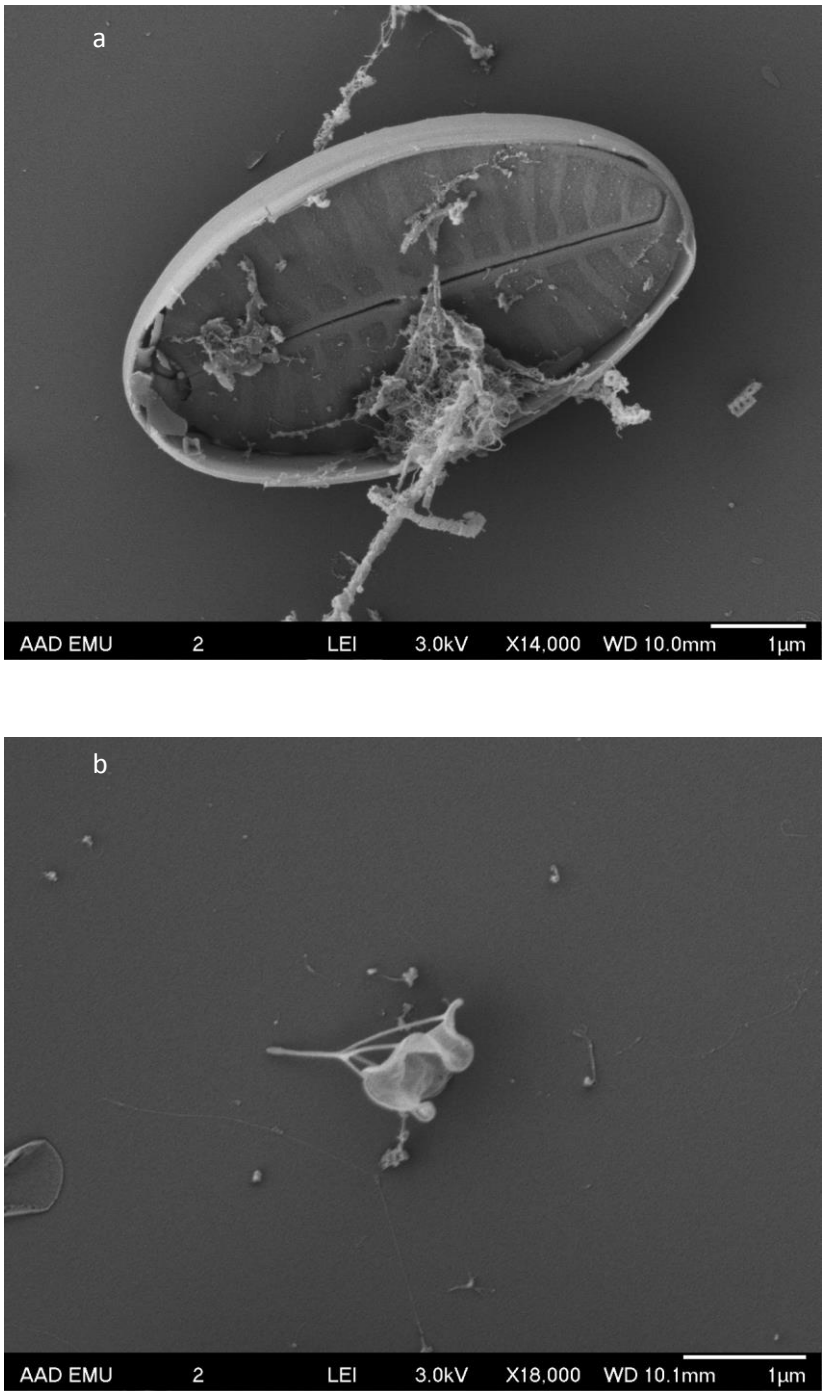


Fig. 4.5. a) *Cocconeis* sp. (Diatom) b) unidentified species 1.

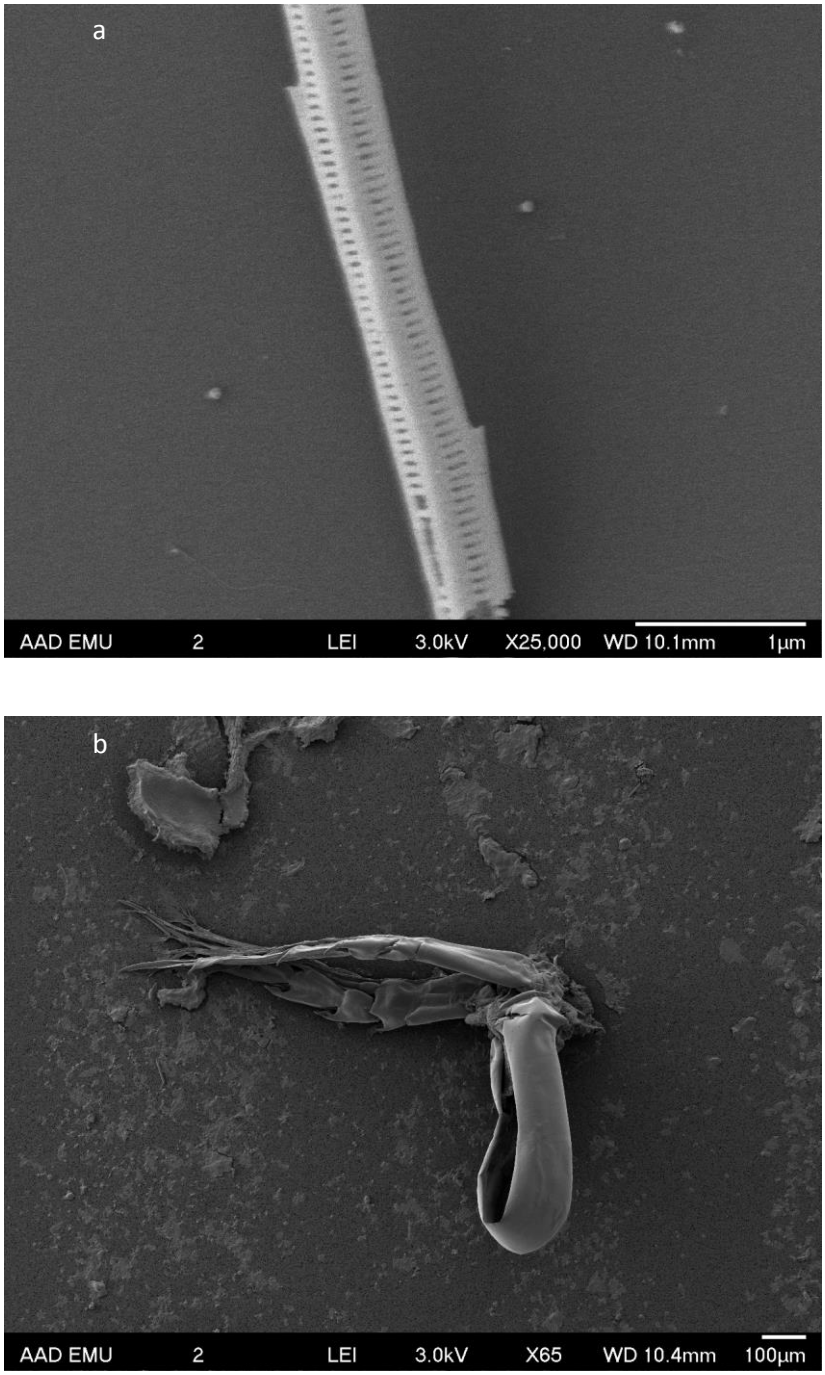


Fig. 4.6. a) unidentified species 2 b) Copepod: A pair of swimming leg.

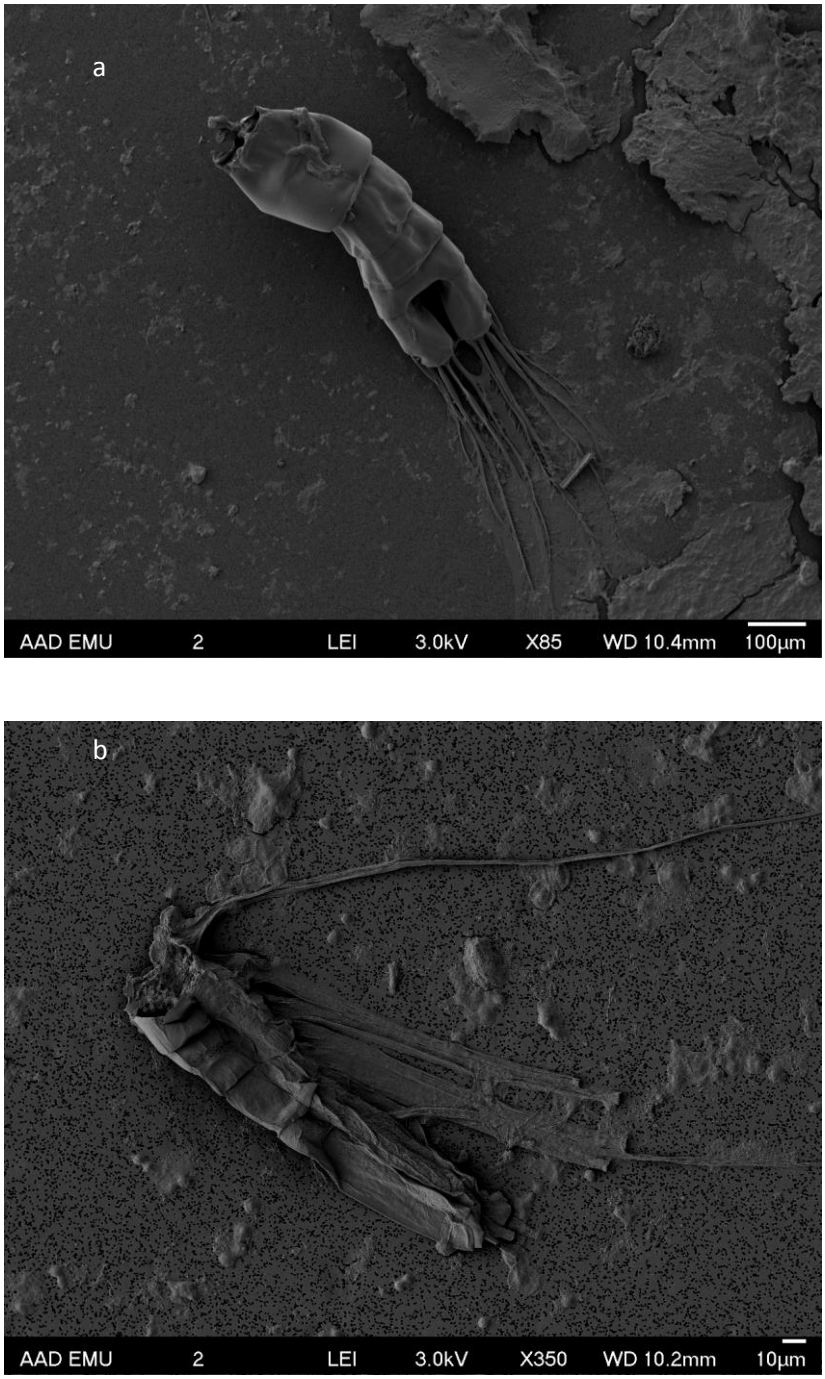


Fig. 4.7. a) Copepod: Caudal ramus and caudal setae. b) Copepod: Antennule.

4.4.1.2 Species observed in the guts of aggregate *T. democratica*

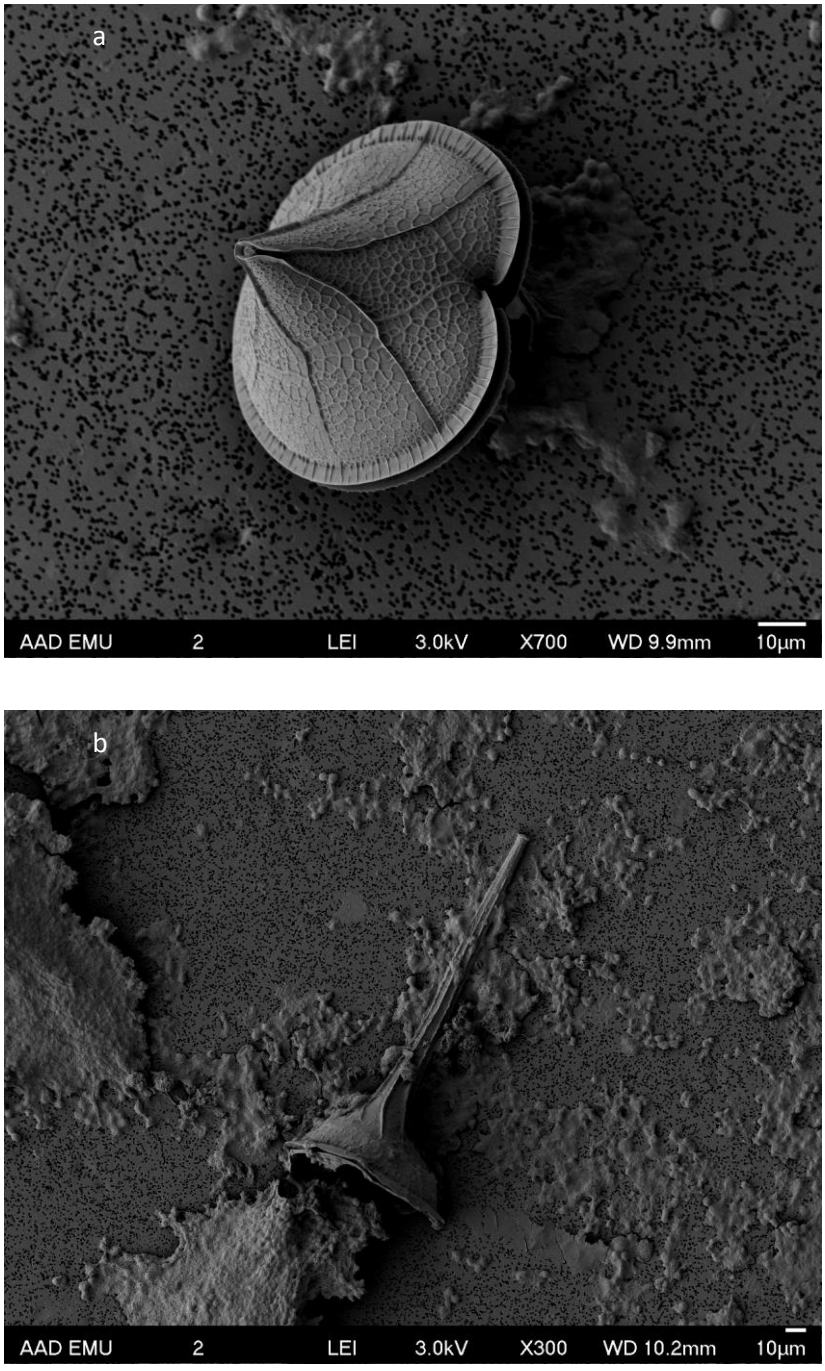


Fig. 4.8. a) *Protoperidinium* sp. (Dinoflagellate) b) Apical horn of *Ceratium* species (Dinophyceae).

