The use of diatoms as biological indicators of water quality, and for environmental reconstruction, in south-east Tasmania, Australia.

by

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Chris Lane

01 / 06 / 05

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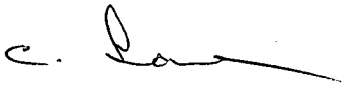

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ABSTRACT

Water quality around the globe has been in serious decline for many decades. To reverse the degradation of waterways there must be a significant improvement in the way the coastal zone is managed. The effective management of the coastal zone requires the ability to effectively monitor and assess changes in water quality, and the ability to identify past, current and potential impacts on water quality. In recent years, water quality monitoring and assessment programs have been significantly improved by the inclusion of biological indicators. Diatoms have been used extensively as biological indicators in water quality monitoring and assessment studies, and in palaeo-environmental reconstruction of water quality, in many areas of the world. This study documents the use of diatoms as biological indicators of water quality, and for environmental reconstruction, in south-east Tasmania, Australia.

The biomass (chlorophyll *a*) of marine benthic algal mats was determined along a depth gradient at two sites within the near-shore marine environment approximately fortnightly for 3 months, to determine whether depth significantly influenced biomass. Average chl *a* levels ranged from approximately 9 to 60 mg/m^2, and varied inconsistently with depth. Physical disturbance of the substrata may account for the greatest variations in biomass observed. Diatoms were found to contribute significantly to the productivity of the near-shore, subtidal marine environment of south-east Tasmania, comprising approximately 95% of the benthic algal community.

Canonical correspondence analysis (CCA) was used to identify causative relationships between the species composition of diatom communities and the corresponding physical and chemical variables from 51 sites within the near-shore, sub-tidal marine zone of south-east Tasmania. The composition of micro-algal communities within these habitats was found to be most strongly influenced by nutrient concentrations. Transfer functions were generated to infer nitrite/nitrate, silicate and sediment size at other sites within the geographic region of the study area. The determination of environmental optima and tolerance ranges for south-east Tasmanian diatom species, and the generation of transfer functions, provides a valuable water quality monitoring and assessment resource for this region.

The environmental history of Pittwater Lagoon, an impacted Ramsar wetland site, was reconstructed from the late 18th century using sediment-core fossil diatoms, ^{210}Pb dating, transfer functions and historical environmental data. A significant change in the diatom flora of the lagoon was found to have occurred during the past 100 years. The future health of south-east Tasmanian coastal ecosystems will depend on the ability of responsible stakeholders and caretakers to incorporate effective biological monitoring and assessment into their management strategies.

Dedication

This research is dedicated to my parents, Peter Alexander Lane and Alice Lane, for their tireless support and encouragement throughout my years of study.

Acknowledgements

There are many people to whom I am deeply grateful for making this work possible. I would firstly like to thank my supervisors, Associate Professor Andrew McMinn and Dr. Donna Roberts, for the opportunity to undertake this research, and for the encouragement, guidance and support that enabled its completion.

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I would especially like to thank each member of my family for their constant emotional and practical support, provided in so many ways during the past few years, and for helping me get through the more difficult times. I would also like to thank Wayne Appleby for his companionship, Andy Jackson for his novel approach to life, and Francis & Debra Fabian for helping me to keep things in perspective. Finally I would like to thank the staff and students from IASOS and the Antarctic CRC for their support and encouragement throughout this research, especially Dr. Kelvin Michael, Lou Trennery and Margaret Hazelwood.

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GENERAL INTRODUCTION

0.1 Monitoring and Assessing Water Quality

Although water is essential to life on earth and is one of our most precious resources, waterways world-wide have been in serious decline for decades, and in some cases centuries (Chrétiennot-Dinet 1998, Gitau 2005). To reverse this situation and maintain the biological integrity of our waterways firstly requires the regular monitoring and assessment of water quality so that impacts from pollutants and changing land-use practices can be determined and quantified.

Until recently, monitoring and assessment of water quality has relied heavily on the comparison of physical and chemical measurements with predetermined guidelines (e.g. Environmental Protection Agency guidelines). These guidelines are generally derived from toxicological testing, and based on levels known to be toxic to biota. However, different results have sometimes been found between laboratory based testing and field experiments, and the lack of toxicity testing on Australian species has resulted in Australian guidelines being largely derived from overseas information (Chapman & Davies 1993, Norris & Norris 1995).

The trend in recent years has been to take a more holistic approach to determining water quality by including biological assessment. Biological assessment of water quality usually involves looking at one or more biological 'indicator' groups for which preferences and tolerance-ranges are known for specific environmental conditions (such as pH, salinity, or nutrient concentrations). Water quality can then be inferred from the presence, absence, abundance and/or composition of key species within this group. Examples of commonly measured biological indicators of water quality include macroinvertebrates (Chessman 1995, Growns *et al.* 1995, Sheldon & Walker 1998, Barton & Metzeling 2004), fish (Cerling 1979, Gehrke & Harris 1994, Pollard 1994, Whitfield & Elliott 2002, Chen *et al.* 2004), and diatoms (Descy 1979, Hodgkiss & Law 1985, Round 1991, Clerk *et al.* 2004, Ramstack *et al.* 2004), among others.

The use of biological indicators to measure water quality is inherently complex. No two water bodies have exactly the same combination of physical and chemical processes occurring within them, and changes in these processes can affect different parts of the biological community in different ways and over different time scales. Additionally, the presence of one biological species can affect the presence or abundance of another (e.g. fish may reduce invertebrate numbers). Therefore, to be effective biological indicators the group of organisms chosen should ideally be found in large numbers and across a wide range of environmental conditions.

For a group of organisms to be effective biological indicators they must be sensitive to biotic and abiotic change, and must respond in a predictable manner so that causal inferences can be made (Reid *et al.* 1995). In addition, biological indicators should effectively show both degradation and recovery rates in water quality, be applicable over a wide geographic region, and be simple to use (Dixit *et al.* 1992). It is also essential that the biology and ecology of the indicator group is well understood. Studied for over 200 years, one of the most effective biological indicator groups currently in use is diatoms.

0.2 Diatoms as Biological Indicators

0.2.1 Diatom Biology & Ecology

Diatoms are single-celled, photosynthetic algae in the class Bacillariophyceae that have opaline silica shells. The exact number of existing diatom species is unknown, but it is estimated that there are at least 12 000 diatom species, contributing 25% of the world's net primary productivity (Werner 1977).

Diatoms are largely cosmopolitan in distribution, and are ubiquitous and abundant in most aquatic environments (Dixit *et al.* 1992, Reid *et al.* 1995, Wu *et al.* 1997). Diatom reproduction is mostly vegetative, occurring through cell division. During the cell division process, a progressive reduction in cell size occurs over successive divisions (Round 1973). After several vegetative reproductive cycles, sexual reproduction occurs and restores cell size to its larger 'original' condition. The doubling time for diatoms ranges from at least 5 to 60 hours depending on taxa, with smaller taxa usually having higher specific growth rates and shorter doubling times (Round 1981).

Diatom species generally exhibit annual growth cycles in response to seasonal changes in environmental variables (Round 1981), with highest diatom numbers usually occurring during the spring (Werner 1977, Jones 1978). However, many diatom species experience a bimodal abundance pattern, with blooms in both spring and autumn (Round 1981, Chessman 1985). Superimposed on the seasonal cycle of diatom growth is the pattern of succession (a relatively precise repetitive pattern to the seasonal occurrence of species: (Round 1981)). Although the effects of season and succession may result in one or two diatom species experiencing peak growth at any point in time, the dominant diatom species in a system are always present (May 1988, Round 1991).

Many diatom species exhibit clear habitat preferences, and diatom communities are generally defined according to the habitat type they occupy. Within aquatic environments diatoms occupy a variety of natural habitat types, which can be broadly defined as follows:

- epilithon (rock surfaces);
- epiphyton (surface of larger plants);
- epipelon (mud and silt);
- epipsammon (sand);
- euplankton (open water environment); and
- epizoic (attached to animals)

The preference of diatom species for a particular habitat type can greatly affect the characteristics of their immediate environment. For example, euplanktonic taxa may rely heavily on nutrients in the water column, while some benthic taxa can obtain nutrients from the sediments (Werner 1977, Hudson & Bourget 1981, Stevenson *et al.* 1985).

Research has shown that diatoms respond sensitively to changes in many environmental variables, including light (Bothwell *et al.* 1989, O'Donohue & Dennison 1997), moisture (Verleyen *et al.* 2003), temperature (Vyverman & Sabbe 1995, Bloom *et al.* 2003), currents (Reisen & Spencer 1970, Munteanu & Maly 1981, Stevenson 1983), salinity (Cumming & Smol 1993, Gasse *et al.* 1997), pH (Stevenson *et al.* 1989, Dixit *et al.*

1991, Dixit *et al.* 1993), organic and inorganic carbon (Pienitz & Smol 1993), phosphorus (Agbeti 1992, Yang & Dickman 1993), nitrogen (Evans & Marcan 1976, Blanco *et al.* 2004), and silicate (Kilham 1971, Egge & Aksnes 1992, Takano & Hino 1996, Wu & Chou 2003).

0.2.2 Effectiveness of Diatoms as Biological Indicators

There are several reasons why diatoms are particularly suited for use as biological indicators. Diatoms are widespread and abundant in most aquatic environments, diatom taxonomy is well established, the ecological tolerances and optima for many species are clearly defined, and diatoms are sensitive to changes in water chemistry (van Dam *et al.* 1981, Reid *et al.* 1995). Because diatoms have a short cell cycle and colonise rapidly, the composition of diatom communities responds quickly to changes in water quality (Fairchild & Lowe 1984, Guzkowska & Gasse 1990, Dixit *et al.* 1992, Reid *et al.* 1995). Many diatom taxa have the ability to attach themselves to substrata by gelatinous pads or stalks (Werner 1977), and are therefore indicators of the immediate surrounding environment. The attached habit of many diatom species also makes them relatively easy to sample.

The opaline silica structure of diatom cells (frustules) means that in some aquatic sediments (such as lakes) diatom fossils are often well preserved and can therefore be used to reconstruct the environmental history of an aquatic ecosystem (Agbeti 1992, Roberts & McMinn 1998). Diatoms have been used extensively to infer pH, total phosphorus, salinity, and saprobity of palaeo-environments (Bennion 1994, Gell 1997, Wu *et al.* 1997, Rott *et al.* 1998). Diatoms have also been used in both neo- and palaeolimnological studies to detect changes such as eutrophication, acidification, UV and climate change (Berge 1979, van Dam *et al.* 1981, Charles 1985, Niederhauser & Schanz 1993, McMinn & Heijneis 1994, Gell 1997, Wu *et al.* 1997, Crosta *et al.* 1998, Rott *et al.* 1998, Chivas *et al.* 2001). Their characteristics and rapid response time to environmental change make them ideal for water quality research.

0.2.3 Relating Diatoms to Water Quality

Using diatoms as water quality indicators involves relating the diatom community composition of a water body to the corresponding physical and chemical variables.

Quantitative data is gathered and statistically analysed to determine the ecological tolerances and optima of diatom species for certain physico-chemical variables along an environmental gradient (Dixit *et al.* 1992). The species composition of the diatom assemblage (rather than a single dominant species) is then used to interpret water quality (use of a single dominant species may be misleading if it has a wide tolerance range).

Although the ecological tolerances and optima for many diatom species is considered universal, water quality assessments within a given geographic region require knowledge of local indicator species (John 1993). Some diatom species in the southern hemisphere do appear to have different ecological tolerances to their northern hemisphere counterparts (Round 1991), and there is evidence of endemic species in Australia (Thomas 1988, Cameron *et al.* 1993, Haworth & Tyler 1993, Vyverman *et al.* 1997). Other researchers have also highlighted the increased accuracy obtained by using regional flora for water quality assessments (Charles 1985, Wu *et al.* 1997).

0.2.4 Diatom Research in Tasmania

Diatoms have been used extensively around the globe for water quality monitoring and assessment studies (Hendey 1977, Lange-Bertalot 1979, Prygiel & Coste 1993), and for palaeo-environmental reconstruction (Flower 1986, Gronlund 1993, Ng & Sin 2003). However, diatom research in Tasmania has mainly focused on freshwater species, and there is very little published work on Tasmanian marine diatoms. Palaeo-environmental reconstruction of Tasmanian freshwater lakes using diatoms is reported by (Cameron *et al.* 1993, Hodgson *et al.* 1996a, Hodgson *et al.* 1998). A reference data set of diatom flora from Tasmanian lakes for environmental reconstruction has also been created (Vyverman *et al.* 1995). Other diatom research in Tasmania has also focused on freshwater species (Croome & A. 1986, Haworth & Tyler 1993, Vyverman *et al.* 1996, Vyverman *et al.* 1997). However, estuarine planktonic diatoms from Storm Bay, Tasmania were included in the work by (Crosby & Wood 1958); estuarine diatoms from the Gordon River estuary were included in the work by (Hodgson *et al.* 1996b); and diatom analysis of late Holocene sediment cores from Macquarie Harbour is reported by (McMinn *et al.* 2003). The marine diatom flora of Tasmania's coastal environment is researched here to investigate relationships between species assemblages and environmental variables .

0.3 The Study Area: South East Tasmania

0.3.1 Geography

The diatom research reported in the following pages was undertaken in the coastal zone of southeast Tasmania, Australia (Figure 1). Within the research area are Tasmania's capital city Hobart, numerous smaller towns and villages, and various commercial activities including aquaculture and forestry. The environmental characteristics of the study area are discussed in more detail below.

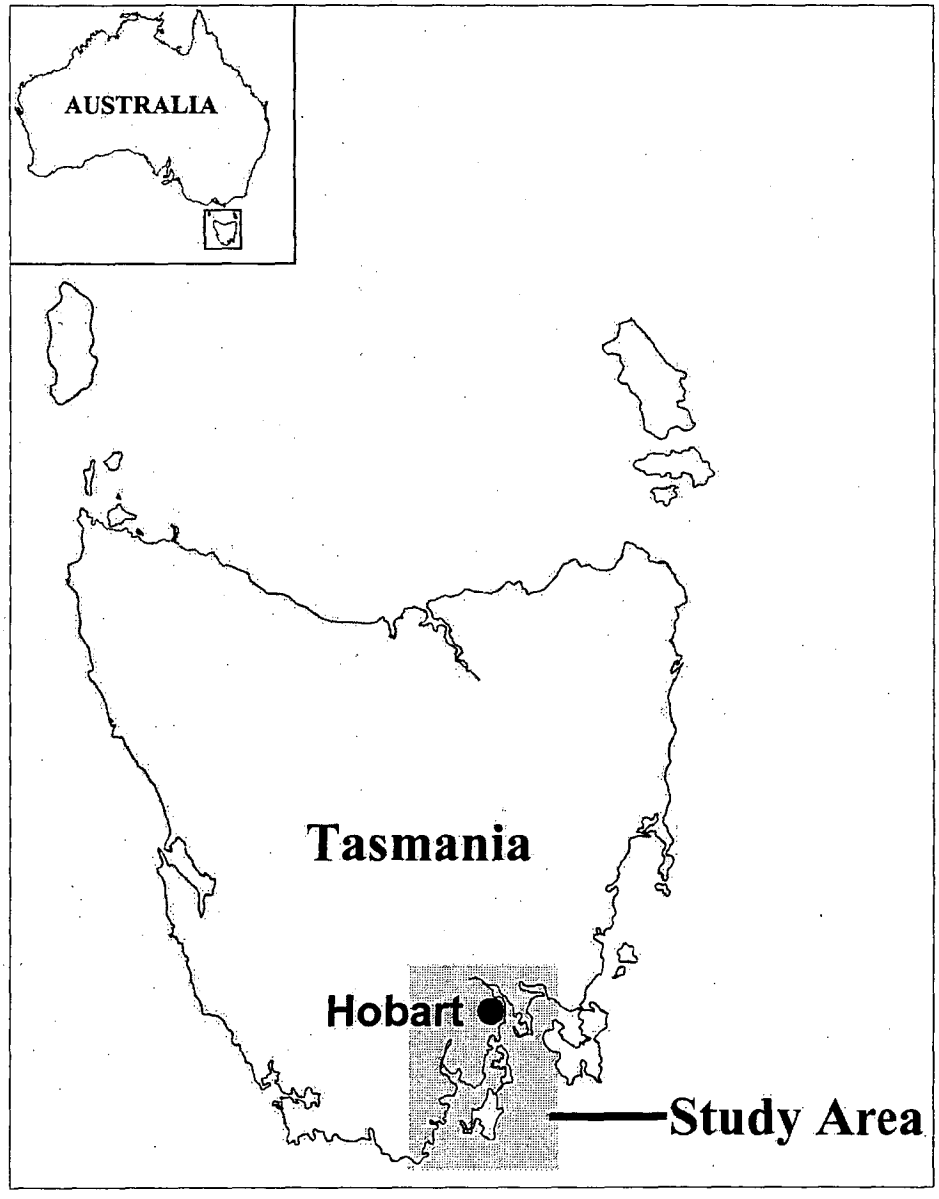


Figure 1: Location of Study Area

0.3.2 Population

Tasmania has a population of approximately 476 000 (March 2003. Source: Australian Bureau of Statistics) with approximately 40% of the population living in the capital, Hobart.

0.3.3 Climate and Hydrology

Tasmania has a predominantly temperate maritime climate, with marked variations of cloudiness, temperature and rainfall due to the prevailing westerly air stream. The east coast experiences milder, drier and sunnier conditions than the generally cool, wet and cloudy west coast. Close to the coast, the daily temperature range is approximately 7°C. The long-term (25yr) average temperature in Hobart ranges from 5.7 - 12.9°C (min. – max.) in winter to 13.3 – 23.2°C in summer. Extremes of temperature recorded for Hobart are –2.8°C and 40.8°C respectively (in 1972 and 1976). Mean annual rainfall within the study area (Hobart) ranges from approximately 495 mm to 576 mm year, and is relatively uniform throughout the year (Australian Bureau of Statistics). The size and flow of water bodies in Tasmania's south-east region is relatively small compared to other regions of Tasmania (DPIWE 2003).

0.3.4 Geology

The geology of the south-east region of Tasmania is complex. It is dominated by Cambrian basalts (and other igneous rocks), Permian to Late Carboniferous undifferentiated glacial, glacio-marine, and non-marine sedimentary rocks (mostly sandstones and mudstones), and Jurassic dolerites with locally developed granophyre (Geoscience-Australia 2004). Along the foreshore, coastal landforms vary considerably and include steep buffs and sea cliffs, rocky shorelines and platforms, sandy and muddy intertidal flats, sandy and pebble beaches (Green & Coughanowr 2003).

0.3.5 Land Use

The Derwent Estuary catchment area of south-east Tasmania comprises approximately one-fifth of Tasmania's land mass, covering an area of approximately 8.900 km^2, and includes the Derwent River catchment, the Jordan River catchment and other areas adjacent to the estuary (Green & Coughanowr 2003). Urban and industrial development occupies less than 1% of the total land area. Approximately 67% of the land area is

covered by forest, scrub and heath; with most of the remaining land being used for agriculture (27%, mostly sheep and cattle grazing) and water storages (3%) (Green & Coughanowr 2003).

0.4 Aims and Objectives of the Research

The future health of Tasmania's waterways (and its associated industries) relies on the effective interpretation and management of water quality conditions within those waterways. This research aims to investigate the use of diatoms for biological monitoring and assessment of water quality, and environmental reconstruction, in the near-shore marine environment of southeast Tasmania.

The objectives of the research are to:

1. Determine whether depth significantly influences the biomass of marine benthic algal mats along a depth gradient at two sites within the near-shore, sub-tidal marine environment of south-east Tasmania;
2. From a diverse range of sites within the south-east Tasmanian near-shore marine environment:
   (i) Identify causative relationships between the species composition of the diatom community and the corresponding physical and chemical water conditions;
   (ii) Develop a transfer function to infer palaeo-environmental conditions at other sites within the study area; and
3. Reconstruct the environmental history of Pittwater Lagoon (part of a listed Ramsar wetland site within the study area) since the late eighteenth-century using sediment-core fossil diatoms;

These objectives are addressed in the following three chapters.

CHAPTER 1: Biomass of Benthic Algae at Tinderbox Marine Reserve and Conningham Beach

1.1 INTRODUCTION

1.1.1 Marine Benthic Algal Mats

Marine benthic algal mats play an important role in coastal ecosystems, contributing significantly to the productivity of coastal areas and influencing sediment transport and erosion patterns (Grant *et al.* 1986, Oppenheim 1988, MacIntyre *et al.* 1996, Austen *et al.* 1999). Benthic algal mats in marine environments are usually dominated by diatoms, although mats dominated by cyanobacteria are also common in extreme environments (de Jonge 1985, Sundbäck & Snoeijs 1991a, Sundbäck *et al.* 1997). However, relatively few studies have been published on benthic algal mats in sub-tidal, near-shore marine environments, and little is known about the productivity of such mats (Cahoon *et al.* 1993).

The near-shore marine environment is often a zone of relatively high energy subject to frequent physical disturbance. As a result of physical disturbance, some benthic algae are resuspended and can constitute a significant proportion of the water-column algal population (Lukatelich & McComb 1986, Bloesch 1995, MacIntyre *et al.* 1996). Resuspended algae provide an important food source for filter-feeders (such as oysters). Benthic algal mats also provide an important food source for many other organisms, including deposit-feeders (such as *Hydrobia ulvae*: (Austen *et al.* 1999)). The biomass of benthic algal mats can greatly exceed the biomass of the entire water column above, even at depths of 200 m (Lukatelich & McComb 1986, Cahoon *et al.* 1990). Benthic algal mats are therefore an important component of the productivity and health of the aquatic system as a whole.

Sediment transport and erosion patterns in the inter-tidal and sub-tidal coastal zones are affected by the presence of benthic algal mats. Benthic diatoms increase the resistance of the sediment surface to erosion by the production of extracellular polysaccharides formed during locomotion and as a means for attaching themselves to substrata (Werner

1977, Austen *et al.* 1999). The presence of this extracellular film also affects the transfer of organic matter between the sediment and water column (Grant *et al.* 1986). Benthic algal mats therefore alter the near-shore marine environment physically, chemically and biologically.

The relationship between benthic algal biomass and water depth in near-shore marine environments is not well established, but must be considered in the study-design of regional calibration sets. Research has shown that benthic algal biomass is reduced by physical disturbance of the substrate (Stevenson & Stoermer 1981, Delgado *et al.* 1991), and it is well recognised that diatoms respond to changes in light (Reynolds 1973, Werner 1977, Round 1981). However, with increasing water depth, physical disturbance is generally reduced, as is photosynthetically available radiation (PAR). Within the near-shore zone (less than 10 m water depth), where the PAR may not be such a limiting factor, the level of physical disturbance may play a more important role.

1.1.2 Study Sites

Tasmania has approximately 5 400 km of coastline, and is situated in a cool temperate climate zone. The coastline is afforded some protection by the surrounding islands (particularly in the southeast), and consists of many bays, cliffs and beaches. The two benthic mat study sites, Tinderbox Marine Reserve (Plate 1.1) and Conningham Beach (Plate 1.2) were chosen as being representative of natural aquatic conditions in Tasmania's south-east marine environment (Figure 1.1) as they are relatively un-impacted by anthropogenic activity. Their waters are protected to a large extent by Bruny Island, and water temperatures in the local area range from approximately 8°C in winter to 20°C in summer (DPIWE 2003).

1.1.2.1 Tinderbox Marine Reserve

Tinderbox was declared a marine reserve in 1991, is one of only 5 marine protected areas in Tasmania, and at 45 ha is also the smallest. Tinderbox beach is a sandy beach bordered by sandstone cliffs. Tinderbox Reserve marine flora and fauna include over 30 species of seaweeds, seagrass, sea dragons and sea horses, bryozoans, filter-feeders, sponges, ascidians, anemones, hydroids, squid and fish. The reserve is regularly used by

divers and snorklers, and a boat ramp provides access to the area outside the reserve.

Plate 1.1: Tinderbox Marine Reserve

Plate 1.2: Conningham Beach

1.1.2.2 Conningham Beach

Conningham Beach (Plate 1.2) is located a short distance W-SW of Tinderbox Marine Reserve in a protected embayment (North West Bay). It is a gently sloping sandy beach, bordered by sandstone cliffs. Although the marine flora and fauna at Conningham Beach is not recognised as being as diverse as Tinderbox Marine Reserve, a wide variety of fish, crabs, squid, urchins and sea stars were observed during sampling dives. A boat ramp at one end of Conningham Beach is regularly used by locals, and the beach by locals and residents of an adjoining caravan park. The relatively cool coastal waters of Tasmania reduce the extent to which the beach is used by swimmers.

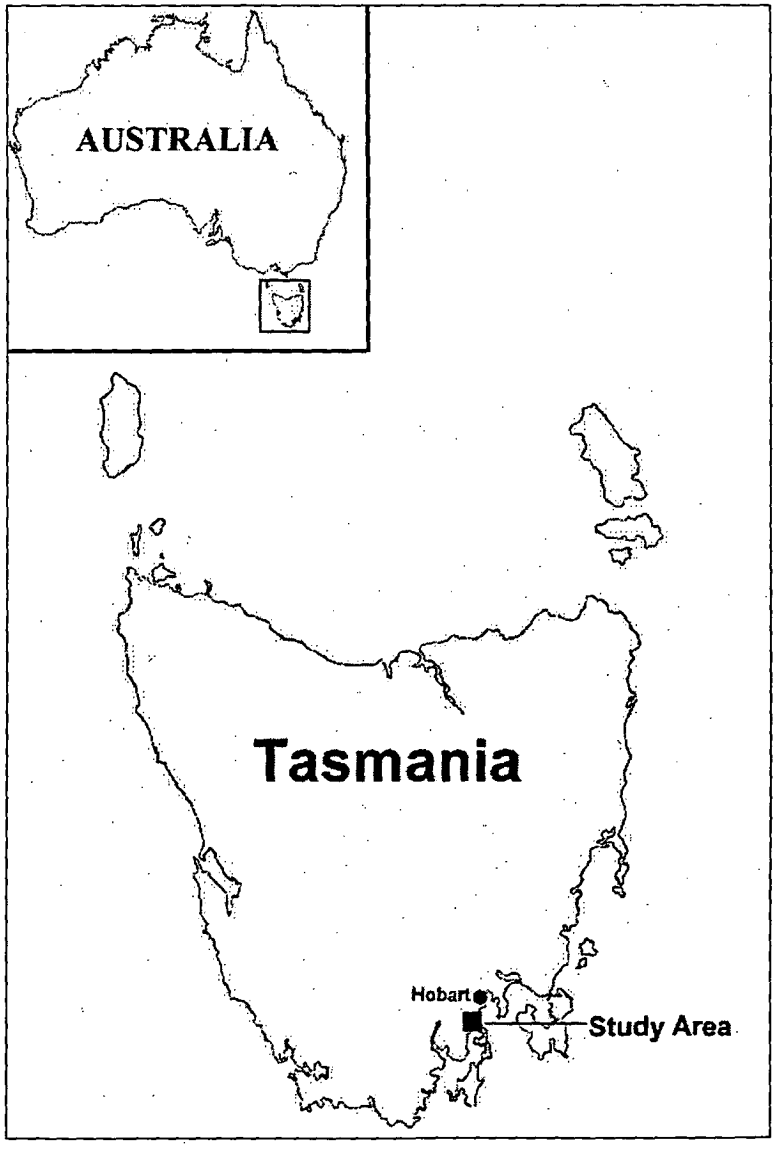


Figure 1.1: Location of Benthic Algal Mat Study Area

1.1.3 Aims and Objectives

Algal biomass measurements provide valuable ecological data on the productivity of aquatic ecosystems, and provide a means for comparing productivity between sites both locally and globally. This study aims to determine the biomass of marine benthic algal mats along a depth gradient at two near-shore, sub-tidal marine sites in southeast Tasmania, Australia.

The specific objectives of the Benthic Algal Mat study are:

- Measure benthic algal biomass along a depth transect at Tinderbox Marine Reserve and Conningham Beach;
- Compare and contrast changes in algal biomass at each site to temporal variation, depth, temperature, and nutrients;
- Compare and contrast the results obtained from spectrophotometry with those obtained from fluorometry; and
- Relate the above findings to research reported in the literature.

1.2 METHODS

Sampling was undertaken along two transects in the northern section of the D'Entrecasteaux Channel, Tasmania (Figure 1.2) on Jan 30, Feb 13, Feb 27, Mar 20, Apr 03 and Apr 19, 2001. Sampling was thus conducted during both summer and autumn, providing contrasting environmental conditions. The sampling transects were located at Tinderbox Marine Reserve (Transect 1), and Conningham Beach (Transect 2), and were oriented due South and 35°E respectively. Sampling at Tinderbox Marine Reserve was conducted under DPIWE Permit no. 1076.

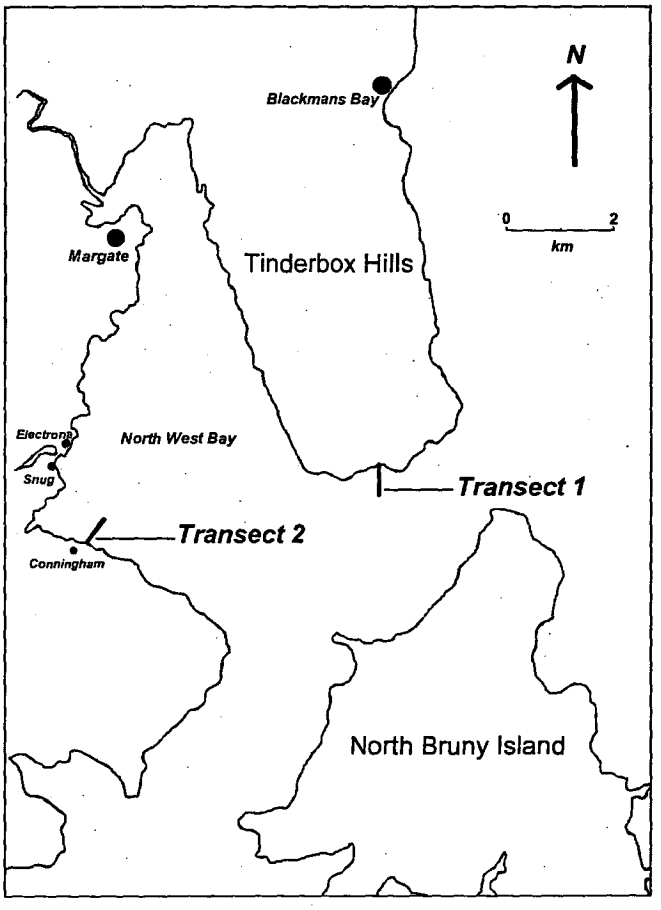


Figure 1.2: Location of sampling transects

At each transect, sampling was undertaken at water depths of approximately 1, 2, 3, 4 and 5 metres mean spring low tide (MSLT) using SCUBA equipment. Depth was measured using a depth gauge attached to the SCUBA equipment, and adjusted relative to fixed starting point noted at low tide prior to the first sampling occasion (a submerged rock outcrop). Underwater, the transect bearing was followed using a compass.

1.2.1 Water Chemistry

During each sampling dive, measurements were taken to determine water temperature, salinity and nutrient levels. Water temperature for each transect was measured at 3 m water depth using a standard mercury thermometer. A 1 litre water sample was collected from 3 m water depth to measure salinity (back on shore) using a ProfiLine Conductivity Meter (Model: TA 197). Water samples for nutrient analysis were collected from the 3 m sampling site by using 50 ml syringes to collect a sample from both 1 cm and 1 m above the sea floor. Nutrient samples were processed at UTAS laboratories using an ALPKEM Auto Analyser, following the ALPKEM Methodology Manual (1992). Samples were analysed for nitrite + nitrate (NO_{2-3}), phosphate (PO_4) and silicate (SiO_2) concentrations.

1.2.2 Algal Mat Sampling

Benthic algal mats generally have a patchy distribution on the ocean floor (Grant *et al.* 1986, Delgado *et al.* 1991). The decision was therefore made to sample the visually thickest algal mats at each sampling depth, so that measured biomass represents approximately maximum levels. Four samples were collected at each depth: three to determine chlorophyll levels and one to inspect the algal flora (i.e. to ascertain whether the algal mats were composed primarily of diatoms). All samples were collected using sterile 70 ml specimen containers modified for minimal disturbance to the algal mats (Figure 1.3).

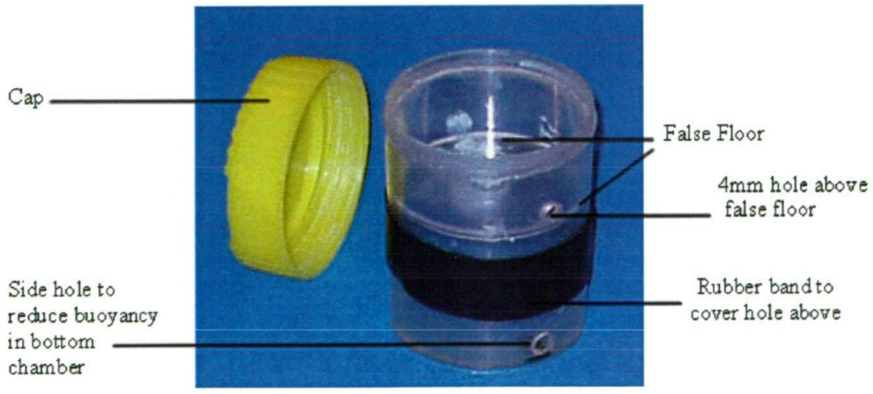


Figure 1.1: Sampling container design

1.2.2.1 Sampling Container Design

The 'false floor' of the sampling container was watertight, and constructed by cutting 1 cm from the base of a 100 ml specimen container, and gluing this section into the top of the 70 ml specimen container (Figure 1.3). A 4 mm hole was drilled through the side (of both containers) immediately above the false floor. Two separate holes were also drilled through the side of the 70 ml container immediately above the original base so that water could freely flow into the bottom of the container, making it less buoyant under the water. The rubber band was cut from a bicycle tube (Figure 1.3).

Prior to sampling, the rubber band was moved down so that the 4 mm hole was exposed. The sampling container was inverted and very gently placed over the algal mat, then gently pushed into the sediment up to the bottom of the hole. While the sampling container was still inserted in the sediment, the rubber band was gently pulled down to cover the hole and effectively seal the container. This process allowed the water overlying the algal mat to escape through the hole as the container was inserted into the sediment (rather than being forced down through the algal mat and sediment below), thereby ensuring disturbance of the algal mat was minimal. The sampling container design ensured a uniform thickness of sediment (1 cm) was obtained for each sample.

A thin, flat steel blade was used to slice through the sediment immediately below the container, and held against the container to seal it while it was removed from the sediment. The container was then turned upright so that the blade was on top. The plastic cap was positioned above the blade so that as the blade was slid out sideways the cap was correctly positioned over the screw thread of the container. The cap was firmly tightened, and the container placed in a mesh diving bag. As samples taken for chlorophyll *a* analyses should be protected from light and warmth at all times during collection and filtration (Wright & Mantoura 1997), upon arriving back on shore all samples were immediately stored in the dark on ice until further processing at UTAS laboratories.

1.2.3 Chlorophyll *a* Analysis

Rather than counting cell numbers, algal biomass is usually reported as a measurement

of the photosynthetic pigment, chlorophyll *a* (Sundbäck & Granéli 1988, Fairchild & Sherman 1992, Peletier 1996, Gibbs 2000). Chlorophyll *a* can be measured using chromatography, spectrophotometry, or fluorometry. The most accurate technique for measuring chlorophyll *a* is High Performance Liquid Chromatography (HPLC) (Mantoura *et al.* 1997a). However, this is also the most expensive of the three methods. Consequently, spectrophotometry and fluorometry methods are commonly employed for the analyses of algal biomass (MacIntyre *et al.* 1996). Fluorometry is the most sensitive but least accurate method (Wright & Mantoura 1997). However, whether chromatography, spectrophotometry, or fluorometry methods are used, the sampling procedure is identical. The samples from Tinderbox Marine Reserve and Conningham Beach were analysed using both spectrophotometry and fluorometry to determine algal mat biomass.

Samples taken for chlorophyll *a* analysis were processed at UTAS laboratories within 4 hours of sampling. Although samples should be extracted as soon as possible, they can be stored in the dark and frozen at -90°C for at least 60 days or at -20°C for less than a week without substantial loss of pigment (Mantoura *et al.* 1997b). All chlorophyll processing was done under low light conditions, with samples being stored in the dark as much as possible. The presence of water can reduce the accuracy of measurements (Porra *et al.* 1989, Jeffrey & Welschmeyer 1997) and was therefore minimised in the final filtered sample. To remove excess water from the sample, filtered seawater was used to wash the entire sample from the original sampling container onto a Whatman GFC filter placed in a vacuum pump. Scanning electron microscopy has shown that combusted GF filters are as efficient as 0.2 µm membrane filters for pigment retention (Nayar & Chou 2003). The sample was filtered under a low vacuum to avoid cell damage (Wright & Mantoura 1997).

The filter and all sediment from the sample were then transferred to a sterile 100 ml container and 60 ml of methanol was added. The sample was shaken vigorously, and stored in a fridge (4°C) for 18 to 24 hours. Although soaking in solvent is the most common chlorophyll extraction technique, grinding and sonication are also common. Sonication in methanol has been strongly recommended for routine field samples, as soaking without mechanical disruption results in recovery which is low and variable

(Wright *et al.* 1997). After overnight storage, the sample was sonicated for 5 minutes to disrupt any remaining resistant cells, then shaken to homogenise the solution. After allowing approximately 5 minutes for settling of the sediment, two 10 ml aliquots were extracted and centrifuged at 2000 rpm for 5 minutes to remove debris from the solution. Solution from one of the 10 ml aliquots was measured for chlorophyll *a* using spectrophotometry, and the other aliquot was measured using fluorometry.

Spectrophotometric readings were taken for wavelengths of 750, 664, 647, and 630 nm, on a Shimatsu 1201 spectrophotometer. Fluorometer readings were undertaken on a Turner 10AU fluorometer. For each sample, after the initial reading was taken 2 drops of 5% hydrochloric acid (HCl) were added to the 10 ml vial of extracted sample. The vial was then inverted 3 times to homogenise the mixture. A second reading was then taken to determine phaeophytin (at 664 nm for the spectrophotometer). Chlorophyll *a* was calculated as the difference between the readings before and after acidification.

1.2.4 Determining the Dominant Algal Flora

On each sampling date, one benthic algal sample from each depth was inspected to determine the dominant algal flora (i.e. to determine whether the majority of the cells were diatoms). Each sample was rinsed into a 250 ml beaker using approximately 50 ml filtered sea water, and stirred 20 times in each direction to dislodge the majority of the algae from the sediment. After allowing approximately 5 seconds for the heavier sediment to settle out, the stirred solution was poured off into a clean beaker, leaving most of the sediment behind. This process was then repeated twice, collecting the stirred solution (approx. 150 ml) in a single beaker. At least two permanent microslides of live material were made from each sample by pippetting 500 µm of the collected solution onto a glass microslide cover-slip and drying for several hours at approximately 60°C on a hotplate. Cover slips were mounted on glass microslides using the mounting medium Naphrax (refractive index 1.72). Microslides were inspected under a Zeiss Standard 20 light microscope at 1000x oil immersion magnification.

1.3 RESULTS

1.3.1 Physical and Chemical Results

1.3.1.1 Temperature & Salinity

Throughout the study period, measured temperature varied by 4.7°C at Tinderbox Marine Reserve and 5.7°C at Conningham Beach, however salinity remained relatively constant. Temperature and salinity results for each sampling date are listed in Table 1.1 below. Salinity measurements are provided hereafter in PSU units.

Table 1.1: Temperature and Salinity

	Tinderbox Marine Reserve		Conningham Beach	
Sampling Date	Temperature (°C)	Salinity	Temperature (°C)	Salinity
30/1/01	18.0	33.7	18.0	33.7
13/2/01	19.6	33.0	20.4	33.8
27/2/01	17.4	34.0	18.8	34.2
20/3/01	18.1	34.0	18.9	34.2
03/4/01	18.2	33.9	17.9	33.9
19/4/01	14.9	33.9	14.7	33.9

1.3.1.2 Nutrients

Nutrient concentrations in 1 cm and 1 m samples taken on the same sampling date were generally very similar. Nutrient levels for both depths are listed below in Table 1.2 for Tinderbox Marine Reserve and Table 1.3 for Conningham Beach.

Table 1.2: Nutrient levels from Tinderbox Marine Reserve

	NO_{2-3} (µmol/L)		SiO_2 (µmol/L)		PO_4 (µmol/L)	
Date	1 cm	1 m	1 cm	1 m	1 cm	1 m
30/1/01	0.05	0.02	1.03	0.94	0.16	0.13
13/2/01	0.08	0.06	3.89	3.86	0.22	0.20
27/2/01	0.45	0.45	4.30	4.18	0.26	0.24
20/3/01	0.04	0.03	0.12	0.08	0.13	0.11
3/4/01	0.04	0.03	0.43	0.40	0.16	0.12
19/4/01	0.05	0.03	2.75	2.68	0.22	0.17

Table 1.3: Nutrient levels from Conningham Beach

	NO_{2-3} (µmol/L)		SiO_2 (µmol/L)		PO_4 (µmol/L)	
Date	1 cm	1 m	1 cm	1 m	1 cm	1 m
30/1/01	0.04	0.03	0.87	0.79	0.13	0.16
13/2/01	0.10	0.10	3.79	2.77	0.31	0.16
27/2/01	0.06	0.04	0.28	0.10	0.09	0.10
20/3/01	0.04	0.03	0.64	0.60	0.16	0.14
3/4/01	0.05	0.04	4.17	4.07	0.22	0.24
19/4/01	0.17	0.20	3.27	3.84	0.22	0.22

Analyses of samples from both sites show little variation between nutrient concentrations at 1 cm and 1 m height above the benthos for NO_{2-3} ($R^2 = 0.988$) or SiO_2 ($r^2 = 0.958$). Phosphate levels vary slightly more between the two depths, the lower correlation found ($R^2 = 0.463$) mainly due to a difference at Conningham Beach on the second sampling date. PO_4 and NO_{2-3} concentrations did not vary markedly over the study period, with the exception of NO_{2-3} levels at Tinderbox in late February. However, at both sites considerable variation occurred between silicate levels on different sampling dates (e.g. >50 fold for Tinderbox 1 m between late February and late March).