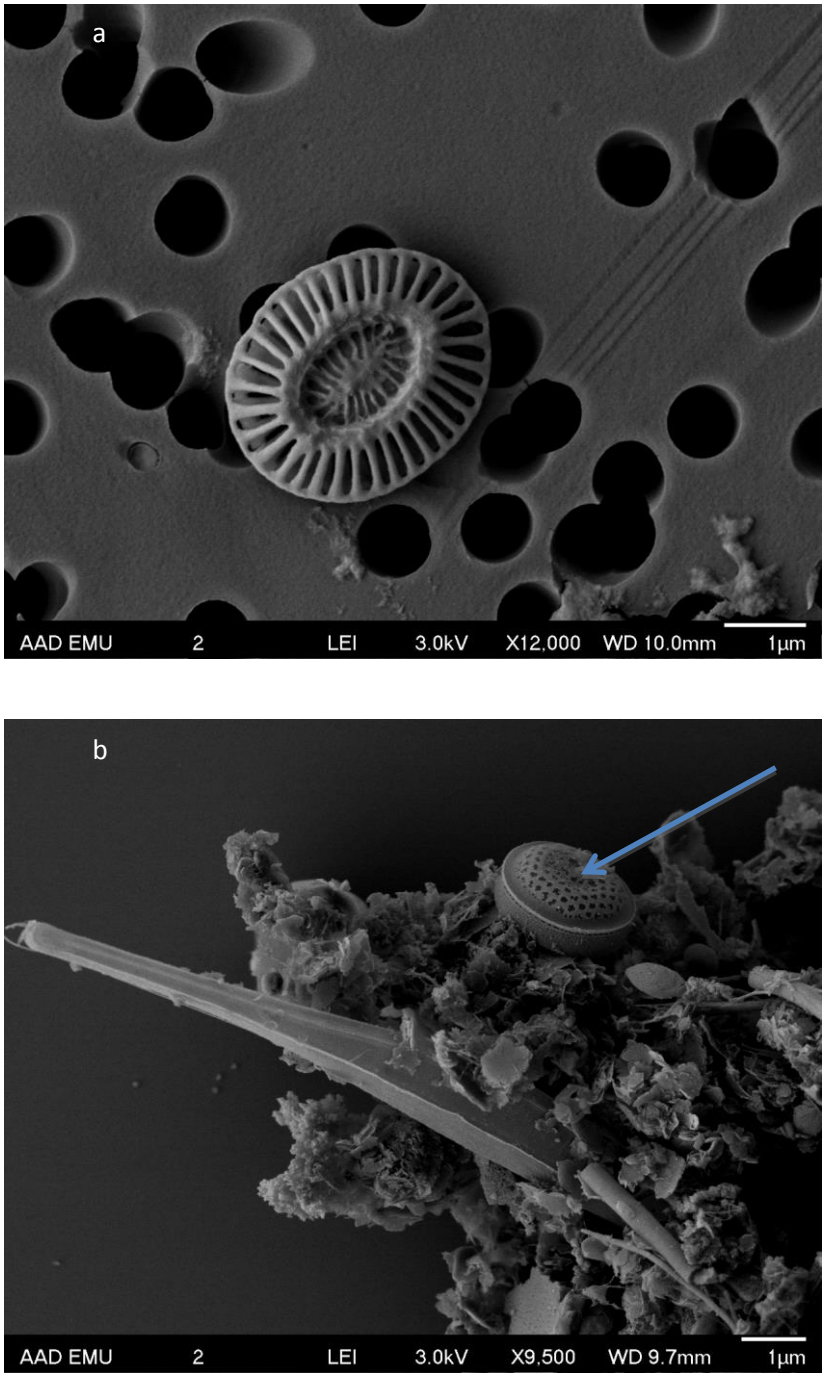
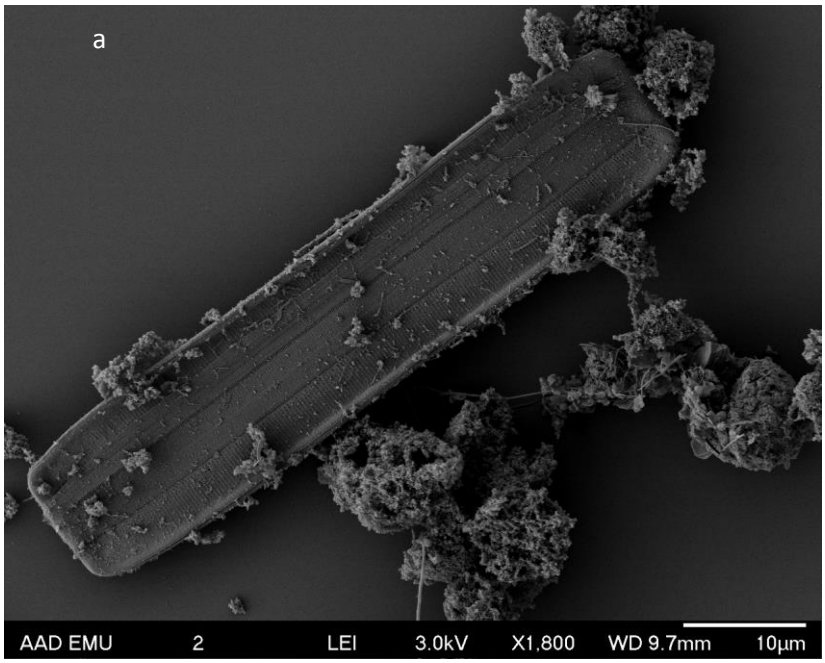


Fig. 4.9. a) *Emiliania huxleyi* (order Coccolithoporales) (Haptophytes) b) *Malacanosma minidiscus* (Diatom).



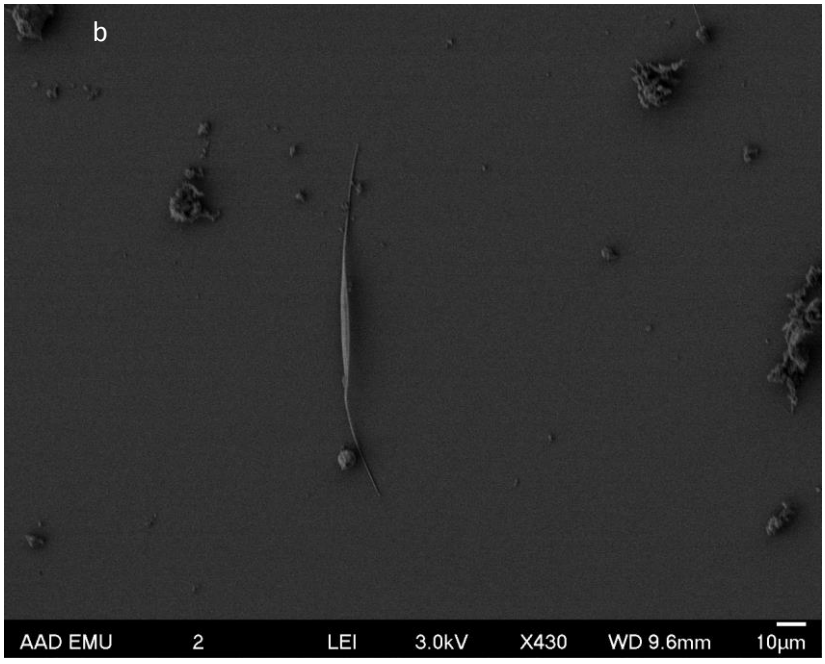


Fig. 4.10. a) unidentified species 3 b) *Cylindrotheca* sp. (Diatom).

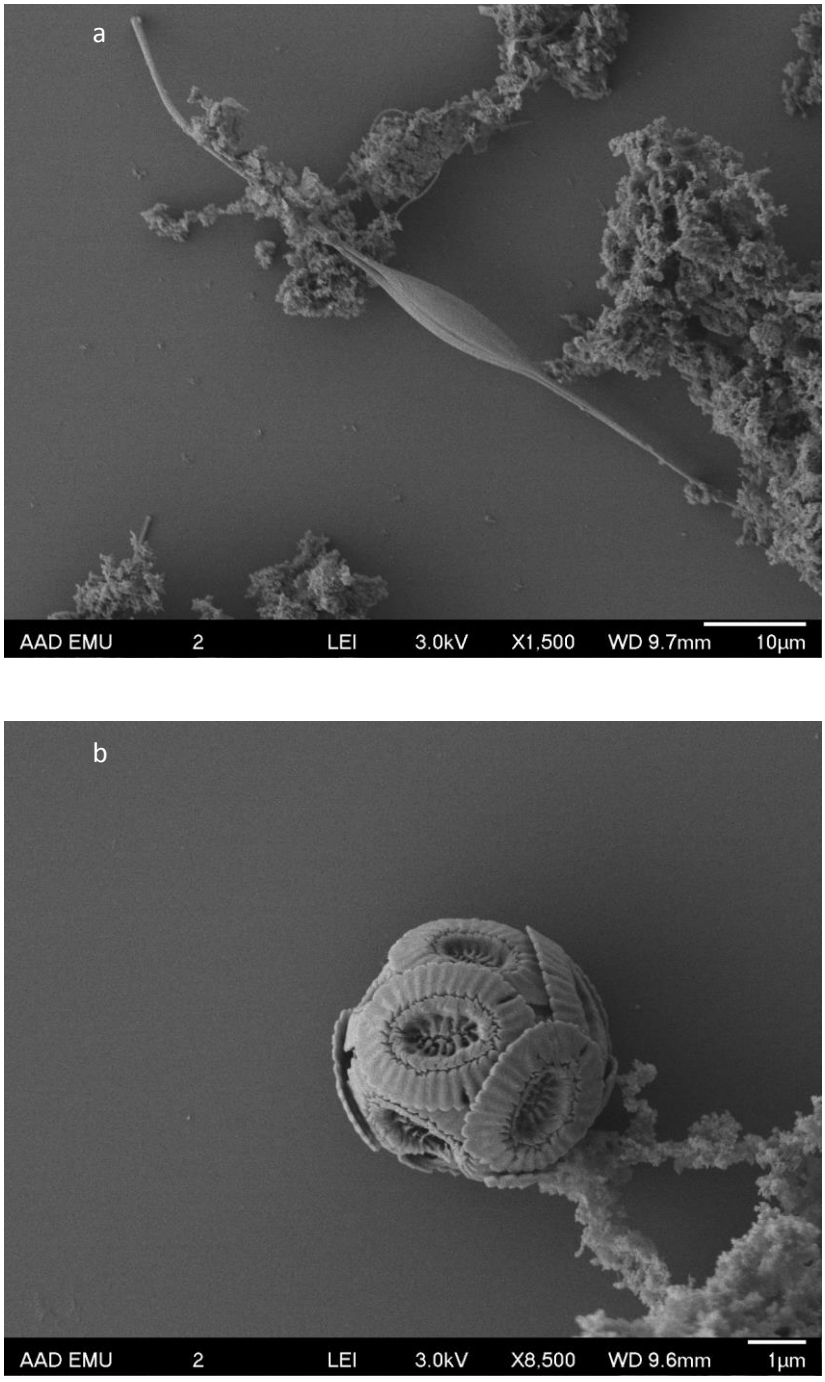


Fig. 4.11. a) *Cylindrotheca closterium* (Diatom) b) *Emiliania* sp. overcalcified type A.

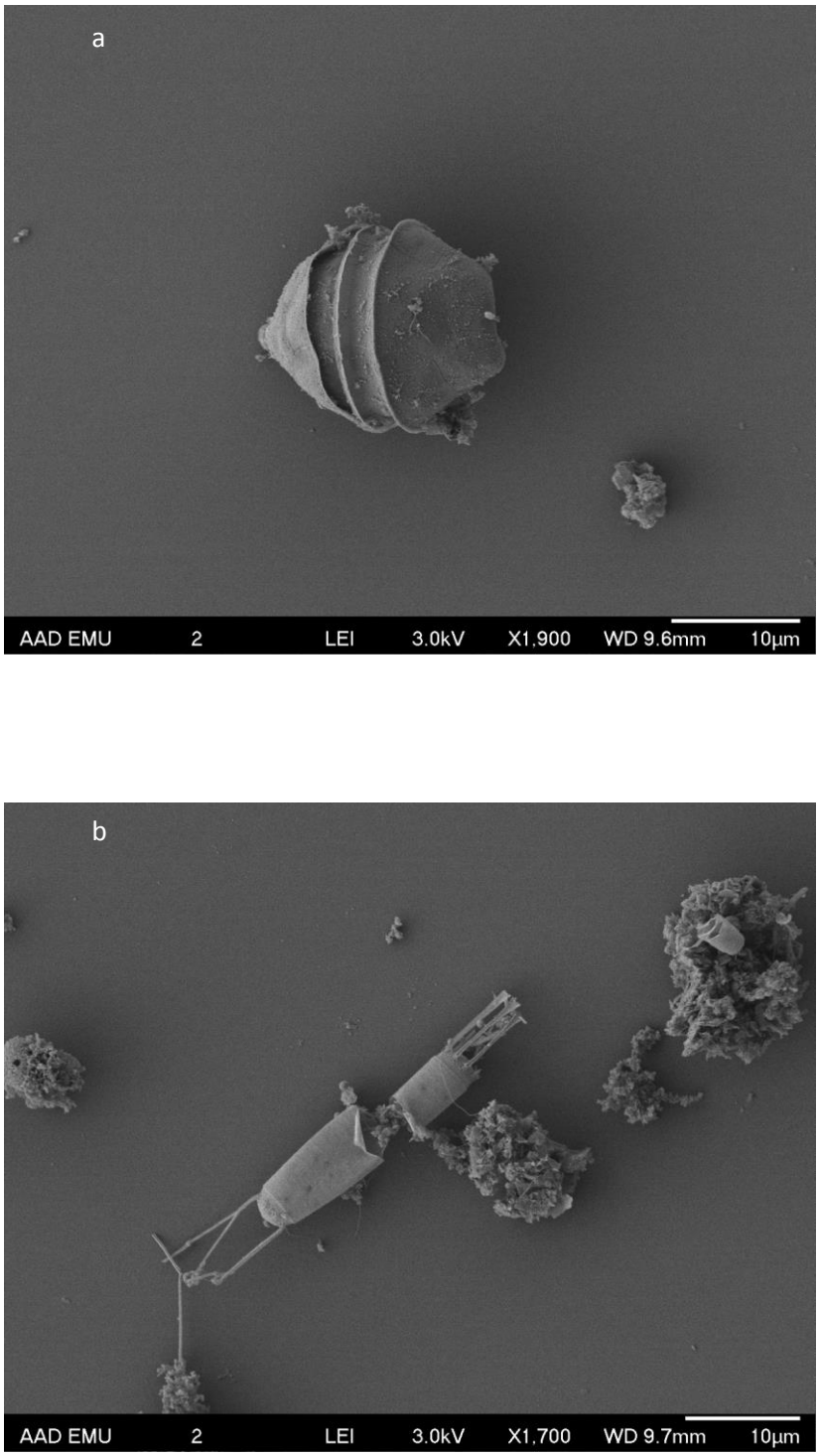


Fig. 4.12. a) *Scripsiella* sp. (Dinoflagellate) b) *Corethron* sp. (Diatom).

4.4.1.3 Species observed in the guts of soliary *S. fusiformis*

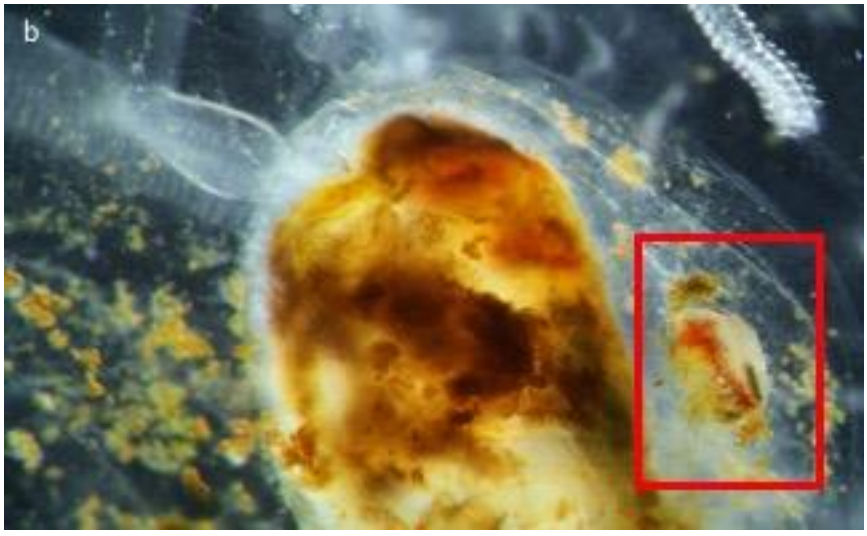


Fig. 4.13. a) Copepod; b) An undigested copepod found in the gut (light microscopy).

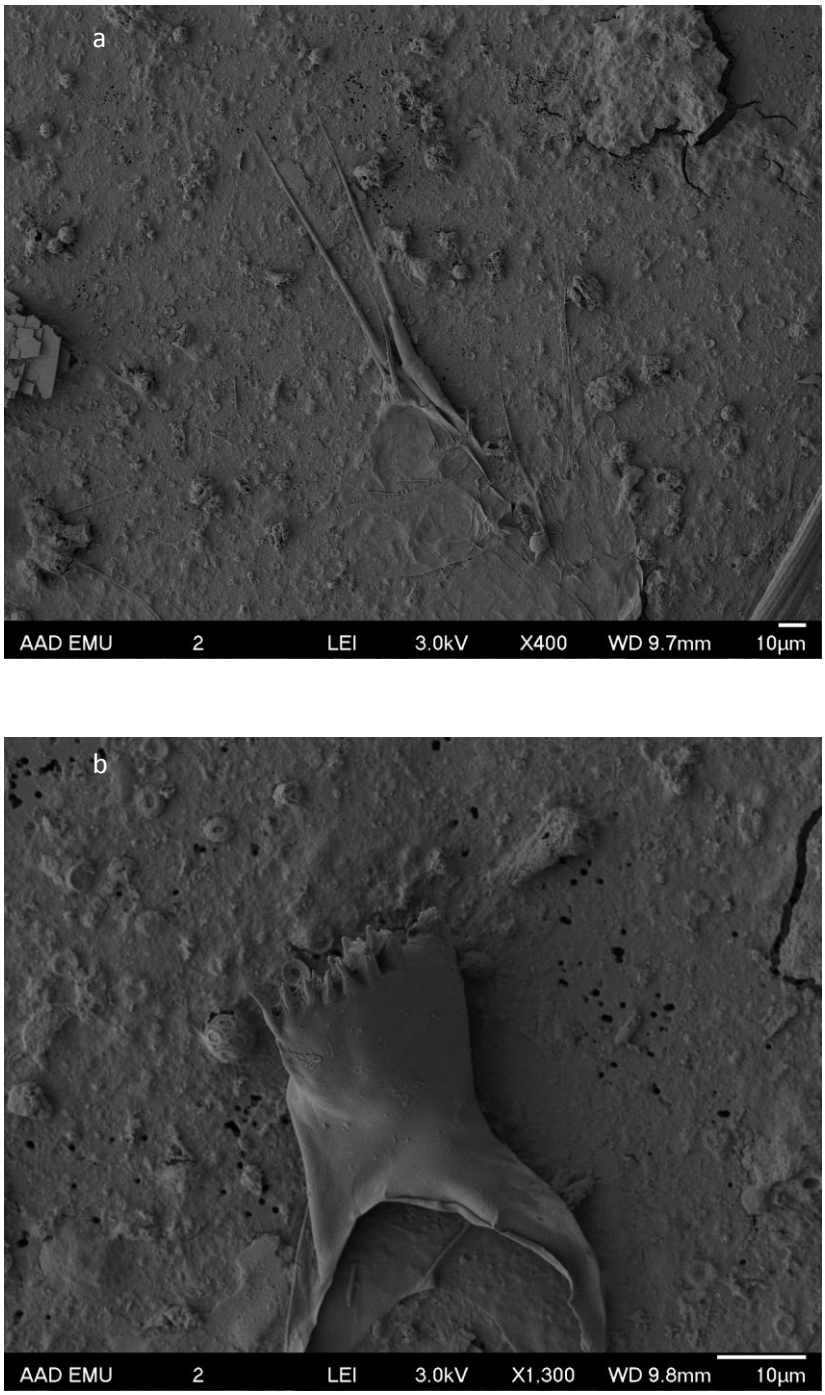


Fig. 4.14. Copepods: a) Caudal ramus and caudal setae b) Mandible (mouthpart).

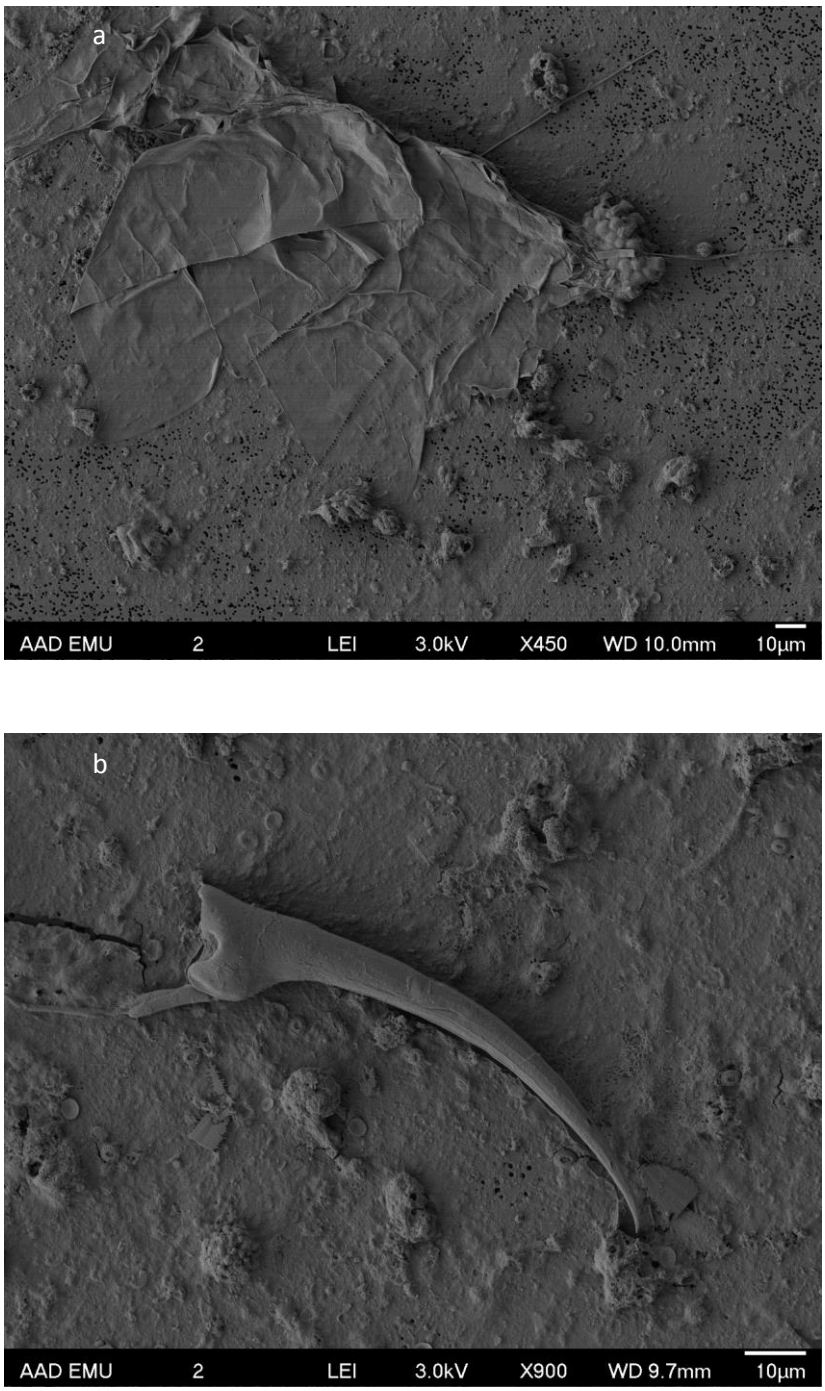


Fig. 4.15. Copepods: a) Metasome with egg sac b) Claw of exopod of leg 1.

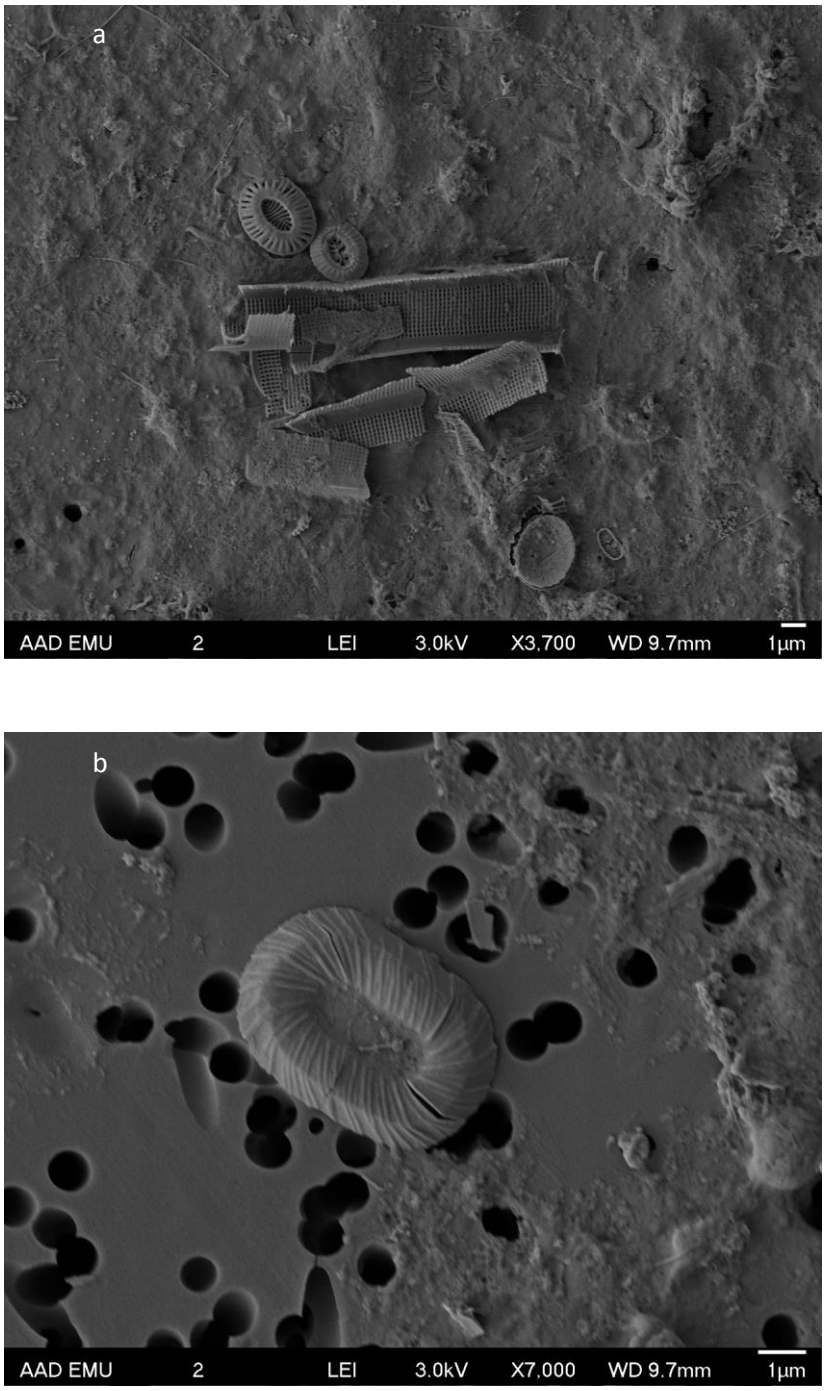


Fig. 4.16. a) unidentified species 4. b) *Cocconeis* sp. 2 (Diatom).

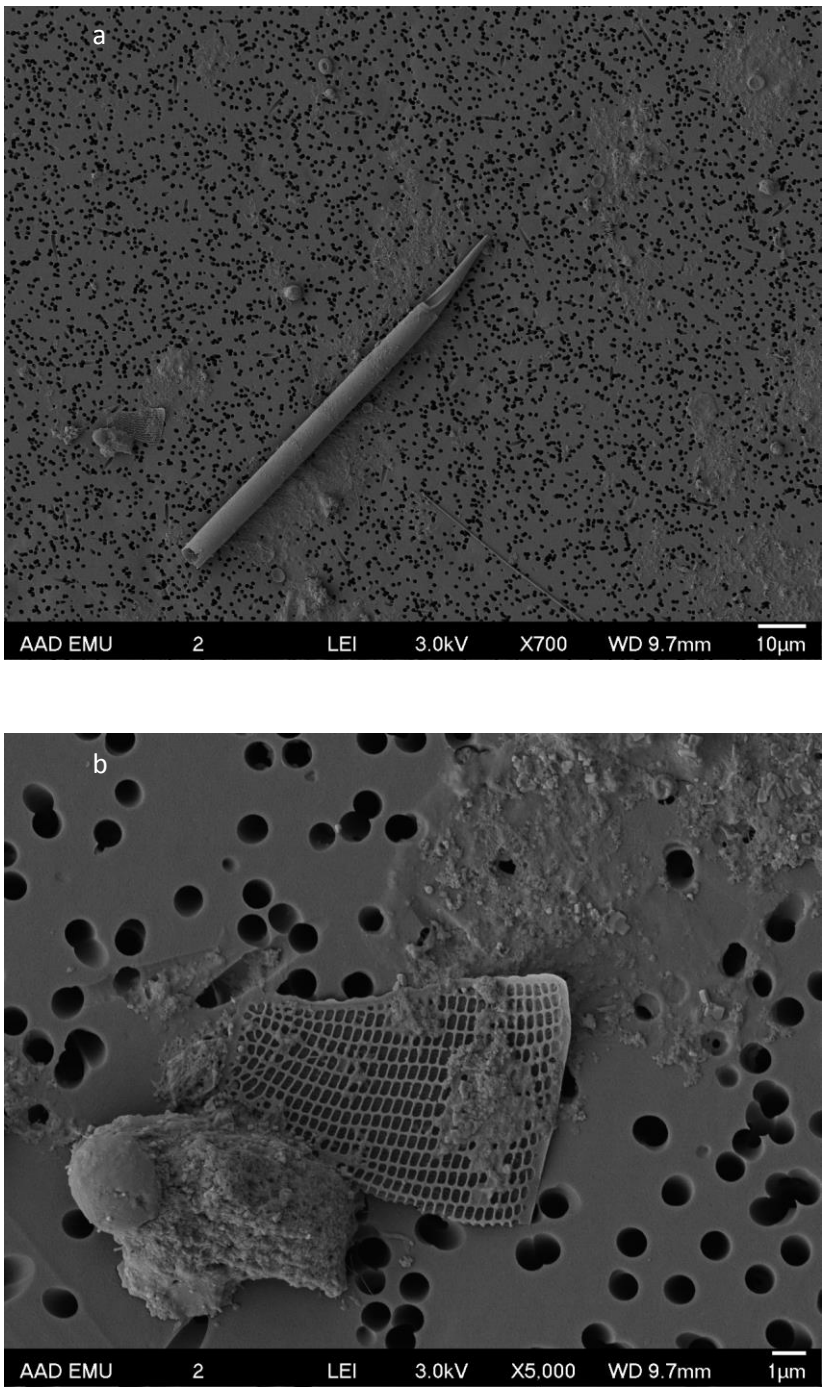


Fig. 4.17. a) unidentified species 5 b) *Rhizosolenia* band (Diatom).

4.4.1.4 Species observed in the guts of aggregate *S. fusiformis*

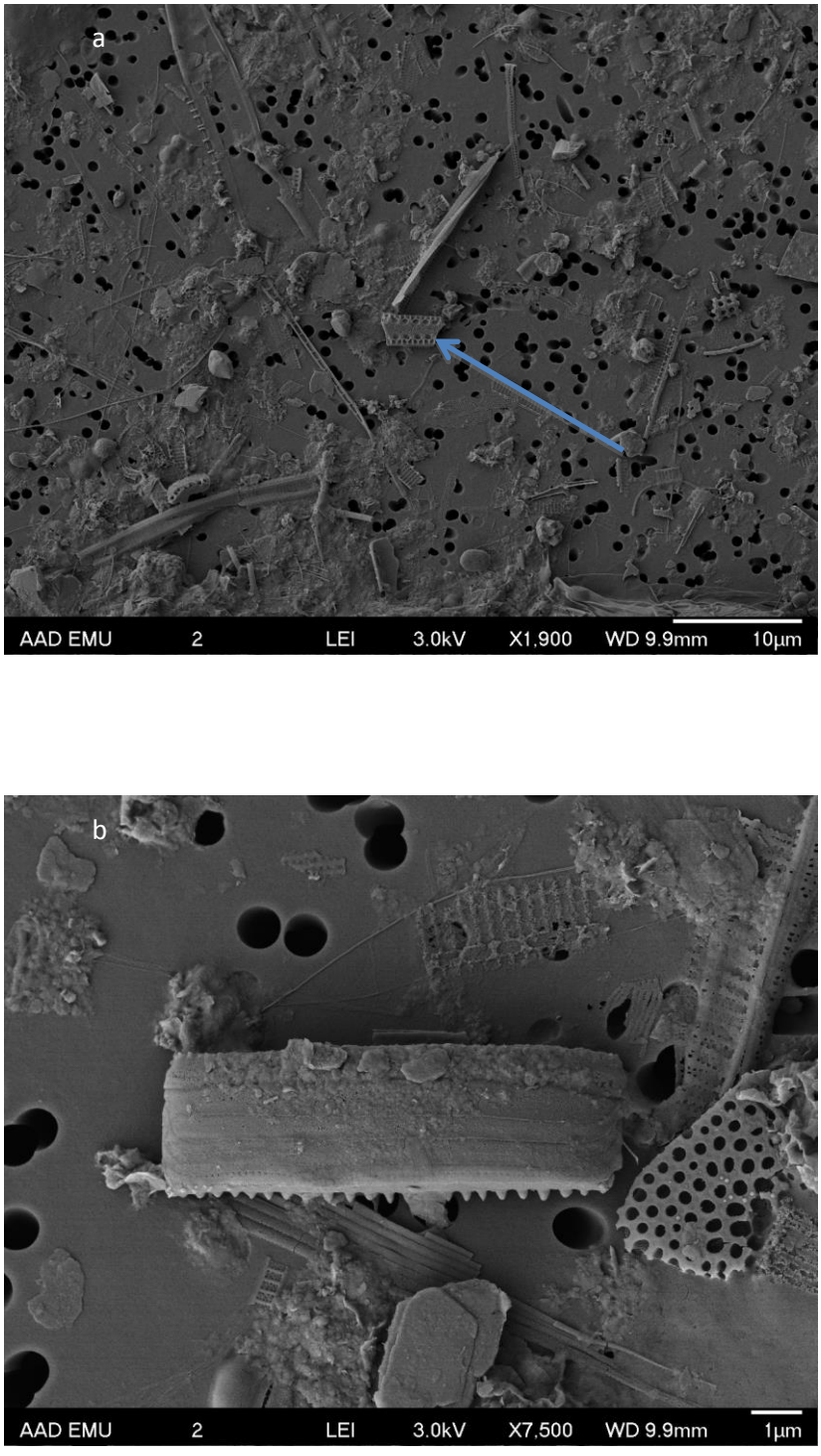


Fig. 4.18. a) *Thalassionema* sp. (Diatom) b) *Fragilariopsis* sp. (Diatom).