1.3.2 Chlorophyll *a* Results

Throughout the sampling period, chlorophyll *a* levels from each depth ranged between 7.3 and 62.7 mg/m^2. Chlorophyll *a* was measured using both spectrophotometry and fluorometry. Results from each method were very similar. The correlation between results from these two methods is shown in Figure 1.4 below.

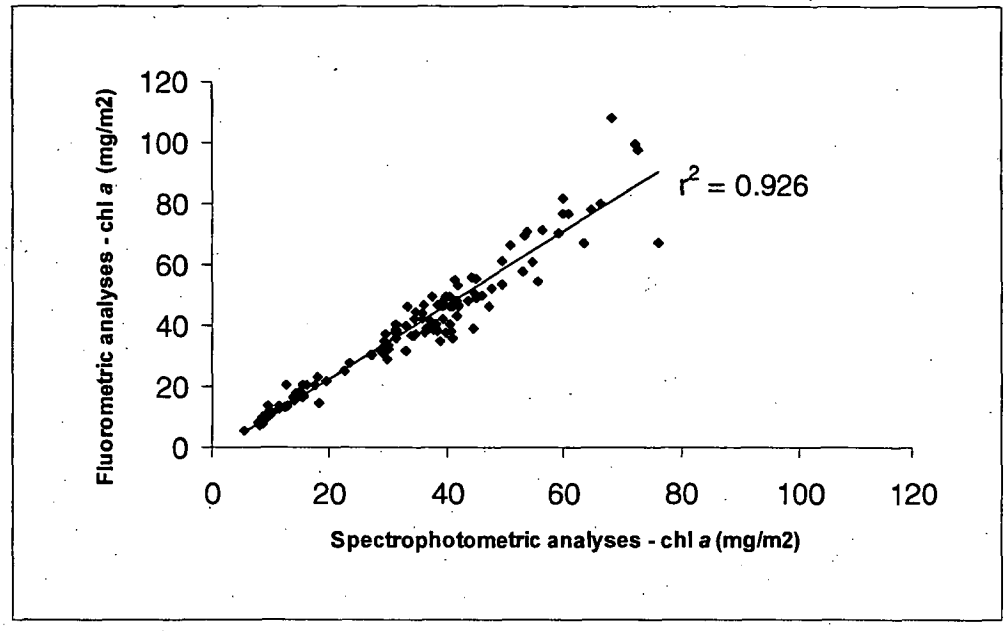


Figure 1.4: Comparison of fluorometric and spectrophotometric results

Although fluorometric and spectrophotometric results were very similar, the fluorometry method on average provided a slightly higher reading. The difference between readings for the two methods also appeared to increase with higher chl *a* levels. These results are shown in the following figure (Figure 1.5).

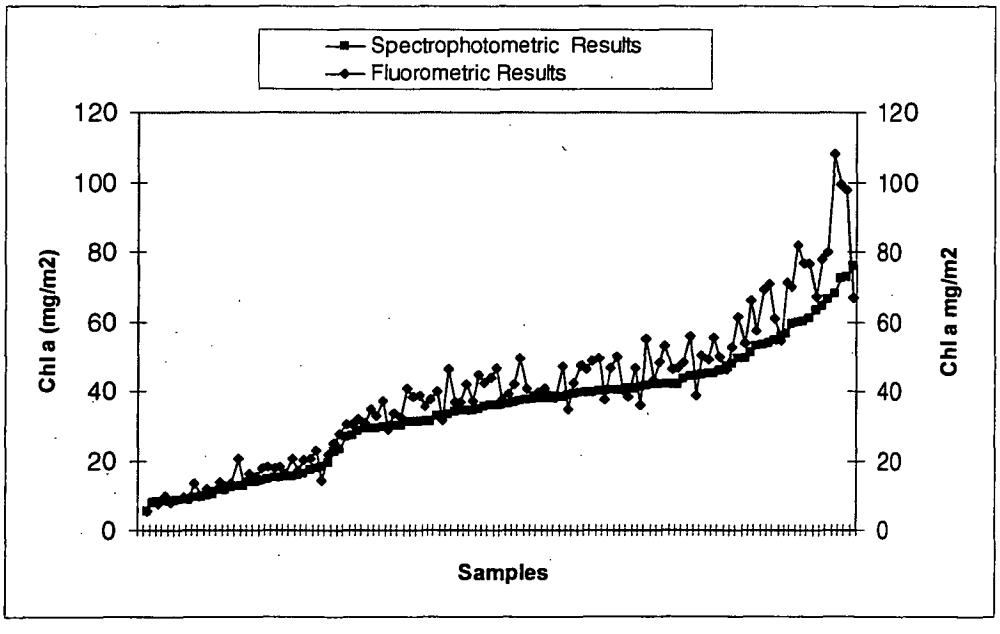


Figure 1.5: Fluorometry results vs. Spectrophotometry results

From here on only the spectrophotometric data is presented, given the close relationship between the two methods ($r^2 = 0.93$), and that fluorometry is generally less accurate (Wright & Mantoura (1997), but the full data set is presented in Table 1.1 as Appendix 1. On each sampling occasion, the average chlorophyll *a* level at each site (all samples averaged) was very similar, but varied over the sampling period (Figure 1.6).

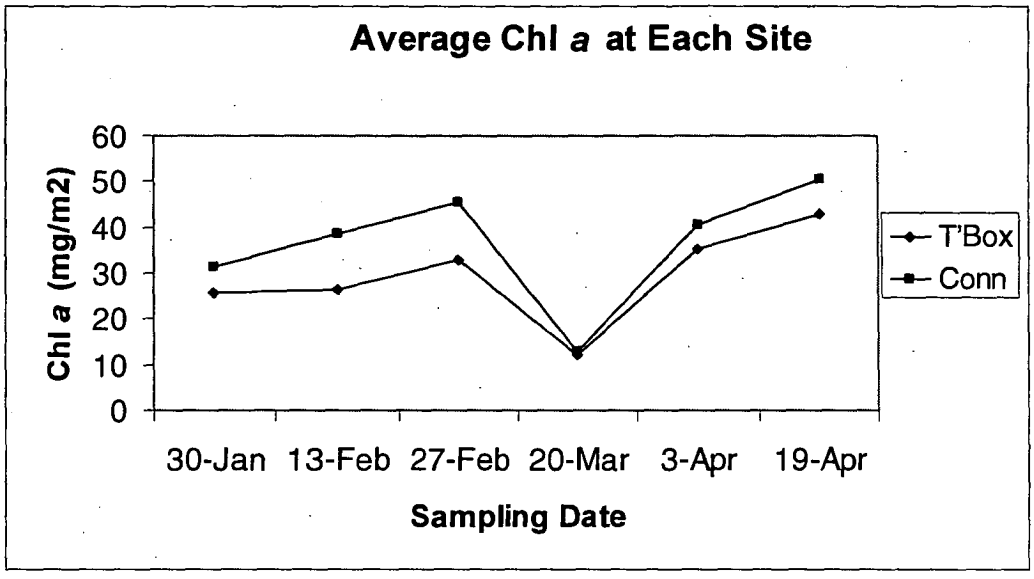


Figure 1.6: Average chlorophyll *a* levels for each site over the sampling period

1.3.3 Algal Biomass and Environmental Variables

No clear trend was discernible between chlorophyll *a* levels and water depth at either site. Chlorophyll *a* levels varied more temporally than spatially. The relationship between chlorophyll *a* levels and depth at each site is shown in Figure 1.7 for Tinderbox Marine Reserve, and Figure 1.8 for Conningham Beach.

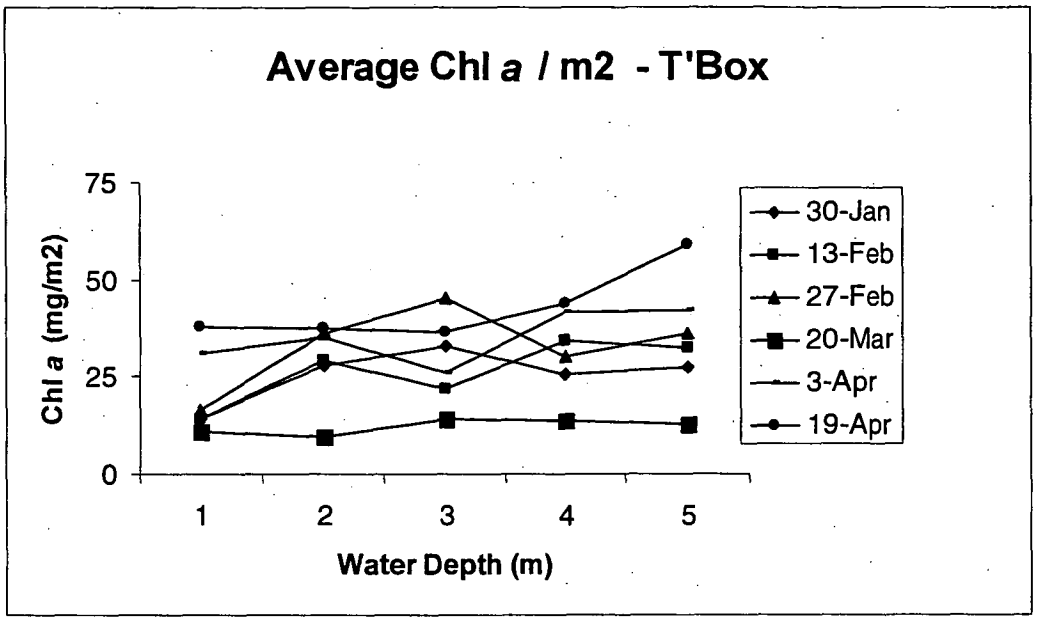


Figure 1.7: Chlorophyll *a* levels for each depth at Tinderbox Marine Reserve

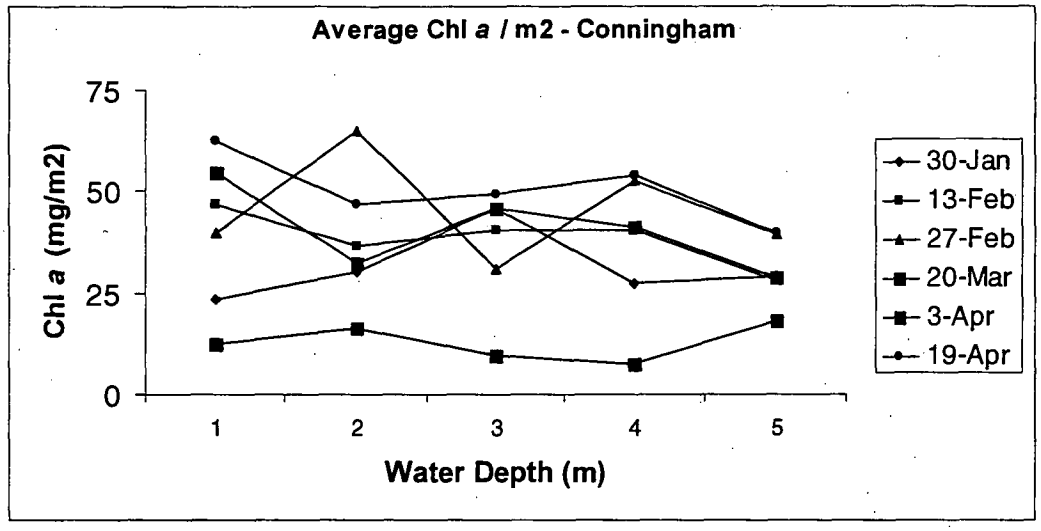


Figure 1.8: Chlorophyll *a* levels for each depth at Conningham Beach

Algal biomass was not strongly correlated with any of the nutrient concentrations (r^2 was < 0.38 for each nutrient at each site on each sampling date). NO_{2-3} and PO_4 did not vary greatly during the study period, however silicate varied considerably and with different temporal trends at each site. The temporal variation in SiO_2 and the corresponding algal biomass at each site is shown in Figure 1.9 and 1.10.

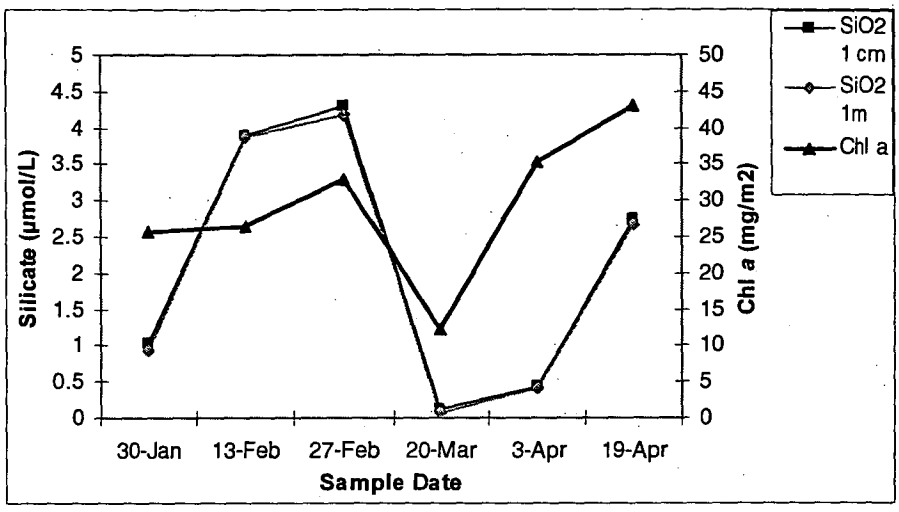


Figure 1.9: Silicate and Chl *a* levels at Tinderbox Marine Reserve

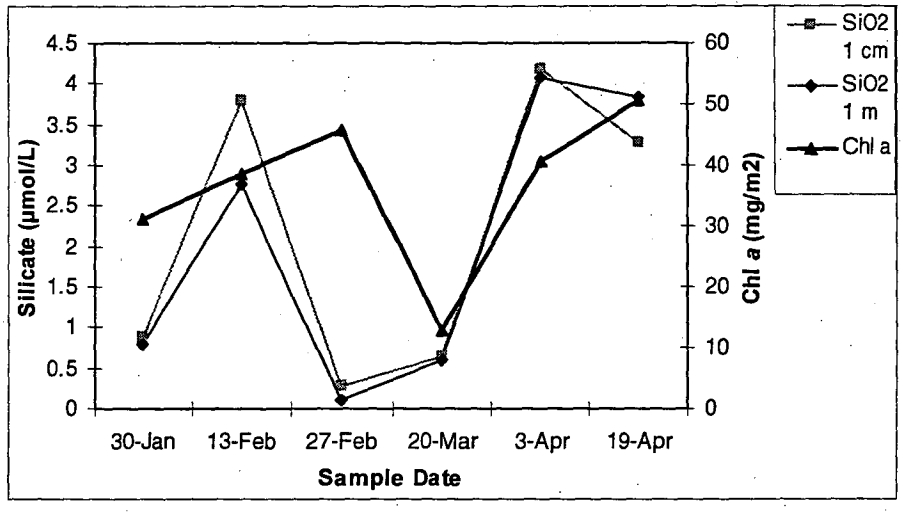


Figure 1.10: Silicate and Chl *a* levels at Conningham Beach

1.3.4 Composition of Benthic Algal Mats

Counts made of approximately 300 cells from the benthic algal samples obtained from each depth determined that algal mats were composed primarily of diatoms (~ 95%). At Tinderbox Marine Reserve, the main diatom species included *Opephora olsenii*, *Nitzschia amphibia*, *Navicula monoculata* var. *omissa*, *Cocconeis peltoides* and *Amphora subturgida*. At Conningham Beach, *Opephora olsenii* and *Nitzschia amphibia* were generally less abundant, and the main diatom species included *Navicula monoculata* var. *omissa*, *Cocconeis scutellum*, *Navicula cancellata* and *Amphora subturgida*.

1.4 DISCUSSION

There are a wide range of sampling devices currently in use across a broad range of scientific disciplines, all of which have inherent design problems that can affect the degree to which samples accurately reflect the features of the sediment under investigation (Lane & Taffs 2002). Careful consideration must be given to the effects that sampling procedure and sampler design have on study outcomes, with acceptable levels of bias being determined by the purposes of the study. The design of the sampling container used in this study was found to be effective for obtaining relatively undisturbed sediment samples of uniform surface area and thickness for chlorophyll *a* analyses. The thickness (depth) of sediment sampled in most chlorophyll studies ranges from 0.5 to 1 cm, because light penetration is largely confined to the top 2 mm of sediment and therefore most chlorophyll can be found within the top few mm (Lukatelich & McComb 1986, MacIntyre *et al.* 1996, Peletier 1996, Masini & McComb 2001). However, because the thickness of sediment sampled varies between studies it must be considered when comparing chlorophyll results reported in the literature.

Tinderbox Marine Reserve and Conningham Beach algal biomass levels (~7.3 to 62.7 mg/m^2) are well within the range of levels commonly reported in the literature for benthic algal biomass, which vary widely. Chlorophyll *a* concentrations of 27 to 558 mg/m^2 were reported from surface sediments in the Peel-Harvey estuarine system, Western Australia, by Lukatelich and McComb (1986), who cited 10 other studies (from estuarine and open marine environments in various locations around the world) across which chlorophyll concentrations ranged from 6 to 770 mg/m^{-2}. Another comparison of 35 studies from various estuarine and open marine environments around the globe showed surface sediment chlorophyll *a* levels ranging from < 1 to 560 mg/m^{-2} (MacIntyre *et al.* 1996). Smaller chlorophyll ranges, such as those recorded from Tinderbox Marine Reserve and Conningham Beach, are also commonly reported – for example, a chlorophyll *a* range of 2.6 to 62.0 mg/m^2 was reported from sediments of Onslow Bay, North-Carolina, by Cahoon *et al.* (1990).

Although fluorometric and spectrophotometric results were very similar ($r^2 = 0.93$), the fluorometry readings were, on average, slightly higher. These results are contrary to

findings reported in the literature that spectrophotometry often provides slightly higher readings than fluorometry (Cahoon *et al.* 1990). The reasons for this discrepancy are not clear, however the variation in measurement between the two methods was relatively small and may simply be due to fluorometer calibration shift. The fluorometric and spectrophotometric methods used in this study provided strongly correlated results, and there is substantial research reporting close alignment between HPLC results and those obtained using spectrophotometric and fluorometric acidification techniques (MacIntyre *et al.* 1996, Mantoura *et al.* 1997a).

The presence of chlorophyll degradation products (phaeopigments) present in natural samples can result in an overestimation of chlorophyll *a* when acidification techniques are used. This is largely because the phaeopigment determination doesn't adequately distinguish between phaeophytin *a* and phaeophorbide *a* derivatives, and the presence of chlorophyll *b* falsely increases the reading for phaeopigments after acidification (Lukatelich & McComb 1986, Mantoura *et al.* 1997a). Chlorophyll *a* correlations with HPLC are significantly improved using the phaeopigment-correcting acidification methods of (Lorenzen 1967) and (Holm-Hansen *et al.* 1965), however these methods do not eliminate interference from phaeophytin and phaeophorbide (Mantoura *et al.* 1997a). Chlorophyll *a* values obtained using spectrophotometric and fluorometric acidification techniques to determine phaeopigments are therefore considered to be an approximation. The results from this study therefore provide a good approximation of maximum biomass of marine benthic algal mats at Tinderbox Marine Reserve and Conningham Beach.

Temporal changes in average algal biomass levels at Tinderbox Marine Reserve and Conningham Beach were remarkably similar throughout the study period. The similarity in algal biomass at each site suggests that the two sites were responding in the same way to significant changes in some environmental variable(s), and further, that this change in environmental variable(s) was very similar at both sites. There are many environmental variables that may affect biomass levels, and the variables measured in this study were not strongly correlated with the variation in measured biomass. Spatial and temporal changes in temperature and salinity do not appear to explain the algal biomass variations recorded. Although algal biomass showed the same pattern of variation at each site, the

nutrients measured (NO_{2-3}, PO_4, SO_2) did not. Silica levels, for example, were approximately 15-40 times higher at Tinderbox Marine Reserve than Conningham Beach in late February (27/02/01). However, by late March (20/03/01), when a sudden decline in biomass was recorded, it was noted that considerable physical disturbance of the sediments had occurred at both sites as a result of a recent storm event. Physical disturbance of the substrata can significantly impact on biomass levels, and is thought to have resulted in the reduced biomass recorded at this time.

With the exception of the above-mentioned sudden decline in biomass in late March, algal biomass levels at both sites increased throughout the study period (i.e. from January to April). The recovery of algal biomass to previous levels following the sudden decline was very rapid (two weeks), suggesting that occasional large-scale physical disturbance of the substrata may not significantly impact on long-term average algal biomass at Tinderbox Marine Reserve and Conningham Beach.

Variations in algal biomass with depth were not consistent temporally or spatially. The depth range investigated (1 to 5 m MSLT) was relatively shallow, and within the euphotic zone of each site. Although physical disturbance of the substrata is more common in shallower water, both sites are relatively protected and the extent of physical disturbance is therefore likely to be relatively small. Longer-term or larger-scale investigation of the relationship between biomass and depth at these sites may reveal a clearer cause-and-effect relationship. The results of this study suggest that algal biomass levels are similar at Tinderbox Marine Reserve and Conningham Beach between the depths of 1 and 5 m MSLT.

1.4.1 Conclusions

Algal biomass levels measured in this study at Tinderbox Marine Reserve and Conningham Beach are broadly similar to levels reported in the literature from other areas of the world. Algae, and in particular diatoms, contribute significantly to the overall productivity of the near-shore, subtidal marine environment of south-east Tasmania. Average algal biomass levels were very similar at the two sites temporally, and appeared to respond similarly to large changes in environmental conditions. It was

found that physical disturbance of the substrata may affect algal biomass more than the effects of depth, temperature, salinity or nutrients at the study sites.

The fluorometric and spectrophotometric methods investigated in this study provided very similar and strongly correlated results, and appeared to provide a good approximation of maximum biomass of marine benthic algal mats at Tinderbox Marine Reserve and Conningham Beach.

CHAPTER 2: Quantifying the Relationship between Water Quality and Diatom Community Composition in South-East Tasmania

2.1 INTRODUCTION

Oceans cover 71% of the earth's surface, yet comparatively little is known about them. As an integral part of the world's ocean ecosystems, coastal marine environments provide feeding, breeding and nursery grounds for many (often commercially important) species, supporting a diverse array of flora and fauna, and provide a buffer zone between human activity and marine biota. With most of the world population living in the coastal zone (Goldberg 1994), the current and potential impacts on coastal aquatic ecosystems are very significant. The greatest impact in many cases is eutrophication, a result of increased nutrient loading (Sundbäck & Snoeijs 1991b, Hallegraeff 1992). However, human occupation of coastal areas also frequently results in physical changes to aquatic environments, such as land fill, chemical contamination, reduced light from turbidity and shading around marinas, construction of rock walls altering sand movement along our beaches, and localized alteration of water temperatures around outlet pipes.

Significant changes in the physical and/or chemical conditions of a water body generally result in changes occurring at the base of the food chain, the algal community (Round 1981). The algal community is a significant and integral part of aquatic ecosystems, and changes in algal composition can have far-reaching impacts on higher levels of the food chain. The long-term benefits of better understanding the interactions between environmental variables and algal communities in the near-shore marine environment are therefore immense, and fundamental to sustainable management of coastal aquatic ecosystems.

Relationships between diatoms and water quality variables have been examined extensively in the recent past (Archibald 1972, Sládeèk 1986, Flower & Nicholson 1987, Royle & King 1992, Roberts & McMinn 1996, Dokulil *et al.* 1997, Rothfritz *et al.* 1997, Mayer & Galatowitsch 1999, Ng & Sin 2003, Crosta *et al.* 2004). Relationships have been examined from a diverse array of aquatic environments, from fresh or estuarine water bodies including lakes (Outridge *et al.* 1989, Guzkowska & Gasse 1990, Fritz *et al.* 1993, Niederhauser & Schanz 1993, Wu *et al.* 1997) and rivers (Stevenson 1984,

Chessman 1985, Chessman 1986, Kutka & Richards 1996, Rott *et al.* 1998) to the near-shore coastal marine environment (Rao & Lewin 1976, Witkowski 1991, Sakson & Miller 1993) and open sea (Cahoon *et al.* 1990, McMinn *et al.* 2001, Barron *et al.* 2004, Crosta *et al.* 2004). Extensive research has also been reported from saline lakes (Gell & Gasse 1990, Cumming & Smol 1993, Juggins *et al.* 1994, Gell 1997, Roberts & McMinn 1998, Roberts *et al.* 2001, Taffs 2001).

During the past two decades, advances in statistical techniques have improved the efficiency and accuracy with which diatom community structure can be related to environmental variables (ter Braak 1986, 1989, ter Braak & Juggins 1993, Juggins 2003). Consequently, there are increasing numbers of studies on the relative influence of various environmental variables on diatom species composition, and the generation of transfer functions to infer environmental variables from palaeo-ecological diatom communities (Bennion 1994, Wilson *et al.* 1994, Jones & Juggins 1995, Vyverman & Sabbe 1995, Roberts *et al.* 2000, Ruhland & Smol 2002, Bloom *et al.* 2003, Yang *et al.* 2003, Tibby 2004). There are, however, comparatively few reported associations between near-shore, sub-tidal, benthic marine diatom assemblages and nutrient concentrations. However, sheltered marine waters such as semi-enclosed or protected embayments provide a similar environment for examining diatom-nutrient relationships to non-marine environments such as lakes and rivers. In semi-enclosed and sheltered marine environments (as opposed to open marine environments) water quality conditions can more readily be affected by the impacts of land-based anthropogenic activity (such as increased nutrients) due to proximity and reduced tidal exchange. Sheltered marine environments are also often heavily utilized (for marinas, aquaculture, industry) because of the fact they are sheltered. Quantitative inference models have been successfully used to infer environmental variables in this type of coastal marine environment (Ng & Sin 2003), and their use to infer nutrients in palaeo-ecological environments has the potential to add significantly to understanding coastal ecosystem processes.

Causative relationships between environmental variables and diatom assemblages are identified for the near-shore marine environment of south-east Tasmania. Investigation of those environmental variables having the greatest influence on diatom community

composition in this area will lead to an improved understanding of the level and type of impact that both natural and anthropogenic activity is having on the coastal aquatic community. Transfer functions for nutrient concentrations are generated for use in these environments as a means of assessing and monitoring the effects of eutrophication. This research therefore:

(i) examines relationships between diatom assemblages and environmental variables in the south-east Tasmanian near-shore, sub-tidal marine environment;

(ii) determines how the diatom community is responding to environmental conditions; and

(iii) establishes a means of monitoring the effects of environmental change on our important coastal marine ecosystems.

2.1.1 Study Area

The coastal environment of south-east Tasmania around the capital city, Hobart, provides a large area of relatively calm waters for much of the year (Figure 2.1). The complex coastline includes many sheltered bays, and is protected to a large extent by the presence of Bruny Island. Between Bruny Island and the main land mass of Tasmania is the D'Entrecasteaux Channel (also referred to simply as the Channel). The study area includes approximately 200 square kilometers of waterway, covering an area extending from the north of Hobart to approximately the southern tip of Bruny Island.

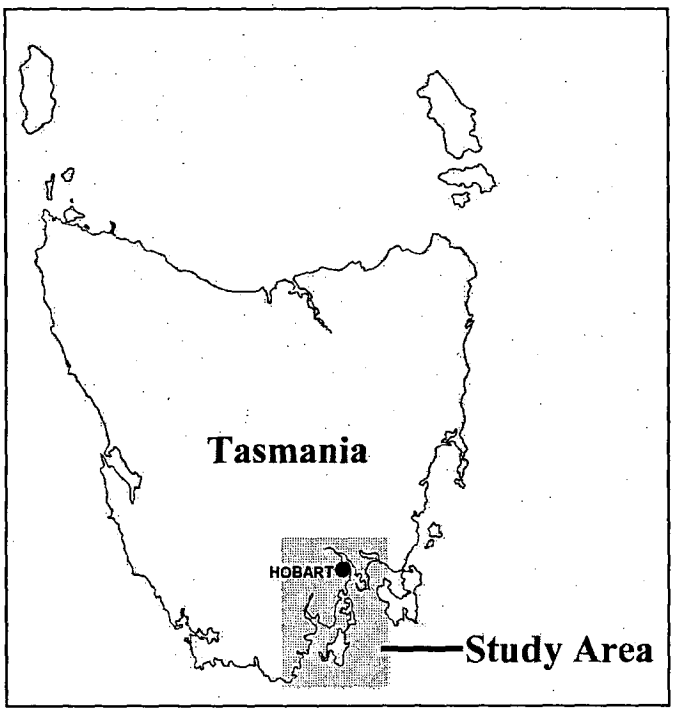


Figure 2.1: Study Area

Because of the protected nature of these waters, a considerable and growing aquaculture industry occurs in the Channel area, particularly salmon farms. These fish farms are comprised of large concave nets, suspended around their outer diameter by a buoyant structure (Plate 2.1), and often contain thousands of fish. The largely unrestricted water flow through the nets prevents the water in which the fish are growing from becoming stagnant or putrefied, thus providing a more natural habitat for the fish to grow in. However, operation of the fish farms requires daily feeding of the fish, and both digested and undigested fish food escapes from the net into the surrounding water, adding substantial nutrients to the system (Coughanowr 2000).

Plate 2.1: Fish farms in the D'Entrecasteaux Channel

Plate 2.2: Industry (Pasminco zinc works) on the banks of the Derwent River

The protected waters of the many bays also provide a safe haven for boats, and there are numerous marinas around the general Hobart and Channel areas. Consequently, antifouling paints and hydrocarbons from the marinas (Volkman *et al.* 1992) are added to the urban run-off already entering these harbours. In addition, industrial activity along the banks of the Derwent River include a pulp-fibre mill, zinc works (Plate 2.2), ship

builders, and numerous factories (e.g. Cadbury's). In the middle and lower reaches of the Derwent estuary, sewage is the main major source of organic pollutant (Green & Coughanowr 2003). Yet, despite the obvious problems accompanying the gradual processes of eutrophication, the Derwent River is still home to rare endemic fish species such as the spotted handfish (*Brachionichthys hirsutus*) and fragile seahorses (such as *Hippocampus abdominalis)*, and supports a diverse array of flora and fauna. Tasmania's estuaries and marine ecosystems are, in fact, amongst the most diverse on earth, and contain many endemic species (Bruce *et al.* 1998, Haddy & Pankhurst 1998, Swain *et al.* 1982). Yet, little is known about Tasmanian marine and estuarine ecology, or about the impacts of anthropogenic activity on these systems.

2.1.2 Aims and Objectives

The need exists for a water quality monitoring system in the lower Derwent and Channel areas, capable of detecting and predicting changes to the microbial aquatic communities so that impacts from pollutants and changing land-use practices can be more fully understood. The aim of this research is to develop a diatom-based water quality monitoring and assessment tool that can additionally be used to infer the palaeo-environmental history of marine sites within the region. The objectives of the research are to:

(i) Determine the main diatom species of the south-east Tasmanian near-shore, sub-tidal marine environment;

(ii) Identify relationships between the species composition of diatom communities and the corresponding physical and chemical water conditions, from a wide range of sites within the study area; and

(ii) Develop a transfer function to infer environmental conditions at other sites within the study area.

2.2 METHODS

2.2.1 Site Selection

Approximately 100 coastal sites were selected from hydrographic maps of south-east Tasmania as potential sampling sites. Site selection aimed to identify sites likely to be relatively physically-protected environments (such as bays), likely to have broadly similar physical characteristics, but spanning a wide environmental gradient of nutrient conditions.

After field investigation of all 100 sites, 51 sites were selected as the sampling set for the study (Figure 2.2; locations of site numbers are listed in Table 2.1). The choice of the final sampling sites was made on the basis of accessibility and site conditions (primarily substrate, flora, depth, and degree of exposure). Common reasons for excluding sites from the sampling set included rocky substrate, dense macrophyte vegetation (especially kelp), water depth greater than 6 m (e.g. at the base of a cliff), and rough water conditions.

2.2.2 Site Sampling

Sampling of sediments for diatoms at all sites was undertaken between 25/10/01 and 02/11/01 (mid to late spring), as highest diatom numbers usually occur during the spring (Werner 1977). However, as many diatom species bloom in both spring and autumn (Round 1981, Chessman 1985), physical and chemical parameters at each site were measured both at the time of diatom sampling and again in mid to late autumn (between 22/04/02 and 04/05/02) to identify environmental conditions during both times of peak growth. The GPS location for each site was recorded at the time of sampling using a Garmin eTrex GPS receiver (Table 2.1).

2.2.2.1 Sediment Samples

It was originally intended that benthic sediment samples for diatom analyses would be collected from all sites using a Glew corer (Glew 1989) operated from a boat. However, while the Glew corer worked well in finer sediments, in substratum consisting of coarse sand it was usually not possible to retrieve a short core intact (the sand fell out of the

bottom of the corer during the retrieval process). Diatom sampling of sites was therefore undertaken using a Glew corer for finer sediments, and by snorkel using 100 ml specimen containers for coarser sediments. Where samples were collected using the Glew corer, the top 1 cm of the core was retained for diatom analyses. Where samples were collected by snorkel, they were obtained using a 100 ml specimen container to collect sediment from the top 1 cm of the substrate, and were immediately capped underwater. A second sediment sample was also obtained from each site for sediment size analysis and total organic carbon measurement. All benthic sediment samples were stored in the dark on ice until processing at University of Tasmania (UTAS) laboratories.

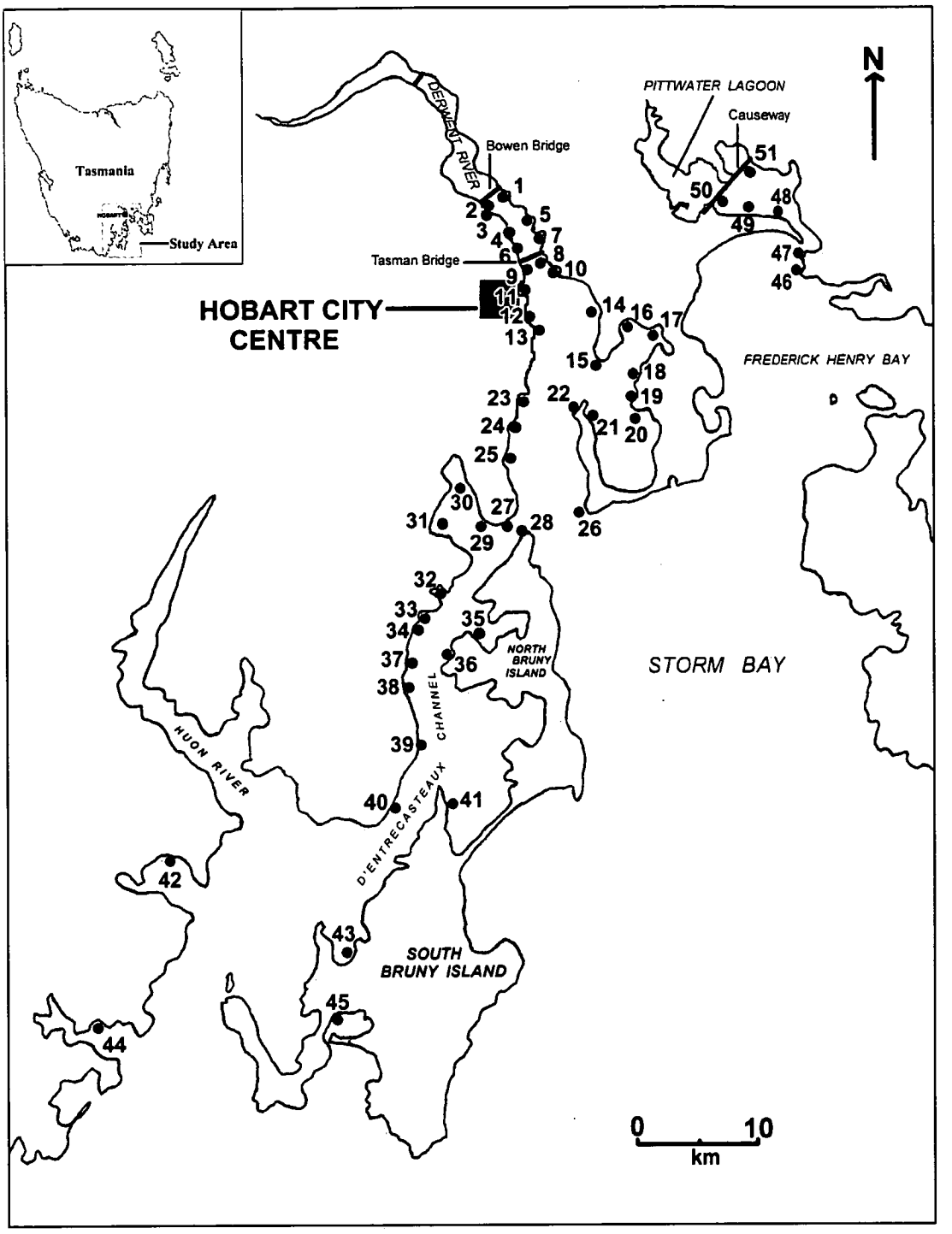


Figure 2.2: Location of Sampling Sites

Table 2.1: Sampling Site Locations

Site no.	Latitude S	Longitude E	Location
1	42 49 055	147 18 901	Risdon Cove
2	42 43 461	147 18 276	Prince of Wales Bay - near entrance
3	42 49 896	147 18 179	Prince of Wales Bay - inside
4	42 50 763	147 19 040	Newtown Bay
5	42 50 314	147 26 606	Geilston Bay
6	42 51 198	147 19 314	Cornelian bay
7	42 50 914	147 21 425	Lindisfarne Bay
8	42 51 942	147 21 274	Montague Bay
9	42 52 548	147 20 294	Ross Bay - southern end toward slipyards
10	42 52 361	147 21 753	Kangaroo Bay
11	42 53 097	147 19 961	Sullivans Cove - near Ferry Wharf
12	42 53 784	147 20 036	Sandy Bay - near Hutchins boat shed jetty
13	42 54 760	147 21 498	Sandy Bay Beach
14	42 54 805	147 24 879	Tranmere - above Tranmere Point
15	42 56 327	147 24 785	Trywork Point
16	42 54 834	147 26 107	Ralphs Bay - Rokeby Beach area
17	42 54 809	147 27 858	Ralphs Bay - Lauderdale area
18	42 55 884	147 27 662	Richardson Beach - Ralphs Bay
19	42 56 890	147 27 274	Huxleys Beach - Ralphs Bay
20	42 58 725	147 26 829	Mortimer Bay- Ralphs Bay
21	42 58 794	147 25 055	Shelley Beach- Ralphs Bay
22	42 58 381	147 24 035	Mary Anne Bay
23	42 57 227	147 21 125	Taroona Beach
24	42 59 012	147 19 594	Kingston Beach
25	43 00 347	147 19 587	Blackmans Bay
26	43 02 311	147 24 346	Beacroft Bay
27	43 03 537	147 19 737	Tinderbox Marine Reserve
28	43 03 959	147 21 003	Dennes Point - near Jetty
29	43 03 713	147 190 19	North West Bay - north-eastern corner
30	43 01 234	147 17 169	North West Bay - north-west corner
31	43 04 623	147 16 943	Conningham Beach
32	43 06 845	147 16 295	Oyster Cove
33	43 07 437	147 15 503	Little Oyster Cove (Kettering)
34	43 07 914	147 15 305	Trial Bay
35	43 08 138	147 13 665	Barnes Bay
36	43 09 501	147 17 166	Apollo Bay
37	43 09 723	147 14 647	Peppermint Bay - Woodbridge
38	43 10 875	147 14 877	Fleurtys Point
39	43 12 362	147 15 582	Whaleboat Rk - between Fluertys Point and Gordon
40	43 16 081	147 14 529	Gordon - near Jetty
41	43 15 883	147 13 209	Simpsons Bay
42	43 19 112	147 01 358	Port Esperance - near Dover Jetty
43	43 22 598	147 13 127	Little Taylors Bay
44	43 25 145	147 12 181	Cloudy Bay Lagoon
45	43 25 991	146 58 496	Southport - near Jetty
46	43 02 119	147 15 278	Between Tiger Hd and Spectacle Isl. - Dodges Ferry
47	42 49 115	147 36 898	Dodges Ferry - south of entrance to Pittwater
48	42 50 166	147 36 284	Pittwater - behind Seven Mile Beach (spit)
49	42 49 095	147 33 966	Pittwater - between spit and island
50	42 48 552	147 01 280	Pittwater - between island and Pittwater causeway
51	42 47 900	147 32 630	Pittwater - near Orielton Lagoon causeway

All samples were collected within the subtidal zone, below 6.5 m water depth (mean tidal height). Based on the biomass results from Chapter 1, samples were generally collected between 1 m and 5 m water depth. However, samples were collected over several days and consequently under various tidal conditions. The depth recorded for each site was therefore corrected relative to the tide level for Hobart at the time of sampling, and adjusted to reflect the mean tidal height over the previous 12 months.

2.2.2.2 Physical and Chemical Sampling

Salinity, temperature and depth were measured using a Conductivity, Temperature and Depth (CTD) meter. Water clarity was measured using a Secchi disc (Tyler 1968). Duplicate 10 ml water samples for nutrient analyses were collected from approximately 2 m water depth using a 2-litre Niskin bottle (General Oceanics Inc.), and were stored in the dark on ice until processing at UTAS laboratories.

2.2.3 Sample Analyses

2.2.3.1 Nutrients

Nutrient samples were processed at UTAS laboratories using an ALPKEM Auto Analyser, following the ALPKEM Methodology Manual (1992). Samples were analysed for NO_{2-3}, PO_4 and SiO_2 concentrations.

2.2.3.2 Total Organic Carbon

Sediment total organic carbon (TOC) was analysed by the Central Science Laboratory at the University of Tasmania, using a Thermo Finnigan Flash Elemental Analyser (1112 Series).

2.2.3.3Sediment Size Analysis

Each sediment sample was divided in half. The weight (g) of each half-sample was then recorded (to 4 decimal places). The lighter of the 2 half-samples was dried in an oven at 60°C for 24 hours and reweighed. The moisture content calculated for the dried half-sample was used to infer the dry-weight of the second half of the sediment sample.