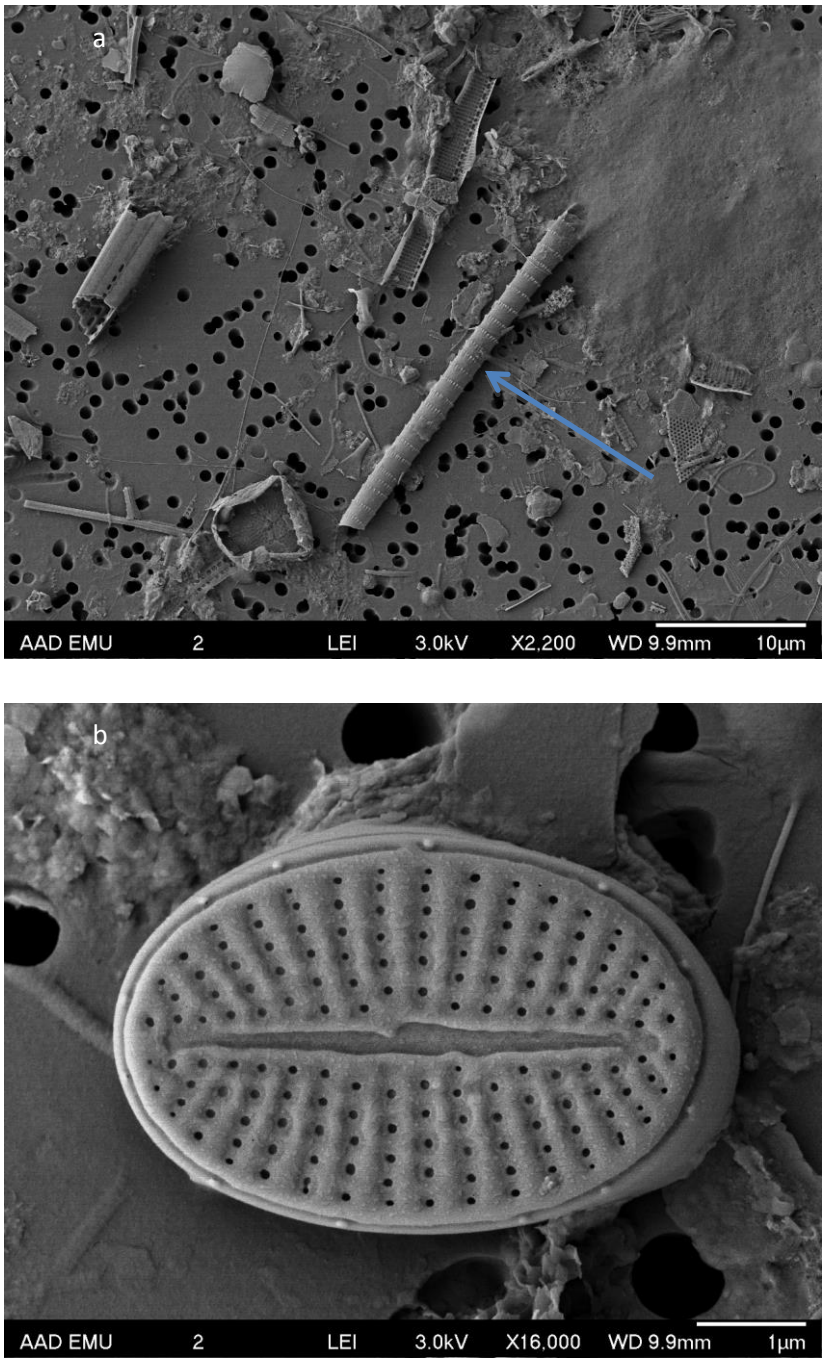


Fig. 4.19. a) *Thalassiosira* sp. (Diatom) b) *Cocconeis* sp. 3 (Diatom).

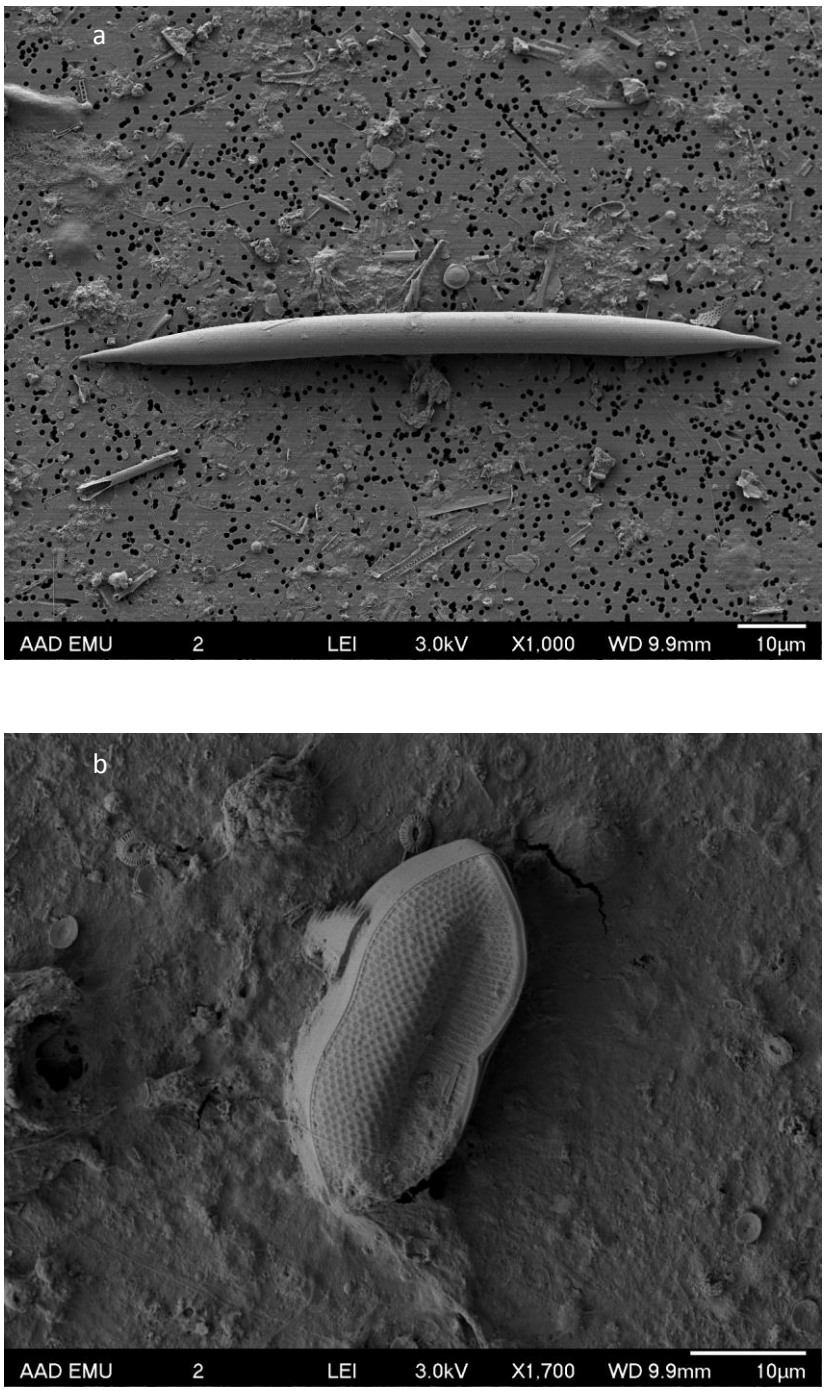


Fig. 4.20. a) *Proboscia* sp. (Diatom). b) *Nitzshia panduriformis* (Diatom).

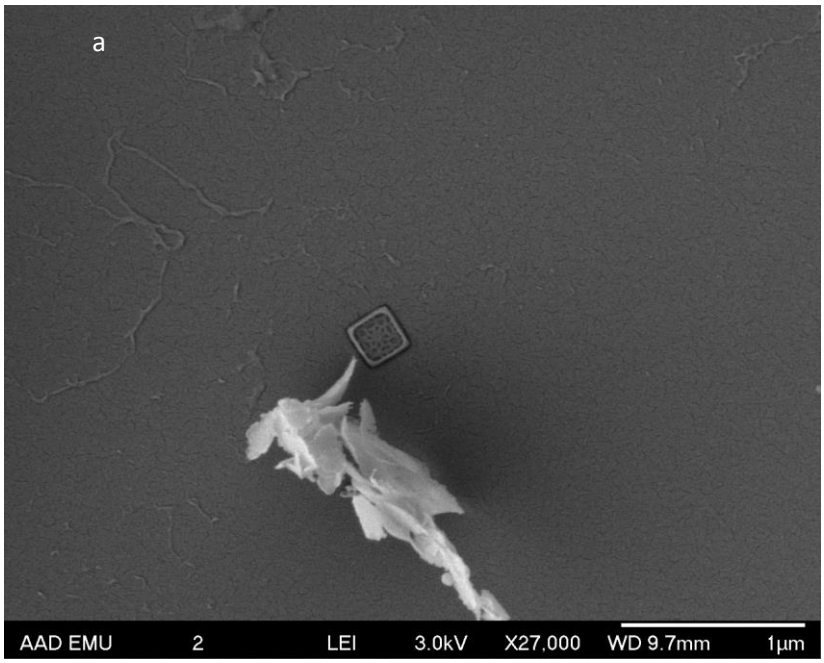


Fig. 4.21. Another box scale of *Pyramimonas grossi* box (Prasinophytes, Order Chlorodendales)

4.4.2 HPLC

The chromatograms of the salp guts were quite cluttered, with many peaks that were not identifiable. This could possibly be due to the gelatinous body material from the specimens being inadvertently added to the extraction process. However, there were several peaks that were identifiable and representative chromatograms for each species and stage are shown in Figs. 4.22-4.25. Descriptions of the pigments observed in this study, and examples of groups where they dominate were shown in Table 4.1.

Table 4.1. Descriptions of the pigments observed in this study, and examples of groups where they dominate.

Pigments observed in this study	**Descriptions**
Fucoxanthin	A yellowish-brown carotenoid pigment called fucoxanthin is confined in the phytoplankton to diatoms, prymnesiophytes, raphidophytes and chrysophytes (Jeffrey and Vesk 1981).
Chlorophyll *a*	Data of Chl *a* decay products would provide a direct biomarker for grazing activity on diatoms, one of the most important trophic pathways in estuarine, coastal, and open ocean food webs (Chen et al. 2003).
Zeaxanthin and Chlorophyll *b*	Zeaxanthin and chlorophyll *b* are typically found in green algae (chlorophytes, prasinophytes), prochlorophytes (Ansotegui et al. 2001; Roy 2009) and cyanobacteria (Roy 2009).
Alloxanthin	Alloxanthin is an indicator of cryptomonads and cryptophytes (Kowzlowski et al. 1995; Roy 2009)
Diatoxanthin	Diatoxanthin could be an indicator for diatoms, dinoflagellates, prymnesiophytes and chrysophytes (Roy 2009).
Astaxanthin	Astaxanthin is the main carotenoid in marine crustaceans the pigment responsible for the pink colour of copepods (Mauchline 2000; Lotocka and Styczynska-Jurewicz 2001; Caramujo et al. 2012). It is also can be found in some forms of green algae.
Antheraxanthin	Antheraxanthin is a light-harvesting carotenoid found in a chromophyte alga (Alberte and Andersen 1986).
β,β-carotene	β,β-carotene is found in many algal groups.

Fig. 4.22 shows a chromatogram of the gut contents of a solitary stage of *T. democratica*. There were few peaks, and the fucoxanthin and fucoxanthin-like peaks suggest the presence of diatoms only (Table 4.1). For aggregates of *T.democratica* (Fig. 4.23), the chromatogram showed a very small peak for

chlorophyll *a* and also a small peak for fucoxanthin (generally used as an indicator for diatoms), but there were several peaks that had absorption spectra similar to fucoxanthin. There were small contributions from antheraxanthin, alloxanthin, and β,β-carotene for *T. democratica*. The pigments identified suggest that the gut content of the *T. democratica* aggregate contained diatoms (fucoxanthin) and cryptophytes (alloxanthin).

For solitary specimens of *S. fusiformis* the chromatogram of the gut contents (Fig. 4.24) showed peaks at 3.647, 5.495, 5.939, 8.551, 17.603 and 22.920 minutes that had absorption spectra that bear no resemblance to any well-known pigment (L. Clementson, personal comment, 2014). There was a very small peak for chlorophyll *a*, and there were peaks for pyro-phaeophorbide and pyro-phaeophytin in the gut contents of solitary *S. fusiformis* , which are degradation products of chlorophyll *a*. This indicates that, in the digestive system, the chlorophyll *a* has been degraded to other products. There was no peak for fucoxanthin, but there were several peaks that had an absorption spectra like that of fucoxanthin, but which had eluted at a different time to fucoxanthin. Again, this may be an example of the digestion process degrading the parent pigment into other forms. There was a peak that appeared similar to a chlorophyll *c*, but with a shift in the absorption spectra that was also possibly due to a change of form due to the digestive processes. It did elute at a similar time to chlorophylls *c*2 and *c*1. The dominant peak in the chromatogram corresponded to alloxanthin (an indicator of cryptophytes), and peaks corresponding to chlorophyll *b* (green algae) and β,β-carotene (not specific to

any one algal group) were also present. Based on this information, it is clear that *S. fusiformis* solitaries had predominately fed on diatoms, cryptophytes and green algae.

Aggregates of *S. fusiformis*, showed peaks at 5.503, 14.784 and 18.0291 minutes that had absorption spectra that bear no resemblance to any well known pigment (Fig. 4.25). There was a very small peak for chlorophyll *a*, and there were peaks for pyro-phaeophorbide and pyro-phaeophytin, which are degradation products of chlorophyll *a.* Again, this may be an example of the digestive process degrading the parent pigment into other forms. There were peaks that eluted at the correct time and had an absorption spectra that matched that of known standards. These were astaxanthin, alloxanthin, diatoxanthin, zeaxanthin and β,β-carotene. Two other peaks eluted at the correct time of a known pigment, but the absorption spectra had significant shifts; these were diadinoxanthin and antheraxanthin. The absorption spectrum for a diadinoxanthin standard has maximum absorption at wavelengths of 445 and 475 nm. In the sample the absorption spectra had the right shape, but the maximum absorption peaks were at 428 and 455 nm. It is possible that the parent pigment has been changed in some way during the digestive process. The pigments identified suggest that the gut content of the salps contained diatoms (fucoxanthin) and cryptophytes (alloxanthin). Astaxanthin is found in some forms of green algae and the pigment is also responsible for the pink colour of copepods. Zeaxanthin is associated with cyanobacteria, but is also an accessory pigment in green algae, so could be from either source. β,β-carotene is found in

many algal groups and may not be from just one source. To provide clearer understanding, a summary of the pigments identified in salps gut is presented in Table 4.2.

Table 4.2. Characteristic pigments of gut contents of salps as identified by HPLC.

Species	**Pigments identified**
T.democratica (solitary)	Fucoxanthin, Fucoxanthin-like, MV chlorophyll-*a*
T. democratica (aggregates)	Fucoxanthin, Fucoxanthin-like, Antheraxanthin, Alloxanthin, MV chlorophyll-*a*, β,β-carotene
S. fusiformis (solitary)	Chlorophyll *a*, Pyro-phaeophorbide and Pyro-phaeophytin, Fucoxanthin-like, Alloxanthin, Chlorophyll *b*, β,β-carotene, Diatoxanthin, Astaxanthin, Zeaxanthin
S. fusiformis (aggregate)	Fucoxanthin, Fucoxanthin-like, Pyrophaeophorbide-a, Astaxanthin, Alloxanthin, Diatoxanthin, Zeaxanthin, MV chlorophyll *a*, Pyrophaeophytin –a, β,β-carotene

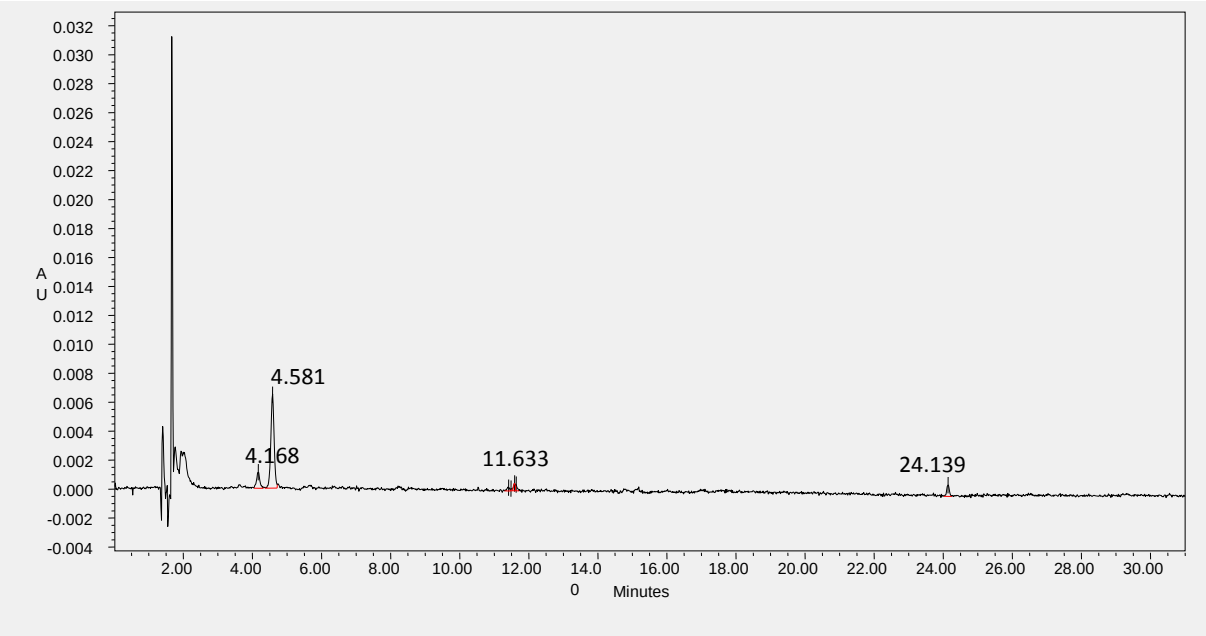


Fig. 4.22. *T. democratica* (solitaries) gut contents; Chromatogram showing the phytoplankton pigments and respective retention times, as separated by HPLC.