The second half of the sediment sample was wet-sieved through 2 mm, 125 μm and 63 μm sieves. Each fraction was dried in the retaining sieve in an oven at 60°C for 24 hours, then removed from the sieve and reweighed. The < 63 μm fraction of the sediment sample was calculated as: Inferred dry weight of sample less the weight of the three retained fractions (2 mm, 125 μm and 63 μm).

2.2.4 Diatom Community Composition

From each sample, approximately 5 - 10 g was placed in a 250 ml beaker with 50 ml 10% hydrochloric acid (HCl) to remove any carbonate material that may be present. Beaker contents were gently simmered on a hot-plate for 2 hours in a fume-cupboard, after which time distilled water was added to bring the beaker volume to 250 ml. The samples were then left to settle for 6 to 12 hours, after which time the supernatant (approx. 70% volume) was carefully poured off. Samples were then rinsed two additional times, repeating the above procedure. Samples were subsequently treated with 100 ml 10% Hydrogen peroxide (H_2O_2) to remove organic matter, using the same method as for the HCl, and rinsed three times.

At least two permanent microslides were made from each sample by pippetting 500 μm of the prepared solution onto a glass microslide cover-slip and drying for several hours at approximately 60°C on a hotplate. Cover slips were mounted on glass microslides using the mounting medium Naphrax (refractive index 1.72). Microslides were inspected under a Zeiss Standard 20 light microscope using 1000x oil immersion optics.

At least 400 diatom species were enumerated from each sample. Species are expressed as a relative abundance (% of counted frustules). Species identifications were based primarily on the works by (Round *et al.* 1990, Krammer & Lange-Bertalot 1991b, 1991a, 1997b, 1997a, Witkowski *et al.* 2000) and particularly the Australian works by (Foged 1978, John 1983).

2.2.5 Statistical Analyses

2.2.5.1 Canonical Correspondence Analysis

Relationships between diatom assemblages and measured environmental variables were examined using Canonical Correspondence Analysis (CCA) to determine which environmental variables best explain variations in the diatom assemblage data. CCA, a direct gradient ordination technique (ter Braak 1986), was performed using Canoco version 4.5.

Measurements for a total of 22 environmental variables were used in the initial CCA analyses, including: site depth; total organic carbon; spring and autumn salinity and temperature at 1 m water depth and 0.1 m above the bottom; spring and autumn Secchi depth; spring and autumn NO_{2-3}, PO_4 and SiO_2 concentrations, and relative percentage of four sediment-size fractions (< 63 um, 63-125 um, 0.125-2mm, >2mm).

All 22 variables in the environmental training set were log transformed ($\log_{10}(x+1)$) prior to statistical analyses to reduce skewness in the data (Verleyen *et al.* 2003). All diatom species that represented ≥ 2% of at least one sample were included in the species training set (111 species) (Katoh 1993). In each CCA, rare species were down-weighted and sample scores were scaled to be weighted averages of species scores. Following preliminary CCA analysis, 2 environmental variables were identified as having a high variance inflation factor (VIF >20) and subsequently removed from the data set. A high VIF value occurs when an environmental variable is almost perfectly correlated with other variables and doesn't contribute additional information to the ordination (ter Braak 1988). The two environmental variables removed were spring temperature at 1 m water depth, and spring salinity at 1 m water depth, which were strongly correlated with spring temperature ($R^2 = 0.88$) and spring salinity ($R^2 = 0.81$) respectively at the sediment-water interface.

Forward selection was used to identify the environmental variables that contributed significantly to explaining the variation in the species data. Unrestricted Monte Carlo permutation tests (499 permutations) were used to judge the statistical significance of each variable. The relative explanatory strength of each environmental variable

identified as significant ($P < 0.05$) in the forward selection process was independently checked in a constrained CCA (ter Braak 1988). In this type of analysis, the variable that explains the most variance in the species data has the highest λ_1/λ_2 ratio (Dixit *et al.* 1991). The λ_1/λ_2 ratios from the constrained CCA analyses were used to determine variables suitable for the development of a transfer function (Dixit *et al.* 1991). In this study, transfer functions were developed for variables with a λ_1/λ_2 ratio >0.5 (Table 2.2). CanoDraw version 4.12 was used to provide graphical representation of the CCA analysis.

Table 2.2: Forward selection results for significant variables with high λ_1/λ_2 ratios

Environmental Variable	P value	λ_1/λ_2 ratio
Spring NO_{2-3}	0.002	0.796
% sediment size < 63μm	0.002	0.734
spring SiO_2	0.002	0.678
autumn NO_{2-3}	0.002	0.502

2.2.5.2 Transfer Functions

Transfer functions were generated for the best explanatory variables in the environmental data set (these variables were identified as contributing significantly to explaining the variation in the species data through Unrestricted Monte Carlo Permutation tests and constrained CCAs, as outlined in the Section 2.2.5.1 above). Transfer functions were generated for: spring NO_{2-3}, % sediment size < 63μm, spring SiO_2 and autumn NO_{2-3} (Table 2.2), using C^2 software (Juggins 2003). Environmental variables included in the transfer function generation were log transformed, and only diatom species representing ≥ 2% of at least one sample were included in the species training set. Weighted averaging with both inverse deshrinking and classical deshrinking, with and without tolerance down-weighting, and with boot-strapping cross-validation was applied to the training set. Simple weighted averaging with inverse and classical deshrinking, as well as with and without tolerance down-weighting, has been shown to be as effective as other methods (e.g. weighted averaging partial least squares) for providing reliable inference models and reconstructions (Köster *et al.* 2004).

2.3 RESULTS

2.3.1 Physical and Chemical Results

2.3.1.1 Salinity, Temperature and Depth

Salinity was generally above 30 (at maximum water depth) on both sampling occasions (spring and autumn). However, although a maximum-depth salinity measurement of above 30 was recorded for each site at least once, salinity did vary widely at some sites during the study period, and maximum-depth salinity ranged from 8.95 (Site 1, Risdon Cove, in spring; 31.6 in autumn) to 34.95 at Site 5 (Geilston Bay, autumn). Surface water (1 m water depth) salinity at each site was more strongly correlated with maximum-depth salinity during spring ($R^2 = 0.81$) than autumn ($R^2 = 0.22$) due to a difference in the amount of overlying fresh water. Full results for spring and autumn salinity are included in Table 2.3. Spring and autumn salinity for the maximum water depth at each study site are shown in Figure 2.3.

Water temperature measurements were generally around 16°C during spring and 14°C during autumn (Table 2.3). Surface water (1 m water depth) temperature at each site was strongly correlated with maximum-depth temperature during both spring ($R^2 = 0.88$) and autumn ($R^2 = 0.91$). Full results for spring and autumn temperature are listed in Table 2.3. Spring and autumn water temperature for the maximum water depth at each study site are shown in Figure 2.4.

Water depth (adjusted to reflect mean tidal height) ranged from 0.44 m (Site 44, Cloudy Bay Lagoon) to 6.41 m (Site 9, Ross Bay) (Table 2.3). The average sampling depth of all sites was 2.93 m (mean tidal height). Water depth at each site is listed in Table 2.3.

2.3.1.2 Light Penetration

Secchi depth measurements at most sites were at least equal to the water depth from which the sample was taken, thus most sampling sites were within the euphotic zone. Site 2 (Dowsings Point) was the only site at which Secchi depth was less than water depth on both sampling occasions (Secchi depth at Site 2 was 74.3% of water depth in spring and 80.3% of water depth in autumn). The lowest recorded Secchi depth measurement was 1.2 m at Site 3 (Prince of Wales Bay) in spring; the highest was 6.41 m at the deepest site (Site 9, Ross Bay) in autumn (Table 2.3).

Table 2.3: Training set site locations, water and Secchi depth, temperature and salinity

Site nos.	**Latitude (South)**	**Longitude (East)**	Water Depth (m)	Spring Secchi (% water depth)	Autumn Secchi (% water depth)	Spring Water Temp °C (1 m)	Spring Water Temp °C (bottom)	Spring Salinity (1 m)	Spring Salinity (bottom)	Autumn Water Temp °C (1 m)	Autumn Water Temp °C (bottom)	Autumn Salinity (1 m)	Autumn Salinity (bottom)
1	42 49 055	147 18 901	1.39	100	100	16.67	16.73	9.18	8.95	15.95	16.00	24.84	31.60
2	42 43 461	147 18 276	2.49	74.30	80.32	14.76	13.97	13.60	29.91	16.03	16.03	30.63	32.64
3	42 49 896	147 18 179	1.98	60.61	100	15.66	15.59	10.53	10.96	16.01	16.00	27.67	32.07
4	42 50 763	147 19 040	2.36	95.34	100	14.06	13.86	30.16	31.24	16.06	16.07	31.14	32.14
5	42 50 314	147 26 606	5.45	40.37	100	13.76	13.31	29.47	33.10	15.63	15.78	23.62	34.95
6	42 51 198	147 19 314	4.27	70.26	100	13.76	13.41	30.25	32.80	16.20	16.20	26.23	26.23
7	42 50 914	147 21 425	1.07	100	100	16.38	16.38	15.06	15.06	16.08	16.08	29.56	30.82
8	42 51 942	147 21 274	3.05	100	75.41	14.74	14.79	25.70	31.65	16.03	16.04	30.55	32.89
9	42 52 548	147 20 294	6.41	54.60	100	13.75	13.29	31.94	33.20	15.90	16.01	30.05	33.41
10	42 52 361	147 21 753	3.34	100	100	14.56	14.48	31.66	31.90	15.92	15.98	32.11	32.78
11	42 53 097	147 19 961	4.84	100	100	14.26	13.49	31.73	33.20	15.74	15.76	29.85	32.53
12	42 53 784	147 20 036	2.87	100	100	14.31	14.21	31.71	32.04	15.80	15.78	30.73	32.09
13	42 54 760	147 21 498	2.42	100	100	14.17	13.83	32.50	32.97	15.70	15.68	32.44	32.57
14	42 54 805	147 24 879	2.91	100	100	12.73	12.75	26.86	26.99	15.46	15.49	27.95	32.27
15	42 56 327	147 24 785	2.95	100	81.36	13.76	13.78	29.01	28.96	15.35	15.44	30.43	31.55
16	42 54 834	147 26 107	3.06	100	100	14.17	14.10	30.89	31.52	15.33	15.32	29.78	30.38
17	42 54 809	147 27 858	2.27	100	100	14.17	14.17	25.85	26.03	15.51	15.60	31.76	32.30
18	42 55 884	147 27 662	2.71	100	88.56	14.05	14.05	26.90	26.86	15.38	15.60	31.56	32.44
19	42 56 890	147 27 274	3.13	100	100	13.86	13.86	27.27	27.37	15.36	15.39	31.84	32.11
20	42 58 725	147 26 829	2.95	100	100	13.78	13.79	27.65	27.82	15.60	15.64	32.26	32.54
21	42 58 794	147 25 055	2.48	100	100	14.09	14.07	28.29	28.57	15.38	15.41	32.33	32.47
22	42 58 381	147 24 035	3.19	100	100	13.93	13.94	29.25	29.92	15.53	15.54	31.65	32.64
23	42 57 227	147 21 125	3.21	100	100	15.57	15.33	30.73	31.76	15.82	15.83	33.05	33.34
24	42 59 012	147 19 594	3.03	100	100	15.16	15.10	31.98	32.03	15.70	15.72	33.17	33.47
25	43 00 347	147 19 587	5.42	100	100	13.69	13.42	33.44	33.62	15.58	15.69	33.04	33.38
26	43 02 311	147 24 346	2.72	100	100	14.07	14.06	30.66	30.63	15.39	15.62	31.90	33.53

Continued.....

Table 2.3 (continued): Training set site locations, water and Secchi depth, temperature and salinity

Site nos.	**Latitude (South)**	**Longitude (East)**	Water Depth (m)	Spring Secchi (% water depth)	Autumn Secchi (% water depth)	Spring Water Temp °C (1 m)	Spring Water Temp °C (bottom)	Spring Salinity (1 m)	Spring Salinity (bottom)	Autumn Water Temp °C (1 m)	Autumn Water Temp °C (bottom)	Autumn Salinity (1 m)	Autumn Salinity (bottom)
27	43 03 537	147 19 737	4.27	100	100	13.99	13.66	32.45	32.49	15.75	15.73	33.67	33.69
28	43 03 959	147 21 003	1.02	100	100	14.78	14.78	32.54	32.54	15.53	15.53	33.73	33.68
29	43 03 713	147 190 19	2.89	100	100	15.03	14.34	32.10	32.31	15.74	15.70	33.72	33.69
30	43 01 234	147 17 169	4.30	100	100	15.01	14.23	32.31	32.07	15.73	15.29	33.46	33.20
31	43 04 623	147 16 943	2.82	100	100	14.54	14.33	32.24	32.30	15.58	15.59	33.74	33.65
32	43 06 845	147 16 295	4.49	100	100	14.42	13.63	32.18	32.63	15.90	15.89	33.42	33.31
33	43 07 437	147 15 503	2.88	100	100	14.81	14.69	31.92	31.87	15.60	15.56	33.46	33.45
34	43 07 914	147 15 305	1.85	100	100	15.29	15.38	31.79	31.68	15.63	15.63	33.41	33.45
35	43 08 138	147 13 665	2.69	100	100	15.20	14.52	32.05	31.79	15.61	15.60	33.62	33.57
36	43 09 501	147 17 166	2.34	100	100	14.79	14.79	32.06	32.10	15.60	15.60	33.58	33.56
37	43 09 723	147 14 647	5.06	100	100	14.96	14.91	31.94	32.10	15.62	15.60	33.44	33.41
38	43 10 875	147 14 877	5.36	100	70.90	14.59	13.54	32.01	33.43	15.60	15.60	33.39	33.39
39	43 12 362	147 15 582	5.10	100	100	14.54	14.01	32.47	33.02	15.54	15.54	32.47	33.02
40	43 16 081	147 14 529	1.66	100	100	15.52	15.53	31.37	31.32	14.95	14.95	33.40	33.36
41	43 15 883	147 13 209	1.32	100	100	14.63	14.64	32.01	32.04	15.29	15.28	33.32	33.19
42	43 19 112	147 01 358	4.08	100	100	14.42	14.28	33.20	33.28	14.88	14.87	33.24	33.49
43	43 22 598	147 13 127	1.35	100	100	12.58	12.60	31.88	31.88	15.39	15.39	33.75	33.75
44	43 25 145	147 12 181	0.44	100	100	13.86	13.86	33.37	33.37	15.60	15.60	33.37	33.37
45	43 25 991	146 58 496	3.02	100	100	13.78	13.75	34.03	34.07	15.12	15.15	32.93	32.91
46	43 02 119	147 15 278	1.11	100	100	13.64	13.64	33.04	33.04	15.50	15.59	33.04	33.04
47	42 49 115	147 36 898	2.22	100	100	13.58	13.58	32.89	32.76	15.30	15.29	32.89	32.76
48	42 50 166	147 36 284	1.19	100	100	13.47	13.48	32.81	32.78	15.46	15.47	32.81	32.78
49	42 49 095	147 33 966	3.72	100	100	13.52	13.52	32.67	32.65	15.32	15.31	32.67	32.65
50	42 48 552	147 01 280	2.04	100	100	13.27	13.27	31.67	31.79	15.50	15.59	31.67	31.79
51	42 47 900	147 32 630	0.46	100	100	13.63	13.63	31.47	31.47	15.44	15.47	31.47	31.47

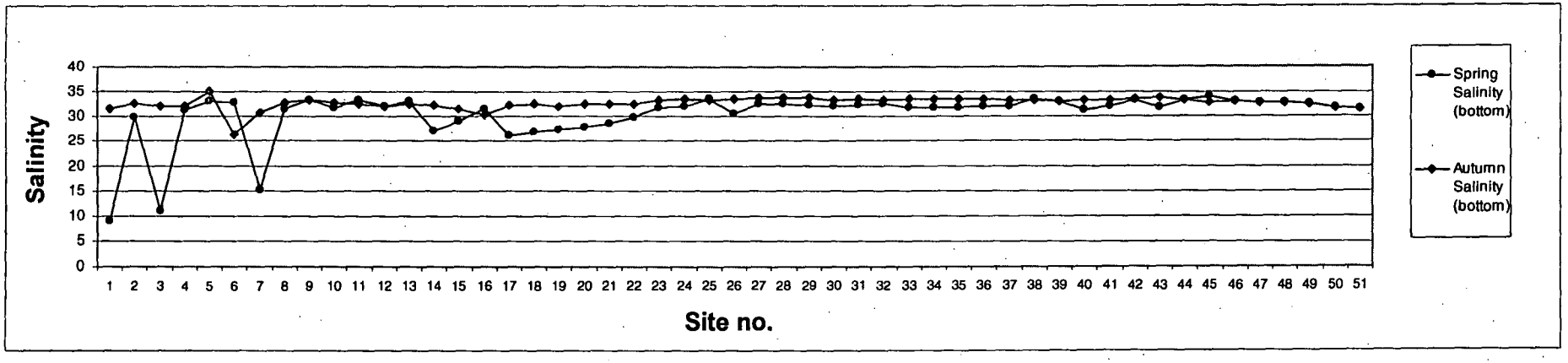


Figure 2.3: Salinity in spring and autumn at training –set sites

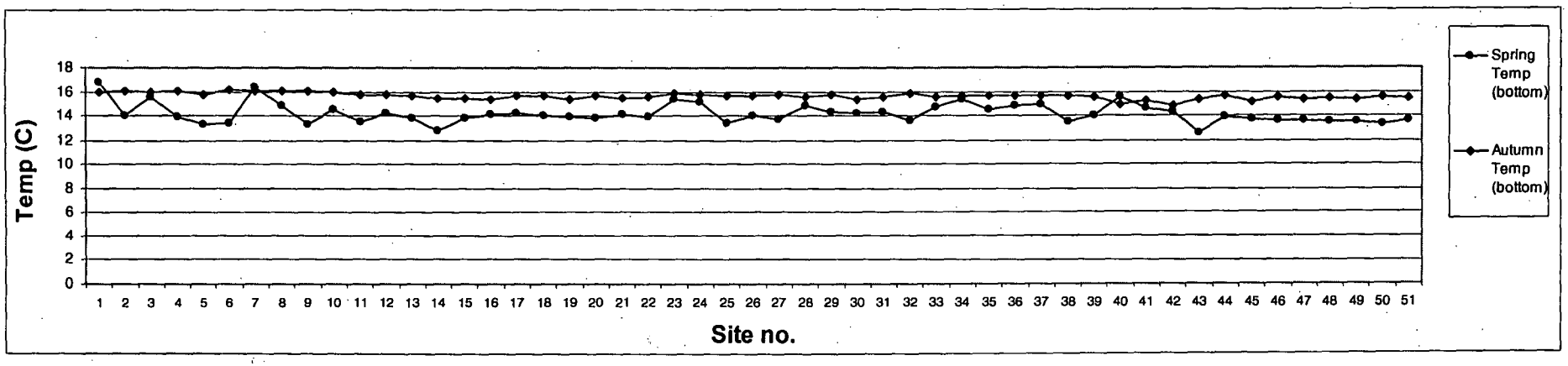


Figure 2.4: Water temperature in spring and autumn at training –set sites

2.3.1.3 Nutrient Concentrations

Concentrations of NO_{2-3} at most sites was low (<0.5 µmol/L) for both spring and autumn samples, and at 16 sites was below the detection limit of 0.01 µmol/L on at least one sampling occasion. However, several sites experienced much higher NO_{2-3} levels (up to > 8 µmol/L), particularly sites in the Derwent Estuary immediately above and below Hobart during spring (Sites 1 to 9, and Sites 10 to 14, respectively) (Figure 2.6). Average NO_{2-3} concentrations were slightly lower during autumn (0.18 µmol/L) than spring (0.76 µmol/L). Recorded levels for all nutrients are listed in Table 2.4.

Phosphate was closely correlated with NO_{2-3} ($R^2 = 0.87$) during spring (Figure 2.5), but to a much lesser extent during autumn ($R^2 = 0.57$), and ranged from 0.10 to 1.21 µmol/L. Phosphate was marginally higher during spring at Sites 1 to 9, and up to 4 times the average spring level for all sites of 0.29 µmol/L.

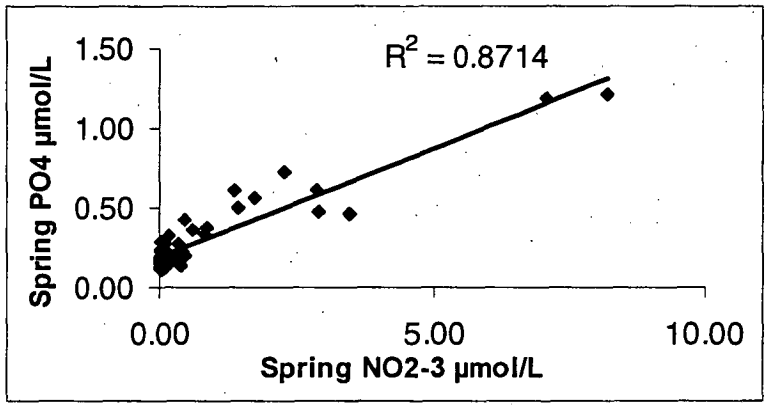


Figure 2.5: Correlation between Spring PO_4 and Spring NO_{2-3}

Average silicate levels were much higher in spring than in autumn (up to 45 times greater at Site 22, Mary Ann Bay), and overall were highest in the Derwent Estuary above Hobart (Sites 1 to 9) (Figure 2.7 and Table 2.4). Silicate levels ranged from 0.16 µmol/L (Site 24, Kingston Beach, autumn) to 64.16 µmol/L (Site 2, entrance to Prince of Wales Bay, spring).

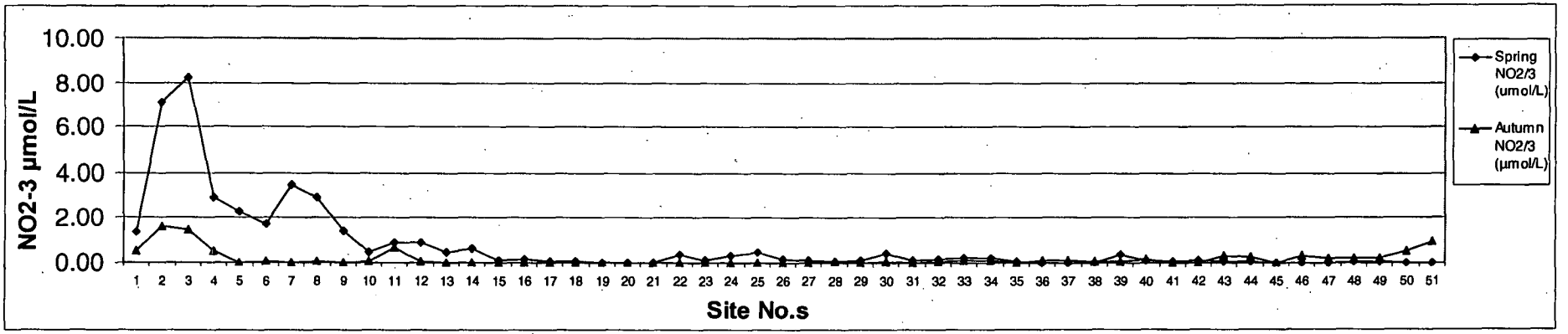


Figure 2.6: NO_{2-3} levels in spring and autumn at training-set sites

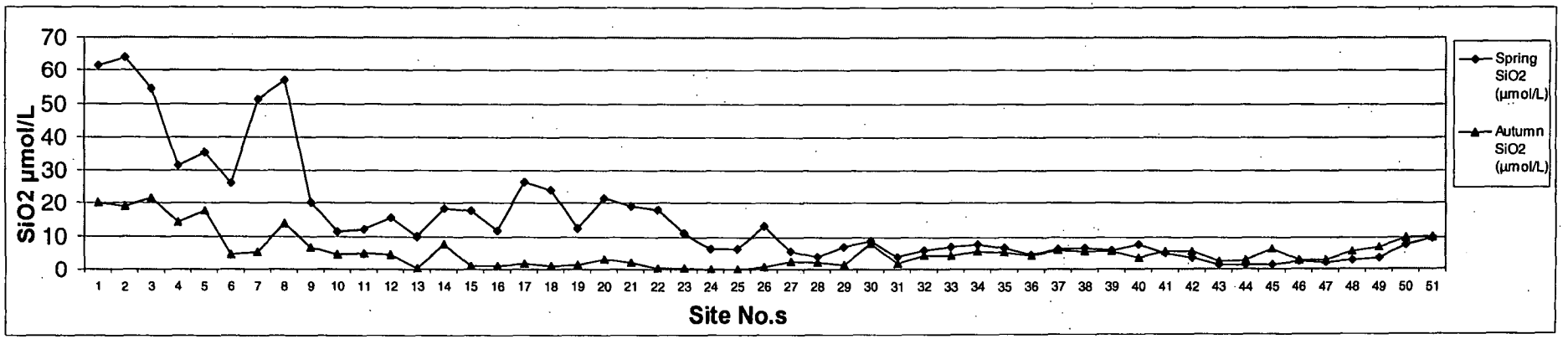


Figure 2.7: SiO_2 levels in spring and autumn at training-set sites

Table 2.4: Nutrient levels at training set sites

Site nos.	Spring NO_{2-3} (µmol/L)	Spring PO_4 (µmol/L)	Spring SiO_2 (µmol/L)	Autumn NO_{2-3} (µmol/L)	Autumn PO_4 (µmol/L)	Autumn SiO_2 (µmol/L)
1	1.36	0.61	61.65	0.54	0.49	20.30
2	7.09	1.19	64.16	1.59	0.88	19.10
3	8.24	1.21	54.37	1.46	0.97	21.71
4	2.88	0.61	31.43	0.53	0.65	13.97
5	2.27	0.73	35.33	0.02	0.39	17.61
6	1.71	0.56	26.02	0.03	0.32	4.66
7	3.45	0.46	51.40	0.01	0.40	5.38
8	2.90	0.48	57.02	0.07	0.47	13.64
9	1.42	0.50	20.04	0.01	0.37	6.79
10	0.46	0.43	11.44	0.04	0.41	4.74
11	0.87	0.37	11.96	0.67	0.40	4.86
12	0.86	0.34	15.58	0.06	0.31	4.42
13	0.45	0.23	9.73	0.00	0.20	0.48
14	0.63	0.36	18.51	0.02	0.26	7.88
15	0.12	0.28	17.59	0.00	0.22	1.11
16	0.18	0.32	11.78	0.00	0.15	1.02
17	0.03	0.29	26.50	0.00	0.35	1.76
18	0.03	0.18	24.07	0.00	0.22	1.07
19	0.00	0.13	12.43	0.00	0.25	1.36
20	0.02	0.22	21.44	0.00	0.24	3.27
21	0.02	0.23	19.09	0.02	0.21	2.14
22	0.35	0.27	18.03	0.00	0.13	0.40
23	0.09	0.21	11.07	0.00	0.27	0.37
24	0.30	0.20	6.41	0.00	0.22	0.16
25	0.47	0.20	6.39	0.00	0.27	0.17
26	0.16	0.23	13.02	0.00	0.19	0.64
27	0.10	0.15	5.64	0.01	0.29	2.37
28	0.05	0.24	3.94	0.07	0.25	2.21
29	0.11	0.12	6.97	0.00	0.18	1.28
30	0.39	0.14	8.78	0.06	0.32	8.04
31	0.10	0.14	3.96	0.00	0.22	1.79
32	0.18	0.15	6.01	0.06	0.24	4.24
33	0.20	0.16	7.23	0.08	0.24	4.16
34	0.19	0.16	7.70	0.10	0.31	5.75
35	0.03	0.11	6.68	0.02	0.30	5.44
36	0.01	0.16	4.58	0.10	0.25	4.16
37	0.01	0.13	6.27	0.11	0.32	5.87
38	0.02	0.13	6.79	0.07	0.28	5.61
39	0.34	0.19	6.05	0.06	0.28	5.71
40	0.08	0.13	7.63	0.15	0.20	3.70
41	0.03	0.20	5.07	0.00	0.24	5.48
42	0.09	0.15	3.64	0.01	0.13	5.79
43	0.07	0.14	1.57	0.29	0.25	2.51
44	0.06	0.15	1.35	0.26	0.10	2.75
45	0.00	0.15	1.31	0.00	0.53	6.36
46	0.00	0.19	2.43	0.33	0.29	2.89
47	0.01	0.12	2.09	0.23	0.28	2.99
48	0.06	0.17	2.91	0.20	0.32	5.66
49	0.03	0.16	3.36	0.23	0.21	7.00
50	0.01	0.15	7.65	0.51	0.27	9.75
51	0.02	0.17	9.88	0.93	0.33	10.39

2.3.1.4 Sediment Size Analyses & Total Organic Carbon

Sediment size analyses of training-set samples determined the relative percentage fractions of < 63 μm, 63-125 μm, 0.125-2 mm and > 2 mm. The range of substrate sediments encountered at sites within the study area was very broad, from ~85% of sediment < 63 μm (Site 3, Prince of Wales Bay), to ~98% of sediment 0.125-2 mm (Site 51, Orielton Lagoon causeway). Sediment analyses results are listed in Table 2.5.

The range of sediment total organic carbon (TOC) also varied widely across the training-set sites, from 0.01% (Sites 25, Blackmans Bay, & Site 26, Seacroft Bay) to 8.85% (Site 3, Prince of Wales Bay). TOC was generally highest at sites within the Derwent Estuary, and was correlated with the % fraction of sediment < 63 μm (R^2 = 0.70) (Figure 2.8). The full range of TOC results are listed in Table 2.5.

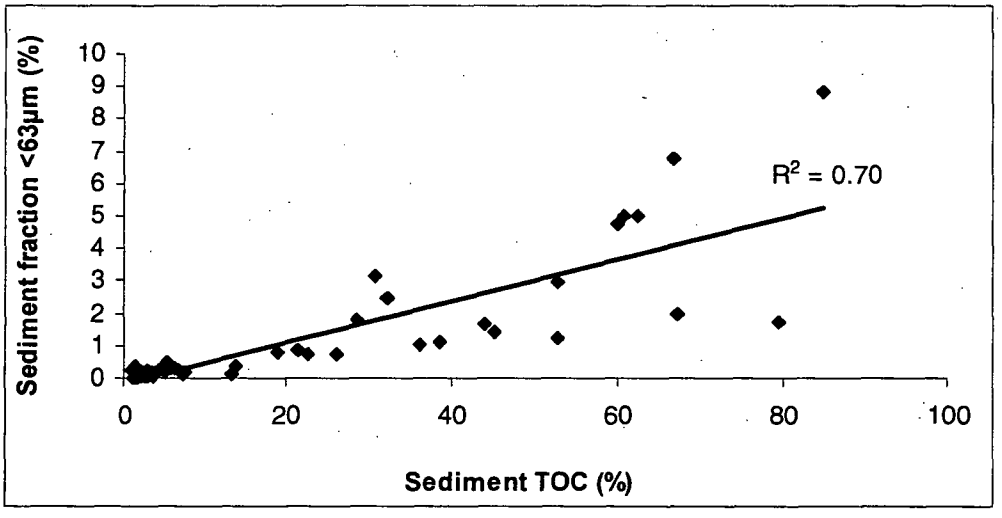


Figure 2.8: Correlation between sediment size and sediment TOC

Table 2.5: Sediment size and TOC for training set sites

Site nos.	< 63 µm	63-125 µm	0.125-2 mm	>2 mm	Sediment % TOC
1	25.92	20.51	52.21	1.36	0.74
2	66.85	18.15	14.99	0.00	6.80
3	84.87	7.21	6.14	1.78	8.85
4	30.66	14.81	54.23	0.29	3.14
5	62.42	8.13	29.46	0.00	5.03
6	18.97	10.45	70.58	0.00	0.80
7	22.62	30.05	47.33	0.00	0.72
8	60.76	18.81	20.00	0.43	5.02
9	32.00	20.25	46.73	1.01	2.46
10	38.50	25.49	35.33	0.67	1.11
11	60.17	19.16	18.50	2.16	4.73
12	43.90	27.88	28.23	0.00	1.64
13	4.35	57.26	38.39	0.00	0.24
14	5.12	10.88	82.68	1.32	0.25
15	3.03	27.10	69.87	0.00	0.08
16	6.85	17.60	75.56	0.00	0.27
17	2.92	18.48	78.60	0.00	0.24
18	3.59	4.97	74.18	17.26	0.15
19	1.77	16.67	81.55	0.00	0.22
20	6.26	35.19	58.56	0.00	0.33
21	1.85	1.10	97.05	0.00	0.21
22	2.25	5.63	91.76	0.36	0.06
23	2.46	25.25	72.25	0.04	0.20
24	1.38	8.45	86.04	4.13	0.36
25	1.15	2.31	96.54	0.00	0.01
26	1.74	30.85	67.10	0.31	0.01
27	2.48	17.15	80.11	0.26	0.14
28	2.70	13.57	80.73	3.00	0.06
29	2.07	6.87	89.96	1.10	0.15
30	45.01	30.73	23.92	0.34	1.41
31	5.15	4.81	87.24	2.80	0.17
32	67.30	22.52	9.73	0.45	1.95
33	52.76	6.64	24.95	15.65	2.96
34	21.45	17.94	40.93	19.69	0.89
35	28.47	13.00	44.94	13.58	1.78
36	3.93	10.07	81.11	4.89	0.21
37	79.55	13.00	7.45	0.00	1.71
38	52.82	37.95	7.86	1.37	1.23
39	36.01	59.12	4.86	0.00	1.08
40	13.37	15.07	71.56	0.00	0.15
41	2.74	1.24	96.02	0.00	0.11
42	7.31	59.92	32.18	0.59	0.13
43	3.97	12.25	83.77	0.00	0.10
44	7.44	29.13	63.43	0.00	0.18
45	5.29	5.44	89.20	0.07	0.51
46	1.93	32.28	64.43	1.37	0.04
47	5.84	29.91	63.90	0.35	0.29
48	3.61	3.65	92.74	0.00	0.06
49	3.44	3.80	92.77	0.00	0.17
50	13.81	14.53	71.65	0.00	0.36
51	0.87	0.37	98.39	0.37	0.23

2.3.2 Diatom Community Composition

All diatom species comprising at least 2% of at least one sample were included in the analyses of the training set data. Together, these species constituted > 90% of the diatom community at every site in the training set except one (Site 30), and accounted for an average 95% of all total diatom counts (at Site 30, Dru Point, these species constituted 84% of the diatom community). The following section focuses on the above-mentioned diatom species from the training set (listed in Table 2.7). Diatom species names and authorities, and diatom abundance and composition for the training set, are listed in Table 2.1 and Table 2.2 respectively, in Appendix 2.

A total of 111 diatom species from 46 genera were recorded at ≥ 2% of at least one sample from the 51 sampling sites. For each sampling site, the seven numerically dominant diatom species from the site constituted between approximately 51% and 91% of the diatom community. The main species from each site are shown in Figure 2.9. On average, the species listed in this figure constitute ~76% of the assemblage at each site (range 51% to 93%, except for site 30 (42%) which contained many species in small proportions).

Navicula monoculata var. *omissa* was the only diatom taxon recorded from all 51 sites, and was the dominant taxon at 16 sites. Across all sites, *N. monoculata* var. *omissa* averaged 14% of diatom community composition (range 0.8 to 56.2%). *Skeletonema costatum* was dominant at seven sites, and *Nitzschia amphibia* was dominant at six sites. The numerically dominant species at remaining sites were *Opephora olsenii*, *Synedra tabulata* var. *tabulata*, *Planothidium delicatulum*, *Plagiogramma staurophorum*, *Navicula arenaria* var. *rostellata*, *Fragilaria pinnata*, *Ehrenbergia granulosa*, *Cyclotella striata*, *Cocconeis carminata*, *Amphora subturgida*, *Amphora laevissima*, *Cymatosira* aff. *belgica*, and two unidentified species (Species 1 and Species 4).

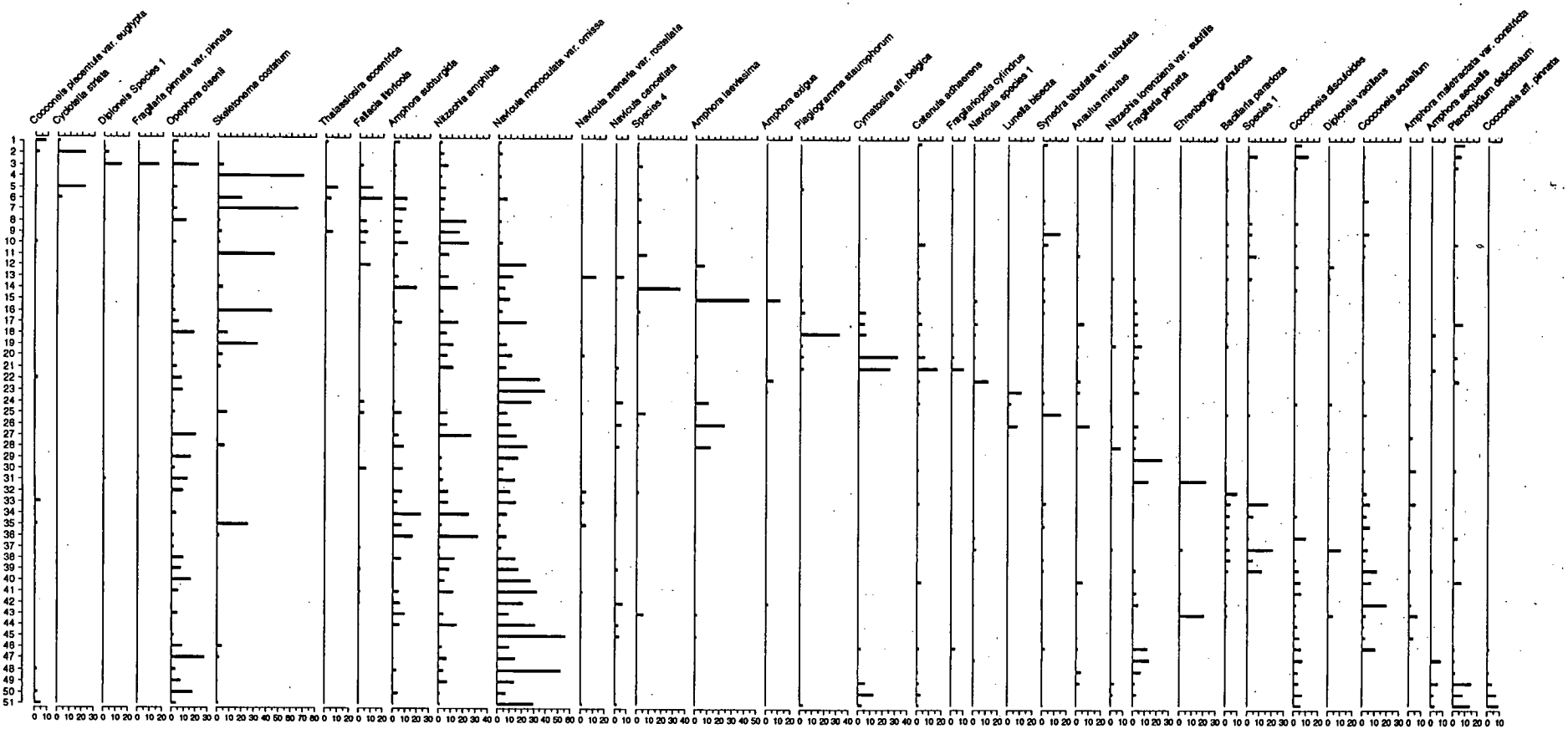


Figure 2.9: Distribution and relative abundance of main diatom taxa at training set sites

2.3.3 Statistical Analyses

Results from the canonical correspondence analysis (CCA) showed that the sum of unconstrained eigenvalues (representing the total diatom variance in the dataset) was 2.86. The twenty selected environmental variables explained approximately 46% of the total diatom variance (combined eigenvalues totalling 1.31). The environmental variables that best explained the variation in diatom community structure were spring $NO_{2\text{-}3}$, spring SiO_2, % sediment < 63µm and autumn $NO_{2\text{-}3}$ (P = 0.002 for all). These four variables together explained ~17.5% of the total variation in diatom community composition. Spring $NO_{2\text{-}3}$ explained approximately 6% of the total variation in diatom community composition (eigenvalue 0.17), while the other three variables (spring SiO_2, % sediment < 63µm and autumn $NO_{2\text{-}3}$) each explained approximately 4% of the variation observed (eigenvalues of 0.11 each). The relationships between the active environmental variables, the sites sampled, and the species assemblage of the diatom communities are shown in the following biplots (Figures 2.10 and 2.11). In the following figures, the arrowed lines for each environmental variable show the relative direction of increasing levels for that variable (these lines can also be extended in the reverse direction through the point of origin (0,0)).

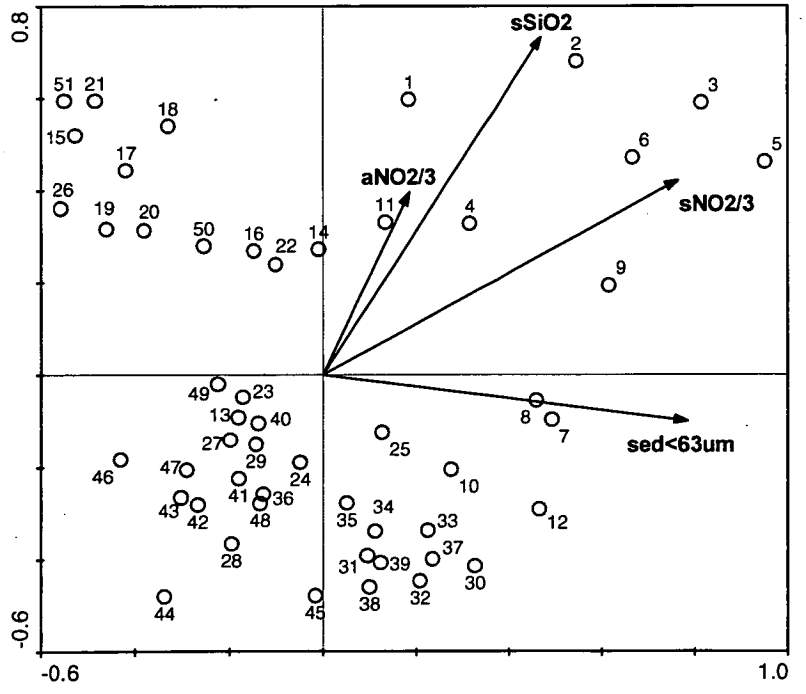


Figure 2.10: CCA ordination biplot of active environmental variables and sampling sites (site numbers are indicated by hollow circles, and are listed in Table 2.1)

The relative position of each sampling site along the gradient for each active environmental variable indicates the relative influence of the environmental variable at the sampling site. For example, Sites 3 and 5 (north-east quadrant, Figure 2.10) are located far along the environmental gradients (arrows) for spring $NO_{2\text{-}3}$, and also for sediment fraction < 63µm, and are therefore fine sediment sites with high $NO_{2\text{-}3}$ concentrations. Conversely, Sites 44 and 46 (south-west quadrant) have low $NO_{2\text{-}3}$ levels and coarser sediments.