Ret. Time (min)	**Pigment**
4.168	Fucoxanthin-like
4.581	Fucoxanthin-like
11.633	Fucoxanthin
24.139	MV chlorophyll-*a*

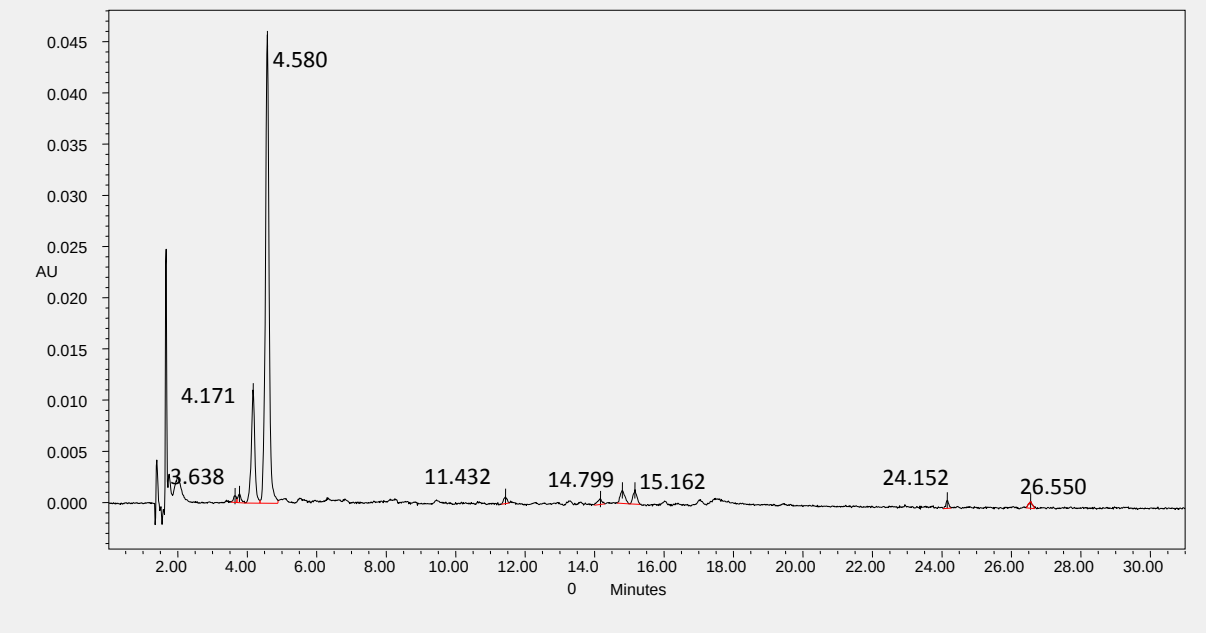


Fig. 4.23. *T. democratica* (aggregates) gut contents; Chromatogram showing the phytoplankton pigments and respective retention times, as separated by HPLC.

Ret. Time (min)	**Pigment**
3.638	Fucoxanthin-like
3.774	Fucoxanthin-like
4.171	Fucoxanthin-like
4.580	Fucoxanthin-like
11.432	Fucoxanthin
14.799	Antheraxanthin
15.162	Alloxanthin
24.152	MV chlorophyll-a
26.550	β,β-carotene

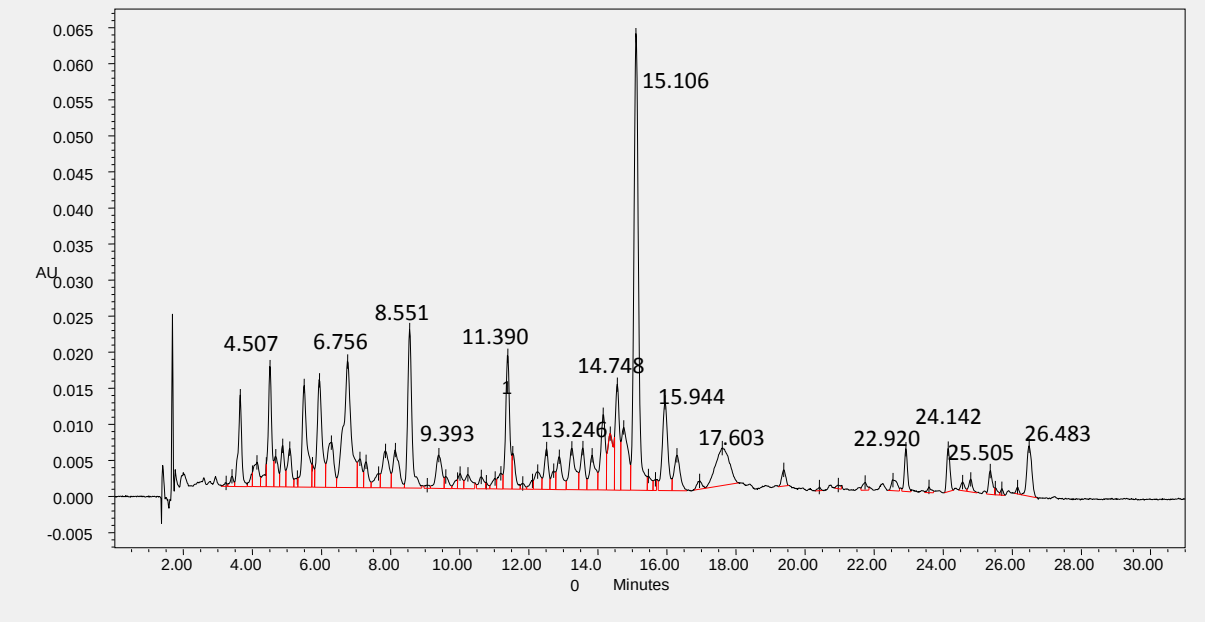


Fig. 4.24. *S. fusiformis* (solitaries) gut contents; Chromatogram showing the phytoplankton pigments and respective retention times, as separated by HPLC.

Ret. Time (min)	**Pigment**
4.507	Fucoxanthin-like
6.756	Chl c-like
9.393	Pyrophaeophorbide-*a*
11.390	Fucoxanthin-like
13.246	Astaxanthin
13.831	possible Diadinoxanthin*
14.748	Antheraxanthin
15.106	Alloxanthin
15.944	Diatoxanthin
16.946	Zeaxanthin
22.545	MV chlorophyll-b
24.142	MV chlorophyll *a*
25.505	Phaeophytin-a
26.150	Pyrophaeophytin-a
26.483	β,β-carotene

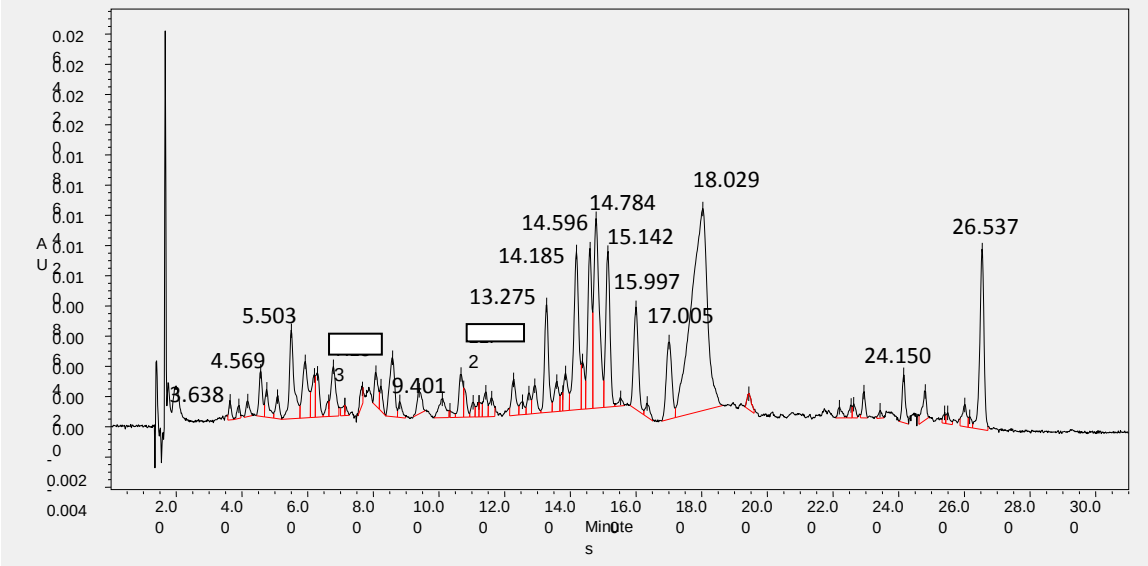


Fig. 4.25. *S. fusiformis* (aggregates) gut contents; Chromatogram showing the phytoplankton pigments and respective retention times, as separated by HPLC.

Ret. Time (min)	**Pigment**
3.638	Fucoxanthin-like
4.569	Fucoxanthin-like
4.751	Fucoxanthin-like
5.084	Fucoxanthin-like
9.401	Pyrophaeophorbide-a
11.605	Fucoxanthin
13.275	Astaxanthin
14.185	possible Diadinoxanthin *
14.596	possible Antheraxanthin *
15.142	Alloxanthin
15.997	Diatoxanthin
17.005	Zeaxanthin
24.150	MV chlorophyll *a*
26.157	Pyrophaeophytin -a

4.5 Discussion

SEM observation obtained 31 images of food particles in salp gut contents

In this study, SEM analyses were used to observe the morphology of food particles in the gut of *S. fusiformis* and *T. democratica*. However, as suggested by Blankenship and Yayanos (2005), consumed organisms may become unidentifiable once partly digested, while those with hard remains (e.g. diatom skeletons) may come to dominate the samples, thus biasing the analysis.

Although both species filter phytoplankton using their mucus net (Silver 1981), examination of their gut contents revealed their diets were slightly different in terms of size particles ingested, despite being collected at the same time and being exposed to the same food sources. Salps are known for being extremely efficient, nonselective gelatinous filter feeders, retaining particles over a wide size range between 1 µm and 1 mm (Harbison and McAlister 1979; Kremer and Madin 1992; Licandro et al. 2006).

A small numbers of salps had copepods present in their guts in summer 2013/2014 field samples. Images of a copepod in the gut of solitary *S. fusiformis* were captured in this study. Specimens of aggregate *Salpa thompsoni* had the copepod *Rhincalanus gigas* in their branchial cavities (Perissinotto and Pakhomov 1997). Their observations showed that the salp's survival was prolonged by exposure to the *Rhincalanus gigas* invasion, and it was suggested that it was actually a type of symbiosis where *Rhincalanus gigas* cleaned the salp's feeding apparatus when it was clogged. The occasional occurrence of copepods in salp guts had also been shown for *Salpa thompsoni* which had

ingested copepod nauplii, and also the genera Oithona and Oncaea (Hopkins and Torres 1989).

In the present study, all particles found in both stages of *T. democratica* and *S. fusiformis* were within the size ranges published for other species. For solitary *T. democratica*, the food particles found ranged in size from 10 µm to 1400 µm (the maximum measurement of the whole body size of a copepod, calculated from those body parts found). This size range does not include free nanoflagellate scales, which can reach 200 nm (Bergesch et al. 2008). For the aggregates, food particles found ranged from 2 µm to 250 µm. For solitary *S. fusiformis*, the minimum particle size found was 8 µm and the maximum was 1400 µm (copepod remains), while the size range of particles found in aggregates was 5-100 µm.

Previously, in Chapter 3, the diatom species in Storm Bay were identified and enumerated. The list was correlated with the abundance of thaliaceans during three summers (2009/2010, 2010/2011 and 2011/2012). When there were occurrences of *T. democratica* and *S. fusiformis*, several species of diatoms were observed regularly. Many of the phytoplankton species observed in the SEM analyses were also species from the list of diatom stocks presented in Chapter 3. They were: *Navicula* sp., *Pseudo-nitzchia* sp., *Leptocylindrus mediterraneus*, *Rhizosolenia* sp., *Thalassionema* sp., *Thalassiosira* sp., *Proboscia* sp., and *Nitzshia* sp. SEM analysis of the gut contents of salps collected during the summer of 2013/2014 revealed that eight species of these diatoms could be the preferred food for these salps.

Interestingly, when *T. democratica and S. fusiformis* occurred in high densities in two summers (2009/2010 and 2011/2012), some flagellates (prasinophytes and prymnesiophytes) and dinoflagellates were recorded from the same sites on the same dates. These included *Ceratium* spp., *Protoperidinium* sp., *Pyramimonas* sp., *Pyramimonas grossi*, *Chrysochromulina* sp., *Phaeocystis* sp. and *Scripsiella* sp. Images of these seven species were obtained through SEM studies in this chapter.

Since salps are non-selective filter feeders*,* vast ranges of sizes of phytoplankton fragments were found in the gut contents. Coccolithopores were observed in abundance in aggregates of both species and the solitary form of *T.democratica,* while partially digested copepods were only found in solitary phases of both *T. democratica* and *S. fusiformis.*

There appeared to be some evidence of differential food selectivity by *T. democratica* and *S. fusisformis*. For example, there were 5 species of Haptophytes (flagellates), 2 Prasinophytes (flagellates), 8 diatoms, 4 dinoflagellates, 3 unidentified species and some copepod body parts observed in images obtained from *T. democratica* guts. In contrast, only 2 species of Haptophytes (flagellates), 6 diatoms, 2 unidentified species were observed in images captured from the guts of *S. fusiformis.*

To my knowledge, only two other studies of SEM analyses have been done on salps (Madin and Purcell 1992; Harbou et al. 2011). These studies have reported several species of diatoms and dinoflagellates in the fresh guts of *Cyclosalpa bakeri*. Species recorded in *C. bakeri* include *Denticulopsis seminae,*

Nitzshia sp., *Corethron hystrix*, *Thalassiosira* spp., *Chaetoceros* sp., *Coccolithus pelagicus*, *Rhizololenia alata*, dinoflagellates, *Emiliania huxleyi* and other several species (Madin and Purcell 1992)

This paucity of studies could be due to similar methodological problems encountered in the present study. Freshly collected specimens are essential for SEM studies. Because they are fragile gelatinous species, mucus from the feeding nets of salps can get incorporated into gut contents if preparations are not done carefully during the gut washing and centrifuging (Madin and Purcell 1992). Improper preservation techniques have also been a problem for SEM examination of gut contents on some other invertebrates. For example, no identifiable organic materials were observed in *Rimicaris exoculata* (a shrimp) as a consequence of the delay in preservation of the material until several hours after collection (Van Dover et al. 1988). Conversely, Harris et al. (1991) observed plant fragments, pennate diatoms, diatom fragments, unidentified coccoid bodies, bacteria and partially digested ciliated protozoans in the guts of two prawns. This could be due to the technique applied where the guts of the prawns were removed within 30 min of collection. Thaliaceans are widely known for their unpredictable occurrence, which might also partly explain why studies of SEM analyses on gut contents of (freshly collected) thaliaceans are scarce.

HPLC confirms the SEM results and provides added information

The chromatograms obtained in this study were noisy, making it difficult to establish a baseline. This could probably be due to the gelatinous matter of the

body walls contaminating the gut content samples. Pigments obtained from salps were slightly different according to species and stage (Table 4.1). *T.democratica* had the least number of pigments identified. This, however, might be due to the small size of the solitary phase specimens that were analyzed.

Fucoxanthin, fucoxanthin-like pigments and chlorophyll *a* were identified in all species and stages of salps. Alloxanthin, astaxanthin and diatoxanthin were presents in both stages of *S. fusiformis*. Meanwhile, β,β-carotene was only present in the guts of aggregate stages of both species. Often carotenoids such as peridinin and fucoxanthin are considered characteristic of dinoflagellates and diatoms respectively, while fucoxanthin derivatives (i.e., 19'hexanoyl-oxy-fucoxanthin) are present in prymnesiophytes, crysophytes, and some dinoflagellates (Kozlowski et al. 1995).

No peridinin or its derivatives (peridinol) were detected in the guts of salps in this study, indicating that dinoflagellates were not an important prey item. Most salps in this study clearly show the predominance of fucoxanthin over other pigments in the guts. The predominance of fucoxanthin in salp guts could be a function of the degree of digestion, as flagellates could be better digested than hard-shelled diatoms. This contrasts with herbivorous copepods, where peridinin content was high in their guts, indicating their feeding preference for dinoflagellates (Claustre et al. 1992).

In Chapter 3, I suggested that *S. fusiformis* favours conditions of high percentages of flagellates over diatoms, based on observation of their response to two environmental parameters: diatom stocks and phytoplankton cell counts.

Here, through HPLC analysis, we can see that only chromatogram profiles of *S. fusiformis* (both stages) showed the carotenoids astaxanthin and zeaxanthin. Studies by Ackman (1989) found that zeaxanthin was one of the carotenoid pigments found in *Pyramimonas grossi*. This finding was supported in my SEM study, where an image of *Pyramimonas grossi* were obtained through SEM in the guts of *S. fusiformis*.

There are several limitations to the HPLC approach to diet analysis. Based on my experience, the separation of a salp's gut from the body must be done extremely carefully, as soft gelatinous parts of the body could contaminate the chromatogram, resulting in many unidentifiable peaks. Since HPLC analysis is expensive, it would be more cost effective if all peaks in a chromatogram were identifiable.

4.6 Conclusion

In total, 31 phytoplankton species were identified from the salp guts, both by scanning electron microscopy and liquid chromatography techniques. As each method has its own strengths and weakness, I suggest using a combination of both methods to get the best results. Taxa identified include diatoms, dinoflagellates, haptophytes, prasinophytes, cryptophytes and green algae. Flagellates are believed to be the best food source for salps, but this study has delivered more details of other dietary items of solitaries and aggregates of *S. fusiformis* and *T. democratica* through ultrastructure images and

chromatogram profiles. Salps were shown to predominantly feed on diatoms and cryptophytes, flagellates and algae. The suggestion in Chapter 3 that *T. democratica* preferred diatoms over dinoflagellates was confirmed using SEM. Additionally, HPLC analysis confirmed that *S. fusiformis* favours the condition of a high proportion of flagellates over diatoms. Future studies (using SEM, HPLC and other techniques) on the gut contents of salps and their relationship to feeding selectivity will provide more insight into the vital food source for salps. Studies on the composition of the phytoplankton are essential to evaluate environment changes, both at short and long-term scales. What's more, by considering a range of organic biomarkers, such studies will contribute to a better understanding of the dynamics of the transformation of the particulate organic matter in Storm Bay, Tasmania ecosystem. In Chapter 5, the trophic linkages of thaliaceans will be further examined by focusing on biochemical composition and the role of salps in the pelagic system in Storm Bay.