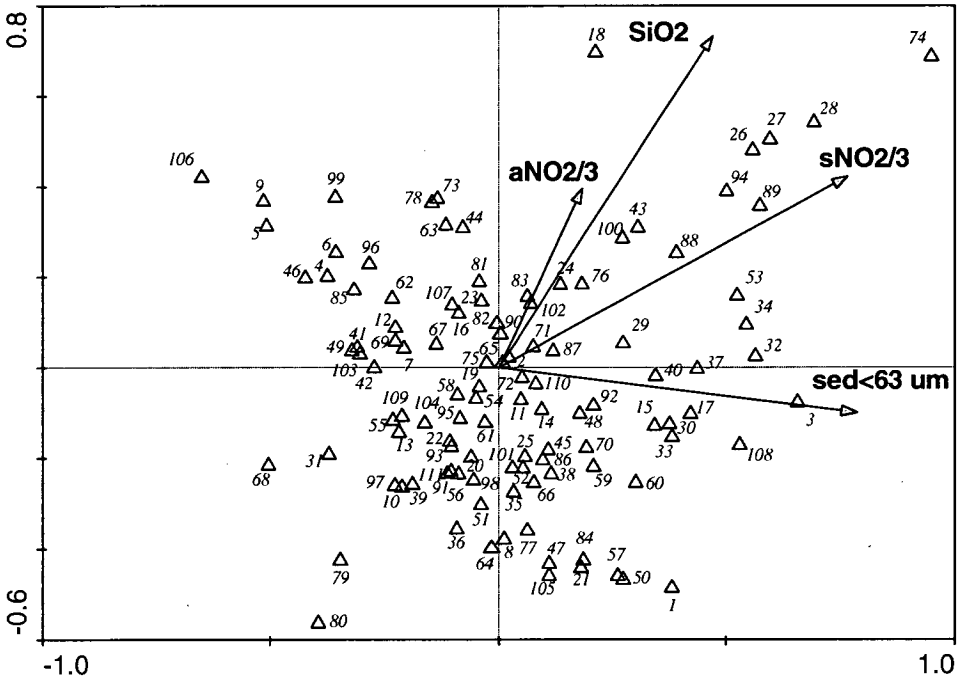


Figure 2.11: CCA ordination biplot of active environmental variables and diatom species (species numbers are indicated by hollow triangles, and are listed in Table 2.6)

The species-environment relationships shown in Fig 2.11 are high: 0.939 for axis one (horizontal axis, Eigenvalue 0.195) and 0.910 for axis two (Eigenvalue 0.167), indicating a strong relationship between diatom species and the active environmental variables. By connecting a line (arrow) from the point of origin (0,0) to a species point, the direction in which the species' abundance value increases at the largest rate across the ordination diagram is indicated (ter Braak & Šmilauer 2002).

2.3.3.1 Transfer Functions

Transfer functions for each of the four active environmental variables (spring NO_{2-3}, spring SiO_2, % sediment < 63 μm and autumn NO_{2-3}) were generated using C^2 software (Juggins 2003) (see Methods Section 2.2.5 re determining 'active' environmental variables). Weighted averaging with both inverse deshrinking and classical deshrinking, with and without tolerance down-weighting, and with boot-strapping cross-validation was applied to the training set.

The best results for spring NO_{2-3}, spring SiO_2 and % sediment <63μm were obtained using weighted averaging with inverse deshrinking. This method provided the highest correlation (0.88, 0.81 and 0.75 respectively), the lowest average and maximum bias, the lowest root mean square of the error (RMSE) (0.08 $\log_{10}$ μmol/L, 0.16 $\log_{10}$ μmol/L and 0.26 respectively), and the lowest or second lowest RMSE of prediction (RMSEP) (0.15 $\log_{10}$ μmol/L, 0.26 $\log_{10}$ μmol/L and 0.38 respectively) (Table 2.6). The training set was checked for outliers for each variable, identified as those samples having a residual greater than the standard deviation of the environmental variable in the training set (Jones & Juggins 1995). Weighted averaging with inverse deshrinking showed no outliers for the above three environmental variables. The strength of the relationships between observed and diatom-predicted values for spring NO_{2-3}, spring SiO_2 and % sediment size <63μm are shown in Figures 2.12 to 2.14.

The best results for autumn NO_{2-3} were also initially obtained using weighted averaging with inverse deshrinking. However, most of the autumn NO_{2-3} samples in the data set were identified as outliers using either inverse or classical deshrinking without tolerance downweighting. Tolerance downweighting was therefore investigated for autumn NO_{2-3}. However, the squared correlation between bootstrap predicted and observed values for autumn NO_{2-3} was so low (<0.01) that developing a transfer function for this variable was not a viable option (Table 2.6). This variable was therefore omitted from further analyses.

Table 2.6: Summary statistics showing performance of Transfer Function models (see key below table for codes)

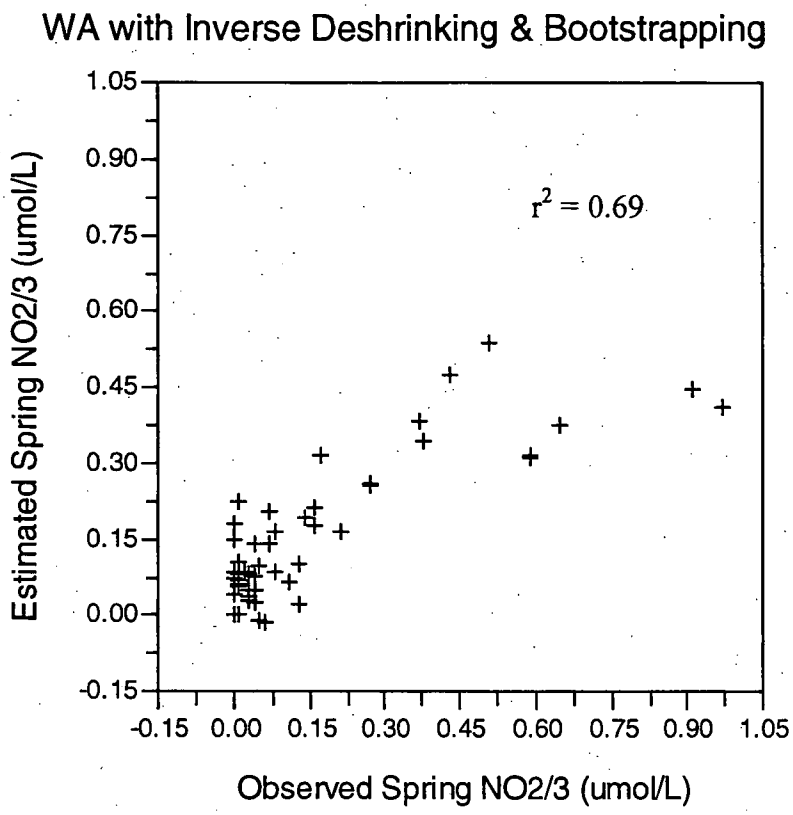
Variable	Model	R2	Average Bias	Max. Bias	RMSE	Boot R2	RMSEP
Spring NO_{2-3}	WA_Inv	0.877	-1.98813E-16	0.157	0.081	0.686	0.151
	WA_Cla	0.877	-2.87624E-16	0.221	0.087	0.696	0.146
	WATOL_Inv	0.864	-9.1192E-17	0.209	0.085	0.545	0.209
	WATOL_Cla	0.864	-9.37431E-17	0.195	0.092	0.557	0.210
Spring SiO_2	WA_Inv	0.815	6.85726E-16	0.256	0.161	0.610	0.262
	WA_Cla	0.815	1.08192E-15	0.431	0.178	0.614	0.256
	WATOL_Inv	0.757	3.04767E-17	0.311	0.184	0.562	0.310
	WATOL_Cla	0.757	-5.00689E-17	0.574	0.212	0.562	0.318
Sed<63um	WA_Inv	0.745	-3.06944E-16	0.381	0.262	0.529	0.381
	WA_Cla	0.745	-8.42463E-16	0.403	0.304	0.535	0.382
	WATOL_Inv	0.719	1.11022E-15	0.298	0.276	0.516	0.424
	WATOL_Cla	0.719	1.31268E-15	0.453	0.325	0.521	0.447
Autumn NO_{2-3}	WA_Inv	0.756	8.27225E-17	0.124	0.047	0.230	0.089
	WA_Cla	0.756	1.39322E-16	0.099	0.054	0.240	0.091
	WATOL_Inv	0.630	-3.42863E-17	0.115	0.057	0.003	0.105
	WATOL_Cla	0.630	-5.65996E-17	0.051	0.072	0.006	0.114

Key:

WA_Inv	Weighted averaging model (inverse deshrinking)
WA_Cla	Weighted averaging model (classical deshrinking)
WATOL_Inv	Weighted averaging model (tolerance downweighted, inverse deshrinking)
WATOL_Cla	Weighted averaging model (tolerance downweighted, classical deshrinking)
RMSE	Root mean squared error for the training set (apparent RMSE)
R2	Squared correlation between inferred and observed values
Average Bias	Average bias in residuals
Max. Bias	Maximum bias in residuals
Boot R2	Squared correlation between bootstrap predicted and observed values
RMSEP	Root mean squared error of prediction (s1 + s2) (bootstrap RMSEP)

2.3.3.2 Species Optima and Tolerances

Optima and tolerances of the training set species for spring NO_{2-3}, spring SiO_2 and % sediment < 63 μm are shown in Figures 2.15 to 2.17. Species optima and tolerance for all three variables show a logged distribution. A complete list of optima and tolerance ranges for all species in the training set is provided in Table 2.7 at the end of the results section.



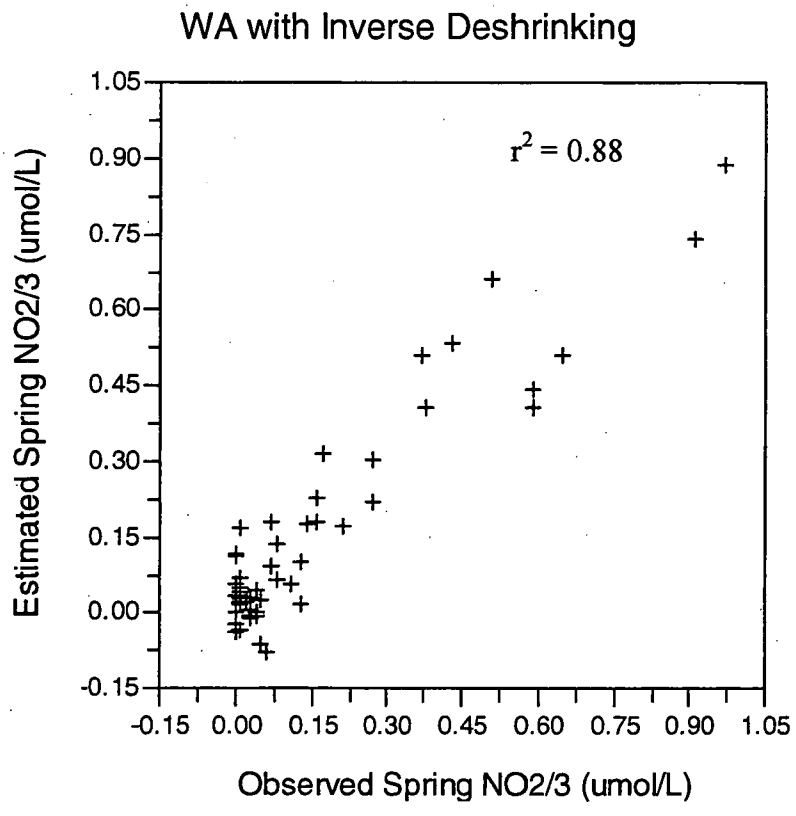


Figure 2.12: Relationship between observed and diatom-predicted values for spring $NO_{2\text{-}3}$, showing bootstrapped r^2 values.

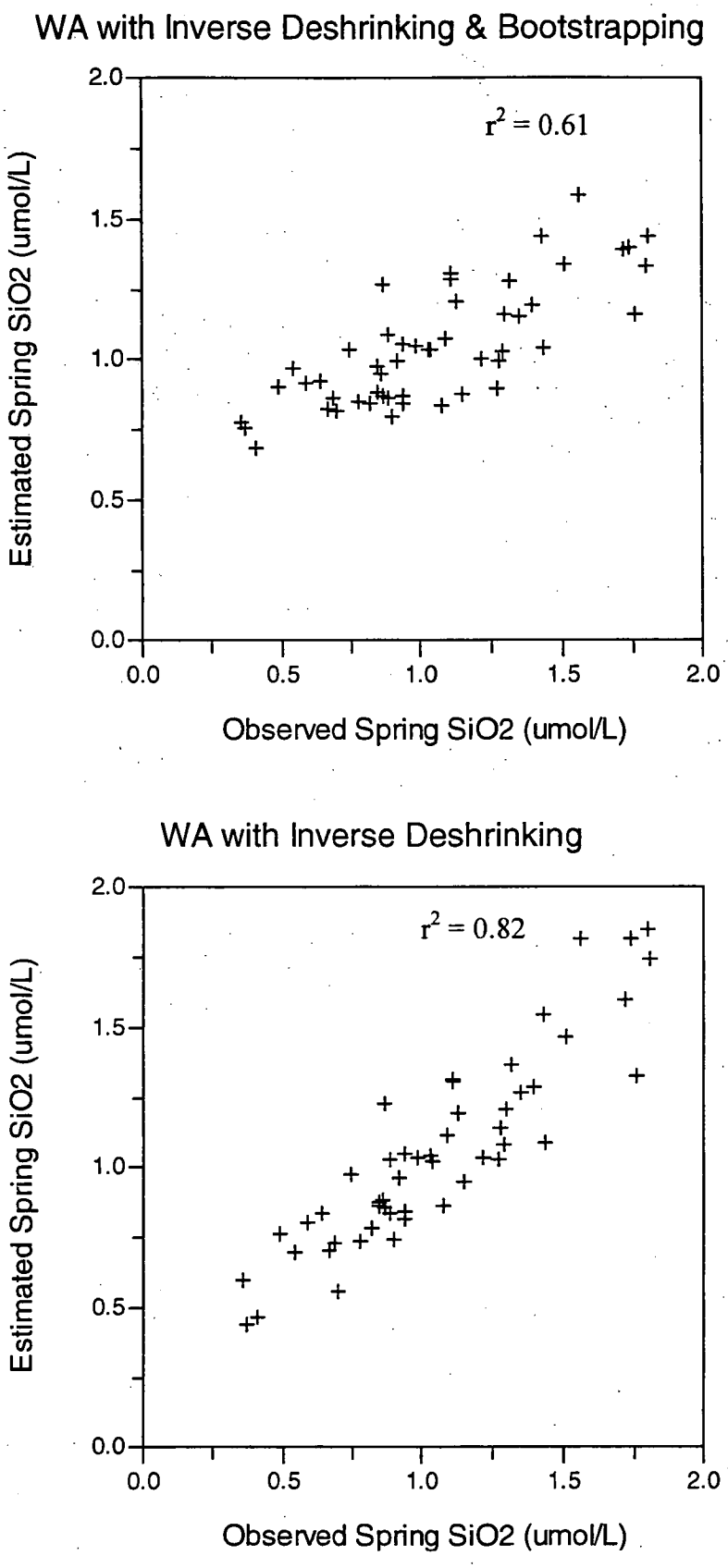
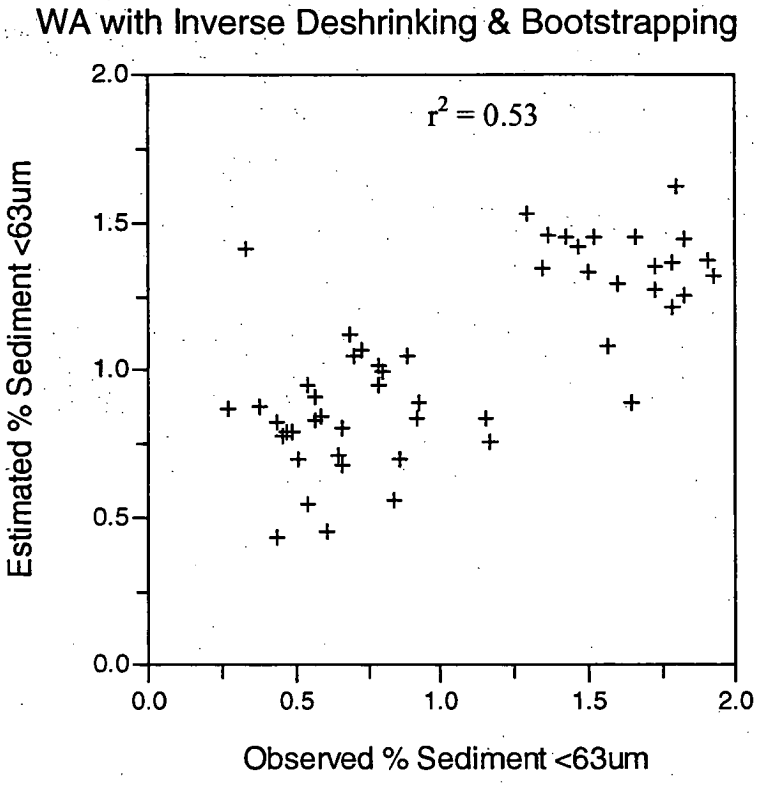


Figure 2.13: Relationship between observed and diatom-predicted values for spring SiO_2, showing bootstrapped r^2 values



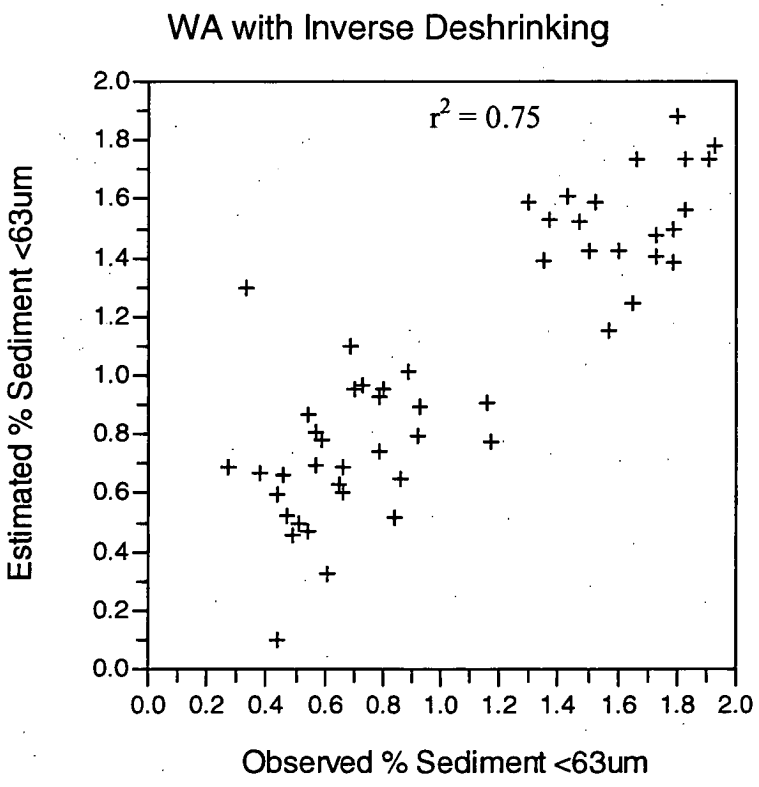


Figure 2.14: Relationship between observed and diatom-predicted values for % sediment size <63μm, showing bootstrapped r^2 values

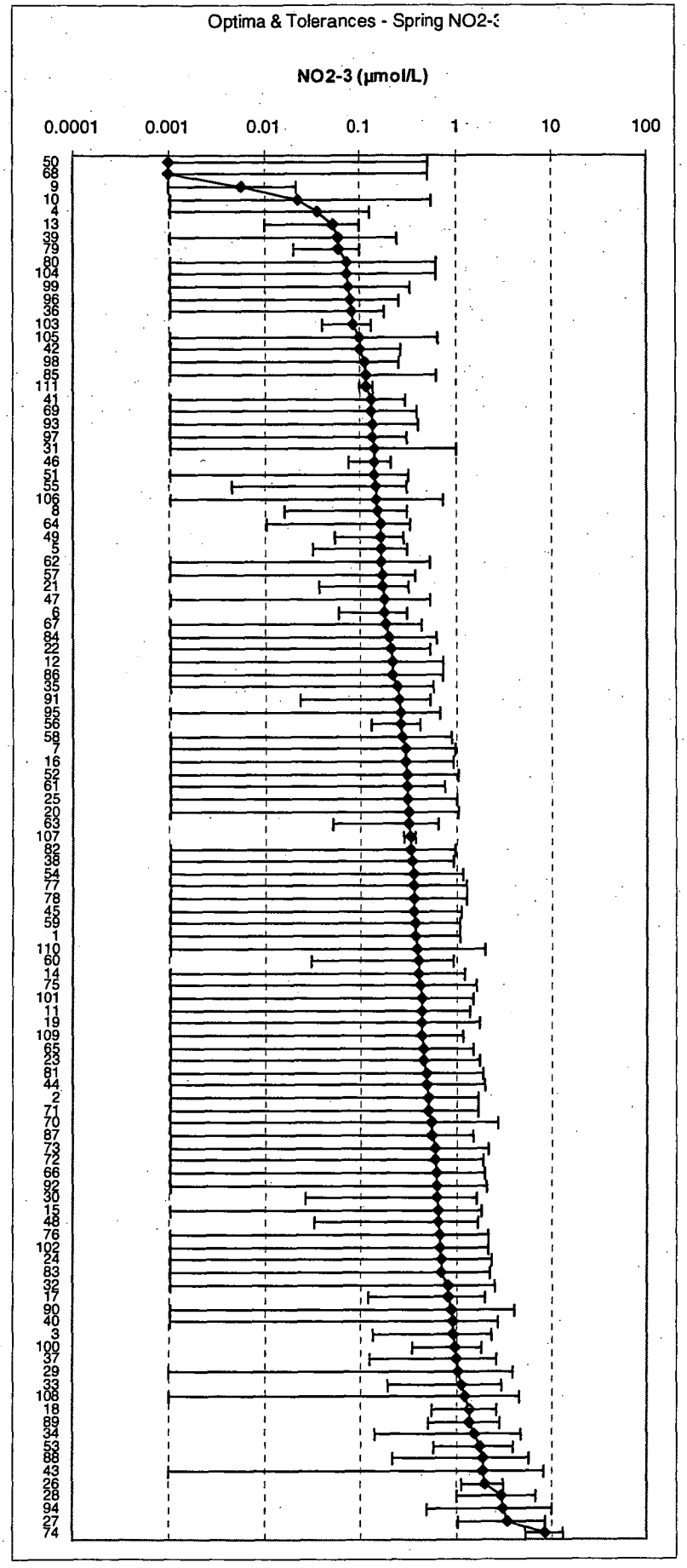


Figure 2.15: Optima and tolerances of the training set species for spring NO_{2-3}

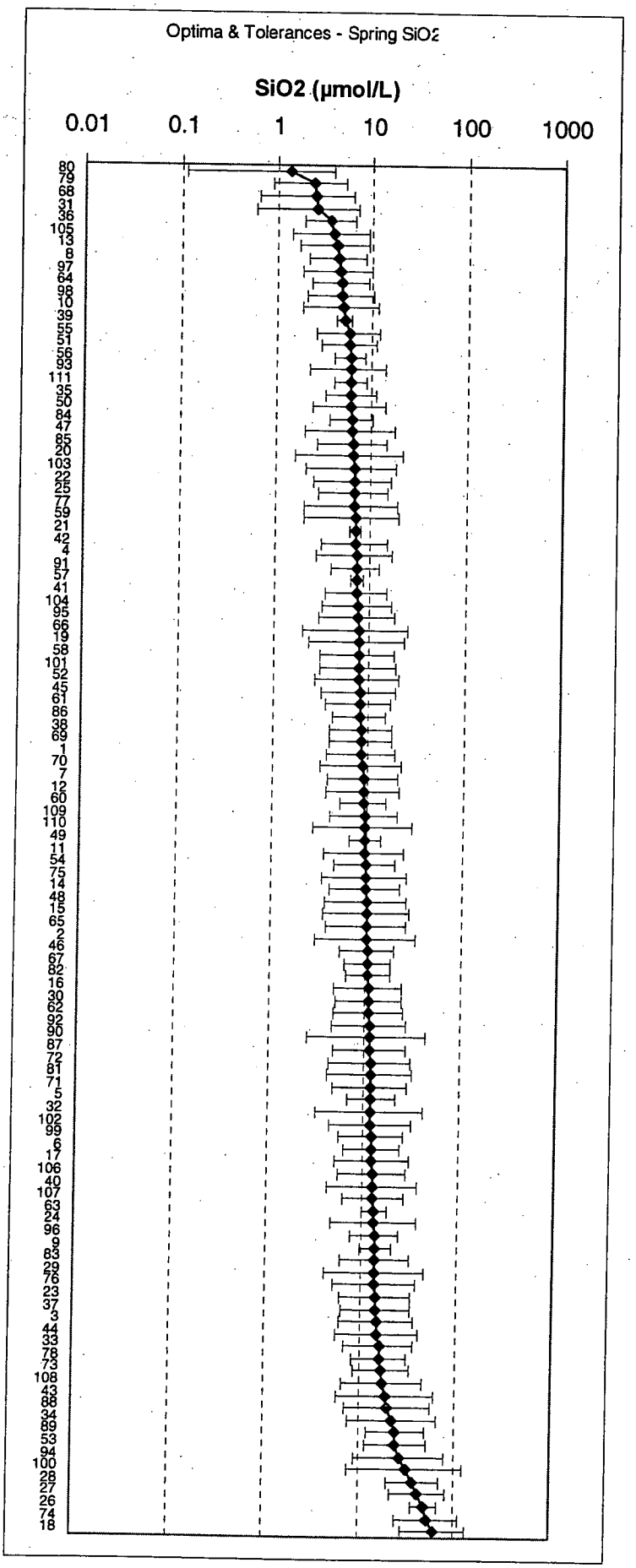


Figure 2.16: Optima and tolerances of the training set species for spring SiO_2

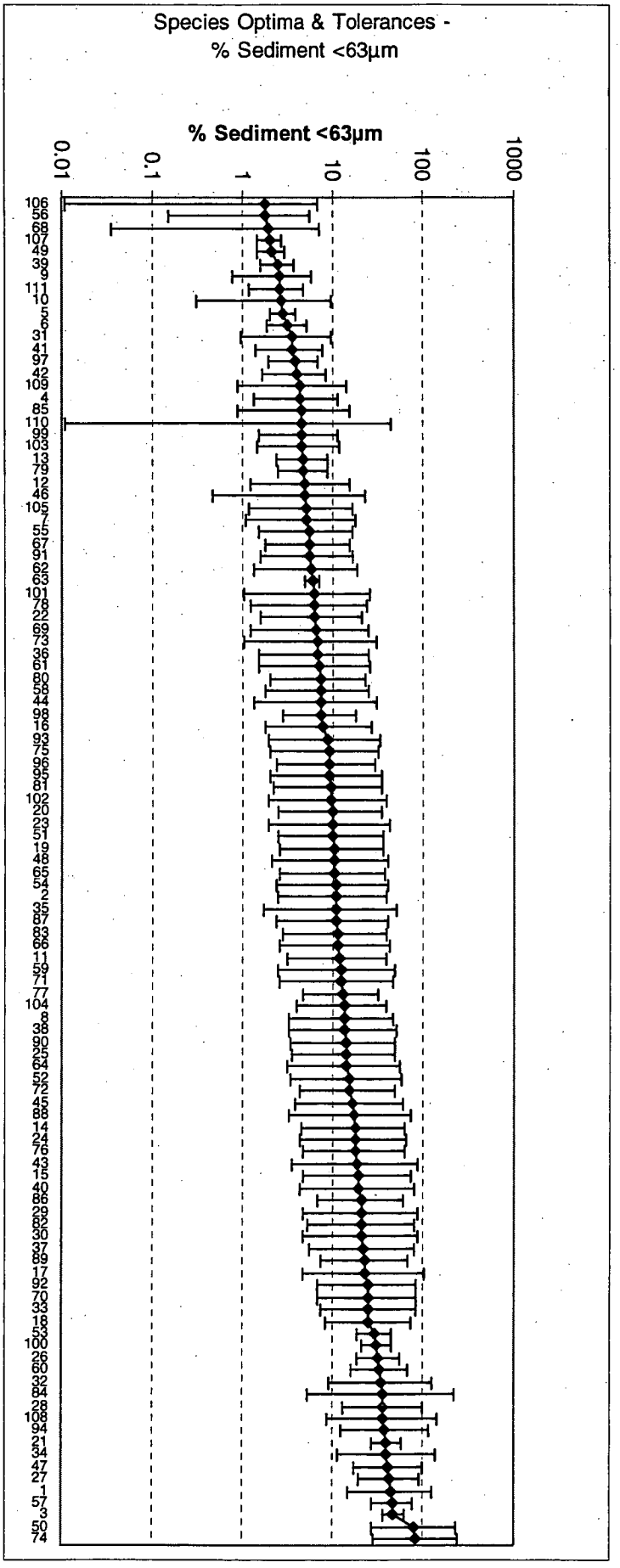


Figure 2.17: Optima and tolerances of training set species for % sediment < 63 µm

Table 2.7: List of training-set species, species code number used in analyses, number of occurrences (N), effective number of occurrences (N2 – (Hill 1973)), maximum relative abundances (% of diatom community composition at each site), and optima & tolerance range for spring NO_{2-3}, spring SiO_2 and % sediment < 63 μm

Species Code	Species Name	N	N2	Maximum Relative Abundance (%)	Optima & Tolerance Range NO2-3 (μmol/L)			Optima & Tolerance Range SiO2 (μmol/L)			Optima & Tolerance Range % Sediment <63μm		
					Optimum	Min.	Max.	Optimum	Min.	Max.	Optimum	Min.	Max.
1	*Achnanthes brevipes*	1	1.0	5.25	0.380	0.001	1.086	8.77	3.63	19.61	44.71	15.02	129.41
2	*Achnanthes residensis*	28	21.0	5.29	0.508	0.001	1.688	10.66	2.92	33.66	10.88	2.47	39.66
3	*Achnanthes oblongella*	3	2.2	2.00	0.935	0.134	2.303	15.31	6.20	35.95	48.41	36.94	63.34
4	*Amphora aequalis*	10	8.5	8.35	0.037	0.001	0.126	7.30	2.72	17.50	4.39	1.31	11.60
5	*Amphora decussata*	4	3.1	4.09	0.163	0.032	0.309	12.41	6.82	22.00	2.84	1.97	3.97
6	*Amphora exigua*	4	3.4	10.82	0.178	0.059	0.311	12.89	6.46	24.87	3.21	1.85	5.22
7	*Amphora laevissima*	20	10.5	43.75	0.292	0.001	0.964	9.31	3.78	21.24	5.30	1.09	17.99
8	*Amphora maletractata* var. *constricta*	12	9.6	7.56	0.152	0.016	0.306	4.54	2.15	8.77	13.52	3.25	48.57
9	*Amphora* species 1	2	1.6	12.38	0.006	0.001	0.022	14.33	9.71	20.93	2.52	0.77	5.96
10	*Amphora submontana*	1	1.0	2.27	0.023	0.001	0.546	5.03	1.86	11.71	2.72	0.30	9.60
11	*Amphora subturgida*	42	34.5	23.68	0.442	0.001	1.363	9.87	3.56	24.88	11.93	3.11	39.67
12	*Anaulus minutus*	22	16.2	10.37	0.213	0.001	0.738	9.40	3.67	22.14	5.01	1.19	15.48
13	*Anorthoneis vortex*	11	6.5	6.56	0.053	0.010	0.097	4.30	1.70	9.41	4.81	2.36	9.05
14	*Bacillaria paradoxa*	34	25.9	9.40	0.407	0.001	1.228	10.11	4.08	23.28	17.92	4.59	62.98
15	*Cocconeis stauroneiformis*	14	10.8	4.97	0.657	0.001	1.822	10.58	3.55	28.50	20.13	4.80	75.99
16	*Catenula adhaerens*	39	29.7	16.46	0.295	0.001	0.929	11.39	4.86	25.22	7.90	1.78	27.54
17	*Chaetocerus resting spores*	9	6.2	8.95	0.821	0.119	1.963	13.04	5.20	30.78	23.57	4.72	104.56
18	*Cocconeis carminata*	1	1.0	10.90	1.344	0.551	2.543	62.10	28.92	132.06	25.92	8.43	75.79
19	*Cocconeis disculoides*	33	26.2	10.95	0.443	0.001	1.722	8.09	2.28	24.15	10.54	2.56	36.36
20	*Cocconeis disrupta*	15	9.0	3.38	0.314	0.001	1.039	6.75	1.56	22.39	10.20	2.42	35.75
21	*Cocconeis molesta* var. *crucifera*	7	6.8	2.00	0.170	0.038	0.319	7.06	6.23	7.97	40.21	27.19	59.26
22	*Cocconeis peltoides*	22	17.3	4.81	0.206	0.001	0.538	6.82	2.46	16.64	6.56	1.56	21.35
23	*Cocconeis placentula*	15	11.5	3.47	0.459	0.001	1.727	14.82	6.12	34.14	10.31	1.88	43.46
24	*Cocconeis placentula* var. *euglypta*	19	15.0	8.58	0.690	0.001	2.286	14.10	4.87	37.88	17.94	4.32	66.34
25	*Cocconeis scutellum*	33	25.3	20.09	0.313	0.001	0.981	6.85	2.78	15.30	14.08	3.56	48.82
26	*Cocconeis scutellum* var. *parva*	3	2.7	5.80	1.951	1.143	3.062	50.37	36.93	68.57	32.89	19.07	56.23
27	*Cyclotella stelligera*	8	5.8	6.22	3.332	1.036	8.219	42.39	21.70	81.93	43.48	20.07	92.89

Continued....

Table 2.7 (continued)

Species Code	Species Name	N	N2	Maximum Relative Abundance (%)	Optima & Tolerance Range NO2-3 (µmol/L)			Optima & Tolerance Range SiO2 (µmol/L)			Optima & Tolerance Range % Sediment <63µm		
					Optimum	Min.	Max.	Optimum	Min.	Max.	Optimum	Min.	Max.
28	*Cyclotella striata*	11	5.6	22.89	2.882	1.005	6.519	38.19	20.40	70.77	36.98	13.27	100.10
29	*Cymbella minuta*	8	5.5	2.50	1.060	0.001	3.868	14.46	4.12	45.73	21.55	4.71	88.04
30	*Cymbella sumatrensis*	5	3.5	2.00	0.629	0.027	1.587	11.46	5.10	24.44	21.94	4.77	90.19
31	*Delphineis surirella*	5	2.9	6.83	0.137	0.001	0.985	2.63	0.60	7.24	3.58	0.93	9.83
32	*Dimerogramma minor* var. *nana*	6	3.4	2.27	0.819	0.001	2.515	12.42	3.16	42.28	35.42	9.16	129.51
33	*Diploneis notabilis*	6	3.7	6.61	1.142	0.191	2.852	16.52	7.01	37.33	25.91	7.51	84.10
34	*Diploneis subovalis*	7	5.2	5.68	1.536	0.139	4.650	23.11	7.60	66.55	40.43	11.30	138.56
35	*Diploneis vacillans*	14	10.2	10.32	0.240	0.001	0.573	6.24	3.23	11.38	10.96	1.72	51.50
36	*Ehrenbergia granulosa*	12	6.6	21.71	0.081	0.001	0.174	3.70	1.92	6.56	7.04	1.47	25.10
37	*Fallacia litoricola*	22	15.2	19.19	1.008	0.125	2.583	15.08	6.35	34.18	22.32	5.69	80.26
38	*Fallacia subforcipata*	13	9.5	6.61	0.343	0.001	0.924	8.69	3.95	17.97	13.82	3.24	50.86
39	*Fragilaria atomus*	2	1.3	2.50	0.058	0.001	0.245	5.21	4.36	6.19	2.49	1.56	3.75
40	*Fragilaria crotonensis*	8	6.7	3.67	0.910	0.001	2.681	13.42	4.33	38.07	20.16	4.33	83.08
41	*Fragilaria martyi*	10	9.0	5.34	0.127	0.001	0.299	7.56	3.48	15.35	3.61	1.37	7.96
42	*Fragilaria pinnata*	28	22.1	24.06	0.100	0.001	0.265	7.24	3.07	15.67	4.06	1.62	8.77
43	*Fragilaria pinnata* var. *pinnata*	11	5.6	16.74	1.874	0.001	7.997	19.79	5.89	61.74	19.39	3.63	88.85
44	*Fragilariopsis cylindrus*	19	12.3	9.92	0.499	0.001	1.923	15.65	5.64	40.75	7.61	1.32	30.86
45	*Gramatophora oceanica*	25	17.2	6.63	0.359	0.001	1.115	8.22	3.18	19.34	16.61	3.91	62.21
46	*Gyrosigma fasciola*	7	3.6	5.87	0.139	0.075	0.206	10.88	5.48	20.79	5.03	0.47	23.75
47	*Gyrosigma perthense*	4	3.0	2.06	0.175	0.001	0.534	6.49	1.96	17.96	42.31	17.23	101.88
48	*Hyalodiscus scoticus*	9	6.6	3.18	0.659	0.034	1.662	10.43	3.76	26.44	10.59	2.09	42.53
49	*Lunella bisecta*	5	3.6	11.09	0.162	0.055	0.279	9.81	6.50	14.57	2.09	1.44	2.92
50	*Mastoglia smithii*	1	1.0	2.95	0.001	0.001	0.511	6.24	2.44	14.28	80.28	27.49	230.90
51	*Mastogloia pusilla* var. *pusilla*	12	9.8	3.50	0.141	0.001	0.314	6.00	2.92	11.50	10.34	2.43	36.51
52	*Mastogloia* species 1	20	14.8	5.41	0.309	0.001	1.038	8.14	2.76	21.21	15.34	3.47	58.76
53	*Melosira nummuloides*	8	5.7	3.71	1.767	0.584	3.833	24.98	11.66	52.32	29.67	19.22	45.52
54	*Navicula arenaria* var. *rostellata*	23	15.4	12.50	0.353	0.001	1.160	9.97	4.63	20.41	10.88	2.34	41.25
55	*Navicula cancellata*	23	17.5	7.08	0.147	0.005	0.310	5.99	2.60	12.56	5.71	1.52	16.86
56	*Navicula cryptocephala*	2	1.3	3.83	0.264	0.128	0.417	6.03	4.08	8.73	1.77	0.15	5.67
57	*Navicula menisculus*	6	3.9	7.75	0.169	0.001	0.375	7.43	6.29	8.76	46.96	27.41	79.96

Continued.....

Table 2.7 (continued)

Species Code	Species Name	N	N2	Maximum Relative Abundance (%)	Optima & Tolerance Range NO2-3 (µmol/L)			Optima & Tolerance Range SiO2 (µmol/L)			Optima & Tolerance Range % Sediment <63µm		
					Optimum	Min.	Max.	Optimum	Min.	Max.	Optimum	Min.	Max.
58	*Navicula monoculata* var. *omissa*	51	44.3	56.16	0.270	0.001	0.902	8.09	3.08	19.25	7.55	1.76	25.50
59	*Navicula nyella*	6	4.6	6.16	0.369	0.001	1.067	7.03	1.99	20.60	12.30	2.46	50.09
60	*Navicula pygmaea*	6	4.5	3.69	0.404	0.032	0.910	9.47	5.34	16.29	34.04	16.32	69.90
61	*Navicula salinarum*	13	8.1	5.79	0.312	0.001	0.759	8.23	3.56	17.68	7.23	1.50	26.06
62	*Navicula* species 1	18	12.6	12.36	0.163	0.001	0.530	11.57	4.90	25.76	5.85	1.31	19.30
63	*Navicula* species 2	2	1.9	2.64	0.321	0.052	0.660	13.98	10.18	19.09	6.14	5.06	7.40
64	*Navicula tripunctata*	15	9.7	6.65	0.161	0.010	0.334	4.89	2.34	9.37	14.47	3.10	57.35
65	*Nitzschia amphibia Grun.*	49	41.2	32.23	0.447	0.001	1.493	10.60	3.91	26.41	10.82	2.52	38.69
66	*Nitzschia dissipata* var. *dissipata*	4	3.6	2.00	0.612	0.001	1.952	7.95	2.00	25.66	11.68	2.55	44.34
67	*Nitzschia laevis*	14	10.5	4.44	0.184	0.001	0.436	10.92	6.08	19.07	5.71	1.73	15.51
68	*Nitzschia longissima*	1	1.0	6.83	0.001	0.001	0.511	2.47	0.64	6.31	1.95	0.03	7.42
69	*Nitzschia lorenziana* var. *subtilis*	21	14.6	8.00	0.129	0.001	0.394	8.70	3.95	18.00	6.57	1.20	25.08
70	*Nitzschia ovalis*	5	4.2	2.23	0.555	0.001	2.662	8.88	3.12	22.69	25.66	7.11	86.62
71	*Nitzschia panduriformis* var. *minor*	40	30.8	3.75	0.515	0.001	1.674	12.29	4.89	29.00	12.35	2.61	48.33
72	*Nitzschia* species 1	17	13.1	6.19	0.590	0.001	1.874	12.11	4.40	30.85	15.48	4.43	48.99
73	*Nitzschia* species 2	15	11.9	2.00	0.588	0.001	2.104	17.35	8.75	33.55	7.03	1.02	30.98
74	*Opephora martyi*	1	1.0	3.49	8.333	5.176	13.103	53.95	25.06	114.89	84.11	28.83	241.83
75	*Opephora olsenii*	49	38.8	28.07	0.421	0.001	1.574	10.07	3.44	26.59	9.17	2.02	33.25
76	*Paralia sulcata*	13	11.2	5.04	0.662	0.001	2.127	14.62	5.17	38.56	18.56	4.83	64.68
77	*Parlibellus* cf. *plicatus*	9	7.0	5.02	0.357	0.001	1.245	6.88	1.97	19.88	13.21	4.90	33.22
78	*Plagiogramma staurophorum*	21	13.1	32.71	0.358	0.001	1.288	16.55	8.52	31.38	6.51	1.23	24.33
79	*Plagiotropis* species 2	4	2.7	4.02	0.059	0.021	0.098	2.43	0.89	5.23	4.89	2.48	8.95
80	*Plagiotropis* species 1	1	1.0	2.54	0.072	0.001	0.619	1.34	0.11	3.94	7.51	1.98	23.28
81	*Planothidium delicatulum*	35	22.9	15.16	0.491	0.001	1.883	12.28	4.16	33.18	9.82	2.21	35.42
82	*Pseudonitzschia australis*	9	6.1	8.37	0.336	0.001	0.960	10.97	6.28	18.69	21.92	5.47	80.24
83	*Skeletonema costatum*	29	20.4	70.10	0.700	0.001	2.180	14.45	6.03	32.93	11.48	2.77	40.28
84	*Surirella fastuosa*	3	1.7	6.88	0.200	0.001	0.621	6.37	3.72	10.51	36.40	5.36	218.84
85	*Cocconeis* aff. *pinnata*	14	7.0	9.73	0.113	0.001	0.631	6.70	2.65	15.20	4.51	0.87	15.27
86	*Synedra investiens*	10	6.5	9.50	0.213	0.001	0.741	8.40	4.39	15.41	21.15	6.87	61.32
87	*Synedra tabulata*	34	22.9	15.45	0.563	0.001	1.488	12.09	4.82	28.42	11.15	2.39	42.50

Continued....

Table 2.7 (continued)

Species Code	Species Name	N	N2	Maximum Relative Abundance (%)	Optima & Tolerance Range NO2-3 (µmol/L)			Optima & Tolerance Range SiO2 (µmol/L)			Optima & Tolerance Range % Sediment <63µm		
					Optimum	Min.	Max.	Optimum	Min.	Max.	Optimum	Min.	Max.
88	*Thalassionema nitzschoides*	11	8.5	4.98	1.848	0.212	5.694	20.35	7.09	55.29	17.23	3.35	75.48
89	*Thalassiosira eccentrica*	12	7.0	10.23	1.390	0.513	2.776	24.92	12.33	49.40	23.36	7.52	68.62
90	*Thalassiosira oestrupii*	8	5.8	5.72	0.899	0.001	3.995	11.69	2.55	44.32	14.00	3.45	49.55
91	*Achnanthes* species 1	6	4.6	2.55	0.251	0.024	0.527	7.32	4.01	12.80	5.77	1.55	16.98
92	Species 1	27	18.4	20.88	0.628	0.001	2.000	11.65	4.63	27.41	25.08	6.89	85.23
93	*Navicula halophila*	11	8.3	3.49	0.135	0.001	0.411	6.03	2.20	14.48	9.09	1.90	34.10
94	*Diploneis* species 1	8	5.4	14.19	3.026	0.501	9.799	27.88	9.14	81.22	39.14	12.49	118.41
95	*Cocconeis* species 1	10	6.8	3.21	0.260	0.001	0.681	7.72	2.88	18.60	9.32	1.96	34.96
96	*Navicula* species 3	4	2.7	2.33	0.077	0.001	0.250	14.21	7.87	25.09	9.18	2.36	29.79
97	Species 2	8	6.7	3.59	0.135	0.001	0.310	4.70	1.87	10.34	3.89	1.96	7.08
98	*Fragilaria* species 1	10	7.5	3.73	0.110	0.001	0.254	4.95	2.05	10.58	7.64	2.83	18.47
99	*Cymatosira aff. belgica*	14	9.7	31.93	0.076	0.001	0.326	12.62	5.77	26.39	4.63	1.48	11.75
100	Species 3	2	1.8	6.96	0.951	0.342	1.838	32.08	7.85	122.68	31.33	21.23	46.02
101	*Navicula cincta*	12	9.4	4.72	0.432	0.001	1.479	8.13	3.01	19.78	6.49	1.03	26.61
102	Species 4	24	14.4	34.95	0.680	0.001	2.076	12.54	4.46	32.59	9.90	1.88	40.21
103	Species 6	3	2.5	3.24	0.084	0.041	0.128	6.79	2.07	18.77	4.63	1.41	12.11
104	Species 7	1	1.0	5.78	0.072	0.001	0.619	7.71	3.13	17.37	13.45	4.07	40.24
105	Species 5	1	1.0	12.94	0.096	0.001	0.657	4.01	1.38	9.57	5.17	1.16	16.59
106	Species 8	1	1.0	5.68	0.148	0.001	0.735	13.13	5.70	28.79	1.75	0.01	6.86
107	Species 9	2	1.7	6.37	0.332	0.289	0.376	13.59	6.49	27.45	1.97	1.41	2.67
108	Species 10	3	1.5	9.62	1.239	0.001	4.381	17.88	6.68	45.44	37.32	8.84	148.24
109	Species 11	1	1.0	5.32	0.445	0.001	1.184	9.72	4.08	21.60	4.37	0.88	14.32
110	*Diploneis* species 2	2	1.5	3.54	0.385	0.001	1.928	9.74	2.67	30.46	4.62	0.01	46.47
111	*Fragilaria vaucheriae*	2	1.5	16.98	0.117	0.099	0.135	6.23	4.22	9.01	2.56	1.18	4.80

2.4 DISCUSSION

2.4.1 Nutrient Concentrations in South-east Tasmanian Marine Waters

Nutrients concentrations in the marine environment of south-east Tasmania are comparable with other marine environments around the world. Nutrient concentrations recorded from the south-east Tasmanian sites ranged from < 0.01 to 8.24 µmol/L for NO_{2-3}, 0.10 to 1.21 µmol/L for PO_4, and 0.16 to 61.65 µmol/L for SiO_2. Nutrient concentrations in the surface waters of the Indonesian Archipelago have been reported, with concentrations of NO_3-N ranging from < 0.1 to 1.0 µmol/L (≈ NO_3 of < 0.44 to 4.4 µmol/L), PO_4-P from 0.05 to 0.3 µmol/L (≈ PO_4 of 0.15 to 0.81 µmol/L), and H_4SiO_4 of 1.0 to 3.0 µmol/L, with the higher concentrations occurring during seasonal upwelling (van Iperen *et al.* 1993). (Nilsson *et al.* 1991) reported seawater nutrient concentrations from the shallow (2 m depth) coastal waters of Sweden of NO_{2-3} < 1.0 µmol/L, PO_{3-4} < 0.2 µmol/L, and $Si(OH)_4$ between 2.5 and 7.5 µmol/L.

In Australia, NO_3 concentrations < 2 µmol/L, and PO_{3-4} < 1 µmol/L were reported from Moreton Bay, a large marine embayment on the east coast of the mainland (O'Donohue & Dennison 1997). Nutrient concentrations in Moreton Bay were also reported by (O'Donohue *et al.* 2000), with oceanic samples having NO_3 concentrations of 0.0 to 0.3 µmol/L, and PO_{3-4} of 0.0 to 0.4 µmol/L. From the Gulf of Carpentaria, northern Australia, average nutrient concentrations from four sites on nine sampling occasions showed NO_{2-3} concentrations ranging from 0 to 3.68 µmol/L, PO_4 from 0.09 to 3.0 µmol/L, and SiO_3 from 0.19 to 13 µmol/L (Burford 1995) (six of these sampling occasions were during the monsoonal wet season, when river output increased nutrient loadings and reduced salinity at the sites from 35-36 to 31-32).

The ANZECC guidelines for fresh and marine water quality (ANZECC 2000) include 'trigger values' that are commonly used as a guide to the level at which nutrient concentrations may pose a threat to marine and estuarine water quality in Australia. These guidelines provide figures for individual states of Australia, however they have been determined from geographical regions that do not include Tasmania, and the guidelines therefore recommend caution in applying these trigger values to Tasmanian systems. (Trigger values for the marine environment in the

ANZECC guidelines are provided in µg/L, but have also been converted here to approximate µmol/L for ease of comparison). ANZECC trigger values for nitrogen and phosphorus in Tasmania's marine environment are as follows: NO_x of 5 µg/L (≈ 0.093 µmol/L); and TP of 25 µg/L (≈ 0.807 µmol/L, and is equivalent to PO_4 of ~2.475 µmol/L). The ANZECC trigger values provided for estuarine environments are 3 times higher for NO_x (15 µg/L or ~ 0.28 µmol/L) and 1.2 times higher for TP (30 µg/L or ~ 0.97 µmol/L, or 2.97 µmol/L PO_4). Nutrient concentrations at some sites in the Derwent were therefore above the ANZECC guidelines at the time of sampling` for NO_x but not for PO_4.

The range of nutrient concentrations recorded from the south-east Tasmanian marine environment is therefore similar to concentrations reported from near-shore marine environments in other areas of the world, including mainland Australia, although some NO_{2-3} and SiO_2 concentrations were relatively high at some sites in this Tasmanian study.

Although nitrogen and phosphorus concentrations play a key role in the process of eutrophication, silica also plays a key role, particularly in determining the dominant type of algae present (Kilham 1971, Egge & Aksnes 1992, Wu & Chou 2003). Research has shown that in many aquatic ecosystems diatoms are the dominant species if silica is in sufficient supply, however other taxa such as *Phaeocystis* will often dominate when silica concentrations become low (Egge & Aksnes 1992). Additionally, enrichment with silica may give rise to a greater increase in the biomass of phytoplankton (especially diatoms) than additions of nitrogen and phosphorus (Wu & Chou 2003) depending on the nutrient balance in the system. Therefore, the concentration of silicate in a system plays a very important role in the process of eutrophication, and (Kilham 1971) suggests that some level of 'silica demand' could be used as an index of increasing eutrophication. Silica concentrations at south-east Tasmanian sites were relatively high during spring, especially at sites with high N and P, but were significantly lower during autumn, and should be monitored along with N and P concentrations as the balance between these nutrients is as important as their overall levels (Hecky & Kilham 1988).

2.4.2 Physical and Chemical Conditions within the Study Area

A better understanding of the relationships between the diatom assemblages and their environment can be obtained by a thorough investigation of the physical and chemical characteristics of sites within the study area. The intention of the original site selection was to choose sites that would be broadly similar and only differ in nutrient concentration. The sites were sufficiently similar so that nutrient concentrations, along with fine sediment composition, did explain the greatest variation in diatom assemblages. However, there was also considerable variability between sites, and a more detailed examination shows that within the study area there are various 'environmental zones'. These zones can be used to distinguish between areas that contain sites which are similar either physically or chemically, or both. The following section discusses the grouping of sites into zones, and some of the key inter-site differences and similarities, to provide a more comprehensive picture of the environmental conditions throughout the study area.

Although the near-shore coastal marine sites sampled for the training set had a similar salinity and temperature, the influence of warmer fresh water was clearly evident at Sites 1, 3 and 7 (upper Derwent Estuary) during spring (with temperatures ~ 2°C above average and salinity ≤ 15). Here, low salinity measurements were directly associated with an increase in water temperature. The environmental factor separating these sites from near-by sites is water depth (< 2 m). At surrounding sites (Sites 2, 5, 6 and 8), overlying freshwater was also evident (from 1 m water depth measurements), however these sites were deeper (> 2.4 m) and were more saline at the bottom. The middle to lower reaches of the Derwent Estuary (south of the Bowen Bridge) have been reported as being partially- to well-mixed (dominated by tidal and wind-driven mixing) (Coughanowr 1997). Therefore, it is likely that Sites 1 to 8 would all experience reduced salinity occasionally as tidal and river flows fluctuate, and land-based run-off varies.

Freshwater influence during spring was also evident in Ralphs Bay, where an increasing salinity gradient (from ~26 to 30) was recorded with distance away from the village of Lauderdale toward the entrance to the bay (sites 17 to 22). However, these sites ranged in depth from ~2.3 m to 3.2 m, and spring salinity was almost identical at maximum depth and 1m depth. The reduced salinity at these sites is related to the freshwater flow through the lower Derwent Estuary. In the Derwent

Estuary between Hobart and the northern tip of Bruny Island, sampling sites on the western side of the estuary had noticeably higher spring salinity than sites on the eastern side of the estuary. This suggests that the freshwater leaving the Derwent was flowing on the eastern side of the estuary, and was subsequently well mixed with the more saline marine waters of Ralphs Bay. This finding is supported by lower salinity at 1 m at eastern sites also occurring during autumn, and is consistent with previous findings and hydrodynamic models developed for the Derwent by the CSIRO (1994, cited in (Coughanowr 1997)).

There are thus two zones within the study area in which sites experienced reduced salinity for at least part of the year: (i) the Derwent estuary between the Tasman Bridge and the Bowen Bridge, also including Montague Bay (Sites 1 to 8); and (ii) sites in the northern half of Ralphs Bay (Sites 16 to 21).

Sites in the upper Derwent Estuary (Sites 1 to 9) had higher light attenuation during spring than most other sites in the study area (although Secchi depth was 100% of water depth at sites 1 and 7, they were very shallow – 1.39 m and 1.07 m respectively). The high light attenuation in the upper Derwent sites is partly a result of these sites generally having finer benthic sediments which are more easily suspended, in addition to the sediment-laden overlying freshwater flowing down-river, and input from urban run-off, sewage and industry. Light attenuation has been shown to significantly influence diatom community composition (O'Donohue & Dennison 1997, Whitehead & McMinn 1997). As a consequence of combined differences in salinity, temperature and reduced light, sites in the upper Derwent have significantly different environmental characteristics to other sites within the study area.

Sites in the upper Derwent (Sites 1 to 9) had different nutrient concentrations to other sites, with the overall highest spring concentrations of NO_{2-3}, PO_4 and SiO_2. The most eutrophic bay in the Derwent was Prince of Wales Bay (Sites 2 at the entrance to the bay, and Site 3 well inside the bay). Site 2 had the highest spring SiO_2 and autumn NO_{2-3}, and Site 3 had the highest autumn SiO_2, spring NO_{2-3}, and spring and autumn PO_4. Prince of Wales Bay is a medium-sized, relatively shallow (2-5 m) and morphologically complex bay with a restricted opening into the Derwent. Nutrient input into this bay is considerable. Situated at the entrance to this bay, along and

inside the southern embankment, are the workshops of International Catamaran (Incat), a large (international) commercial-catamaran building company. Further inside the bay along the southern bank is a sewage treatment plant. Numerous boat jetties, residential development and major roads surround the remaining shoreline of the bay. The sediment sample obtained from Site 3 contained a tar-like substance and had a strong unpleasant smell. With such high nutrient inputs, increased light attenuation and restricted flushing due to the relatively narrow entrance, the water quality of this bay is degraded, and threatened by further eutrophication.

It is also possible to group sites according to sediment size. There were two geographic zones within the study area in which all sites contained a high percentage of fine-grained sediments. One zone includes the upper Derwent Estuary down to Sandy Bay (Sites 1 to 12), and the other zone includes all of the sites on the western side of the D'Entrecasteaux Channel from Oyster Cove (Site 32) to Gordon (Site 40) (this is a relatively narrow area of the Channel). Most sites on the eastern side of the Channel had coarser sediments, except for one relatively protected site (Site 35 at Barnes Bay). Differences in the sediment size in various zones are indicative of the difference in the energy of water flow within the area (with finer sediments settling in calmer waters).

Although the entire depth range for all sites (0.44 m to 6.41 m) did not have as significant an influence on diatom community composition as nutrient concentration, many studies report finding differences in diatom community structure associated with depth (Round 1981, Stevenson & Stoermer 1981, Stevenson *et al.* 1985) and transfer functions have been developed to infer depth (Whitehead & McMinn 1997, Yang *et al.* 2003). It is therefore likely that intra-site depth-related differences in diatom assemblages did exist, particularly since co-variables (such as salinity, temperature and light) changed to such a large extent with depth in some areas (e.g. upper Derwent). The fact that associations between depth and community composition were not identified in this study may simply be a consequence of the over-riding influence of nutrients and sediment size on the diatom assemblages.

The physical and chemical differences between sites can be used to qualitatively group sites into the following zones (Figure 2.18):

(i) Zone 1: Derwent Estuary from Site 1 to 8 – Fine to medium grain–size sediments, high to very high nutrient concentrations, low light, and variable salinity and temperature;

(ii) Zone 2: Derwent Estuary from Site 9 to 12 – Fine to medium grain–size sediments, moderate to high nutrient concentrations, moderate light; relatively stable salinity;

(iii) Zone 3: Ralphs Bay (Sites 16- 21) Medium grain-size sediments, low to moderate nutrient concentrations, variable (reduced) salinity;

(iv) Zone 4: Lower Derwent to the Channel (Sites 13 to 31 - excluding Zone 3 & Site 30) Medium grain- size sediments, low to moderate nutrient concentrations, stable salinity;

(v) Zone 5: Western side of the D'Entrecasteaux Channel from Oyster Cove to Gordon (Site 30 to 40, excluding Sites 31, 36) Fine to medium grain –size sediments, generally low to moderate nutrient concentrations, stable salinity;

(vi) Zone 6: Bruny Island and Lower Channel (Sites 36, 41 to 45) Medium grain-size sediments, generally very low nutrient concentrations, salinity slightly higher and more stable;

(vii) Zone 7: Pittwater area (Sites 46 to 51) Medium grain- size sediments, low to moderate nutrient concentrations (increasing toward the causeway), stable salinity.

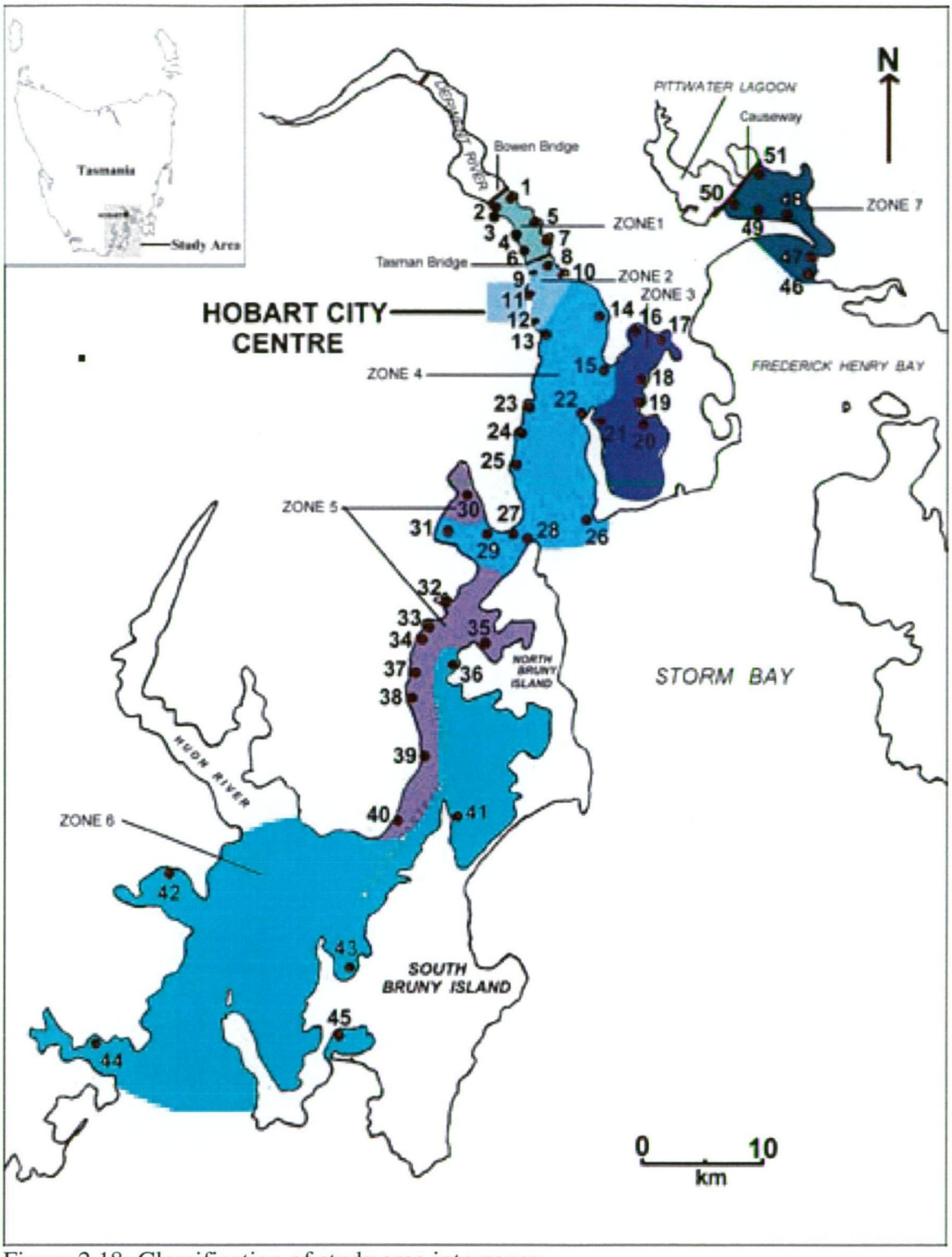


Figure 2.18: Classification of study area into zones

The above classification divides the study area into zones in a linear fashion (excluding zone 7) so that changes can be more easily identified across a continuum (Figure 2.18). This highlights a key point in regard to environmental conditions within the study area. Although nutrient concentrations generally decrease southwards from sites in the Derwent down through the D'Entrecasteaux Channel, individual sites located adjacent to more densely populated areas still showed

elevated nutrient concentrations (e.g. at Kingston Beach, Blackmans Bay and North West Bay – Sites 24, 25 and 30, respectively), and the highest nutrient concentrations overall were from the most densely populated areas. This direct link between human occupation and increased nutrient concentrations highlights the need for ongoing monitoring and assessment of the impacts on the micro-algal community in the south-east Tasmanian marine environment.

Surprisingly, at two sites in close proximity to fish farms (within ~ 100-150 m at Sites 29 and 38) samples showed particularly low nutrient concentrations. Fish farms are generally located in areas of moderately high water flow to avoid de-oxygenation of the water within the nets. It is possible that increased water flow, or the direction of flow, may be directing the nutrient load away from the sampling area. However, the impact may also be more localised around the nets.

A second point highlighted by the classification of sites into zones is the broad range of environmental conditions at sites across the study area as a result of variations in only a few environmental variables. Within each zone, inter-site differences are still considerable, and it could therefore be expected that the composition of diatom assemblages would reflect this.

2.4.3 Diatom Assemblages

The dominant diatom species at each site varied both between environmental zones, and between sites within environmental zones. This reflects the wide range of environmental factors combining in different ways to affect the floral composition at each site. However, certain trends in the distribution of individual species were evident. The dominant diatom species from each of the identified environmental zones are discussed below.

The most widespread diatom species was *Navicula monoculata* var. *omissa*, which was recorded from every site and numerically dominant at 16 sites. Although *N. monoculata* var. *omissa* was widespread, it was recorded in very low numbers in the Derwent Estuary from sites 1 to 12, and also more generally from sites with fine sediments. *Navicula monoculata* var. *omissa* showed a preference for coarser sediments and low to moderate nutrient concentrations, although it was recorded in

high abundance at sites with fine to medium grain-sized sediments and moderate nutrients. The fact that *N. monoculata* var. *omissa* was present at all sites indicates the wide environmental tolerance range of this species. Although species with wide tolerance ranges may generally not be as useful as environmental indicators, the absence of this species in any future sampling would be noteworthy as this would suggest a very significant change in environmental conditions.

Sites in the Derwent Estuary (Zone 1) were dominated by *Skeletonema costatum*, *Opephora olsenii*, *Fragilaria pinnata* var. *pinnata*, *Cyclotella striata*, *Cocconeis placentula* var. *euglypta*, and *Nitzschia amphibia*. Sites in Zone 2 of the Derwent were dominated by *S. costatum*, *N. amphibia* and (at Site 12) *Navicula monoculata* var. *omissa*. Commonly found in coastal environments, these are cosmopolitan species that have been reported as dominating or being abundant in brackish and/or marine diatom assemblages from many areas of the globe, and are discussed below.

Skeletonema costatum is common in the marine environment (Round 1981) and has been widely reported from many areas including False Bay, Washington (Rao & Lewin 1976), coastal deposits of the Netherlands (Vos & de Wolf 1988, 1993a), the Northern Adriatic Sea (Thornton & Thake 1998), Netarts Bay, Oregon (Whiting & McIntire 1985) and northern Australia (Hallegraeff & Jeffrey 1984). *Skeletonema costatum* was also reported by (Twomey & John 2001) as periodically dominating the diatom flora in the lower Swan-Canning estuary in Western Australia. *Skeletonema costatum* is often associated with low light conditions (Pratt 1965) and high nutrient concentrations (Round 1981). The abundance, and at some sites the dominance, of this species in the Derwent is therefore consistent with reports on its autecology.

Opephora species are also common epipsammic diatoms in the marine environment (Werner 1977, Round 1981), and *Opephora olsenii* has been reported from many coastal environments including the coastal shallows of Puck Bay, Poland (Witkowski 1991) and the west coast of Sweden (Sundbäck & Snoeijs 1991b). Witkowski (1991) described *O. olsenii* as a species typical of coastal shallow sediment, and indeed this species was recorded from most of the shallow coastal sites in south-east Tasmania. However, whereas Witkowski (1991) also reports an increase in the abundance of *O. olsenii* coinciding with higher organic matter content of the sediment, at south-east

Tasmanian sites *O. olsenii* was recorded in high proportions from sites with either high or low sediment TOC.

Fragilaria species are common in both fresh water and marine environments (Round 1973). Although varieties of *Fragilaria pinnata* are commonly reported from marine environments (Rao & Lewin 1976, Palmer 1978, Whiting & McIntire 1985, Vos & de Wolf 1993a), *F. pinnata* var. *pinnata* has not been reported as widely. However, this variety has been reported from Basin Head Harbour, Prince Edward Island (Atlantic Canada) (Palmer 1978), and categorised as oligohalobian (normally freshwater). In the Derwent, *F. pinnata* var. *pinnata* was abundant at Site 3 (Prince of Wales Bay) which had low salinity during spring, but was uncommon and recorded only in very small numbers elsewhere. This suggests that *F. pinnata* var. *pinnata* may have a similar tolerance for salinity in south-east Tasmania to that reported by Palmer (1978).

Cyclotella striata is generally a brackish species (Round 1981), and has been reported from a wide range of brackish and marine environments including the Baltic Sea (Grönlund 1993, Bianchi *et al.* 2000), coastal deposits of the Netherlands (Vos & de Wolf 1993a) the Indonesian Archipelago (van Iperen *et al.* 1993), continental shelf waters of north and north-west Australia (Hallegraeff & Jeffrey 1984), eastern Australia (Foged 1978) and the Swan-Canning Estuary from Western Australia (John 1983, Twomey & John 2001). The presence of *Cyclotella striata* at south-east Tasmanian sites was mostly restricted to sites higher up the Derwent, where it was recorded at > 20% relative abundance at Sites 2 and 5, and in smaller abundance at other sites. The presence of *C. striata* at these sites is therefore consistent with reports on its preference for brackish environments.

Cocconeis species are commonly epipsammic flora in marine environments, however *Cocconeis placentula* var. *euglypta* is an epiphytic form (Round 1981). *Cocconeis placentula* var. *euglypta* has been reported from coastal areas including the north-western Baltic (Miller & Risberg 1990), False Bay, Washington (Rao & Lewin 1976), Netarts Bay, Oregon (Whiting & McIntire 1985), and was reported by John (1983) as a common epiphytic species in the upper reaches of the Swan River, Western Australia. *Cocconeis placentula* var. *euglypta* has been categorised as a dominant epiphytic diatom on the seagrass species, *Zostera marina* (Edsbagge

1966b, 1966a) cited in (Werner 1977). In the Derwent and Channel areas, small beds of the seagrass *Heterozostera tasmanica* still exist around Cornelian Bay (Site 6), the northern part of Halfmoon Bay and Opossum Bay (both between Sites 22 and 26), as well as smaller patches in some locations further north in the Derwent (Green & Coughanowr 2003). Historic aerial photographs indicate that seagrass beds were formerly much more widespread, and abundant in Ralphs Bay (Sites 16 to 21) (Rees 1993). The occurrence of *Cocconeis placentula* var. *euglypta* in the Derwent, Channel and Pittwater areas may therefore be associated with remaining patches of the seagrass *Heterozostera tasmanica*. Further research on this matter may provide a means for reconstructing the environmental history of seagrass distribution in south-east Tasmania.

Nitzschia amphibia is a cosmopolitan species reported from many estuarine and coastal environments, including sediments of the north-western Baltic (Miller & Risberg 1990), estuaries of South Africa (Watt 1998), Western Australia (John 1983) and eastern Australia (Foged 1978). It has been suggested that *Nitzschia amphibia* may avoid high nutrient concentrations (Seenayya 1972). However, *N. amphibia* occurred at most of the study sites in south-east Tasmania, and was the dominant species at six sites, including two sites with particularly high nutrient concentrations in the upper Derwent (Sites 8 and 9) and one site with moderate nutrient concentrations (Site 10). The other three sites at which this species was dominant (Sites 27, 34 and 36) had particularly low nutrient concentrations. Nutrient optima and tolerance ranges calculated for *N. amphibia* in south-east Tasmanian waters show an optimum for moderate nutrient concentrations (e.g. $NO_{2\text{-}3}$ of 0.447 µmol/L, Table 2.6) but a wide tolerance range. This highlights the importance of using optima and tolerance ranges for species that have been derived from the geographical region in which they are to be used.

Ralphs Bay (Zone 3) was dominated by *Skeletonema costatum*, *Navicula monoculata* var. *omissa*, *Plagiogramma staurophorum*, and *Cymatosira* aff. *belgica*. *Plagiogramma* and *Cymatosira* species (also common epipsammic diatoms in brackish and marine environments) are non-motile or only slowly motile genera (Round 1981). *Plagiogramma staurophorum* has been reported from False Bay, Washington (Rao & Lewin 1976), coastal areas of the Netherlands (Vos & de Wolf 1988, 1993a), shallow coastal waters of Sweden (Sundbäck & Snoeijs 1991b), the

Swan River Estuary in Western Australia (John 1983), eastern Australia (Foged 1978) and Macquarie Harbour, Tasmania (McMinn *et al.* 2003). The abundance of *Plagiogramma staurophorum* at south-east Tasmanian sites was greatest in Ralphs Bay and the north-eastern side of the causeway at Pittwater lagoon. Apart from being the dominant species at Site 18 in Ralphs Bay, *P. staurophorum* was generally recorded in relatively small proportions at other sites, as was the case in south-western Tasmania (McMinn *et al.* 2003) and Western Australia (John 1983). There were no single outstanding differences measured between Site 18 and other nearby sites, so the reasons for the abundance of *P. staurophorum* at Site 18 (~33%) are unclear. However, this species is reported as being very common in some areas, as was reported in epipsammic samples from False Bay, Washington (Rao & Lewin 1976).

Cymatosira belgica is a common marine tychoplanktonic species reported from estuarine and costal environments from areas including the coastal wetlands of the Netherlands (Vos & de Wolf 1993b, 1993a) and the Ems Estuary, Wadden Sea (de Jonge 1985). However, *Cymatosira* species have not previously been reported from Tasmania, and were not recorded in the survey of diatoms from the Swan River Estuary in Western Australia (John 1983), nor in the bibliotheca of diatoms in eastern Australia by Foged (1978). *Cymatosira lorenziana* was recorded from the Gulf of Carpentaria, northern Australia by (Hallegraeff & Burford 1996) (and previously by (Hasle *et al.* 1983)), who describe this species as widely distributed on warmer coasts. The presence of *Cymatosira* in south-east Tasmania is somewhat surprising, however the morphology of the Tasmanian *Cymatosira* species varies from the nominal variety (e.g. in its shorter length - generally < 10 μm), and may have different ecological requirements. *Cymatosira* aff. *belgica* was restricted to two areas within the study region – Ralphs Bay (where it was the dominant species at Site 20 and 21, constituting 32% and 26% of community composition respectively), and sites in Pittwater (~ 4 to 13%) . Very small proportions (< 1%) of *Cymatosira* aff. *belgica* were also recorded from Sites 5, 9 and 10 in the Derwent (close to Ralphs Bay), possibly as a result of resuspension and deposition, and at both Site 46 (< 3%) and Site 47 (< 1%) at the mouth to Pittwater. Salinity was reduced during spring in Ralphs Bay (~28, at Sites 20 and 21) but not in Pittwater, which suggests that *Cymatosira* aff. *belgica* may be euryhaline.

The lower Derwent to Channel area (Zone 4) was dominated by *Navicula monoculata* var. *omissa*, *Navicula arenaria* var. *rostellata*, *Amphora laevissima*, *Synedra tabulata*, *Nitzschia amphibia*, *Fragilaria pinnata*, *Ehrenbergia granulosa* and an unidentified species (Species 4).

Navicula arenaria var. *rostellata* (synonym: *N. rostellata*) has been reported as a marine, probably cosmopolitan species common on the North Sea coasts and in the Baltic Sea (Witkowski *et al.* 2000). It also was reported as being very common as epipelon in False Bay, Washington (Rao & Lewin 1976) and Netarts Bay, Oregon (Whiting & McIntire 1985). Although *Navicula arenaria* var. *rostellata* was recorded from 23 of the 51 training-set sites, its abundance was generally < 2%. However, at Site 13 its relative abundance of 12.5% made it the dominant species.

Amphora laevissima is a brackish/marine species reported from the Finmark, Scotland and England (Cleve 1965), and in small proportions from False Bay, Washington (as *Amphora laevis* var. *laevissima*) (Rao & Lewin 1976). (Witkowski *et al.* 2000) report that *Amphora laevissima* is a widespread marine species known from the Arctic to the tropics. At south-east Tasmanian sites *A. laevissima* was recorded from 21 of the training-set sites, and was particularly abundant at Site 15 with a relative abundance of 44%.

Synedra tabulata is a very widely reported, cosmopolitan species (Witkowski *et al.* 2000), that has been recorded from the coastal shallows of Puck Bay, Poland (Witkowski 1991), north-western Baltic (Miller & Risberg 1990), Gulf of Riga, eastern Baltic (Sakson & Miller 1993), Gotland Basin, Baltic Sea (Grönlund 1993), the north coast of Cornwall (Hendey 1977), False Bay, Washington (Rao & Lewin 1976) and eastern Australia (Foged 1978). This species was common at south-east Tasmanian sites, however its relative abundance was generally < 3%. The greatest relative abundance of *Synedra tabulata* (15.5%) occurred at Site 25 in the Channel.

Fragilaria pinnata is a cosmopolitan species that is often reported from freshwater environments (Agbeti 1992, Pienitz & Smol 1993, Bloom *et al.* 2003). However, this species has also been reported from Netarts Bay, Oregon (Whiting & McIntire 1985), and False Bay, Washington (Rao & Lewin 1976), and was common in small proportions at south-east Tasmanian sites, and the dominant species at Site 29 (24%).

Ehrenbergia granulosa has been reported from areas including tidal flats of the North Sea and Atlantic coasts in Europe, the western Baltic Sea, New Caledonia, and South Africa (Witkowski *et al.* 2000). *Ehrenbergia granulosa* was recorded from only 12 of the 51 training-set sites in south-east Tasmanian sites, and was generally in very low proportions (< 2%), but was the dominant species at Site 31 (21%). Site 31, and the only other site at which *Ehrenbergia granulosa* was recorded in abundance (Site 43, also 21% relative abundance) both had particularly coarse sediments (> 83% > 0.125 μm). However, other researchers have reported *Paralia sulcata* from a wide range of sediment types (within the one study), including fine muds and coarse sands (Whiting & McIntire 1985, Huang 1990).

On the western side of the D'Entrecasteaux Channel, northern sites (Zone 5) were dominated by *Amphora subturgida*, *Navicula monoculata* var. *omissa*, *Nitzschia amphibia* and *Skeletonema costatum*. The Bruny Island and lower Channel areas (Zone 6) were dominated by *Nitzschia amphibia*, *Navicula monoculata* var. *omissa* and *Ehrenbergia granulosa.*

Amphora subturgida was reported as being found in large numbers from the Swan River Estuary in Western Australia by John (1983). At the training-set sites in south-eastern Tasmania, *Amphora subturgida* was very widespread, being recorded from most sites with a relative abundance generally around 5-10%, and recorded from Site 34 with an abundance of 23.6%

Sites around Pittwater area (Zone 7) were dominated by *Navicula monoculata* var. *omissa*, *Opephora olsenii*, *Planothidium delicatulum* and *Fragilaria pinnata.* Witkowski *et al.* (2000) describe *Planothidium delicatulum* (synonym: *Achnanthes delicatula*) as being one of the most common inhabitants of sandy sediments of world-wide occurrence on marine and brackish water coasts, that is sometimes abundant at places affected by waste water outflow. This species has been reported from the north-western Baltic (Miller & Risberg 1990), estuaries from South Africa (Watt 1998), the west coast of Sweden (Sundbäck & Snoeijs 1991b), coastal wetlands of the Netherlands (Vos & de Wolf 1993a), the United Kingdom (Oppenheim 1988), coastal shallows of Poland (Witkowski 1991), and the Swan River, Western Australia (John 1983). At south-east Tasmanian sites, *Planothidium*

delicatulum was widespread (occurring at 35 sites), but was most abundant at sites in Pittwater where its maximum relative abundance reached ~15% at Site 49. *Planothidium delicatulum* clearly has a wide tolerance range for environmental conditions, also occurring in proportions around 5-10% in Pittwater and sites higher in the Derwent (esp. Sites 1, 2 and 3). The range of nutrient concentrations and sediment size across these sites was the maximum range encountered throughout the study. The occurrence of *Planothidium delicatulum* at south-east Tasmanian sites is therefore consistent with its wide occurrence elsewhere, including its abundance at sites affected by waste water outflow (Witkowski *et al.* 2000).

Many of the dominant diatom species recorded from the training set (discussed above) show similar environmental preferences to those reported in the literature from other areas of the world. However, some differences also exist. There is a paucity of data available on the autecology of coastal marine species, and differences in the environmental requirements of diatom species from different areas of the world are not uncommon. The use of diatoms as water quality indicators therefore requires the use of local indicator species. The calculation of species optima and tolerances in this study, and the generation of the transfer functions to infer conditions at other sites (including palaeo-environmental conditions), is therefore integral to the effective use of diatoms as biological indicators in coastal south-east Tasmanian ecosystems.

2.4.4 Transfer Functions

The development of transfer functions for inferring nutrients in the near-shore coastal environment of south east Tasmania will provide coastal managers with a significant additional resource for monitoring and assessing water quality. Additionally, the ability to reconstruct the environmental history of sites within the study area using diatoms means that historical impacts on water quality can be more easily assessed and the likely consequences of continuing current practices can be more accurately predicted.

The spread of species across the logged range of nutrient concentrations indicates the strong effect that small variations in nutrient concentrations can have on the composition of diatom assemblages. Given the variability of environmental

conditions across the range of study sites, the fact that nutrients are having the greatest influence on diatom community composition indicates that anthropogenic activity is having a strong impact on the micro-algal community. This situation highlights the need for effective coastal management strategies for the region. This also highlights the success of using diatoms as biological indicators of nutrient concentrations in the south-east Tasmanian near-shore marine environment.

2.4.5 Conclusions

A diverse array of environmental conditions exists within the near-shore marine environment of south-east Tasmania. The composition of micro-algal assemblagess within these habitats is most strongly influenced by nutrient concentrations. Although nutrient concentrations generally decrease with distance away from the upper Derwent, an increase in nutrient concentrations is evident around more densely populated areas. Nutrient concentrations at some sites in the Derwent are particularly high, for example at Prince of Wales Bay. The proportion of fine benthic sediments at some sites also strongly influences diatom community composition, and is indicative of the physical energy associated with the hydrology in the area. Sites can be grouped environmentally according to differences in their physical and chemical characteristics.

Although many of the main diatoms species in south-east Tasmania show similar environmental preferences to those reported from other areas of the world, differences in these preferences also exist. The determination of environmental optima and tolerances for south-east Tasmanian diatom species, and the development of transfer functions to infer nutrient concentrations at other sites, provides a valuable water quality monitoring and assessment resource that can also be used for environmental reconstruction of palaeo-marine sites within the study area.

2.4.6 Recommendations

The micro-algal community of south-east Tasmanian can now be used as biological indicators of nutrient concentrations in this region. The inclusion of diatoms in water quality monitoring and assessment programs is recommended for the Derwent River and D'Entrecasteaux Channel areas. The inclusion of SiO_2 measurements in the chemical analyses of water within these areas is also recommended.

CHAPTER 3: Environmental Reconstruction of Pittwater Lagoon, South-east Tasmania

3.1 INTRODUCTION

One of the most informative ways we can improve our knowledge of the ecological consequences of environmental impacts is to investigate these consequences retrospectively. The development of transfer functions to infer palaeo-environmental history from coastal habitats using diatoms provides a means of doing this. By calibrating palaeo-sediment data using the ecological optima and tolerances of diatom species, the environmental history of the aquatic environment from which a core is taken can be reconstructed (Stevenson *et al.* 1989). Long-term trends and fluctuations in environmental variables can thus be identified, and the long-term ecological consequences of environmental change can be more accurately determined (Dixit *et al.* 1991).

Environmental reconstruction using diatoms has been undertaken extensively around the globe from a wide range of environments, including the Arctic (Ruhland & Smol 2002), Antarctic (McMinn & Heijneis 1994, Crosta *et al.* 1998, Roberts & McMinn 1999, Taylor & McMinn 2001), Australia (Gell *et al.* 1994, Taffs 2001), Africa (Gasse 1987), America (Fritz *et al.* 1991, Dixit *et al.* 1993), the Baltic sea (Morris *et al.* 1988, Miller & Risberg 1990, Korhola & Blom 1996), Canada (Palmer 1978, Cumming & Smol 1993), the U.K. (Juggins *et al.* 1996) Kenya (Cerling 1979, Barker *et al.* 1990), Siberia (Flower *et al.* 1995), and many other countries.