CHAPTER 5

LENGTH-WEIGHT RELATIONSHIPS AND C/N COMPOSITION OF *Thalia democratica and Salpa fusiformis* IN STORM BAY

5.1 Highlights

- Three methods for preparing salps for carbon and nitrogen analyses were tested in this study, and compared with several methods from the literature. There were differences in the results for fresh and frozen specimens of *T. democratica*, while results for *S. fusiformis* were less affected by the different preparation methods.
- Freshly collected and incised salps, rinsed with small volumes of Milli-Q water, provided the best method and is thus recommended for future elemental analyses.
- Carbon (8 – 10%) and nitrogen (~2%) concentrations of *T. democratica* were higher than those of *S. fusiformis* (~6% C and ~1% N)
- There were differences in the elemental concentrations of the two life stages, whereby solitary *T. democratica* had higher carbon and nitrogen concentrations than aggregates, while solitary *S. fusiformis* had lower carbon and nitrogen content than aggregates.

5.2 Introduction

In marine ecosystems, trophic pathways can be characterised by using nitrogen and carbon stable isotope ratios as food web tracers (Fleming et al. 2011). Based on elemental content, marine zooplankton can be distinguished into three groups, the gelatinous plankton (cnidarians, ctenophores, larvaceans, salps), the non-gelatinous group (crustaceans, larvaceans) and the semi-gelatinous plankton (mollusks, chaetognaths). Of all these groups, the gelatinous plankton was observed to have very low and variable nitrogen and carbon percentages (Larson 1986).

Knowing the elemental chemical composition of the plankton is essential for understanding the production and the biogeochemical circulation of components in the ocean. Previous studies on salps provide an insight into how these organisms function in their environment. For example, in the Lazarev Sea of the Southern Ocean, *Ihlea racovitzai* showed high carbon and protein content, leading to the conclusion that they were an important component in the pelagic food webs of the Antarctic and represent a notable energy source to invertebrate predators (Dubischar et al. 2012).

Stable isotope ratios, primarily $\delta^{13}C$ and$\delta^{15}N$, play an important role and provide powerful tools for estimating trophic positions and carbon flow in food-web studies (Post 2002; Volkman et al. 2009). $\delta^{15}N$ values highlight the trophic level at which an organism feeds, whereas $\delta^{13}C$ values are often used to investigate energetic pathways through food webs and can be used to determine the source of food for each trophic level (DeNiro and Epstein 1978; Wada et al.

1987; Post 2002; Volkman et al. 2009). Due to the differences in atomic mass, animals generally have greater concentrations of ^{13}C and ^{15}N in their body than is present in their food (Volkman et al. 2009).

One continuing concern for salps is that, although they are characterised by very high water content, their carbohydrate, protein and lipid concentrations are very low (Dubischar et al. 2006). In that study, the three components and ash free dry weight explained only about two thirds of the dry weight of *Salpa thompsoni*. It was possible that groups of proteins could have not been detected by the measurements. Though the missing components were previously believed to be partly due to residual water (Larson 1986), and lost during the ashing process, the "missing components" were possibly due to other unidentified organic compounds (Dubischar et al. 2006).

More recently, stable isotope analysis has been found to be a useful tool for the study of dietary relationships in marine food webs (Schmidt et al. 2003; Stowasser et al. 2012). Carbon (13C/12C) and nitrogen (15N/14N) isotopes are commonly used to provide insight into food-web structure (Sydeman et al. 1997; Post 2002). Carbon isotope ratios of animal tissues are close to those in their diet, and, therefore, can be useful for tracing carbon pathways and sources of primary productivity (Hobson and Welch 1992; Post 2002). Dietary analyses of salps have been performed based on the determination of their $^{13}C/^{12}C$ ratio (Huntley et al. 1989; Dubischar et al. 2012). Further, dry weight and elemental compositions (carbon, nitrogen, protein, lipid, and carbohydrate) have been measured to characterize the potential value of salps as a food source for higher-

trophic levels in the Antarctic ecosystem. Chapter 3 highlighted that changes to environmental conditions could result in significant changes to the Storm Bay ecosystem, whereby gelatinous organisms occur more frequently and in higher abundance. However, at present there is no information on these local species to help determine whether a salp-based food web in Storm Bay would have ramifications for species further up the chain.

The aims of this study were to:

1. Compare three methods of preparation for determining the carbon and nitrogen content of salps and establish the most effective method.
2. Investigate the relationships between weight and carbon/nitrogen content for the solitary and aggregate phases of *T. democratica* and *S.fusiformis*.
3. Measure baseline isotopic signatures to assist with the evaluation of where each species fits within the marine food web in Storm Bay.

5.3 Materials and Methods

To compare results with those obtained via SEM and HPLC, specimens used for stable isotope analysis were collected at the same time as the specimens that were analyzed using SEM and HPLC: in the summer of 2013/14.

5.3.1 Analytical method development for salps

In the present study the carbon and nitrogen content of the dominant salps in Storm Bay were measured, with species separated into the solitary and aggregate phases. Trophic relationships were determined using i) carbon-nitrogen content, ii) stable isotope analysis and iii) stable isotope analyses of seawater.

Ninety-eight individual salps were used for carbon-nitrogen elemental analyses. Specimens used to compare all methods of processing were placed into cryovials after wet masses (mg) were obtained. After being freeze-dried at -40°C overnight, they were stored in a desiccator until further processing. Samples were homogenized in the cryovial using a small spatula, and encapsulated in tin cups, with dry weights of material used for elemental analysis falling between 0.7 and 1.7 mg.

Three different methods of preparation were tried. These included the physical condition of the salps after collection, as both fresh and frozen specimens were tested. They were then separated based on species and stage, and lengths and weights were measured. Specimens were rinsed either with Milli-Q, deionized water or ammonium formate prior to analysis. Data are presented as percentages of dry weights, since most literature values have been reported in terms of dry weight (Bailey et al. 1995).

i) Preserved by freezing in filtered seawater, rinsed with Milli-Q

Twenty-three specimens of *S. fusiformis* and twenty-nine *T. democratica* that were kept frozen in GF/F filtered seawater were rinsed with Milli-Q without going through the cutting process (Table 5.1). This was to determine if the seawater that was trapped in the salps' bodies would affect the results of elemental analysis. This method was also tested to determine if there were significant differences between fresh and frozen samples that were treated with the same methods of preparation.

ii) Fresh salps cut and rinsed with ammonium formate

Only five *S. fusiformis* (three solitaries and two aggregates) were tested with ammonium formate (Table 5.1). In order to avoid interference from salt in seawater, the seawater was flushed from the internal cavity of the salps with a microsyringe containing isotonic ammonium formate so that the salt was squeezed out naturally without damaging the salp's tissue. Ammonium formate solution washes and displaces unwanted material such as saltwater and it is believed that ammonium formate then evaporates leaving no residue.

iii) Fresh salps cut and rinsed with Milli-Q

Forty-one fresh salps (32 *T. democratica* and 9 *S. fusiformis*) were processed (Table 5.1). Small parts of each salp's body were cut with a small, fine scalpel to let the seawater drain out. Next, salps were rinsed with Milli-Q. This was to release the seawater trapped in the internal organs of salps.

5.3.2 Biochemical analysis of salps

Irrespective of the initial preparation method, all salp samples were freeze-dried (JAVAC SB9) to constant mass at -40°C for 24 h. Each salp was weighed and transferred into a pre-weighed tin cup. Specimens beyond the required weight were homogenised to fine powder and a subsample was taken from the powder. If necessary, small individuals of the same species were pooled together to obtain sufficient material for analysis (0.7 to 1.7 mg dry sample weight). Salps were not acidified because acidification treatments are still under debate and their effect on both carbon and nitrogen isotopic compositions are still not clear (Mintenbeck et al. 2008; Brodie et al. 2011). Carbon and nitrogen contents were determined at the Central Science Laboratory, University of Tasmania, using a Thermo Finnigan EA 1112 Series Flash Elemental Analyser.

Carbon and nitrogen stable isotopes were analysed using an Iso-Prime100 mass-spectrometer coupled with an elemental analyser (Elementar vario PYRO cube, Germany) at the Central Science Laboratory, University of Tasmania. Samples were flash combusted, when they were burnt in an excess of oxygen and converted into N_2 and CO_2. These gases were then introduced into the elemental analyser to obtain the quantitative data on element content in the samples. The purified gases were sequentially released into the mass spectrometer to perform the isotopic measurements. Isotope ratios were reported as parts per thousand (‰) deviations from the conventional standards, Pee Dee Belemnite (PDB) for carbon and atmospheric nitrogen gas for nitrogen.

Stable isotope concentrations are expressed in delta (δ) notation as parts per thousand according to the following equation:

$$\delta^{13}C\ (\delta^{15}N) = [(R_{sample}/R_{standard}) - 1] \times 1{,}000 \qquad (5.1)$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Internal laboratory standards with known isotopic composition were run after every 5th sample. The stability of the instrumentation, analytical precision, drift correction and linearity performance was calculated from the repetitive analysis of these standards.

Table 5.1. Carbon and nitrogen values as percentage of dry weight (DW), as measured using three methods of preparation in the present study.

Methods of preparations					Species	Stage	n	Carbon (% DW)	Nitrogen (% DW)
Fresh	Frozen in filtered seawater	Cut	Rinse						
			Milli-Q	Ammonium Formate					
	X		X		*S. fusiformis*	Sol	9	4.26	0.99
	X		X		*S. fusiformis*	Agg	14	5.13	1.24
	X		X		*T. democratica*	Sol	10	4.18	0.96
	X		X		*T. democratica*	Agg	19	2.28	0.53
X				X	*S. fusiformis*	Sol	3	8.21	4.45
X				X	*S. fusiformis*	Agg	2	5.69	2.90
X		X	X		*S. fusiformis*	Sol	3	5.22	1.20
X		X	X		*S. fusiformis*	Agg	6	6.78	1.23
X		X	X		*T. democratica*	Sol	8	10.70	2.32
X		X	X		*T. democratica*	Agg	24	8.17	1.70

5.3.3 Seawater stable isotope analysis

Quartz-microfibre filters (47 mm in diameter) were pre-combusted at 450°C for 24 hours. Seawater samples of a known volume (generally 1 to 2 L) were filtered through the filters to trap the particulate matter. In the laboratory, filters were freeze-dried overnight, and then were demineralized in concentrated HCL (1%) fumes for 24 h to remove inorganic carbon. Filters were then dried at 60°C for at least 24 h. Subsamples were taken from each filter with syringe-punches and packed in a pre-weighed silver cups for stable isotope analysis. Blank filters were also treated the same way to confirm that the filters were not contaminated with any elements. For seawater samples, I could not retrieve $\delta^{15}N$ values because the amount of nitrogen retained on the filters was below the precision of the instrument.

5.3.4 Statistical analysis

The preparations of fresh and frozen samples that were treated with Milli-Q were compared with a t-test to test for differences in the lengths, weights and carbon and nitrogen contents of *T. democratica* and *S. fusiformis*. The non-parametric Mann-Whitney U test was applied when the data did not meet the assumptions for parametric analysis.

5.4 Results

5.4.1 Comparison of preparation methods

Carbon and nitrogen content, as estimated by three preparation methods and for both stages, were compared (Table 5.1). The preparation procedure that used a Milli-Q rinse on freshly collected specimens resulted in slightly higher contents than those measured for *T. democratica* by Heron et al. (1988). However, carbon and nitrogen weights for *S. fusiformis* in the current study were found to be lower compared to the literature (Curl 1962; Le Borgne 1982; Small et al. 1983; Clarke et al. 1992).

The carbon and nitrogen contents, and lengths and weights measured for fresh and frozen samples of each species were compared with t-tests or Mann-Whitney U test. From the results (Table 5.2), it can be observed that the two preparations yielded statistically different results when comparing fresh and frozen specimens of *T. democratica*, but not for *S. fusiformis*.

5.4.2 Length-weight relationships

The relationships between wet weight and length, and dry weight and length for both species were expressed as a power equation, $W=aL^b$, where W is weight in mg (wet/dry), L is length in mm and a and b are the coefficients (Fig. 5.1). The slopes for the fresh and frozen specimens ranged from 0.88 to 1.49.

Table 5.2. Comparison of the fresh and frozen methods of prepartion for each species with significant differences indicated by *. T-test applied when data met assumptions of normality and heteroscedasticity, Mann-Whitney U nonparametic statistic applied when parametric assumptions were not met.

	T. democratica		***S. fusiformis***	
Wet weight (mg)	Mann-Whitney U Statistic= 181.0 P <0.001*		Mann-Whitney U Statistic= 74.0 P = 0.224	
Dry weight (mg)	Mann-Whitney U Statistic= 44.000 P <0.001*			t_{30} = 1.249; P = 0.221
Length (mm)		t_{59} = -1.584; P = 0.119		t_{30} = -1.474; P = 0.151
Carbon (mg)	Mann-Whitney U Statistic= 95.5 P <0.001*		Mann-Whitney U Statistic= 66.0 P = 0.121	
Nitrogen (mg)	Mann-Whitney U Statistic= 119.5 P <0.001*			t_{30} = -0.676; P = 0.504

The corresponding statistically significant r^2 values ranged from 0.271 to 0.793 (Fig. 5.1). Frozen *T. democratica* were heavier per unit length than fresh

specimens for both wet and dry weight, whereas fresh specimens of *S.fusiformis* had higher values of wet weight per unit length than the frozen specimens.

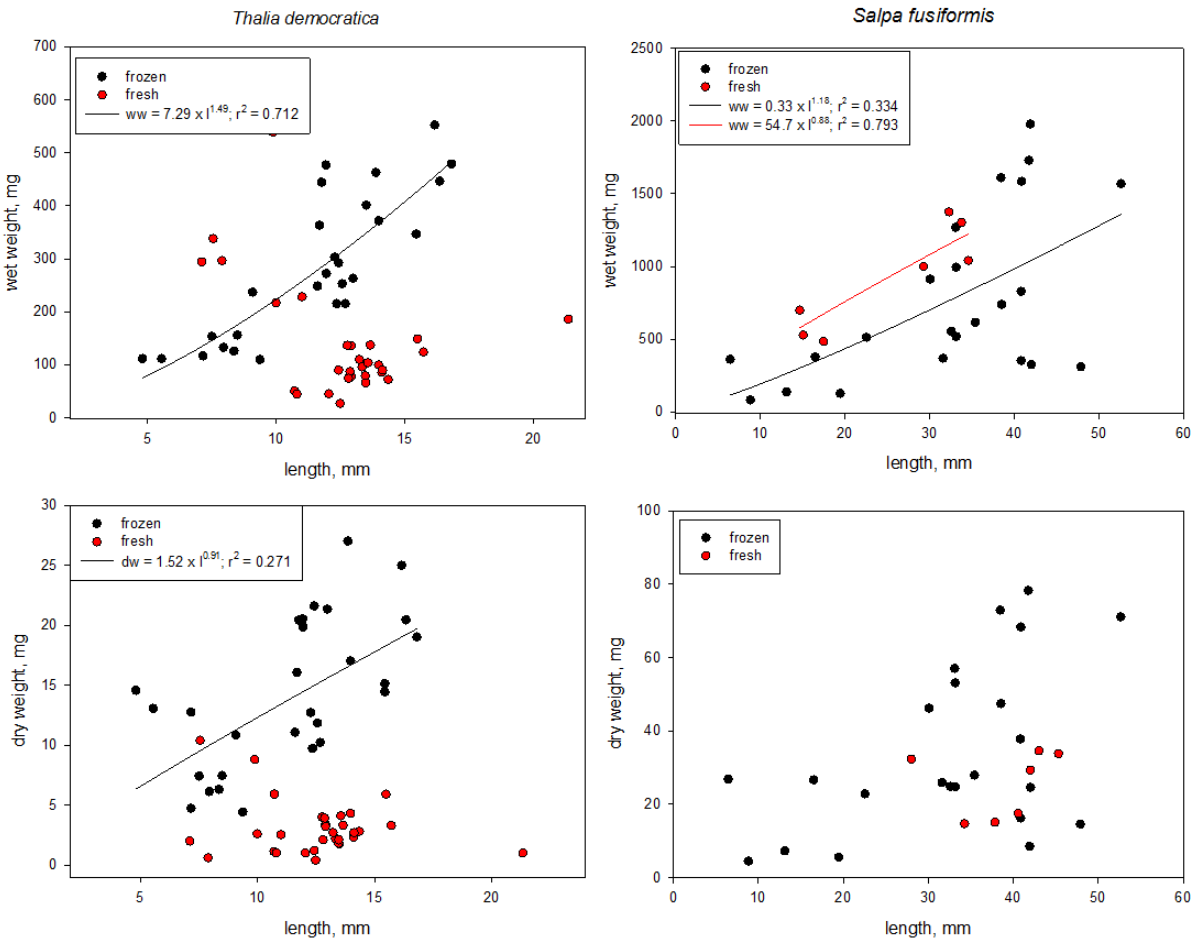


Fig. 5.1. Length/wet weight and dry weight relationships of *T. democratica and S.fusiformis.* Significant regressions are presented.