In Tasmania, environmental reconstruction using diatoms has recently been undertaken in Lake Fidler to determine the history of meromixis (Hodgson *et al.* 1996a, Hodgson *et al.* 1998), in coastal lagoons for salinity (Saunders 2002), and in Macquarie Harbour to examine the effects of mine development (McMinn *et al.* 2003). The palaeolimnology of Lake Nicholls has also been reported by (Cameron *et al.* 1993). However, no work has been reported to date on reconstruction of nutrient conditions in Tasmania's coastal environment.

European settlement of Tasmania has resulted in a significant change in land-use practices and resource-use of marine environments over the past two hundred years. The long-term effects of these changes on the ecology of Tasmania's marine environment are not well understood, although several results have been well

documented, such as the loss of seagrass and kelp beds (Rees 1993), introduced marine pests including the northern Pacific seastar (*Asterias amurensis*) (Green & Coughanowr 2003) and toxic dinoflagellates (*Gymnodinium catenatum*) (McMinn *et al.* 1997), and the contamination of waterways from heavy metals and organic nutrients (Green & Coughanowr 2003). Very little is known about the historical impacts of anthropogenic activity on the micro-algal communities from Tasmania's coastal waters.

Five marine ecosystems have been identified within Tasmania's coastal environment as being of high ecological importance and subsequently protected as special reserves. These include the marine reserves at Nine Pin Point, Tinderbox, Mariah Island, Governor Island and Macquarie Island. However, aside from Macquarie Island, only 0.06% of Tasmania's coastal waters are protected and it is estimated that only 0.05% of Tasmania's marine species occur in reserves (Resource Planning and Development Commission, 2003). There are many ecologically important sites in Tasmania's marine environment that are either wholly or relatively unprotected. One such area of high ecological importance is Pittwater Lagoon, identified as a wetland of international importance to migratory birds under the international Ramsar agreement in 1982, and one of only ten Ramsar sites in Tasmania (Ramsar 2005). Although listed as a Ramsar site, Pittwater Lagoon is used for aquaculture (oyster farming) and receives considerable nutrient input from anthropogenic sources (e.g. surrounding residential development and urban run-off) (Wetlands International 2005).

A better understanding of the ecological changes that have occurred in Pittwater will assist not only for the better protection and management of Pittwater, but also in understanding the ecological changes occurring elsewhere in the region. This study examines historical changes in the micro-algal community of Pittwater Lagoon, with the aim of improving understanding of the long-term effects of anthropogenic activity on the ecology of south-east Tasmanian marine environments.

3.1.1 Study Area

Pittwater lagoon (Figure 3.1) is a Ramsar listed wetland site, which has restricted tidal flow as a result of a causeway originally constructed in 1868, and modified in

1906, 1953 and 2003 (Plate 3.1). The causeway has a narrow opening under the eastern end which allows restricted tidal exchange.

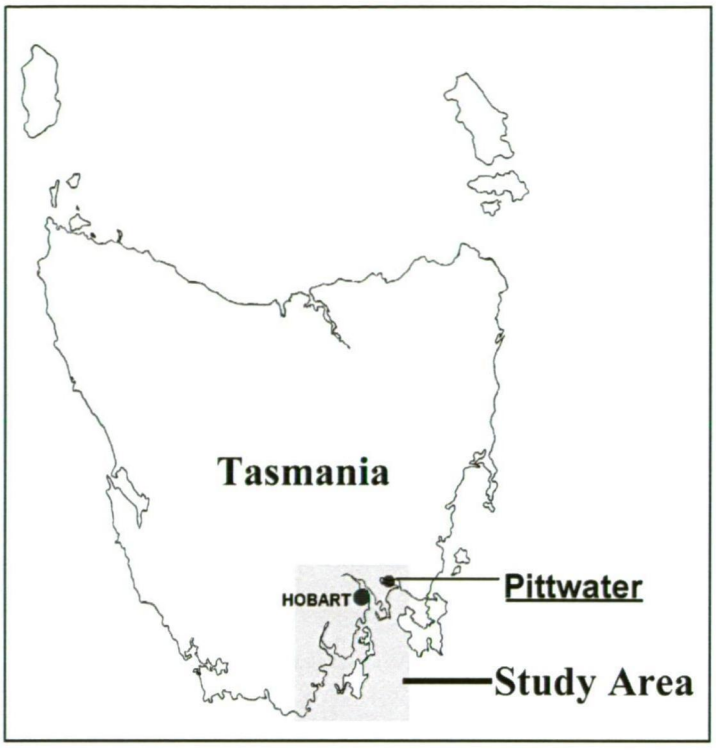


Figure 3.1: Location of Pittwater lagoon

Plate 3.1: Pittwater causeway separating Pittwater Lagoon (on left) from the open marine environment

Pittwater is very important ecologically, being a major feeding area for a large range of migratory bird species that come from as far away as the Arctic (Wetlands International 2005). In addition, six species of threatened plants are found around the lagoon, which also contains the highest known concentration of the threatened starfish, *Patriella vivipara* (Green & Coughanowr 2003).

The main tributary entering Pittwater is the Coal River. The catchment for the Coal River is 541.6 km^2, has an average annual rainfall of 631 mm, and its geology is predominantly sedimentary rocks, with the high surrounding ridges and rounded hills formed from Jurassic dolerite intrusives (DPIWE 2003). Land within the catchment is predominantly privately owned and much of it has been cleared for agriculture, rural-residential settlements and townships. Pittwater has considerable nutrient input as it's surrounded by farms and the townships of Sorrel and Midway Point. Water quality issues within the catchment include vegetation clearing, soil erosion, urban run-off, sewage discharge, leachates from septic systems, fertiliser run-off, stock access to streams and altered flow regimes (DPIWE 2003). In the 1930s a weir was constructed across the Coal River near the bridge at Richmond, causing a barrier to upper tidal influence in the estuary. Construction of the Craigborne Dam in the Coal River in 1986 (Crawford & Mitchell 1999) altered flows into Pittwater Lagoon, decreasing flows in winter and increasing flows in summer (DPIWE 2003). A second weir constructed 0.5 km below the original weir at Richmond further reduced freshwater input into Pittwater. A 94% decline in seagrass in Pittwater (equating to 1201 ha) occurred between approximately 1948 and 1990 (Rees 1993). Pittwater is currently used for oyster farming.

3.2 METHODS

3.2.1 Coring Site

Pittwater is a large and relatively shallow water body that is partially enclosed by a causeway. Toward the causeway, the passage of water into and out of Pittwater is largely directed along a deeper channel which flows through openings constructed at one end of the causeway wall. The site chosen for coring was away from this channel (north-east) toward a more sheltered area of the lagoon. The coring site and the approximate location of the channel are shown in Figure 3.2.

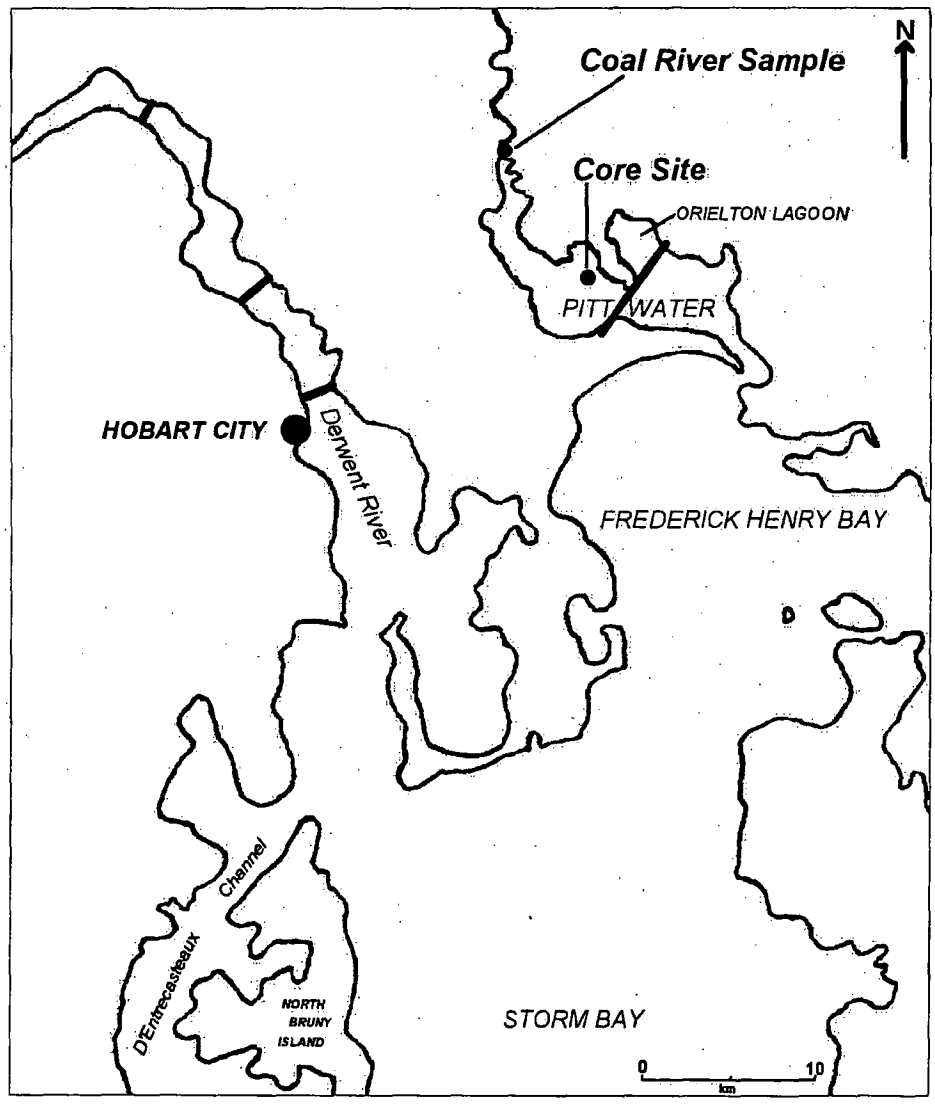


Figure 3.2. Site map showing location of core sample from Pittwater Lagoon and Coal River sample.

3.2.2 Core Collection

From approximately 4.2 m water depth, a 69 cm long sediment core was collected from Pittwater on November 22, 2001, using a purpose-built diver-operated coring device (Plate 3.2) (constructed by Iona Mitchell from the Tasmanian Fisheries and Aquaculture Institute – TAFI, Hobart). The coring device consists of a clear Perspex tube (9 cm outside diameter, 80 cm long) with a handle attached on 2 sides near the top. The corer was pushed into the sediment almost up to the handles. A waterproof cap was then placed over the top of the tube providing a vacuum seal, so that as the core was extracted the sediment sample remained intact. A waterproof cap was placed over the bottom of the tube as soon as the corer was fully extracted from the sediment. The core was maintained in a vertical position and removed from the water.

Back on shore, the core was extracted by removing the base cap, inserting the plunger, removing the top cap, and pushing the sediment up through the core using the plunger. The core was sectioned as it was removed by placing a 1 cm Perspex ring (cut from the original tube) on top of the corer and pushing the sediment up until it was level with the top of this ring. A thin steel blade was used to slice through the sediment between the corer and the top ring, providing sediment sections of approximately 1 cm thickness. Each section was halved diametrically to provide 2 samples for various analyses, bagged in 'zip-lock' bags, labelled, and stored in the dark on ice for later processing at UTAS laboratories.

Plate 3.2: Pittwater core

3.2.2.1 Coal River Sediment Sample

A surface sediment sample was collected from 1.5 m water depth in the lower reaches of the Coal River, above where this tributary enters Pittwater lagoon, on the

same day that coring was undertaken (22/11/01). The purpose of this sample was to provide data on the composition of diatom species that may be entering Pittwater from the Coal River, to aid in the interpretation of species assemblages in the core. Using SCUBA equipment and a 100 ml specimen container, the sample was collected from the top 1 cm of the substrate at 1.5 m water depth, and was immediately capped underwater. The sample was stored in the dark on ice for later processing. The location of this sampling site is shown in Figure 3.2.

3.2.3 Physical and Chemical Sampling

Physical and chemical parameters at both sites were measured at the time that sediment samples were collected. Salinity, temperature and depth were measured using a CTD meter. Water clarity was measured using a Secchi disc (Tyler 1968). Duplicate 10 ml water samples for nutrient analyses were collected by the divers (prior to collecting the core) from approximately 2 m water depth (for the core), and from approximately 1 m water depth for the Coal River sample. Water samples were stored in the dark on ice until processed at UTAS laboratories.

3.2.3.1 Sample Analyses

Methods for the analysis of water samples for nutrients, and sediment samples for TOC and grain size analyses were followed as in Chapter 2. All core samples are treated as described for the surface sediment samples.

3.2.4 Sediment Dating

To be able to reconstruct the environmental history of an aquatic environment it is necessary to determine the chronology of changes that have occurred. Dating of the Pittwater core was undertaken to determine the chronology of the core and relate any changes that may have occurred in the microflora. ^{210}Pb is the principal isotope for dating on the time scale of 100-150 years (Appleby *et al.* 1990, El-Daoushy 1990). Dating of the Pittwater core sediments using ^{210}Pb isotopes was undertaken at the Australian Nuclear Science and Technology Organisation (ANSTO) in Sydney using alpha spectrophotometry. Excess ^{210}Pb was determined by subtracting individual ^{226}Ra values. Age depth relationships were obtained using the modified CIC technique (Brugam, 1978) allowing for non-linear sedimentation rates. The ^{210}Pb dating data is provided in Table 3.1 in Appendix 3.

3.2.5 Diatom Community Composition

Methods for the analyses of diatom community composition were followed as in Chapter 2.

3.2.6 Statistical Analyses

The transfer functions created for NO_{2-3} and SiO_2 (see Chapter 2) were applied to the species training set from the Pittwater core using C^2 software (Juggins 2003).

3.3 RESULTS

3.3.1 Physical and Chemical Results

3.3.1.1 Salinity, Temperature & Depth

The sediment core was collected from approximately 4.02 m water depth (adjusted to reflect mean tidal height at Hobart). Salinity was relatively constant throughout the depth profile (measurements ranged between 30.03 and 30.30). Water temperature decreased from approximately 16.5°C at the surface to 15.8°C at maximum depth.

3.3.1.2 Light Penetration & Nutrient Concentrations

Light penetration through the water column was relatively low, with Secchi depth measured at 1.55 m. Nutrient analyses of water samples collected immediately prior to core collection showed NO_{2-3} concentrations of 0.03 µmol/L, PO_4 at 0.19 µmol/L and SiO_2 at 18.61 µmol/L.

3.3.1.3 ^{210}Pb Dating & Sedimentation Rates

The ^{210}Pb chronology dates the bottom of the sediment core back to approximately the early 1880s (Table 3.1). As the sectioned core provided samples of approximately 1.2 cm thickness, the chronological age from the top to the bottom of an individual section (slice) varies. Therefore ^{210}Pb results provide a ± date range. Sedimentation rates, calculated from the relationship between core depth and ^{210}Pb dated age, were applied to the core to determine the age of each sediment section. The sedimentation rate increased very slightly in the top half of the core (Table 3.1).

Table 3.1: ^{210}Pb chronology of Pittwater Core, and sedimentation rates

Depth (cm)	~Year	Plus/minus (years)	Sedimentation Rate (cm/yr)
0	2001	0	
0.0 to 4.3		Mixed surface layer	
9.6	1991	3	0.542
17.2	1977	3	0.542
26.4	1960	4	0.542
37.2	1939	6	0.536
43.2	1929	6	0.536
49.2	1918	7	0.536
57.6	1902	8	0.536
67.2	1884	9	0.536

3.3.1.4 Sediment Size Analysis

Sediment size analysis was undertaken on sections (slices) from various depths of the core (Figure 3.3). Each core section was approximately 1.2 cm thick – in the following pages the use of a single measurement refers to the base of that section. During the late 1800s, the sediment fraction < 63 μm increased by almost 12%, with a corresponding decrease in the percentage of fractions 63 -125 μm and (to a lesser extent) 0.125 μm – 2 mm. During the 1900s sediment size remained relatively constant, with a small variation evident in the mixing layer of the top few cm. The core did not contain sediment > 2 mm.

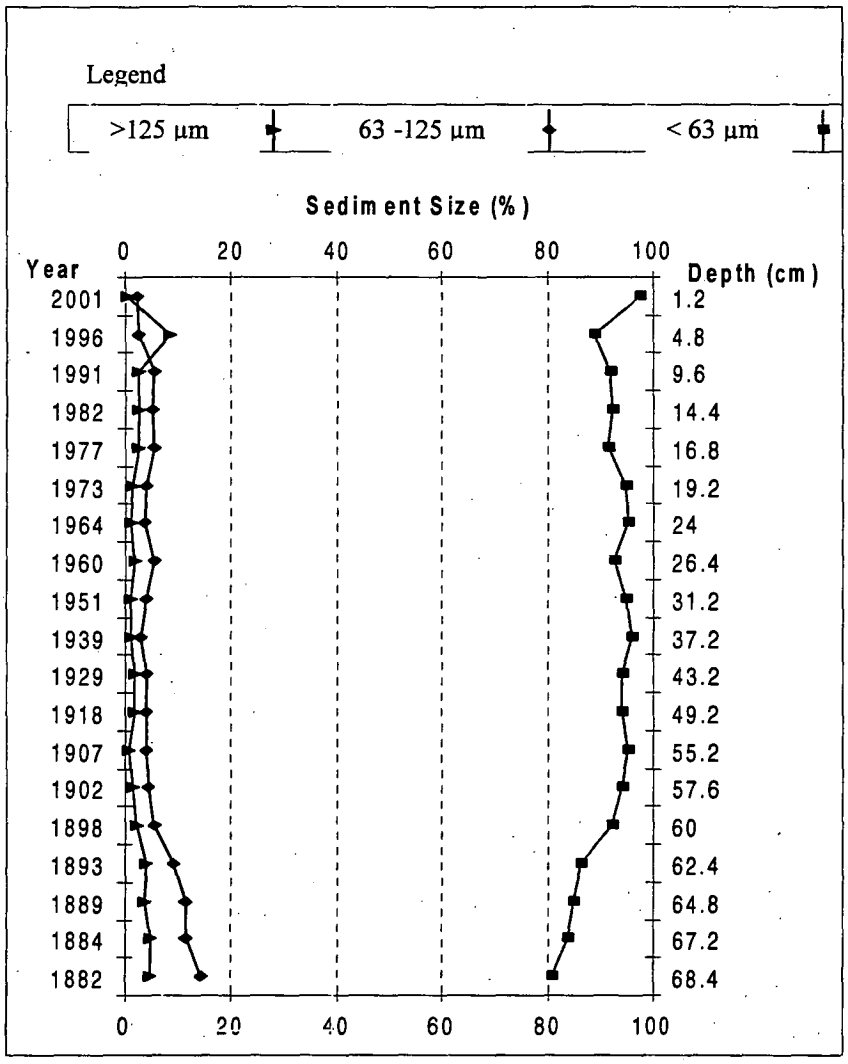


Figure 3.3: Sediment size analyses of the Pittwater Lagoon core

3.3.1.5 Total Organic Carbon

Total organic carbon (TOC) analyses of the core sediments shows that TOC increased sharply by almost 50% during the late ~1800s (Figure 3.4). A similar rise in TOC levels is evident in the top 6 cm of the core (from ~1994). TOC was weakly correlated with the percentage of sediment fraction <63 μm ($r^2 = 0.55$).

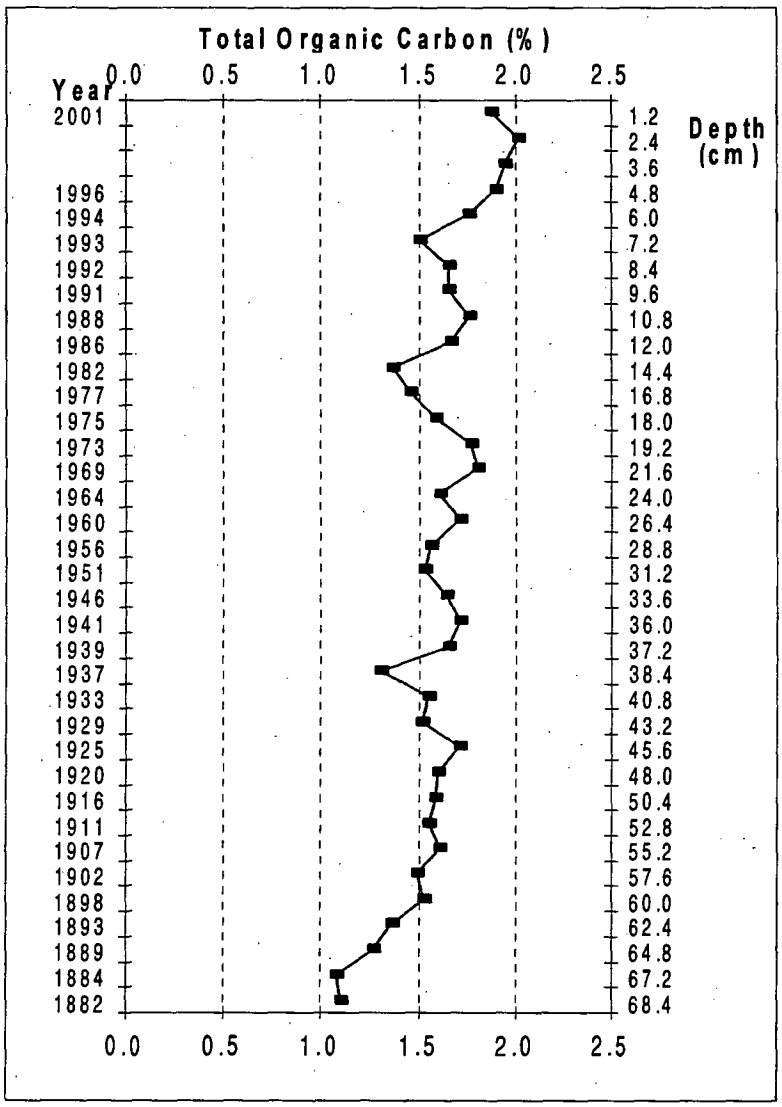


Figure 3.4: Total Organic Carbon in the Pittwater Lagoon core

3.3.2 Application of the Transfer Functions

The transfer functions generated from the training set (Chapter 2) were applied to the sediment core from Pittwater Lagoon to reconstruct the nutrient history of the lagoon. The number and effective number of diatom species in the fossil data set, as well as the number of species in the fossil data set present in the training set, are listed in Table 3.2 in Appendix 3). Reconstructed NO_{2-3} concentrations for the Pittwater core are shown in Figure 3.5 (reconstructed Pittwater core values for NO_{2-3} and SiO_2 are listed in Table 3.3 in Appendix 3).

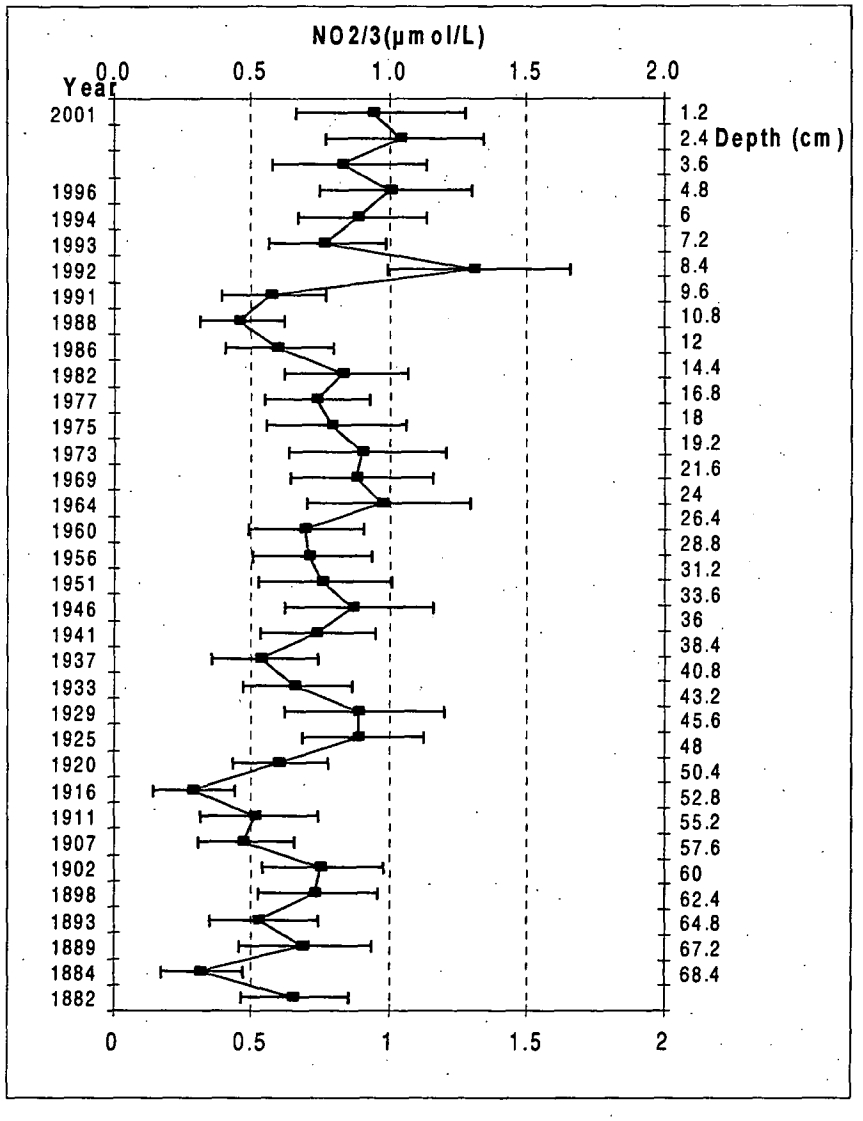


Figure 3.5: Reconstructed spring NO_{2-3} concentrations and calculated RMSEP errors for Pittwater core

Inferred NO_{2-3} concentrations in Pittwater show low to moderate concentrations with peaks occurring in the mid-late ~1920s, mid-late ~1960s, and early ~1990s.

Reconstructed SiO_2 concentrations for Pittwater core are shown in Figure 3.6.

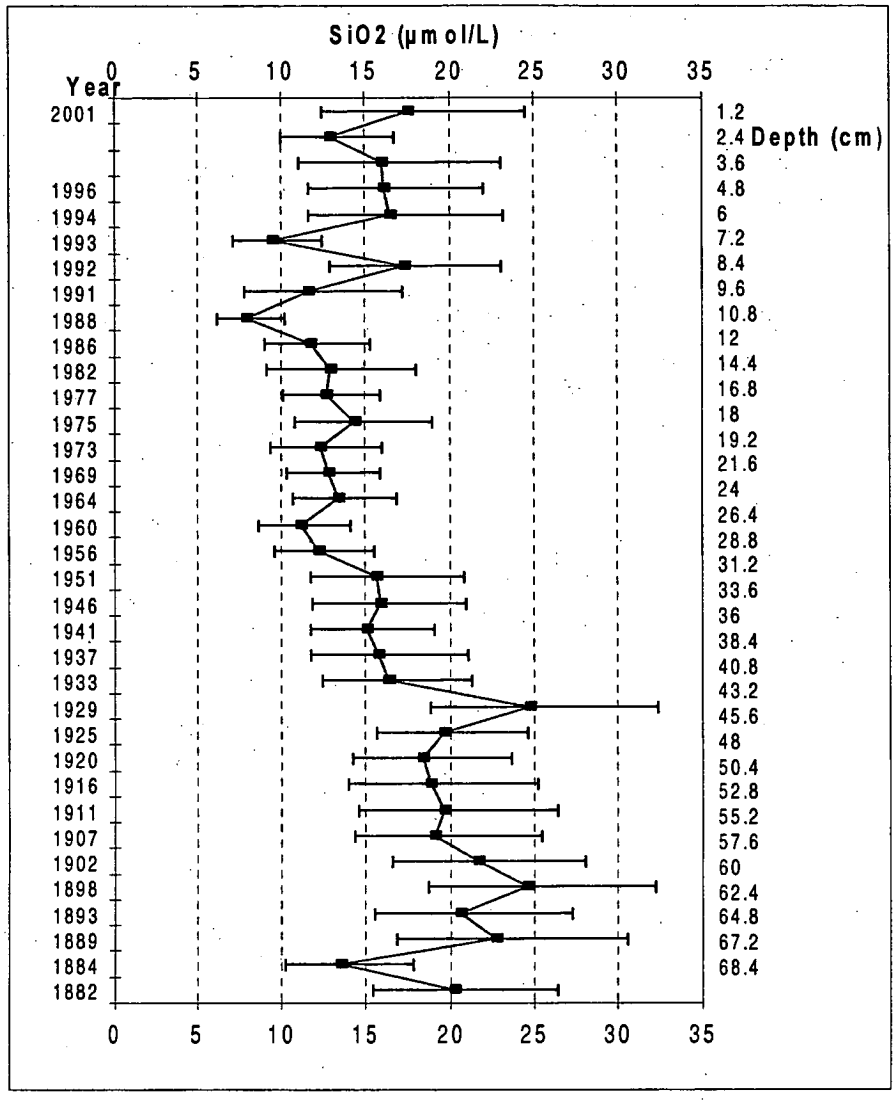


Figure 3.6: Reconstructed spring SiO_2 concentrations and calculated RMSEP errors for Pittwater core

Inferred SiO_2 concentrations in Pittwater show low to moderate concentrations that have overall decreased since the early ~1930s, with peaks occurring in the late ~1890s, mid-late ~1920s, and early ~1990s.

3.3.3 Diatom Community Composition

All diatom species comprising at least 2% of at least one sample were included in the analysis of the core data. Together, these species averaged > 93% of the diatom community from every sample (Range 88% to 99%). The following section focuses on the above-mentioned diatom species from the core. Diatom species names and

authorities, and abundance and composition for the core, are provided in Tables 3.4 and 3.5 respectively, in Appendix 3.

A total of 36 diatom species from 23 genera were recorded at ≥ 2% of at least one sample from the core. The species composition of diatom assemblages changed significantly over the length of the core. From the base of the core (~1882) up to approximately 1951 (31cm sample), diatom assemblages were strongly dominated by the centric tychoplanktonic species *Paralia sulcata* and *Ehrenbergia granulosa*, which together constituted >50% of all diatom assemblages (averaging 65%) (Figure 3.7). However, the abundance of these two species declined rapidly after this date, constituting only 31% of assemblages in ~1956 (28.8 cm sample), 7% of the assemblage in ~1960 (26.4 cm sample), and averaging 3.2% of remaining samples to the top of the core (2001).

The middle section of the core, from ~1956 to ~1977 (samples 28.8 cm up to 16.8 cm), covers the transition zone between the decline of *Paralia sulcata* and *Ehrenbergia granulosa*, and the emergence and dominance by *Cymatosira* aff. *belgica*. Diatom assemblages in this middle section were not as strongly dominated by individual species, and consisted mostly of *Catenula adhaerens*, *Glyphodesmis distans*, *Plagiogramma staurophorum*, *Gramatophora oceanica* and *Cocconeis* aff. *pinnata*.

Diatom assemblages from ~1982 (from the 14.4 cm sample up) were strongly dominated by *Cymatosira* aff. *belgica* (averaging 45% of the assemblages). This species first appeared in significant proportions (15.4%) in approximately 1975 (in the sample at 18 cm). Prior to this time, the only recording of this species was two specimens from ~1960 (26.4 cm section). However, the complete absence of this species between ~1960 and ~1975 (26.4 and 18 cm) suggests that the two individual specimens may have been the result of contamination as the sediment was extruded through the coring chamber during sectioning. Other species in abundance in the top sections of the core include *Cocconeis* aff. *pinnata*, *Opephora olsenii*, and *Catenula adhaerens*.

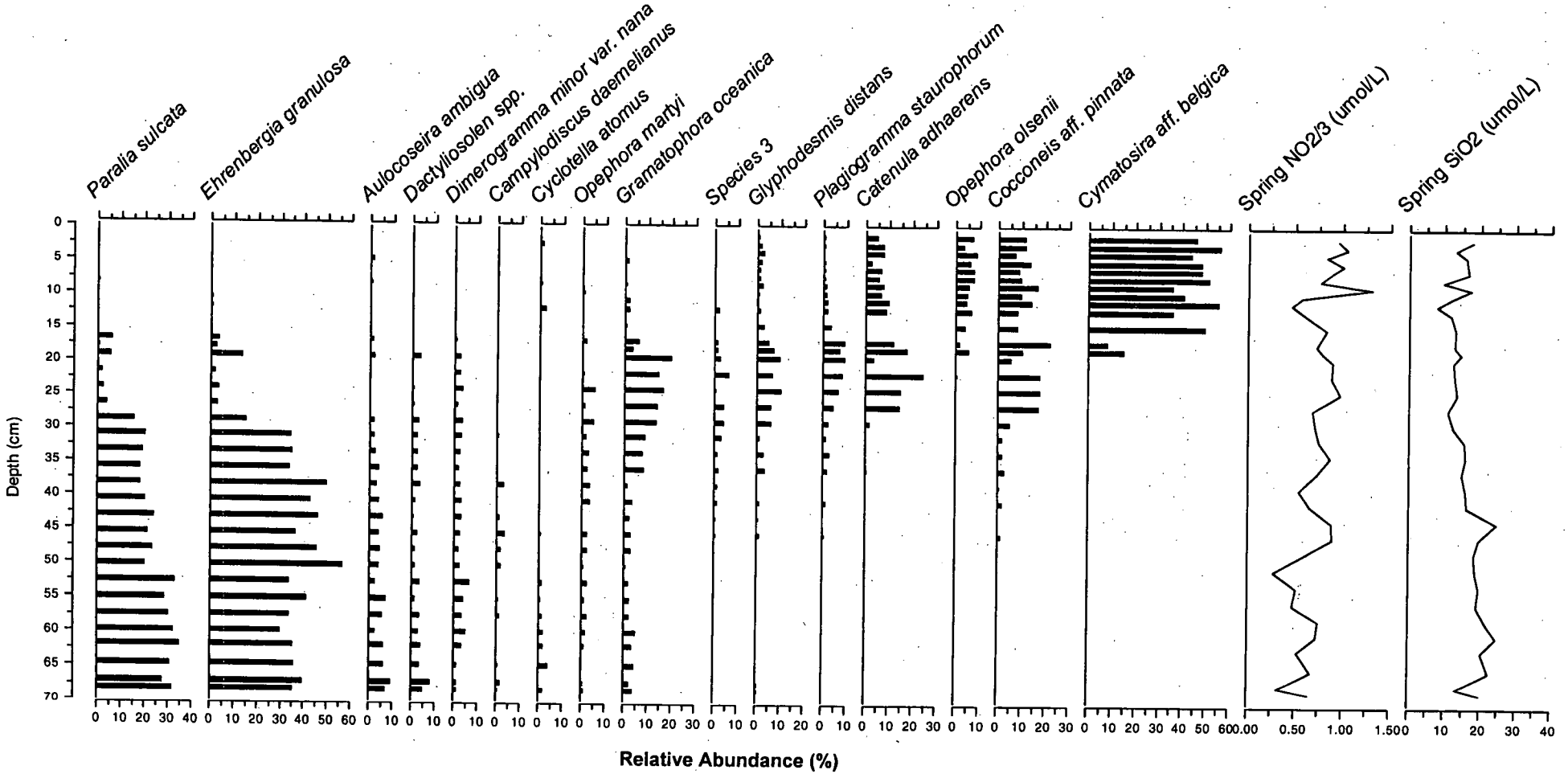


Figure 3.7: Diatom stratigraphy showing the most abundant species in the Pittwater core, and transfer function diatom-inferred NO_{2-3} and SiO_2.

3.3.4 Coal River Sample

Physical and chemical measurements taken at the time of collecting the surface sediment sample (22/11/01) showed that temperature was 17.8°C at the surface and 18°C at the bottom (depth 1.5 m), salinity was 20.0, and Secchi depth was 0.65 m.

Nutrient concentrations were as follows:

NO_{2-3} = 0.68 µmol/L; PO_4 = 0.71 µmol/L; SiO_2 = 78.52 µmol/L.

Sediment size composition was as follows:

< 63 µm = 96.53% 63 - 125 µm = 2.17% 0.125 µm -2 mm = 1.30

There were only six diatom species from this sample that constituted ≥ 2% of the assemblage. These species were:

Navicula arenaria var. *rostellata*	37.0%
Navicula species 1	12.4%
Nitzschia amphibia	12.5%
Nitzschia species 1	5.3%
Navicula monoculata var. *omissa*	4.6%
Cymatosira aff. *belgica*	2.7%

Sampling at this site was undertaken to provide additional relevant information regarding the possible input of allochthonous species to the Pittwater core to aid in interpreting the core data. Data from this site has therefore not been included in any of the statistical analyses in this study.

3.4 DISCUSSION

3.4.1 Physical and Chemical Parameters of Pittwater

3.4.1.1 Salinity and Temperature

Salinity at the Pittwater coring site (~30) was very similar at the time of sampling to salinities measured several weeks earlier at training-set sites on the other side of the causeway. The small change in salinity (0.27) that occurred throughout the 4 m depth profile shows that the water was well mixed. Previous research has shown that salinity near the Pittwater causeway generally ranges between approximately 30 and 37, averaging 33 to 35 (over a 2 year period), the waters are well mixed, and salinity tends to increase with distance up the Coal River Estuary due to evaporation and low freshwater input (as a result of the Craigbourne Dam, and weirs between the dam and Pittwater) (Crawford & Mitchell 1999). Pittwater is therefore typically marine, with reductions in salinity occurring only every few years after exceptionally heavy rainfall and extensive flooding (Crawford & Mitchell 1999).

Water temperature at the time of sampling (~16°C) was toward the high end of the temperature range normally experienced in Pittwater (~7 to 18 °C, depending on season: (Crawford & Mitchell 1999).The Secchi disc measurement in Pittwater at the time of sampling (1.55 m) was low in comparison with other marine sites in the region. However, a thick algal mat was visible on the sediment surface (and surface of the core), and Secchi depth measurements of almost 4 m (maximum site depth) were recorded on the other side of the causeway from sites in the training-set. This indicates that light penetration at the coring site is usually sufficient for algal growth, and suggests that the Secchi depth measurement at the site may not always be so low. Sampling at the coring site was undertaken around the turn of the tide, which may have resulted in increased turbidity of the water column.

3.4.1.2 Sediments

Sediment size analysis of the core revealed a gradual increase of > 10% in the proportion of the sediment fraction < 63 μm from the late ~1800s until ~1902, with a corresponding decrease in the percentage of coarser fractions (> 63 to 2 mm). The percentage composition of sediment-size fractions remained relatively constant after this initial change. The gradual reduction of the coarser fractions of the sediment is

thought to be a result of the construction of the causeway, resulting in restricted tidal flow and consequential reduction in the sediment-carrying capacity of the inflowing tide. In the sample at the top of the core (after ~1996) the fine sediment fraction has again increased. However, the top of the core is subject to reworking through bioturbation and resuspension, and this uppermost sample may represent the lighter resuspended sediment that is last to settle after disturbance. Hence, limited information can be drawn from this single uppermost sample.

3.4.1.3 Total Organic Carbon

Total organic carbon (TOC) in the Pittwater Lagoon core sediments increased sharply by almost 50% during the late ~1800s until the 20th century. The results showed that TOC was positively correlated with the fraction of sediment size < 63 μm ($r^2 = 0.55$), which increased during the same period. Hence, the increase in TOC during this period is probably associated with the increase in the fine sediment component and the reduction in tidal flushing resulting from the construction of the causeway. Additionally, the fact that TOC remained relatively stable during the 20th century, and at concentrations above those of the late ~1800s, further suggests that the permanent shift in TOC concentrations was a consequence of the construction of the causeway altering the hydrological processes in Pittwater.

3.4.1.4 Nutrients

Nutrient concentrations in Pittwater at the time of sampling were low except for silicate (NO_{2-3} = 0.03 μmol/L; PO_4 = 0.19 μmol/L; SiO_2 = 18.61 μmol/L), and were very similar to sites from the training-set in the D'Entrecasteaux Channel and Ralphs Bay. Previous research has shown that nutrient concentrations in Pittwater are generally low, with NO_x concentrations averaging less than 4 μg/L (~0.07 μmol/L), but ranging from 0.1 to 34.0 μg/L (~0.002 to 0.63 μmol/L) over a period of > 3 years (1991-94) (Crawford & Mitchell 1999). PO_4 concentrations during the same period were generally in the range of 5 to 15 μg/L (~0.05 to 0.16 μmol/L), and SiO_4 concentrations measured only during the last year of the aforementioned study (1994) ranged from 32.25 to 211 μg/L (approximately equivalent to 0.5 to 3.4 μmol/L of SiO_2). Nutrient concentrations in Pittwater at the time of core collection were therefore similar to previous findings. However, SiO_2 concentrations were relatively high, and were similar to concentrations measured on the eastern side of the causeway

and in Ralphs Bay during spring 2001 (Chapter 2). Nutrient concentrations in Pittwater at the time of coring are also similar to those reported from other coastal areas in Australia and overseas, and are below the trigger values provided in the ANZECC guidelines (see Discussion, Section 2.4.1).

3.4.2 Inferred Nutrient Concentrations in Pittwater

Inferred NO_{2-3} concentrations throughout the Pittwater core ranged from approximately 0.28 to 1.30 µmol/L (average 0.73 µmol/L), with highest overall concentrations occurring toward the top of the core. Inferred concentrations are an estimate of the average nutrient concentrations for each sample, which in this study represents approximately two years. The highest of these inferred concentrations is above the higher limit previously reported for Pittwater, however NO_x concentrations in Pittwater may vary by more than 40 fold in the space of a few months, and average annual concentrations can vary by more than 13 times (Crawford & Mitchell 1999).

Modern NO_{2-3} concentrations, inferred from the NO_{2-3} transfer function applied to the fossil data (0.95 µmol/L) do not closely agree with NO_{2-3} concentrations measured at the time of coring (0.03 µmol/L). However, at the two sites on the eastern side of the causeway NO_{2-3} concentrations were 0.51 µmol/L (Sites 50) and 0.93 µmol/L (Site 51) during autumn sampling for the training set (Chapter 2). These concentrations are very similar to those inferred for the nearby Pittwater coring site on the western side of the causeway, and highlight the variability in the NO_{2-3} concentrations in this system. The range of NO_{2-3} concentrations inferred for the Pittwater core is therefore considered to approximate real values, and this is supported by the strong correlation obtained in the generation of the transfer function ($r^2 = 0.88$).

The inferred NO_{2-3} concentrations in Pittwater show fluctuating, low to moderate concentrations with peaks occurring in the mid-late ~1920s, mid-late ~1960s, and early ~1990s. Following the peak NO_{2-3} concentration in 1964, NO_{2-3} levels steadily declined until the late ~1980s, before peaking at their highest concentration in ~1992 and remaining at relatively high concentrations since. The increase in nutrient concentrations after ~1988 coincides with the construction of the Craigborne Dam in 1986, which decreased flows in winter and increased flows in summer (DPIWE

2003). Since diatom growth is greatest during the warmer months, during which time freshwater flow into Pittwater is also increased, the composition of the diatom assemblages may be reflecting increased nutrient concentrations associated with the increased freshwater input during summer.

Present day SiO_2 concentrations, as inferred from the SiO_2 transfer function applied to the fossil data (17.56 µmol/L), agree closely with SiO_2 concentrations measured at the time of coring (18.61 µmol/L), and are similar to many of the other marine sites in south-east Tasmania. This suggests that that the transfer function generated for inferring SiO_2 can be used to infer past SiO_2 concentrations in this near-shore marine environment of south-east Tasmania. Reconstructed SiO_2 concentrations in Pittwater Lagoon show a significant decline from the early ~1930s to the present day. Although SiO_2 concentrations are still relatively high in Pittwater Lagoon, the reduction in concentrations from the early ~1930s onwards indicates a significant change in the system. This change coincides with the construction of the weir at Richmond in the early 1930s, which altered hydrological flows into Pittwater. However, because silica concentrations are relatively high in Pittwater Lagoon they are unlikely to be a strong limiting factor for diatom growth, and therefore unlikely to be independently and significantly impacting on the diatom community in Pittwater. Nonetheless, the change in SiO_2 concentrations in Pittwater is an example of the impact that anthropogenic changes further upstream can have on our coastal marine systems.

3.4.3 Historical Changes in the Diatom flora

There are profound changes in the diatom flora of Pittwater Lagoon during the last century. From the ~1880s until approximately 1951, *Paralia sulcata* and *Ehrenbergia granulosa* strongly dominated diatom assemblages, constituting > 50% of all diatom assemblages and averaging 65%. However, by ~1956 the relative abundance of these two species had declined to a little over 30%, and from the ~1960s onwards they averaged only 3.2% of remaining samples to the top of the core (2001). The decline in the abundance of these species was very abrupt.

Paralia sulcata (synonym: *Melosira sulcata*) and *Ehrenbergia granulosa* (synonym: *Coscinodiscus granulosus*) are common constituents of marine littoral sediments and are widely reported from coastal diatom communities around the world (Round 1981, Witkowski *et al.* 2000). For example, *Paralia sulcata* has been reported from areas including the north coast of Cornwall (Hendey 1977), coastal deposits of the Netherlands (Vos & de Wolf 1993a), coastal waters of Hong Kong (Ng & Sin 2003), the continental shelf of Taiwan (Huang 1990), upper Florida Bay (DeFelice 1978), Netarts Bay, Oregon (Whiting & McIntire 1985) and the continental shelf waters of north and north-west Australia (Hallegraeff & Jeffrey 1984). *Ehrenbergia granulosa* has been reported from areas including tidal flats of the North Sea and Atlantic coasts in Europe, the western Baltic Sea, New Caledonia, and South Africa (Witkowski *et al.* 2000). However, neither of these two species has previously been reported from Tasmania.

The relatively low abundance of *Paralia sulcata* and *Ehrenbergia granulosa* in modern sediment samples from Pittwater is similar to modern sediment samples from elsewhere in the south-east Tasmanian coastal region. *Paralia sulcata* was recorded from 13 sites throughout the training-set study area, but only in small proportions, with the maximum abundance of 5% recorded from Site 50 on the eastern side of the Pittwater causeway. There is therefore a significant difference between the modern-day abundance of *Paralia sulcata* at all south-east Tasmanian marine sites, and the abundance of *Paralia sulcata* in the Pittwater core up to ~1960. A similar situation exists regarding the abundance of *Ehrenbergia granulosa*, which was recorded in surface sediment samples from only 12 of the 51 training-set sites in abundances of < 3%, except for Sites 31 and 43 in the Channel. At these latter two sites, *Ehrenbergia granulosa* was the dominant species with a relative abundance of 20-21%. However, these two sites are on opposite sides and at opposite ends of the Channel, and have little in common apart from both containing a high proportion of coarse sediments (> 80% > 0.125 μm), which is the opposite of the fine sediment composition throughout the Pittwater core.

The middle section of the Pittwater core (approximately 1956 to 1977) represents a transition zone between the decline of *Paralia sulcata* and *Ehrenbergia granulosa*, and modern day assemblages which have been relatively stable in Pittwater since ~1982. Dominant species in this middle section include *Gramatophora oceanica*, *Glyphodesmis distans*, *Plagiogramma staurophorum*, *Catenula adhaerens*, and *Cocconeis* aff. *pinnata*.

Gramatophora oceanica is a cosmopolitan species in brackish water and marine coasts (Witkowski *et al.* 2000), and is widely reported from areas including Atlantic Canada (Palmer 1978), coastal waters of Hong Kong (Ng & Sin 2003),the north-western Baltic (Miller & Risberg 1990), the north coast of Cornwall (Hendey 1977), the continental shelf waters of north and north-west Australia (Hallegraeff & Jeffrey 1984), and the Swan River Estuary in Western Australia (John 1983). *Gramatophora oceanica* was present throughout the core profile in small proportions, and was found at many of the training-set sites (again, in small proportions). The relative abundance of *G. oceanica* in the core increased from approximately 1964 as the abundance *Paralia sulcata* and *Ehrenbergia granulosa* began to decline, and abruptly declined after ~1973 to ~pre-1964 levels as the abundance of other species in modern day assemblages increased.

Glyphodesmis distans (synonym: *Dimeregramma distans*) was recorded in small numbers throughout the core, and showed a very similar increase and subsequent decline in relative abundance to that of *Gramatophora oceanica*. *Glyphodesmis distans* is a widespread marine species in the littoral sediments of coastal environments, including the Baltic Sea (Witkowski *et al.* 2000), and has previously been reported from Australia in the Swan River Estuary by John (1983). However, *Glyphodesmis distans* was not recorded from any of the training-set sites in south-east Tasmania, and its relative abundance in the Pittwater core was generally < 2%, although reached 10.3% in the middle section of the core before declining again in more recent assemblages.

Plagiogramma staurophorum first appeared in the core in approximately 1920, and was recorded in relatively small abundances from this time on. *Plagiogramma staurophorum* showed a very similar increase and subsequent decline in relative abundance to that of *Gramatophora oceanica* and *Glyphodesmis distans*. *Plagiogramma staurophorum* is a common epipsammic diatom in brackish and marine environments, and has been widely reported from coastal environments around the world (discussed in Chapter 2). The abundance of *Plagiogramma staurophorum* at south-east Tasmanian sites from the training-set was greatest in Ralphs Bay and the north-eastern side of the causeway at Pittwater lagoon, and was generally recorded in relatively small proportions, as was the case in south-western Tasmania (McMinn *et al.* 2003) and Western Australia (John 1983).

Catenula adhaerens first appeared in the Pittwater core in approximately 1933, but was present in proportions of < 2% until becoming relatively abundant (> 10%) in ~1960 until the late ~1970s, and has averaged approximately 6% of diatom assemblages since that time. Although *C. adhaerens* shows a generally similar increase and decline in relative abundance to the above-mentioned species in the middle section of the core, its relative abundance has generally been higher during the past 20 years than before it became abundant in ~1960. *Catenula adhaerens* is a common species in marine littoral zones, often recorded from the epipsammon (Witkowski *et al.* 2000), and has been reported from the Gotland Basin in the Baltic Sea (Grönlund 1993), the Ems Estuary, Wadden Sea (de Jonge 1985), coastal wetlands of the Netherlands (Vos & de Wolf 1993a), and the west coast of Sweden (Sundbäck & Snoeijs 1991b). However, *Catenula adhaerens* has not previously been reported from Tasmania, and Australian records of this species are scarce or non-existent. *Catenula adhaerens* was widespread throughout the south-east Tasmanian training-set sites, occurring at 39 out of the 51 sites, mostly in relatively small proportions (< 2%). It was most abundant in Ralphs Bay, where the maximum abundance of *Catenula adhaerens* (~16.5%) occurred at Site 21, and was recorded from all sites within Pittwater (maximum abundance < 4%).

Cocconeis aff. *pinnata* was present throughout the Pittwater core, however its relative abundance was < 2% until approximately 1941, after which time its relative abundance was always > 2% and averaged 10.8%. The greatest abundance of *Cocconeis* aff. *pinnata* occurred within the middle section of the core, however the abundance of this species significantly increased in ~1960 and remained comparatively high ever since. *Cocconeis pinnata* is a marine littoral species (Witkowski *et al.* 2000) and was rare at south-east Tasmanian sites, occurring at less than 2% at all sites except those near Pittwater, where its abundance still remained at < 10%.

From ~1982, the top sections of the core were dominated by *Cymatosira* aff. *beligica*, which averaged > 45% of diatom community composition (range 36 to 56.3%). The first appearance of *Cymatosira* aff. *beligica* in the Pittwater core was in approximately 1960. However, only two specimens were recorded from this sample, and the next appearance was four samples above this (in ~1975), at which point *Cymatosira* aff. *beligica* constituted 15.4 % of the diatom assemblage. In ~1982, *C.* aff. *beligica* constituted 50.1% of the diatom assemblages. The appearance and subsequent dominance of *Cymatosira* aff. *beligica* in the sediment core is of particular interest as it represents a significant change and is a recent introduction to the microflora in the Pittwater core. *Cymatosira* aff. *beligica* is discussed further in the following section on interpretation of the changes in Pittwater.

3.4.4 Coal River Diatoms

Diatoms in the Coal River sample differed considerably from those in the Pittwater core, however all diatom species from the Coal River present in ≥ 2% relative abundance were also recorded in training-set samples. *Paralia sulcata* and *Ehrenbergia granulosa* were not recorded from the Coal River. None of the main diatom species from the Pittwater core were present at ≥ 2% abundance in the Coal River sample, with the exception of *Cymatosira* aff. *belgica.* However, *C.. belgica* did not constitute a significant proportion of the diatom assemblage (only 2.7%), suggesting the Coal River is not a major allochthonous source for the Pittwater site.

3.4.5 Interpretation of Historical Changes in the Microflora

3.4.5.1 Base Sections of the Core: ~1882 - ~1960

There is strong evidence indicating that the rapid decline in the abundance of *Paralia sulcata* and *Ehrenbergia granulosa* in Pittwater Lagoon is a result of modifications to the causeway in the 1950s. Historical photographs of the section of the Pittwater causeway that separates Pittwater Lagoon from the main water body of Pittwater (i.e. from Pittwater Bluff to Midway Point) show that the previous structure (fundamentally a bridge supported by pylons: State Library of Tasmania 2004) allowed the relatively free flow of water under the causeway for most of its length. The replacement structure was a solid wall of earth and rock, with an opening under the road at one end to allow water exchange. This replacement structure therefore significantly reduced tidal exchange between the eastern and western side of the causeway.

Modification to the causeway began in 1953 and ended in 1957 (Structurae 2004). Prior to these causeway modifications, *Paralia sulcata* and *Ehrenbergia granulosa* constituted over 50% of all diatom assemblages (core samples up to 1951). However, during the period of causeway modification a decline in the abundance of these two species to ~30% occurred (~1956). After ~1960, these two species averaged only 3.2% of remaining samples to the top of the core (2001). This indicates that modifications to the causeway in the 1950s were directly related to the abrupt decline in *Paralia sulcata* and *Ehrenbergia granulosa* in Pittwater Lagoon.

Further evidence suggests that the area from which the core was collected was also an area of deposition of resuspended frustules of *Paralia sulcata* and *Ehrenbergia granulosa*. The factors suggesting this relate to the hydrodynamics within Pittwater, sediment size within Pittwater, and substrate preferences for these species. Wind driven waves are a prominent feature of the hydrodynamics of Pittwater (Crawford & Mitchell 1999), and as the dominant wind direction in Tasmania is from the west, a considerable amount of resuspended material may be naturally transported the eastern side of Pittwater. The natural channel through Pittwater also directs some incoming and outgoing tidal water toward the eastern side of Pittwater (Crawford & Mitchell 1999). Lower Pittwater surveys have shown that the substrate is generally medium to

fine sands (Crawford & Mitchell 1999), as opposed to the very fine silt where the core was taken. Additionally, sediment size analysis of training-set sites on the eastern side of the causeway in Pittwater showed that sediments were ~64 to 99% > 0.125 μm. The core was collected from a relatively sheltered area on the eastern side of Pittwater, and the fine sediment throughout the core indicates that the coring site is an area where fine suspended sediments (and by inference resuspended diatoms) are naturally deposited.

Other researchers have reported *Paralia sulcata* from a wide range of sediment types (within the one study), including fine muds and coarse sands (Whiting & McIntire 1985, Huang 1990). This suggests that *Paralia sulcata* and *Ehrenbergia granulosa* were occurring naturally on the fine sediments at the core site. However, the transfer functions generated from the training-set (Chapter 2) inferred that in south-east Tasmania, *Paralia sulcata* and *Ehrenbergia granulosa* prefer coarse sediments (with optima for sediment < 63 μm of < 20% and < 10% respectively). In fact, the only (two) sites from the training-set in which these species were abundant had coarse sediments that were > 80% > 0.125 μm. Neither *Paralia sulcata* nor *Ehrenbergia granulosa* were recorded from the Coal River sample. This suggests that *Paralia sulcata* and *Ehrenbergia granulosa* may have been widespread (although not necessarily abundant) on the coarser sediments in Pittwater.

If *Paralia sulcata* and *Ehrenbergia granulosa* were more widespread on the coarser sediments of Pittwater, and the coring site is a natural area of deposition of fine material (including resuspended diatoms) in Pittwater, then resuspended tychoplanktonic species in Pittwater would be found in significantly greater proportions at the coring site than would naturally occur there. Similar findings have been reported from Japan, where *Paralia* species were found in large numbers as an allochthonous component in tidal marsh deposits, thought to be a result of the long chains of this species floating more readily and being easily transported by tidal currents (Sawai 2001). Prior to the modifications to the causeway in the 1950s, resuspended frustules of *Paralia sulcata* and *Ehrenbergia granulosa* from the entire larger Pittwater area (and perhaps from the bay outside) may in fact have been deposited at the coring site. Following the causeway modifications, by far the greater part of Pittwater was separated from the coring area, thus reducing the potential

allochthonous input of *Paralia sulcata* and *Ehrenbergia granulosa*. This would account for the significant and abrupt change in the abundance of *Paralia sulcata* and *Ehrenbergia granulosa* at the coring site, and is supported by an additional factor. In other surface-sediment samples from training-set sites within south-east Tasmania, *Paralia sulcata* and *Ehrenbergia granulosa* occur naturally only in relatively small proportions, similar to those proportions recorded from the Pittwater core after modifications to the causeway. This means that the abundance of *P. sulcata* and *E. granulosa* recorded in the core after the causeway modifications in the 1950s are more consistent with other diatom assemblages in south-east Tasmania, and further supports the argument that the abundance of these species in the core prior to 1960 was a result of resuspension and deposition.

3.4.5.2 Middle Sections of the Core: ~1960 – ~1975

The increase in the relative abundance of species within the middle section of the core (~1960 to ~1975) follows the decline in relative abundance of *Paralia sulcata* and *Ehrenbergia granulosa*. Although a higher relative abundance of *Catenula adhaerens* and *Cocconeis* aff. *pinnata* continues to the top of the core, the contribution of these two species to the community composition since ~1960 was significantly outweighed by that of *Cymatosira* aff. *belgica*.

3.4.5.3 Top Sections of the Core: ~1975 - ~2001

The relatively recent appearance and rapid and sustained dominance by *Cymatosira* aff. *belgica* in the top sections of the Pittwater core suggest that this species was introduced to Pittwater. As discussed in Chapter 2, *Cymatosira* species have not previously been reported from Tasmania, and were not recorded in the survey of diatoms from the Swan River Estuary in Western Australia (John 1983) or eastern Australia (Foged 1978). *Cymatosira* aff. *belgica* was restricted to two areas within the study region – Pittwater (including training set sites), and Ralphs Bay (where it was the dominant species at Site 20 and 21). Pittwater opens into Frederick Henry Bay, and Ralphs Bay is separated from Frederick Henry Bay by a narrow strip of land at Lauderdale. This strip of land has a canal which runs most of the distance between the two bays, leaving the bays separated by less than 100 m. This canal has also been deliberately opened between the two bays in the past. The spread of *Cymatosira* aff. *belgica* in considerable proportions from one of these two bays to the other is

therefore quite possible.

Species introduction can occur from a variety of sources, and consequently is reported for a wide range of organisms (including diatoms) from all around the world (Kelly 1993, Ruiz *et al.* 2000, Grosholz & Ruiz 2003, Trowbridge 2004), including Tasmania (Ross *et al.* 2004). Ballast water, migrating birds, fouling organisms, transport vessels, wind and freak meteorological events are only a few of the mechanisms by which species may be introduced from one area to another. The possible introduction of a new diatom species to Tasmanian waters is therefore not an unlikely or necessarily unusual event. It is possible, for example, that the introduction of *Cymatosira* aff. *belgica* may be associated with the introduction of Pacific Oysters (*Crassostrea gigas*) into Pittwater from Japan in 1947-1948, and again in 1951-52 (English *et al.* 2000). Most of these oysters were transferred to Port Sorrell in Tasmania's north in 1953 in the hope of improving growth conditions in the warmer waters, but were re-introduced into Pittwater in 1981 from colonies in the Tamar River (northern Tasmania) (English *et al.* 2000). As *Cymatosira* aff. *belgica* has had such a significant impact on the community composition in Pittwater Lagoon, and is dominating diatom assemblages at two nearby sites in Ralphs Bay, further research on the origin, and current and historical distribution of this species is warranted. The inclusion of a sediment core from one or more of the training-set sites in Ralphs Bay, and investigation of diatom community composition from other Tasmanian coastal sites including Port Sorrel and the Tamar River, may add significantly to understanding the origin and spread of this species.

Pittwater is important both ecologically, as a designated Ramsar wetland site and habitat for rare and threatened marine species, and commercially as an oyster growing area (Crawford & Mitchell 1999). Since European settlement of the area, Pittwater has experienced significant physical, chemical and biological changes. The future health of Pittwater Lagoon ecologically and as a commercial oyster-growing area will depend on the ability of responsible stakeholders and caretakers to incorporate effective biological monitoring and assessment of the area into their management strategies.

3.4.6 Conclusions

Pittwater Lagoon has undergone significant changes since the late 18th century in relation to hydrological flows and freshwater input, organic carbon content of the sediments, species introduction, and to a lesser extent nutrient concentrations and sediment size composition. The dramatic changes that have occurred in the microflora throughout the core appear to be a result of reduced tidal exchange as a consequence of causeway alterations in the 1950s, and the introduction of a new diatom species to Pittwater. The application of the transfer-functions to infer nutrient concentrations in Pittwater show that nutrient concentrations have altered in Pittwater since the late 18th century (with an overall increase in $NO_{2\text{-}3}$ and decrease in SiO_2). However, other physical and biological changes have had a greater impact than nutrient changes on the microflora in this system. However, the generation and practical application of the transfer functions in Pittwater now means that $NO_{2\text{-}3}$ and SiO_2 can be reconstructed from south-east Tasmanian near-shore marine environments to help water quality managers assess impacts and determine best rehabilitation and maintenance routines for these sensitive coastal ecosystems.

3.4.7 Recommendations

Ongoing biological monitoring and assessment, in conjunction with chemical measurements, is essential for maintaining the ecological integrity of Pittwater. Further research on the origin, distribution and historical abundance of *Cymatosira* aff. *belgica* in south-east and northern Tasmania is warranted as this species represents a very significant change in the microflora of at least one site, and its dominance at two other sites indicates that it is a relatively competitive species in south-east Tasmania. The investigation of a sediment core from Ralphs Bay would add significantly to understanding the origin and spread of this species.

4.0 CONCLUDING GENERAL DISCUSSION

The issues relating to water quality have been significantly increasing on a global scale for many decades. Eutrophication, anthropogenic alterations to the physical structure of waterways, and the introduction of exotic species have resulted in changes globally to natural aquatic ecosystems (Sundbäck & Snoeijs 1991b, Grosholz & Ruiz 2003, Trowbridge 2004). Simultaneously, growth in our understanding of the primary issues relating to maintaining the biological integrity of our waterways, and the ecological complexities of species interactions with their environment and each other, combined with recent improvements in statistical techniques and technological advances that have improved the dissemination of information, have increased our awareness and ability to address these issues.

The near-shore coastal marine environment is one of the areas that have been receiving increasing attention in recent times. With most of the world's population living in the coastal zone, significant impacts on coastal ecosystems are continually being identified, and there is growing public awareness of the limited capacity of coastal aquatic systems to cope with these anthropogenic impacts. This study aimed to identify some of these impacts on the micro-algal community in the south-east Tasmanian near-shore coastal marine environment, provide a means for future monitoring and assessment of these impacts, and provide a tool for identifying the historical impacts that these changes have had. To achieve these aims:

(i) The biomass of the benthic microflora in south-east Tasmania has been measured and related to depth, temperature, nutrients, temporal variation, and algal biomass from other areas of the globe;

(ii) Causative relationships have been identified between the species composition of diatom communities and the corresponding physical and chemical conditions from 51 south-east Tasmanian sites;

(iii) Transfer functions have been developed to infer NO_{2-3}, SiO_2, and sediment size for palaeo-reconstruction of near-shore marine sites in south-east Tasmania; and

(iv) The environmental history of an impacted Ramsar wetland site in south-east Tasmania has been reconstructed to identify historical anthropogenic impacts from changes in the microflora.

This research has shown that, both historically and currently, the diatom communities in south-east Tasmania are significantly affected by anthropogenic activity. The diatom assemblages in south-east Tasmania are generally similar to many other areas of the globe and, as has been shown to be the case in many other areas, change in response to altered nutrient concentrations, physical modifications to hydrological regimes, and the introduction of exotic species.

The development of transfer functions to infer nutrient concentrations in the coastal marine environment has not previously been undertaken in Australia. Very little research has previously been undertaken on Tasmanian marine diatoms, and consequently most of the species recorded during this study have not previously been reported from Tasmania, and several have not previously been reported from Australia. The results of this study therefore contribute significantly to the body of knowledge on Australian diatoms and their causative relationships with environmental variables, and provide a valuable resource for management of Tasmania's coastal marine environment.

The future health of Tasmania's coastal environment relies on the regular monitoring and assessment of the water quality in the region, and on improving our understanding of the ecological consequences of anthropogenic activities. Since the micro-algal community constitutes the major component of the base of the food chain in most marine systems, it is imperative that changes in the micro-algal community are regularly monitored and assessed, and that our understanding of the interactions involved is continually improved. Hence, a number of recommendations are made here regarding the use of diatoms in water quality research in south-east Tasmania, and their inclusion in water quality monitoring programs.

4.1 Recommendations

Effective water quality assessment requires the inclusion of biological indicators in water quality programs. The research presented here highlights the value of using diatoms as indicators of nutrient concentrations in the south-east Tasmanian coastal environment, and provides the necessary ecological data for their inclusion in future

water quality programs and environmental impact assessments in this region. Diatoms are particularly valuable as biological indicators of water quality, and should be included in water quality monitoring and assessment programs in south-east Tasmania. Both the abundance and community composition of micro-algal assemblages provide valuable information on changes occurring in response to altered environmental conditions. Specifically, substantial benefit would be gained from the following:

(i) Regular monitoring of benthic algal biomass in selected areas of Tasmania. This would provide valuable information on the long-term trends of algal productivity in this region, and thus be useful in detecting the onset of eutrophication or the impact of contaminants;

(ii) Regular assessment of diatom community structure at selected sites within Tasmania. This would provide valuable data on changes occurring in micro-algal community structure over time in response to changing nutrient concentrations. This data would provide a direct measure of the effect that changes in nutrient concentrations are having on the base of the food chain, and should therefore be a fundamental component in the decision-making processes of coastal managers;

(iii) The inclusion of diatoms in environmental impact assessments of projects within the coastal environment of south-east Tasmania. This would improve identification of those projects and activities having adverse short or longer-term impacts on the marine microflora, and thus contribute significantly to coastal management in this region;

(iv) Further research to improve knowledge of diatom species abundance and distribution in Tasmania's coastal environments. This will add significantly to our ability to use diatoms as biological indicators in south-east Tasmanian coastal ecosystems. Additional palaeo-environmental reconstructions from this region will significantly add to the understanding of the long-term impacts of environmental change in our waterways.

(v) Including measurement of silica concentrations in water quality assessment regimes. Although nitrogen and phosphorus are key components of eutrophication processes, silica also plays a key role in determining community composition, particularly at times of maximum algal growth when algal blooms may pose a threat. Measurement of silica concentrations should

therefore also be included when determining nutrient concentrations, to provide a more comprehensive picture of the processes occurring in algal communities.

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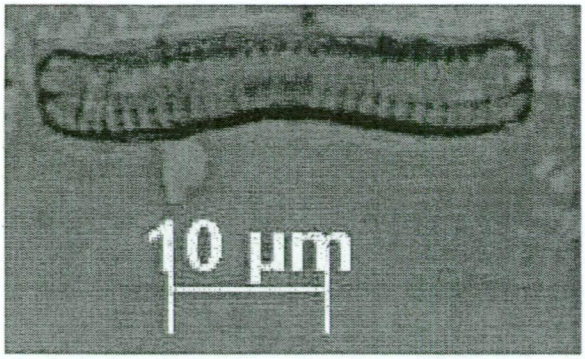

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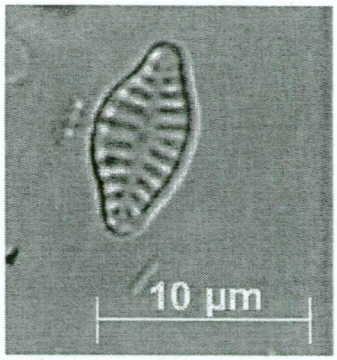

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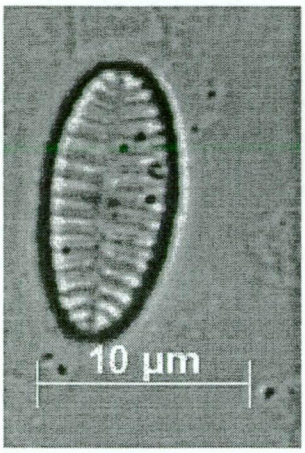

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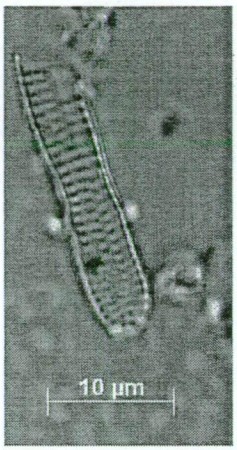

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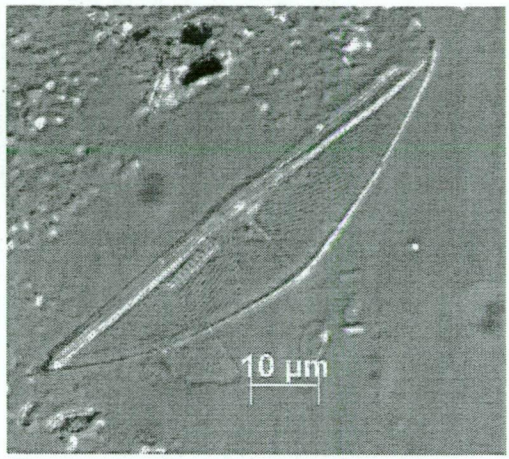

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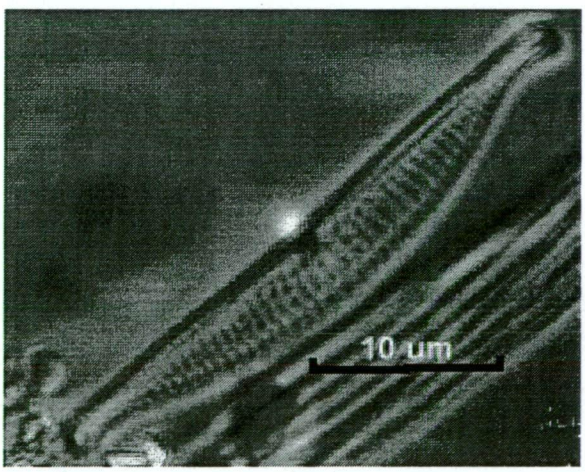

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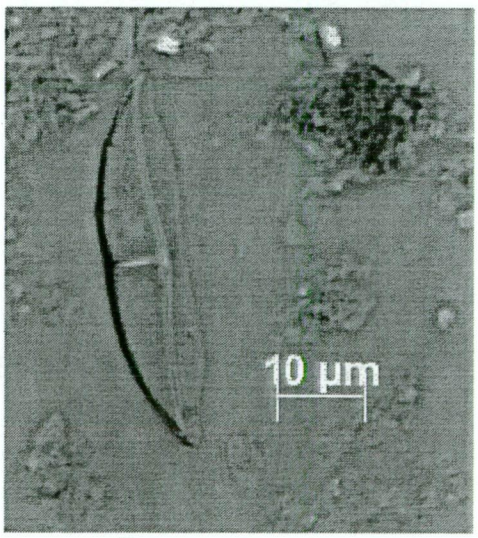
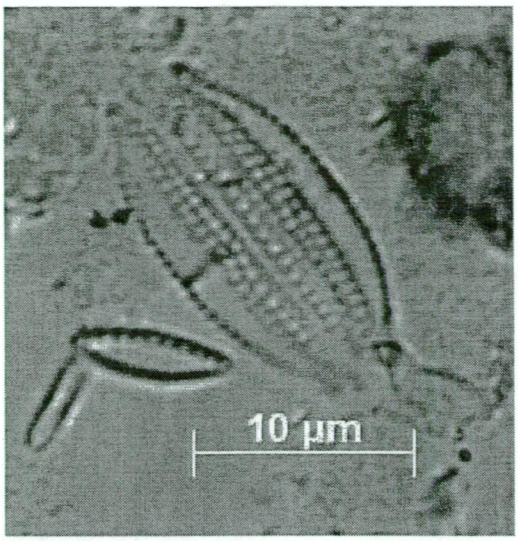


7

Plate 1: Diatom Photos

1. *Achnanthes brevipes* Agardh
2. *Achnanthes residensis* Foged
3. *Achnanthes oblongella* Østrup
4. *Achnanthes* sp. 1
5. *Amphora decussata* Grunow
6. *Amphora exigua* Gregory
7. *Amphora laevissima* Gregory



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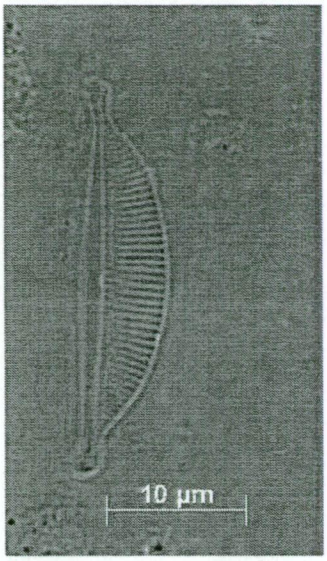

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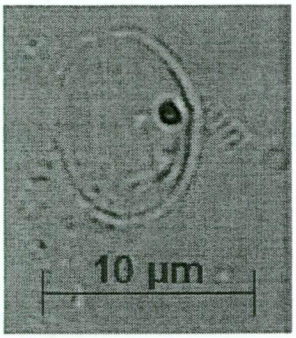

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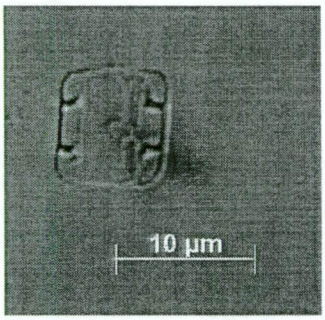

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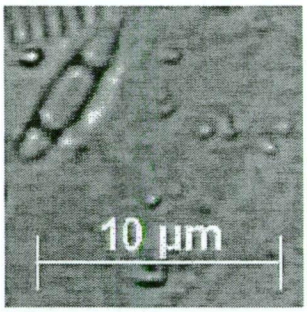

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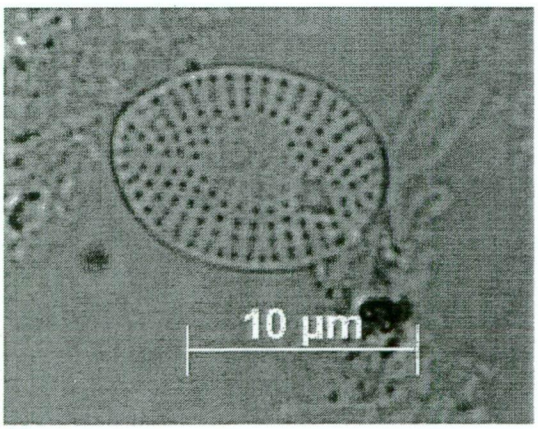

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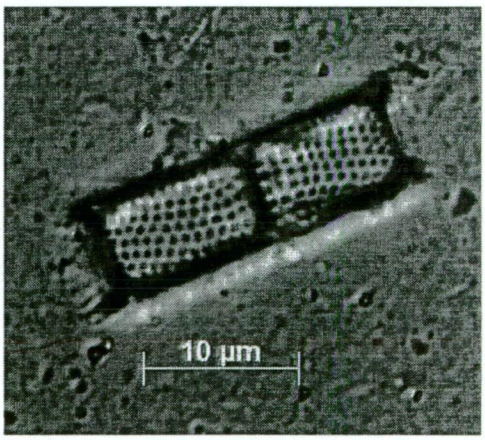


14

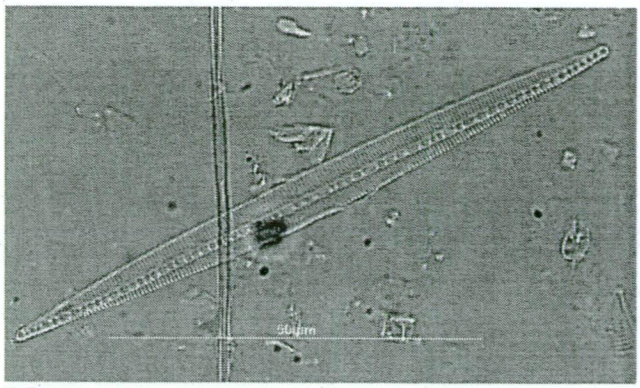
Plate 2: Diatom Photos

8 *Amphora maletractata* var. *constricta* Heiden Simonsen

9. *Amphora subturgida* Hust.

10. *Amphora* sp. 1 11 & 12. *Anaulus minutus* Grun. in Van Heurck

13. *Anorthoneis vortex* Sterrenburg 14. *Aulocosiera ambigua* (Grun.) Simonsen



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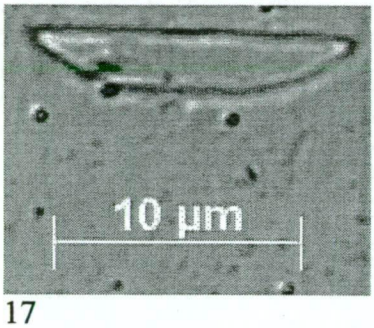

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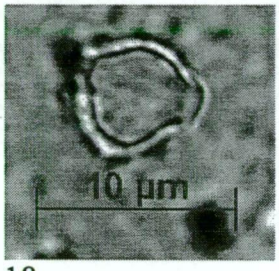

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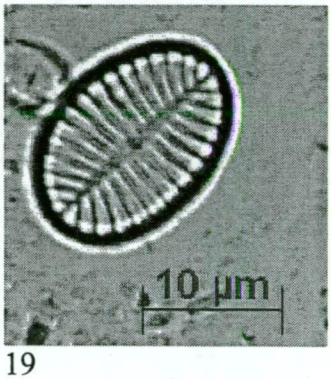

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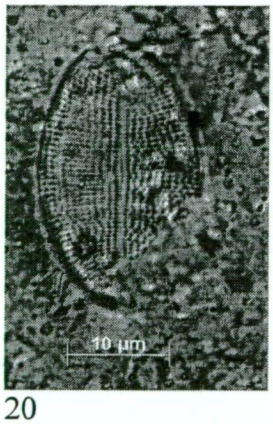

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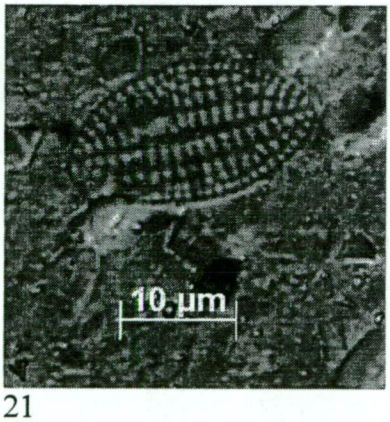

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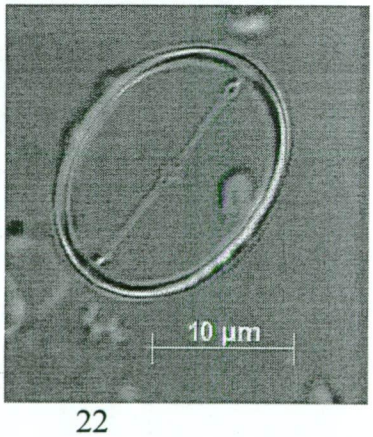


22

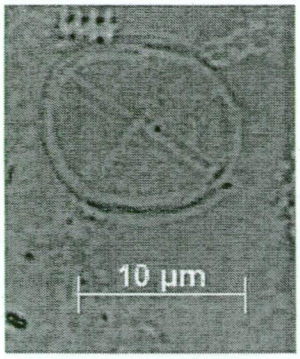
Plate 3: Diatom Photos

15. *Bacillaria paradoxa* Gmelin 16. *Campylodiscus daemelianus* Grun.

17. *Catenula adhaerens* Mereschkowsky 18. *Chaetocerus* resting spore

19. *Cocconeis* aff. *pinnata* Gregory ex Greville 20. *Cocconeis carminata* Cholnoky

21. *Cocconeis disculoides* Hust. 22. *Cocconeis disrupta* Gregory



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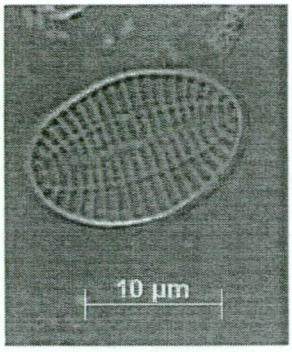

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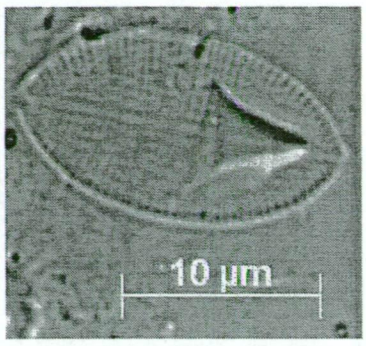

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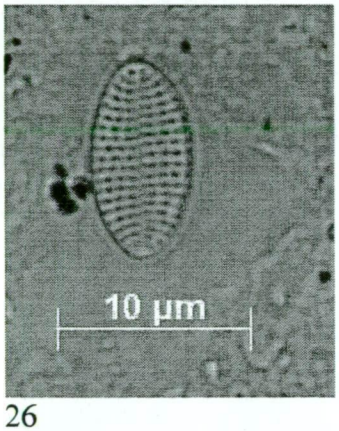

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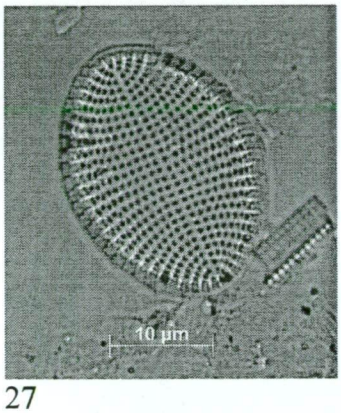

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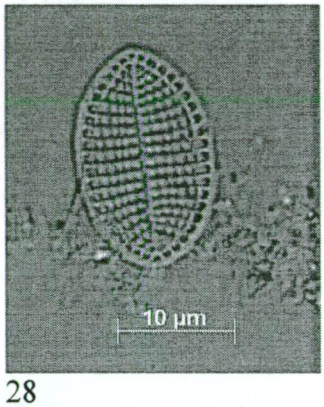

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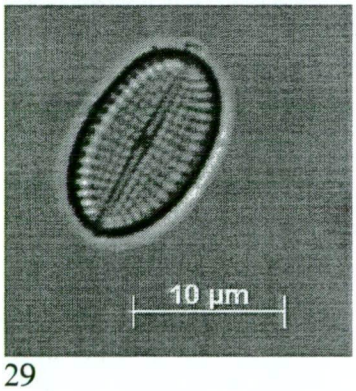

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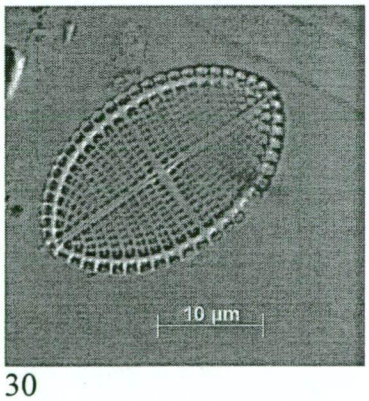


30

Plate 4: Diatom Photos

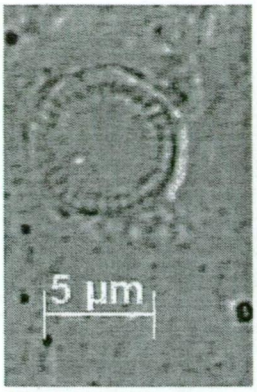
23. *Cocconeis molesta* var. *crucifera* Grun.in Van Heurck

24. Cocconeis peltoides Hust. 25. *Cocconeis placentula* Ehr.

26. *Cocconeis placentula* Ehr. var. *euglypta* (Ehr.) Grun.