5.4.3 Carbon and nitrogen content

Although there were three different methods of preparation tested in this study, all results were consistent in terms of the range of values for each species' elemental compositions, when data were normalised to dry weight (Fig. 5.2). Fresh samples that were cut and rinsed with ammonium formate showed the

highest elemental composition, (C: 127.87-573.26 mg: N: 60.08-248.04 mg), followed by thawed and unincised specimens that were frozen in filtered seawater (C: 9.48-376.02 mg; N: 0.59-91.72 mg). Finally, the fresh salps that were incised and rinsed with Milli-Q showed C: 0.54-156.78 mg and N: 2.74-37.30 mg. C/N ratios for the ninety eight specimens fell between 1.83 and 5.39 (mean ~ = 4.4), comparable to the ratios reported for other salps (Table 5.3). The only exception were the aggregates of *S. fusiformis*, where the C/N ratio was closer to 2.

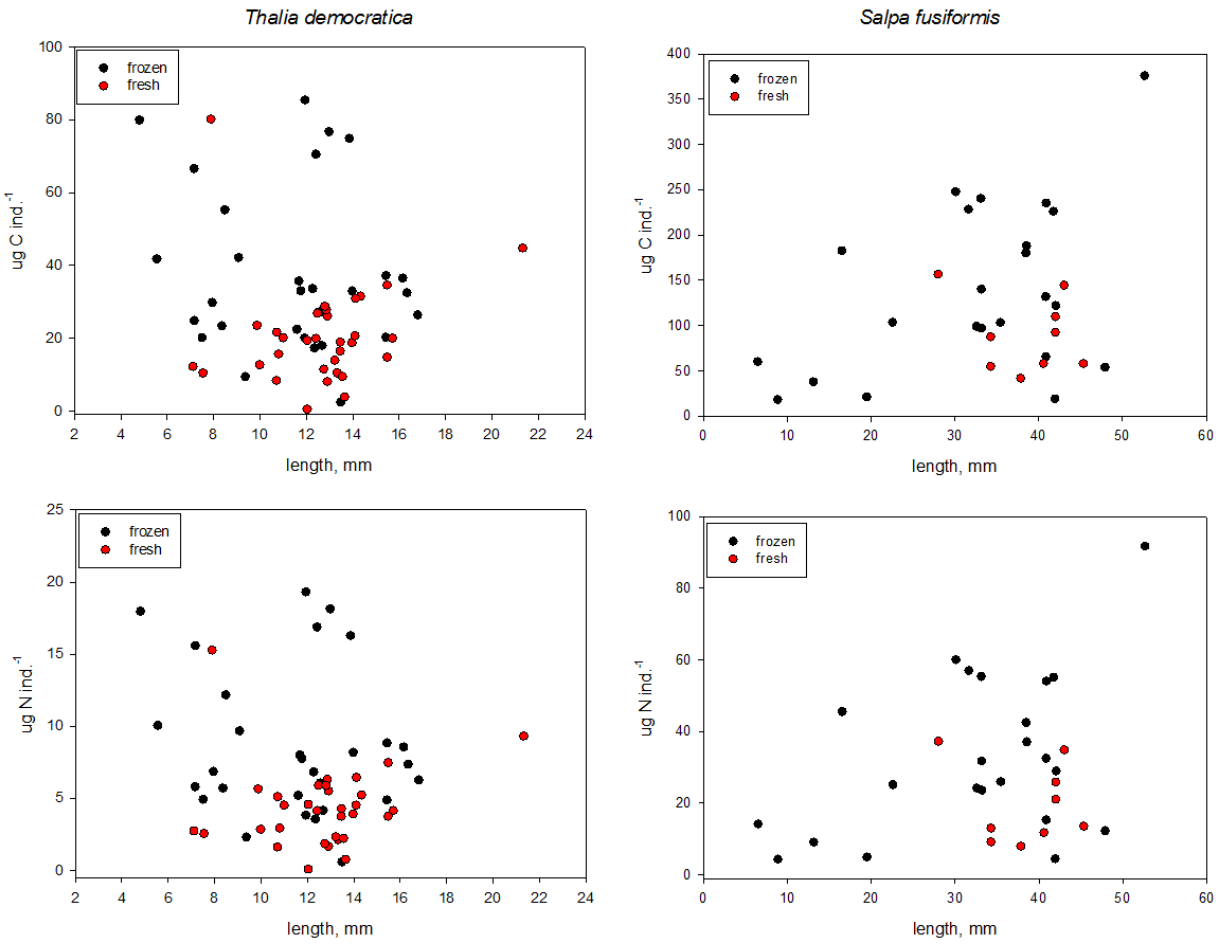


Fig. 5.2. Relationship between carbon and nitrogen content and lengths of both species of salps.

Table 5.3. Comparisons of C/N ratios from this study and those from the literature.

Methods of preparation	Species	Stage	n	C/N	References
Rinsed in distilled water and frozen in cleaned and acid-rinsed glass vials. Large specimens were frozen individually and smaller ones in groups of up to six.	*T. democratica*	Sol	12	4.4	Madin et al. 1981
	T. democratica	Agg	12	4.0	
	Salpa maxima	Sol	6	4.4	
	Salpa maxima	Agg	30	4.6	
	Salpa cylindrica	Sol	19	4.4	
	Salpa cylindrica	Agg	4	4.1	
	Pegea bicaudata	Sol	8	4.1	
	Pegea bicaudata	Agg	23	4.4	
	Pegea confoederata	Sol	26	4.7	
	Pegea confoederata	Agg	23	4.8	
	Pegea socia	Sol	2	4.6	
	Pegea socia	Agg	18	4.6	
	Ihlea asymmetrica	Sol	10	3.7	
	Ihlea asymmetrica	Agg	10	4.2	
	Cyclosalpa affinis	Sol	16	4.1	
	Cyclosalpa affinis	Agg	19	4.7	
	Cyclosalpa floridana	Sol	9	4.9	
	Cyclosalpa floridana	Agg	9	5.2	
	Cyclosalpa pinnata	Sol	18	4.1	
	Cyclosalpa pinnata	Agg	11	4.5	
	Cyclosalpa polae	Sol	8	4.3	
	Cyclosalpa polae	Agg	6	4.6	
	S. fusiformis	Sol		4.0	Small et al. 1983

	S. fusiformis	Sol		4.3	Clarke et al. 1992
	Salpa thompsoni	Agg Sol		3.96 3.90	Iguchi and Ikeda 2004
	Salpa thompsoni	Agg		4.24	Huntley et al. 1989
	Salpa thompsoni	Agg Sol		8.1 8.9	Reinke 1987
	Salpa thompsoni	Sol		3.92	Ikeda and Mitchell 1982
	Salpa thompsoni	Sol		4.7 4.2	Ikeda and Bruce 1986
	Salpa thompsoni	Sol		4.6 4.5	Dubischar and Bathmann 1997
Preserved by freezing in filtered seawater, rinsed with Milli-Q	*T. democratica* *T. democratica* *S. fusiformis* *S. fusiformis*	Agg Sol Agg Sol	19 10 14 9	4.38 4.32 4.15 4.32	This study
Fresh salps cut and rinsed with ammonium formate	*S. fusiformis* *S. fusiformis*	Agg Sol	2 3	1.94 1.83	This study
Fresh salps cut and rinsed with Milli-Q	*T. democratica* *T. democratica* *S. fusiformis* *S. fusiformis*	Agg Sol Agg Sol	24 8 6 3	4.89 4.55 5.39 4.26	This study

5.4.4 Life stages

Generally, solitaries of *S. fusiformis* were found to be lower in dry weight than the aggregates. When fresh and incised specimens were rinsed with Milli-Q, carbon contents were 5.22 mg (solitary), 6.78 mg (aggregates), while nitrogen contents were 1.20 mg (solitary) and 1.23 mg (aggregates). Similar to thawed specimens, carbon content of solitaries of *S. fusiformis* were 4.26 mg and 5.13 mg for aggregates, while mean nitrogen content of solitaries was 0.99 mg compared to 1.24 mg for aggregates. Solitaries of *S. fusiformis* only showed higher values of carbon (8.21 mg) and nitrogen (4.45 mg) compared to aggregates (5.69 mg- carbon and 2.90 mg- nitrogen) when treated with ammonium formate.

Carbon and nitrogen contents of *T. democratica* solitaries were slightly higher than the aggregates when fresh and thawed specimens were treated with Milli-Q. Fresh solitaries of *T. democratica* that were treated with Milli-Q had higher carbon and nitrogen contents than aggregates: 10.70 mg of carbon for solitaries compared to 8.17 mg for aggregates and 2.32 mg of nitrogen for solitaries compared to 1.70 mg for aggregates.

5.4.5 Stable isotopes

A summary of $\delta^{15}N$ and δ13C values that were measured on seawater and salps in Storm Bay are presented in Fig. 5.3. $\delta^{13}C$ for seawater particulate matter fell within the range of -25.43 to -29.09. Unfortunately the levels of nitrogen in the seawater samples were too low to be recorded by the instrument.

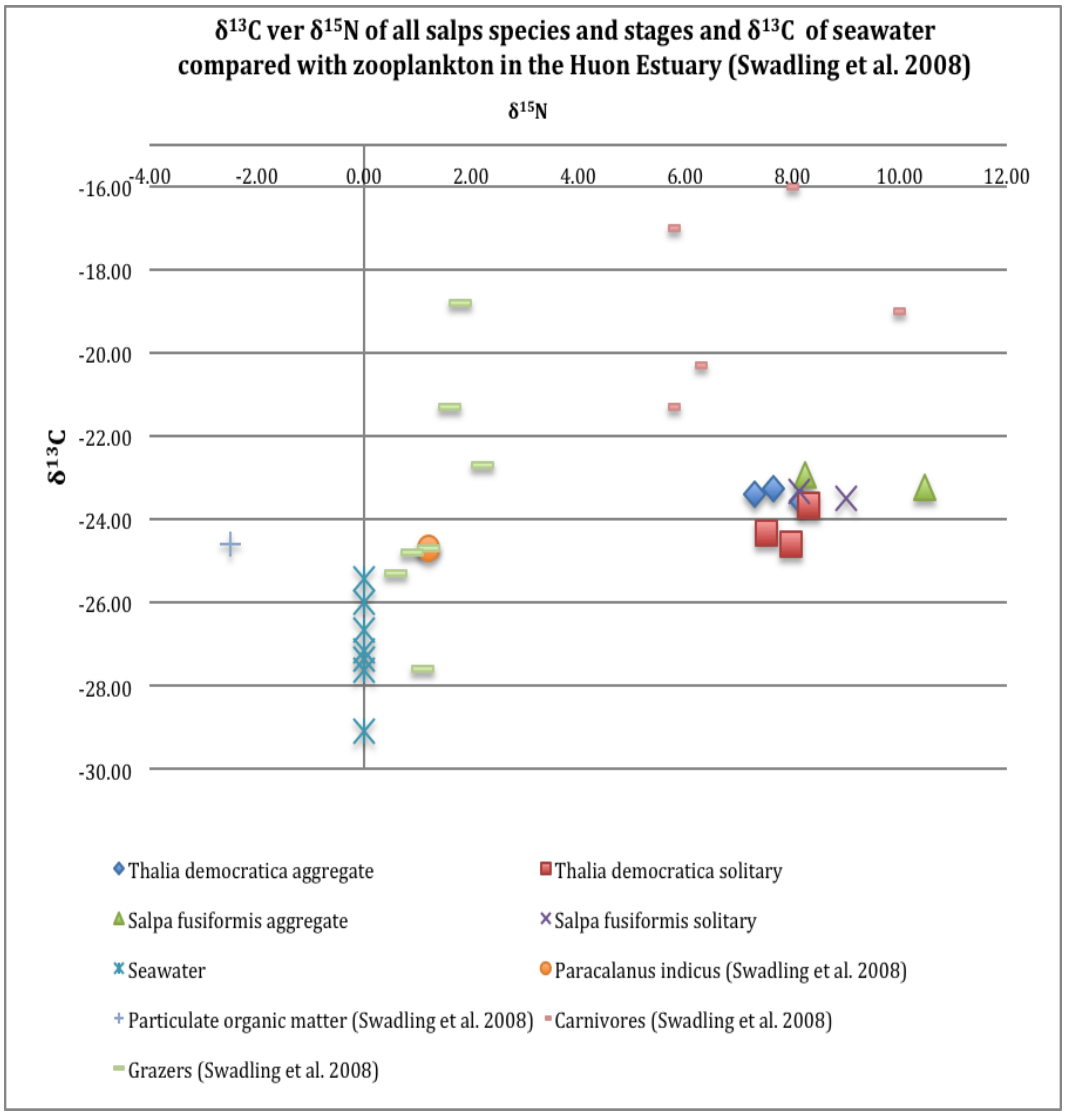


Fig. 5.3. $\delta^{15}N$ and $\delta^{13}C$ values that were measured on seawater and salps in Storm Bay. $\delta^{15}N$ could not be measured for seawater. The copepod *Paracalanus indicus*, grazers and carnivores from Huon estuary are shown for comparison.

$\delta^{13}C$ values for salps were slightly enriced compared to those for the particulate matter, suggesting that the diets of the salps might not have been ingesting particulate matter completely indiscrimately. $\delta^{15}N$ values suggested that both stages of both species where ingesting food at a similar trophic level,

with *S. fusiformis* occupying slightly higher position in the food web than *T.democratica*.

Table 5.4: Comparison of isotope values in present study and previous work.

Species	**References**	**$\delta^{13}C$**	**$\delta^{15}N$**
T. democratica	This study (aggregate)	-23.27	7.63
	This study (solitary)	-23.67	8.29
	De Lecea et al. 2013	-23.48	5.44
S. fusiformis	This study (aggregate)	-23.22	10.47
	This study (solitary)	-23.48	8.99

5.4.6 Nitrate and nitrite (NOx) in seawater

In Storm Bay, water samples for analyses of NOx concentrations were collected using Niskin bottles. NOx concentrations from summer 2009/2010 to summer 2011/2012 ranged between 0.00 to 8.48 μM. Generally, the concentrations were lowest during summers (Fig. 5.4).

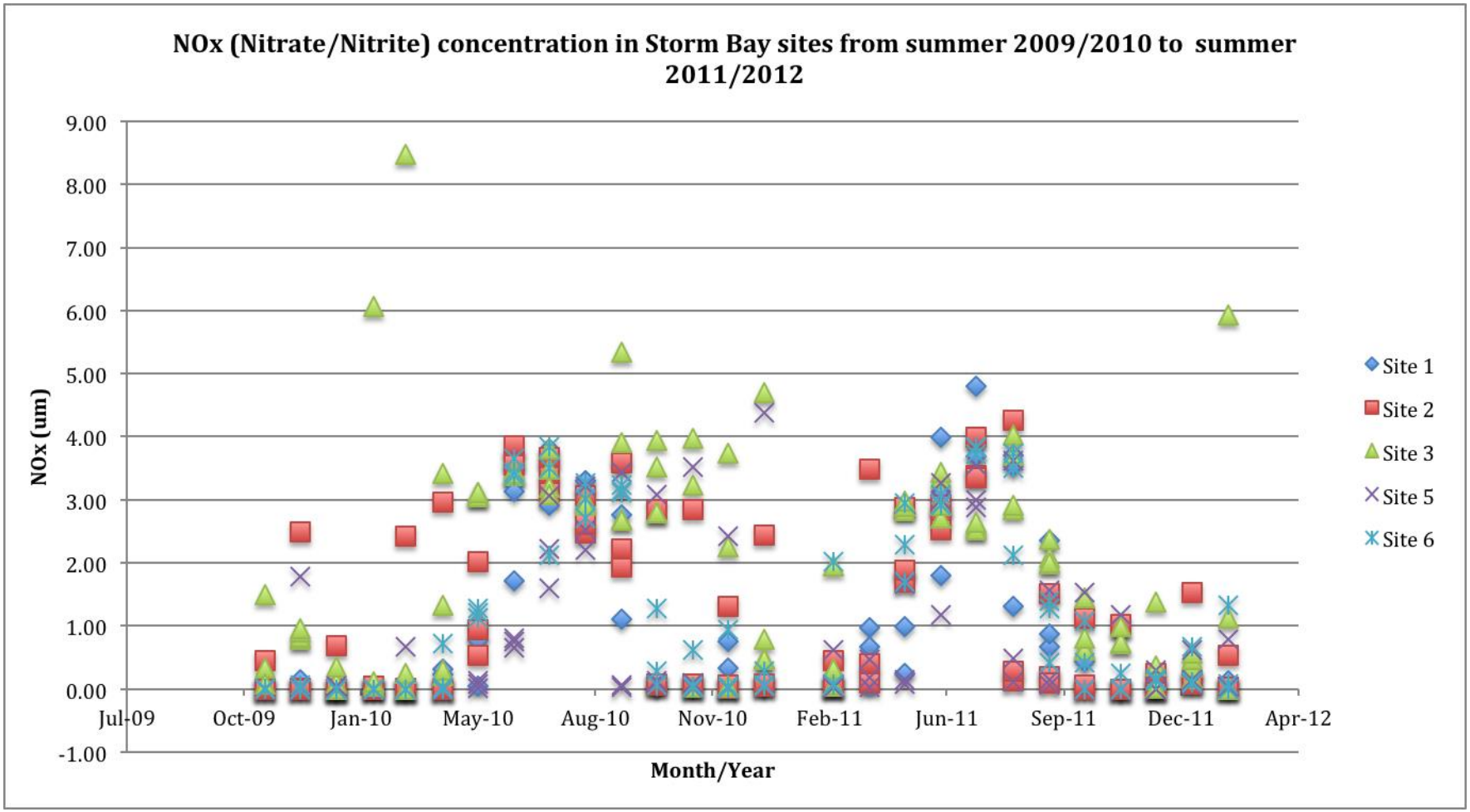


Fig. 5.4. Nitrate and nitrite (NOx, in μM) concentration in Storm Bay sites from summer 2009/2010 to summer 2011/2012.

5.5 Discussion

Effects of preservation on body size

The limitations to obtaining consistent results of elemental analysis are generally well understood, especially when dealing with tissue shrinkage of gelatinous zooplankton in preservatives (Nishikawa and Terazaki 1996). Due to the significant amount of water that is common in their tissues (Clarke et al. 1992), gelatinous animals are known to rapidly lose physical integrity (Fleming et al. 2011). Preserved gelatinous zooplankton are more prone to shrinkage than other zooplankton due to their high water content and the absence of strong structural features or hard covering (Thibault-Botha and Bowen 2003). For instance, *Salpa thompsoni* was shown to shrink by as much as 14% after many months of preservation (Reinke 1987). In my experience, the two species of salps (*T.democratica* and *S. fusiformis*) that were collected from Storm Bay, and preserved in 70% ethanol for up to 24 months before elemental analysis, underwent severe shrinkage. Other studies have also shown that preservation with formaldehyde results in considerable shrinkage (Madin et al. 1981; Heron et al. 1988; Nishikawa and Terazaki 1996). Therefore, as morphological alteration (shrinkage) might also affect the biochemical composition of specimens, it was thought best to steer clear of preservatives of any type when undertaking elemental analysis of gelatinous organisms. Because my specimens that were collected from 2009-2012 had been fixed with formaldehyde then preserved in ethanol they could not be used for the analyses presented in this chapter. Thus I was restricted to using salps that were collected and processed immediately

during the summer of 2013/2014. Unfortunately, doliolids were absent in that summer, so could not be analysed along with the salps. Both the solitary and aggregate life stages of the salps were analysed, although it had been suggested that there was no significant difference between solitary and aggregate stages of *T. democratica* (Heron et al. 1988).

Fresh salps that were cut and rinsed with Milli-Q were found to be the best method of preparation for C and N elemental analysis

In this study, I trialled two different conditions of specimens that had been freshly collected: kept fresh or immediately frozen. Freshly collected samples were weighed and prepared within 3 to 6 hours of collection, while frozen samples were analyzed within 1 month of collection.

Salps close their valves when removed from seawater, and, as a result, they trap a volume of seawater that is large relative to their tissue volume (Heron et al. 1988). Therefore, I trialled two techniques for removing the water: either (i) cutting into the tissue with a small, sharp scapel and allowing the trapped water to drain, or (ii) using a microsyringe filled with ammonium formate to gentley replace the trapped seawater.

The notion behind rinsing specimens with isotonic ammonium formate was to remove any sodium chloride adhering to the specimens (Parsons et al. 1961). This procedure was adopted by Heron et al. (1988) in their study of *T. democratica*. Likewise, in the present study, seawater was flushed from the internal cavities of five speciments of *S. fusiformis* with a microsyringe containing

isotonic ammonium formate, without cutting the bodies of the salps. Ammonium formate is most likely the source of the high nitrogen content of the *S. fusiformis* specimens used in that method trial. Although Heron et al. (1988) concluded that all the ammonium formate was evaporated by drying, and did not contribute to the weight estimates, the present study indicated that excess nitrogen from the ammonium formate was present in the samples. Ammonium formate is a salt with a melting point of over 100 °C, and it was suggested many years ago (Omori 1978) that chaetognaths and copepods used for biochemical studies should be not be rinsed with isotonic ammonium formate.

In the past, several different methods have been used to prepare salps elemental analyses and 17 methods, dating back to the 1960s, have been summarised in Table 5.5. Most of the studies were focused on the genus *Salpa*, a few on the genus *Ihlea* two on *T. democratica* and only one on *Tethys vagina*. Limited data on elemental composition of *T. democratica* and *S. fusiformis* have been published in previous studies (Table 5.5). Carbon and nitrogen content of the solitary *S. fusiformis* in the present study were generally within the range of those for other solitary *S. fusiformis* in the literature.

Based on the present study and the data in the literature I can conclude that, for both life stages of *T. democratica* and *S. fusiformis*, fresh samples that were incised and rinsed with Milli-Q was the best method for carbon-nitrogen elemental analysis; this method produced the most reliable results when compared with the literature and when based on C/N ratios. As highlighted above the nitrogen values in the ammonium formate preparations were high,

producing C/N ratios that were approximately half of those normally recored (~4). Except for *S. fusiformis* (solitaries), the value of Carbon and Nitrogen were a bit lower from the literature (Curl 1962; Le Borgne 1982; Small et al. 1983).

Table 5.5. Comparison of carbon and nitrogen values as percentage of dry weight (DW) from different methods of preparation (literature).

Methods of preparation	Species	Stage	n	DW Carbon	DW Nitrogen	References
Fresh specimens, seawater was removed by gentle suction on a cintered glass filter and the animals were rinsed with freshwater	Thaliaceans			4-10	1-3	Beers 1966; Larson 1986
Fresh specimens were dipped in Bouin's solution and washed 3 times with distilled water	*Ihlea punctata*		6	6.4	1.7	Gorsky et al. 1988
Salps were thawed in the fridge, and each salp was rinsed with distilled water.	*Ihlea racovitzai*				21.5	Dubischar et al. 2012
Freshly collected salps	*S. fusiformis*		5	11.90	2.74	Clarke et al. 1992
**methods were not clarified*	*S. fusiformis*	Sol	6-7	8.2	2.1	Le Borgne 1982
**methods were not clarified*	*S. fusiformis*	Sol		7.8		Curl 1962
**methods were not clarified*	*S. fusiformis*	Sol		3.9	1.0	Small et al. 1983
Preserved in filtered seawater	*Salpa thompsoni* *Salpa thompsoni*	Sol Sol	3 1	1.48 2.25	0.37 0.59	Dubischar and Bathmann 1997
Rinsed with distilled water, blotted on filter paper and kept frozen	*Salpa thompsoni*	Agg		6.02	1.52	Iguchi and Ikeda 2004
Thawed overnight in refrigerator	*Salpa thompsoni*	Agg	50	17.4	3.53	Dubischar et al. 2006
Thawed overnight in refrigerator	*Salpa thompsoni*	Sol	4	22.3	4.93	Dubischar et al. 2006
Frozen salps	*Salpa thompsoni*	Agg		3.69	0.84	Huntley et al. 1989

Specimens were measured within 1 hour of capture, wet weight was determined after removing seawater from the salps. Ammonium formate used	*T. democratica*			5.45	1.430	Parsons et al. 1961; Heron et. al. 1988
**methods were not clarified*	*T. democratica*			1.91	0.67	Le Borgne and Moll, 1986
**methods were not clarified*	*Tethys vagina*		68	18.77-42.68	1.52- 8.09	Henschke et al. 2013

Stable isotopes and the role of salps in the Storm Bay ecosystem

Stable Isotope Analysis (SIA) has enabled ecologists to observe the role of various species in biogeochemical cycles (Aita et al. 2011). Moreover, the structure of food webs and the interactions between organisms in an ecosystem can be determined through variation in carbon and ntrogen isotope ratios (Wada 2009).

In this study, I used isotopic ratios to examine where salps fit in the Storm Bay ecosystem (Fig. 5.5). The isotopic ratios of salps placed them in a separate group to the other grazers in Storm Bay, as depicted by Swadling et al. (2008). Previously, the copepod *Paracalanus indicus* showed an estimated value of $\delta^{15}N$ 1.2 and $\delta^{13}C$ -24.7, while other grazers in the system had $\delta^{15}N$ signatures ranging between 6 and 7 (Swadling et al. 2008). Average $\delta^{15}N$ values of the salps found in this study were 6.09‰ to 9.27‰, indicating that there trophic level was slightly above that of the herbivorous crustaceans. Interestingly, $\delta^{13}C$ values suggest that solitary *T. democratica* were ingesting *Paracalanus indicus* available in the Storm Bay environment; this finding is supported by SEM micrographs that revealed a copepod's body parts in the gut contents of solitary *T. democratica* (Chapter 4).

It is not possible to make firm conclusions about the exact trophic position of the salps, because I was unable to detect nitrogen stable isotopic values in the Storm Bay seawater. Detection ranges in mass spectrometers differ between laboratories and normally the detection ranges are narrower for nitrogen and hydrogen compared to carbon. The present study had an issue with the precision and sensitivity of the analyser available, whereby the minimum value that could be detected by the equipment was above 40 μg nitrogen. Our elemental analyser

(Elementar vario PYRO cube attached to IsoPrime 100) was able to detect carbon in all samples but could not detect the very low nitrogen values present in Storm Bay waters in the summer of 2013/2014.

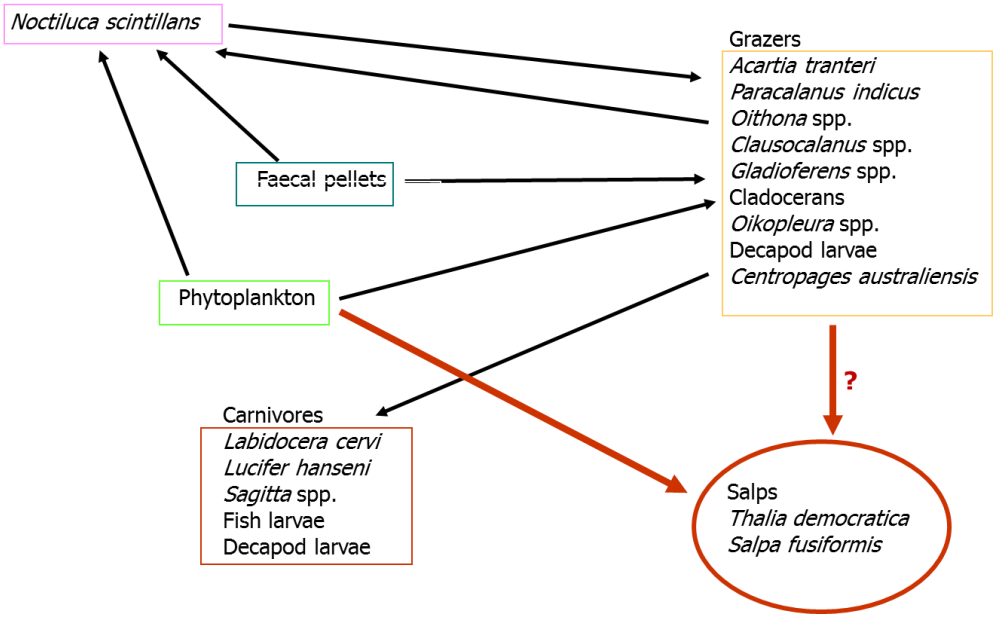


Fig. 5.5. Role of salps in the Storm Bay ecosystem, as based on SIA. Modified from original food web constructed by Swadling et al. (2008).

Nitrogen in Storm Bay, measured in the form of nitrate + nitrite, is very low in the summer months. This is due to uptake by phytoplankton during the spring bloom, but is also a feature of the low nutrient EAC waters that are a feature of Storm Bay in the summer. Subsequent studies must take this into account and ensure that adequate volumes of seawater are filtered for future analyses.

5.6 Conclusion

From this study I learned that different methods of preparation of salps for elemental analysis will produce different results. When compared to previous works, it showed that even a small change in the technique used will contribute to more reliable results. Three methods of sample preparation were compared i) fresh specimens, incised and rinsed with Milli-Q ii) frozen specimens rinsed with Milli-Q iii) fresh specimens rinsed with ammonium formate. I found that the best method for preparing samples was fresh specimens that were incised and rinsed with Milli-Q. This is an important result from this chapter because often small salps were observed with low elemental contents. Therefore, techniques that improve accuracy and yield are to be preferred. In particular, it is advised against using frozen specimens, especially in the case of *T. democratica*, and the use of ammonium formate as a rinsing solution is not advised because of adverse effects on nitrogen analysis. Significantly, this study observed higher values of carbon and nitrogen content in *T. democratica* compared to previous studies. Obtaining accurate measurements is important for assessing the role of salps in trophic webs and their potential value as food sources for higher trophic level organisms.

Once I had clarified the optimal method for preration I undertook a smaller study of the stable isotopic signatures of salps. It was clear that both stages of both species were occupying similar trophic states, though *S. fusiformis* might be occupying a slightly higher level, as it appears that they are ingesting

copepods, at least in small amounts. The evidence to support the finding of copepods in the guts of *S. fusiformis* were recorded in both analyses of SEM and HPLC (astaxanthin) whereas for *T.democratica* (solitary), partly digested copepods were only found in SEM analysis. This result was consistent with direct observations of gut contents of this group (Chapter 4).

CHAPTER 6

CONCLUSIONS

Salps and doliolids (thaliaceans) are important grazers of phytoplankton and are useful indicators of climate change as their physiology is strongly coupled to temperature. Worldwide they are known to form sporadic 'blooms' that appear to relate to periods of increased water temperatures. Under favourable conditions some salps can double their population in 24 hours. In this study I investigated for the first time the dynamic blooms of thaliaceans in Storm Bay, south-east Tasmania, and the environmental conditions influencing them. The dietary habits of salps were determined through their gut content analysis. Finally, their role in pelagic food web was evaluated by interpreting their isotopic compositions.

Through examining abundance and distribution patterns associated with seasonal environmental changes in Storm Bay, the most significant drivers in structuring thaliaceans assemblages were: salinity, followed by the presence of diatoms, seawater temperature, local rainfall events, chlorophyll *a* concentration, *Noctiluca scintillans* and abundance of phytoplankton stocks. According to BEST analysis, of those 7 environmental variables, the top 3 drivers that best explained thaliacean abundance in Storm Bay were salinity, diatom concentrations and temperature. These results are consistent with earlier

studies that hightlight the importance of temperature and phytoplankton concentration in thaliacean blooms. Salps generally prefer to avoid large quantities of diatoms, as their feeding apparatus are adversely affected by high levels on primary production. One important result from Chapter 3 was that doliolids were not exhibiting a similar negative response to diatoms; conversely, they appeared to do well when diatoms were abundant. Differences in feeding modes between salps and doliolids are clearly important and point to the fact that environmental drivers need to be assessed separately for the two groups.

The dietary preferences of salps were further elucidated by examining the contents and pigments of salp guts using both SEM and HPLC. To my knowledge, this is the first time both have been used together to describe salp diets. This resulted in a clear picture of the feeding selectivity of salps in Storm Bay. Thirty-one species of phytoplankton were observed from the imagery, which is significantly greater than previous studies, and in much greater detail. The size range of food particles found in solitary stages of *Thalia democratica* and *Salpa fusiformis* were similar, while a smaller size range of food particles was found in aggregates. The pigments identified suggest that the gut content of the salps contained fucoxanthin, alloxanthin and astaxanthin, thus this confirmed that the salps had predominately fed on diatoms, cryptophytes and green algae.

Carbon and nitrogen elemental and stable isotopic composition were measured to explore further the potential role of salps in the Storm Bay environment. Three methods of preparation were trialled, allowing for comparison. Freshly collected and incised salps rinsed with a small volume of

Milli-Q water provided the best method and is thus recommended for future elemental analysis. *T. democratica* had higher nitrogen and carbon contents than *S. fusiformis*. The solitary phase of *T. democratica* had greater carbon and nitrogen content than aggregates, while solitary *S. fusiformis* had lower carbon and nitrogen content than aggregates. These differences between the life stages of each species warrants further investigation. It might be that the differences were not large enough to be considered significant, or it might relate to some fundamental differences in ecology or physiology that are not yet understood. Not surprisingly, aggregate forms were the dominant stage in Storm Bay so these are the individuals most likely to be preyed upon by fish and other species. The present study does point to the need to consider the life stages separately for any research, e.g. ecosystem modelling, that is attempting to produce realistic carbon budgets for a system.

The three chapters of this thesis combined to produce a strong picture of the dietary and environmental preferences of thaliaceans in Storm Bay, however, there are still many aspects to be examined. For example, nothing is known about feeding rates and how these groups interact with the crustacean plankton that is resident in the bay. The role of the EAC, and how it is influenced by ENSO events, is of fundamental importance to understanding thaliacean dynamics in Storm Bay. In years when the EAC flowed further south and persisted for longer periods, salp blooms were encountered both in the bay and in surrounding coastal sites. However, it is still not understood how the blooms persist in the bay: are they generated purely within the bay over time, or are they replenished

from stocks that are flowing from the north with the EAC? Further, while salps and doliolids were encountered at the same sites and times on occasion, there was generally a spatial or temporal separation in the peak abundances of both groups. Experimental studies designed to examine direct completion between salps and doliolids would be of great interest, though not trivial to undertake in a laboratory setting. Finally for the Storm Bay system there is almost no information on the species that might prey on thaliaceans. This information is important, particularly if blooms are likely to continue to grow in frequency. Though not presented in this thesis, I observed that in 2013/2014 the presence of *T. democratica* persisted throughout the bay and coastal regions well into autumn. The bloom drew media attention and public awareness was markedly higher than earlier summers. Thus it appears that blooms of thaliaceans, particularly salps, are likely to continue and warrant further investigation in Tasmania.

Storm Bay has proven to be useful site for environmental studies, so extending this time series will reap further benefits for understanding the drivers of salps and doliolids, allowing for better predictability of bloom events of thaliaceans.

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