27. *Cocconeis scutellum* Ehr. 28. *Cocconeis scutellum* var. *parva* Grun.

29. *Cocconeis* sp. 1 30. *Cocconeis stauroneiformis* (Van Heurck) Okuno



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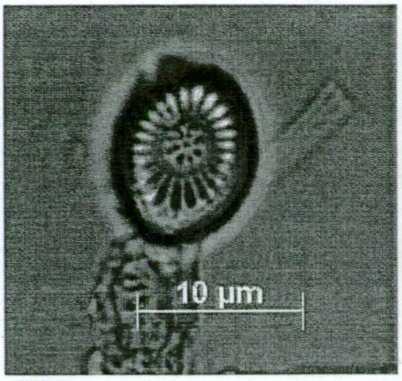

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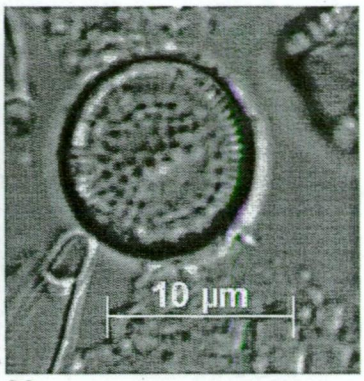

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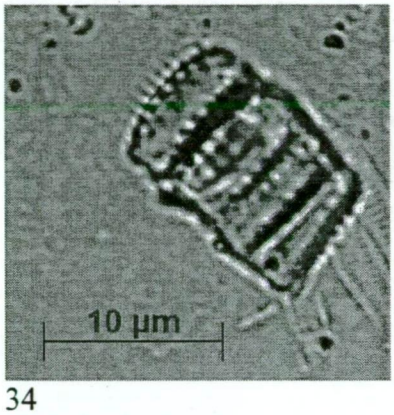

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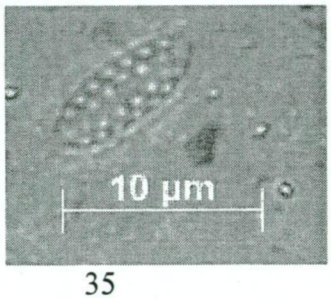

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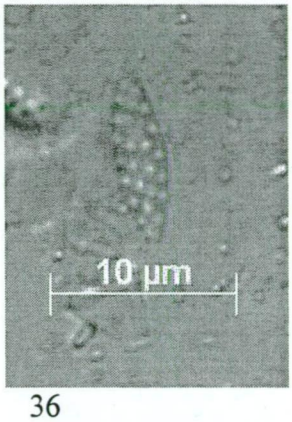

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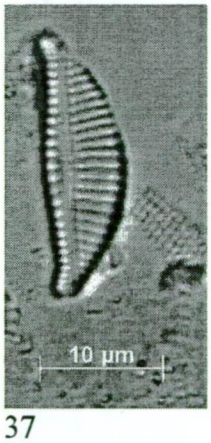

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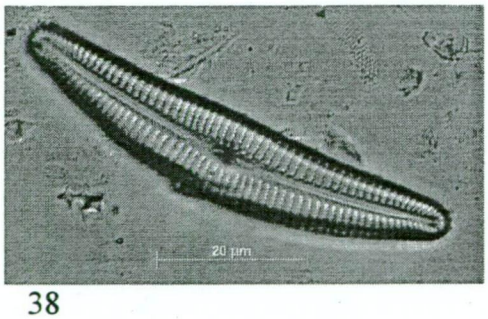

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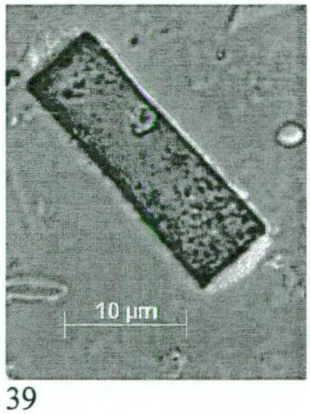

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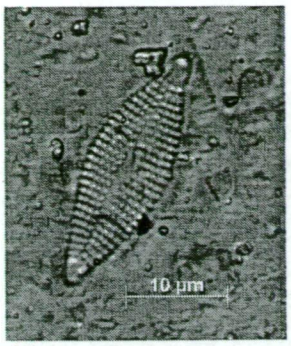
Plate 5: Diatom Photos

31. *Cyclotella atomus* Hust. 32. *Cyclotella stelligera* Cleve & Grun.

33. *Cyclotella striata* (Kütz.) Grun. 34, 35, 36. *Cymatosira* aff. *belgica* Grun.

37. *Cymbella minuta* Hilse ex Rabh. 38 *Cymbella sumatrensis* Hust.

39. *Dactyliosolen* spp.



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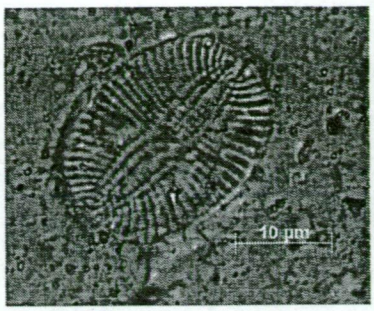

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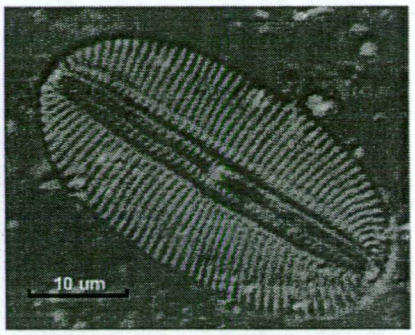

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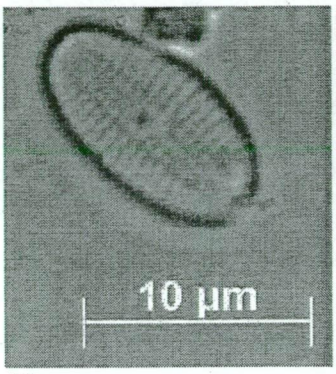

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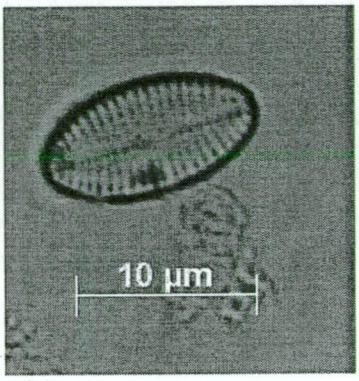

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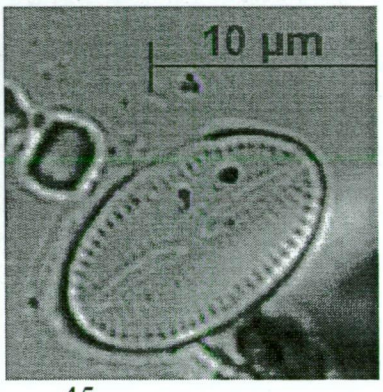

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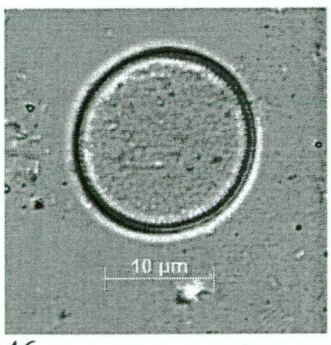

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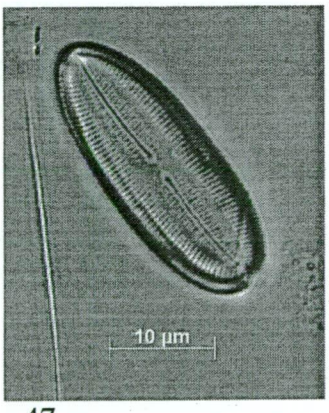

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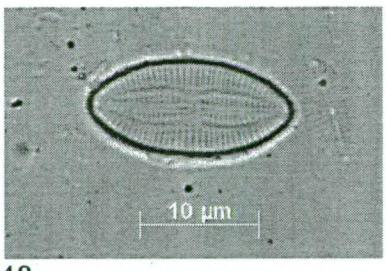


48

Plate 6: Diatom Photos

40. *Dimerogramma minor* var. *nana* (Greg.) Van Heurck

41. *Diploneis notabilis* (Grev.) Cleve

42. *Diploneis subovalis* Cleve

43. *Diploneis vacillans* (A. Schmidt) Cleve

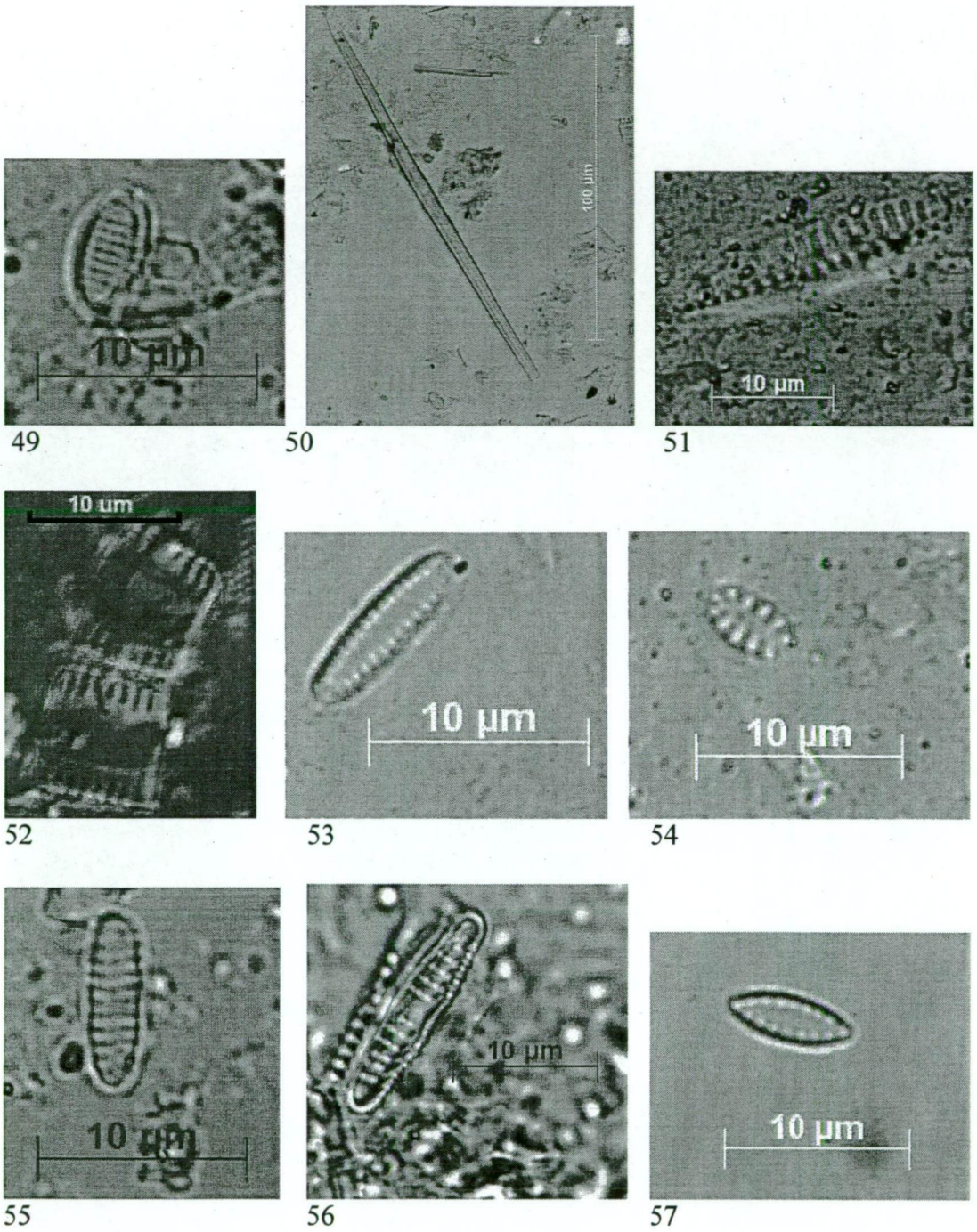
44. *Diploneis* sp. 1

45. *Diploneis* sp. 2

46. *Ehrenbergia granulosa* (Grun.) Witkowski

47. *Fallacia litoricola* (Hust.) D. G. Mann

48. *Fallacia subforcipata* (Hust.) D. G. Mann



49 50 51

52 53 54

55 56 57

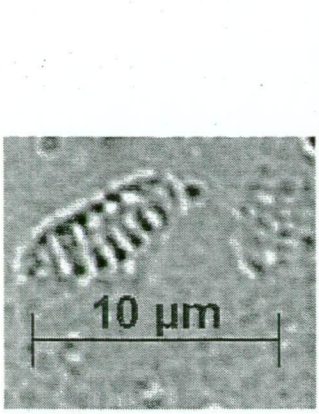
Plate 7: Diatom Photos

49. *Fragilaria atomus* Hust. 50. *Fragilaria crotonensis* Kitton

51. *Fragilaria martyi* (Heribaud) Lange-Bertalot

52 & 53. *Fragilaria pinnata* Ehrenberg 54 *Fragilaria pinnata* Ehr. var. *pinnata*

55 & 56 *Fragilaria vaucheriae* Kützing Petersen 57. *Fragilaria* sp. 1



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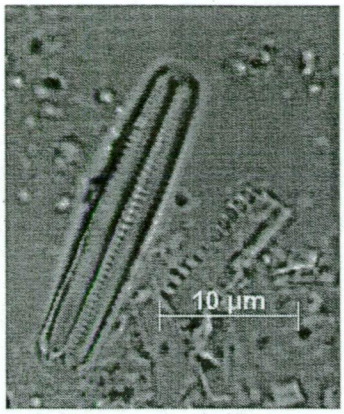

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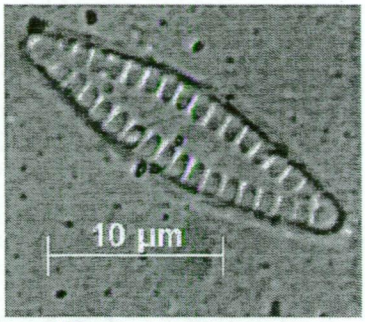

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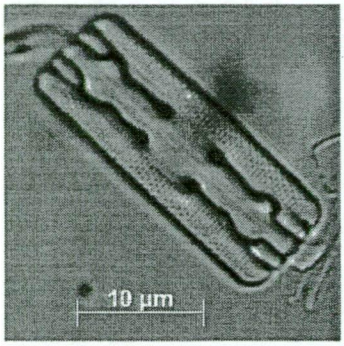

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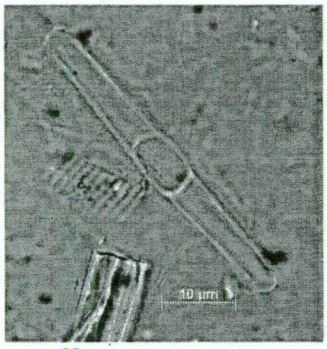

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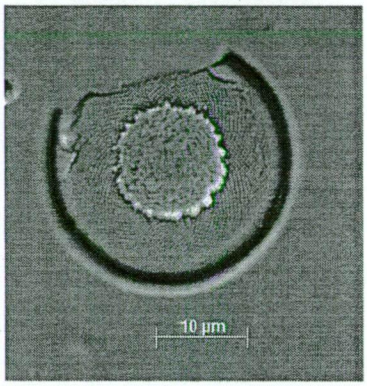

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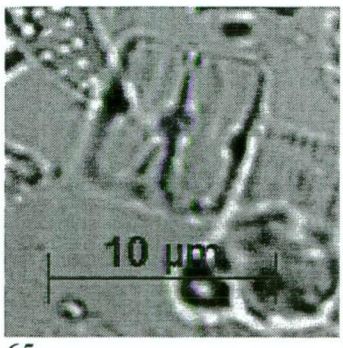


65

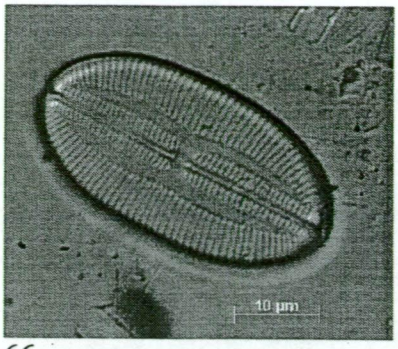
Plate 8: Diatom Photos

58. *Fragilaria* sp. 2 59. *Fragilariopsis cylindrus* (Grun.) Krieger

60. *Glyphodesmis distans* (Greg.) Grun. 61 & 62. *Gramatophora oceanica* Ehr.

63. *Hyalodiscus scoticus* (Kütz.) Grun. 64. *Gyrosigma perthense* John

65. *Lunella bisecta* Snoeijs



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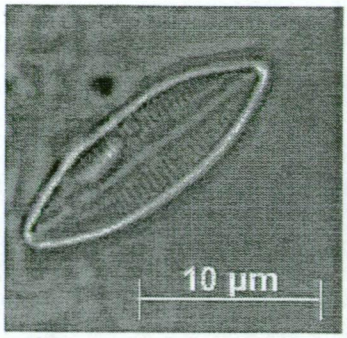

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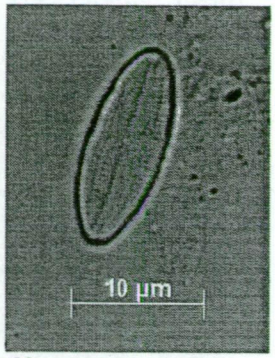

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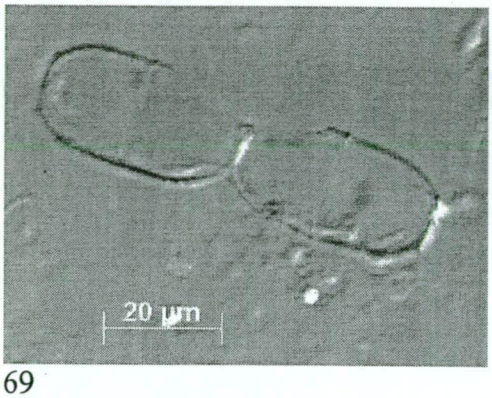

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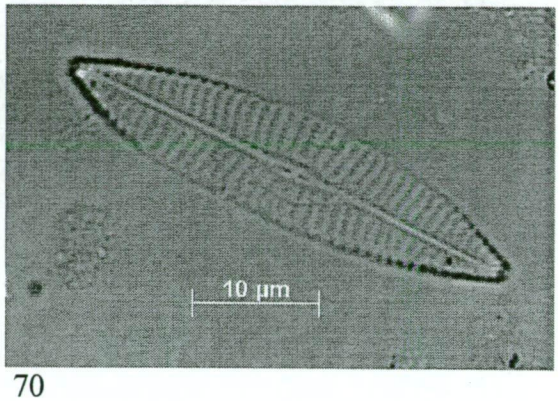

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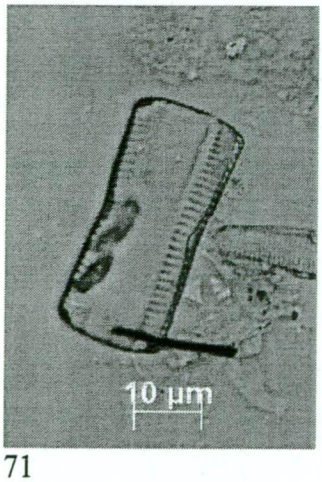

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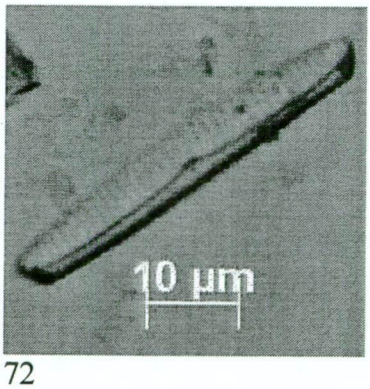

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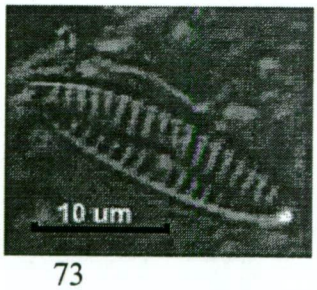


73

Plate 9: Diatom Photos

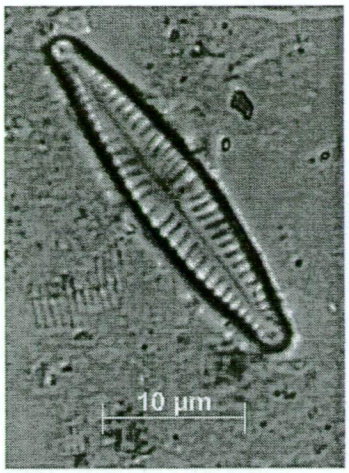
66. *Lyrella david-mannii* Witkowski

67. *Mastogloia pusilla* (Grun.) Cl. var. pusilla 68. *Mastogloia* sp. 1

69. *Melosira nummuloides* Agardh

70. *Navicula arenaria* Donken var. *rostellata* Lange-Bertalot

71 & 72. *Navicula cancellata* Donkin 73. *Navicula cincta* (Ehr.) Ralfs in Pitchard



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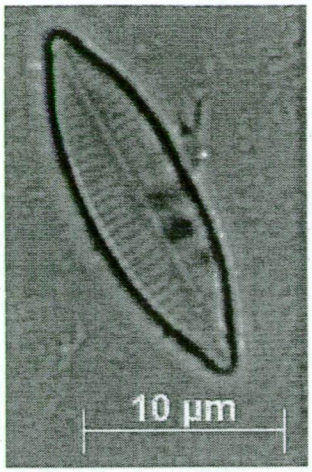

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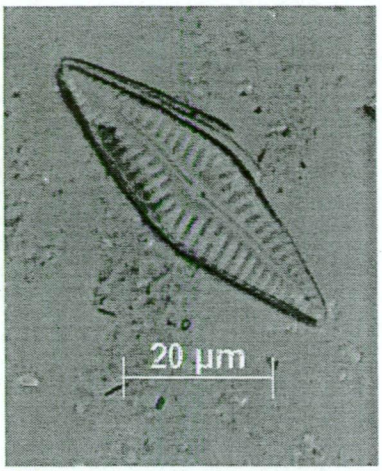

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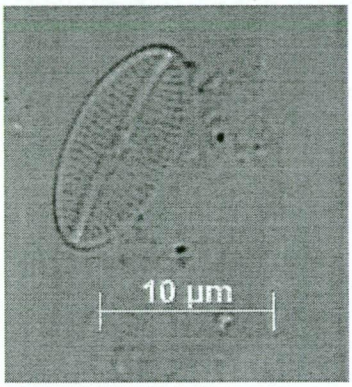

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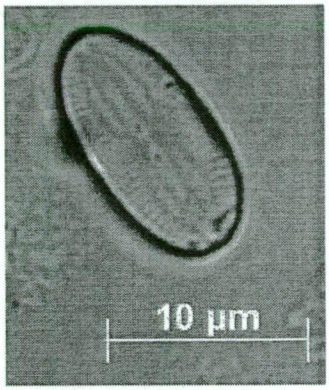

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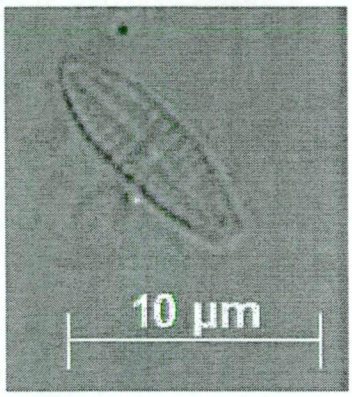

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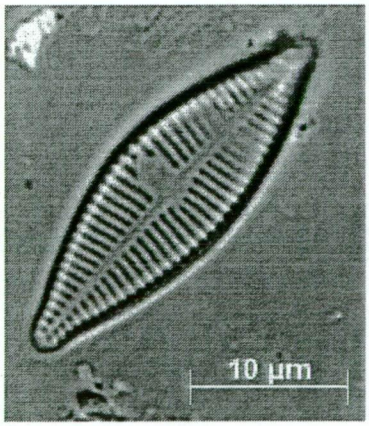

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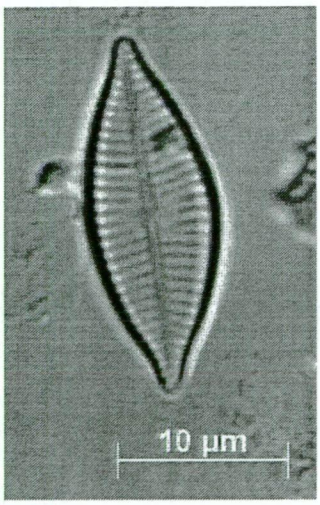

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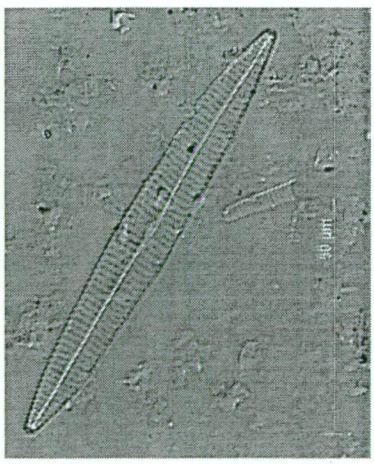

82

Plate 10: Diatom Photos

74. *Navicula cryptocephala* Kütz. 75. *Navicula halophila* (Grun.) Cl. fo. *robusta*

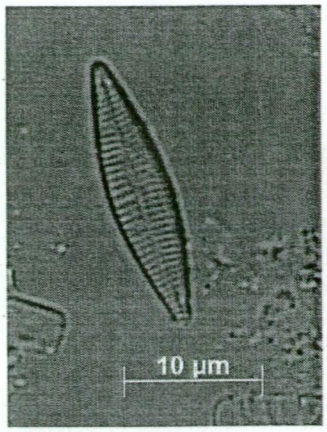
76. *Navicula menisculus* Schumann

77. *Navicula monoculata* var. *omissa* (Hust.) Lange-Bertalot

78. *Navicula nyella* Hust. 79. *Navicula pygmaea* Kütz.

80 & 81. *Navicula salinarum* Grun. in Cleve & Grun. var. *salinarum*

82. *Navicula tripunctata* (O. F. Muller) Borg.



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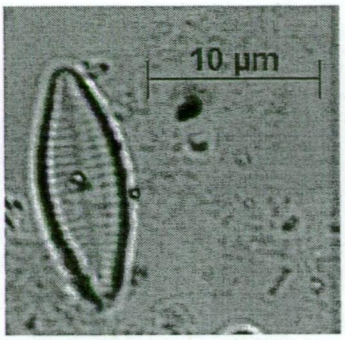

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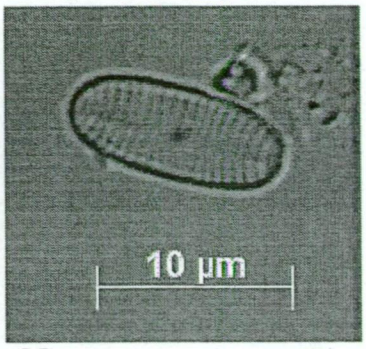

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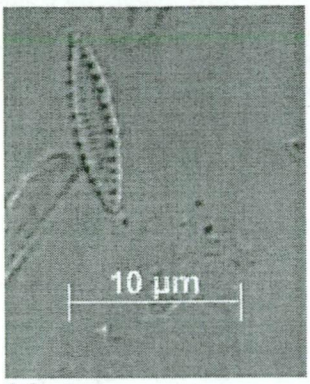

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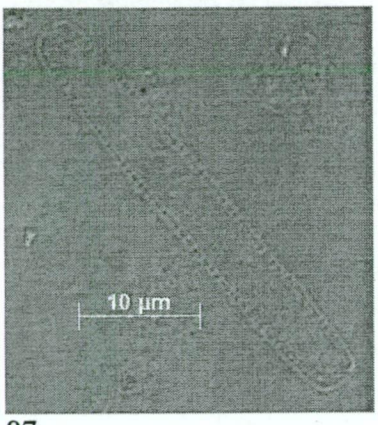

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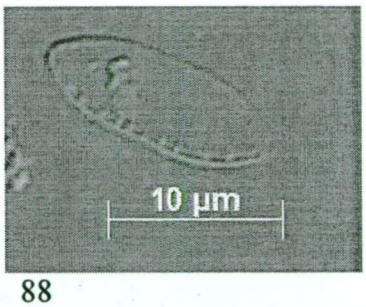

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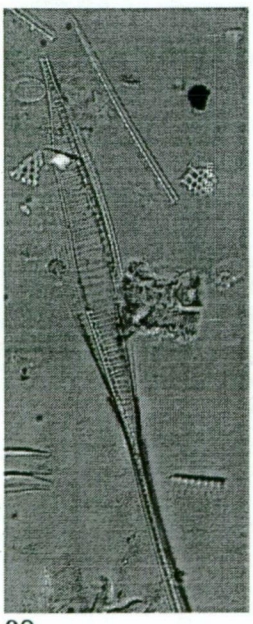

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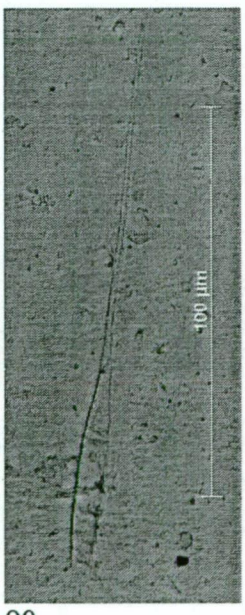

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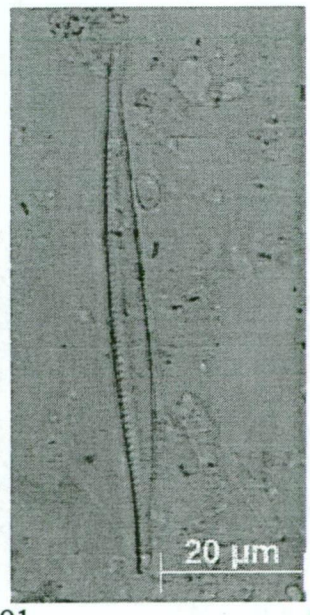

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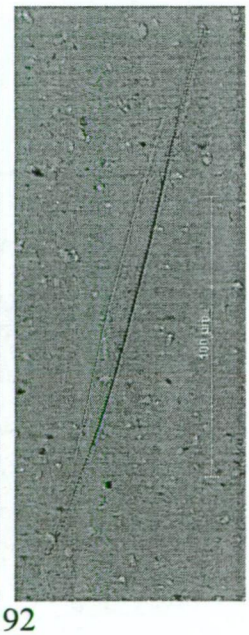

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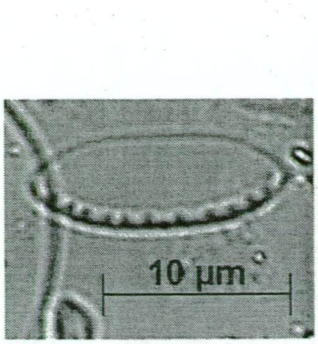
Plate 11: Diatom Photos

83. *Navicula* sp.1. 84. *Navicula* sp.2 85. *Navicula* sp. 3

86. *Nitzschia amphibia* Grun. 87. *Nitzschia dissipata* (Kütz.) Grun. var. *dissipata*

88. *Nitzschia laevis* Hust. 89 & 90. *Nitzschia longissima* (Bréb.) Ralfs

91 & 92. *Nitzschia lorenziana* Grun. var. *subtilis* Grun.



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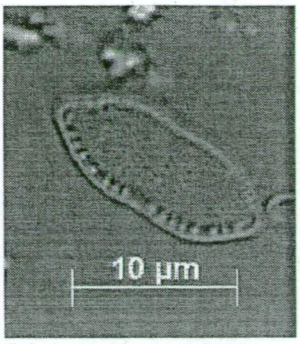

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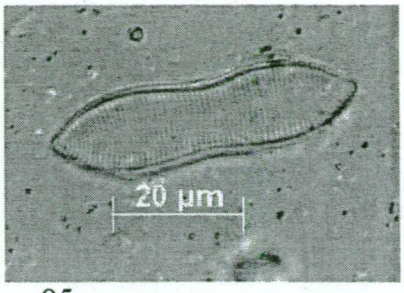

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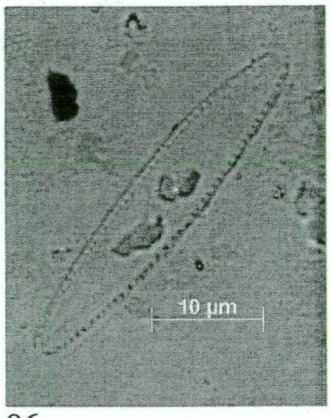

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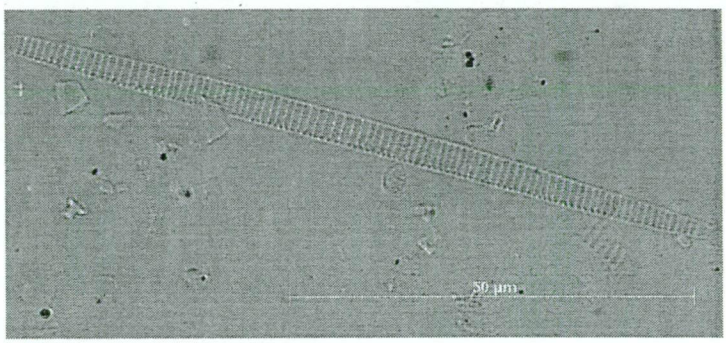

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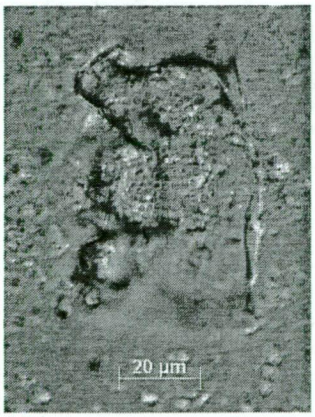

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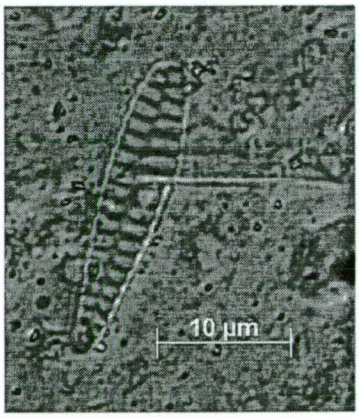

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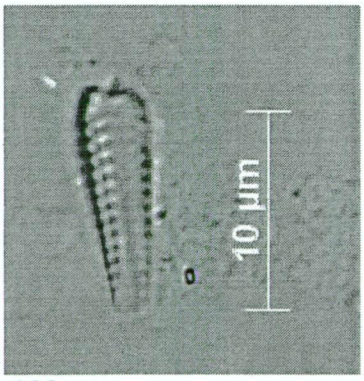


100

Plate 12: Diatom Photos

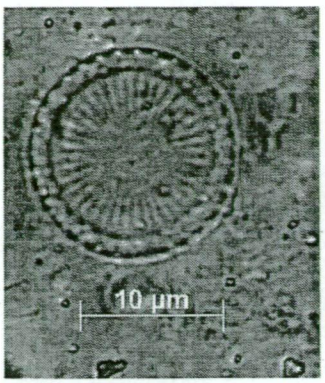
93. *Nitzschia ovalis* Arnott ex Grunow in Cleve & Grunow

94. *Nitzschia panduriformis* Greg. var. *minor* Greg.

95. *Nitzschia punctata* var. *coarctata* (Grun.) Hust. 96. *Nitzschia* sp. 1

97. *Nitzschia* sp. 2 98. *Odontella aurita* (Lyng.) Ag.

99. *Opephora martyi* Herib. 100. *Opephora olsenii* Möller



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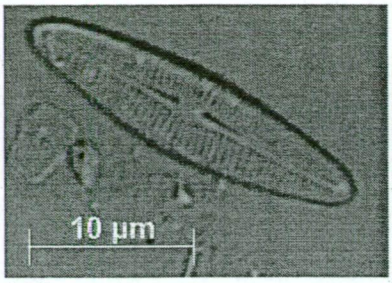

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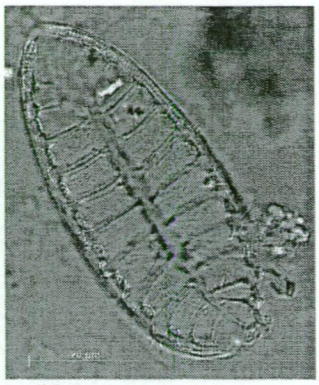

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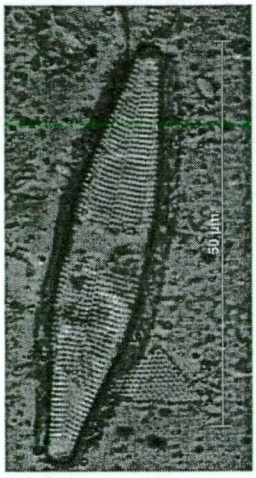

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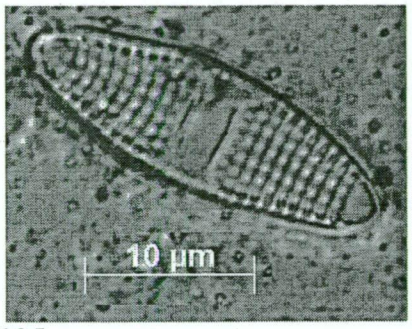

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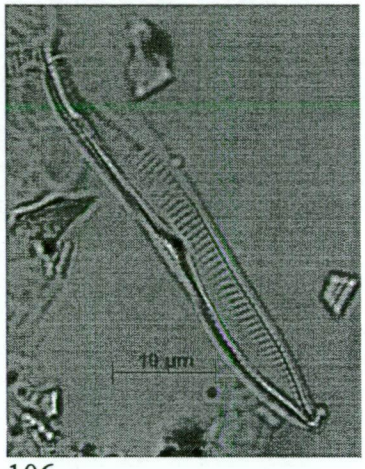

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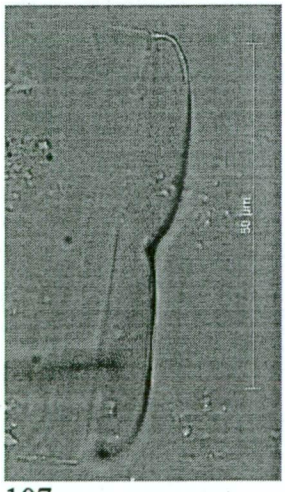

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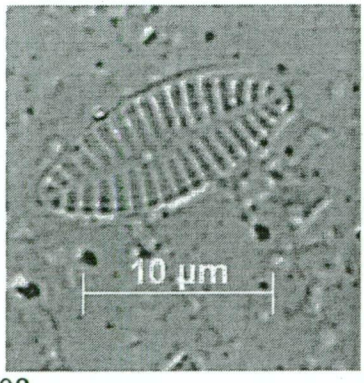

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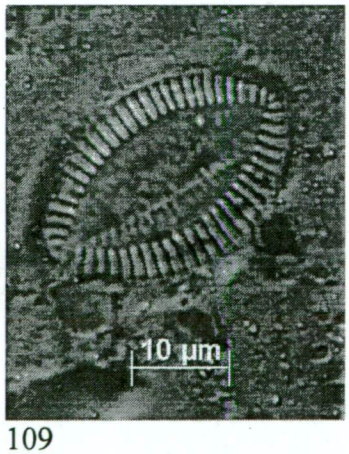


109

Plate 13: Diatom Photos

101. *Paralia sulcata* (Ehr.) Cleve 102. *Parlibellus* cf. *plicatus* (Donkin) Cox

103. *Petrodictyon gemma* (Ehr.) D. G. Mann

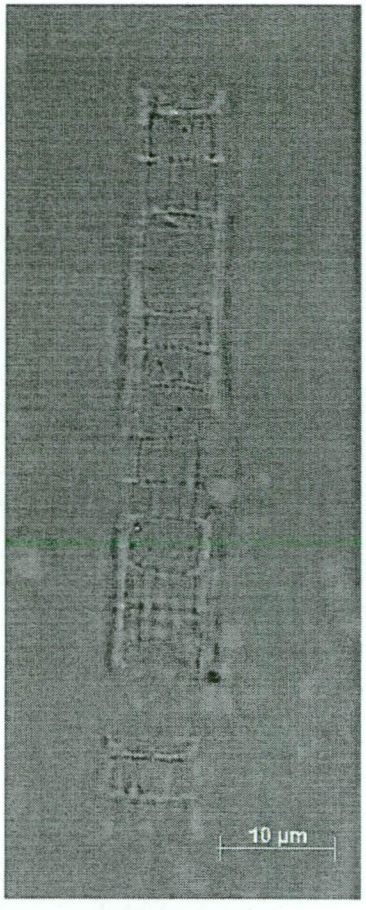
104. *Plagiogramma appendiculatum* Giffen.

105. *Plagiogramma staurophorum* (Greg.) Heiberg 106. *Plagiotropis* sp.1

107. Plagiotropis sp.2 108. *Planothidium delicatulum* (Kütz.) Round & Buktiyarova

109. *Planothidium quarnerensis* (Grun.)

110



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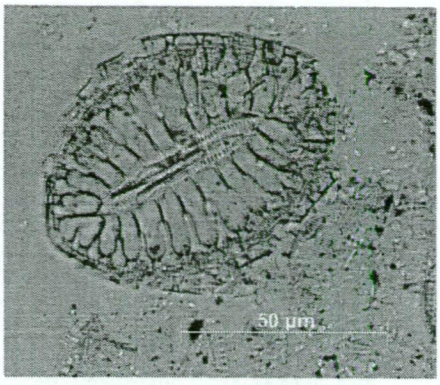
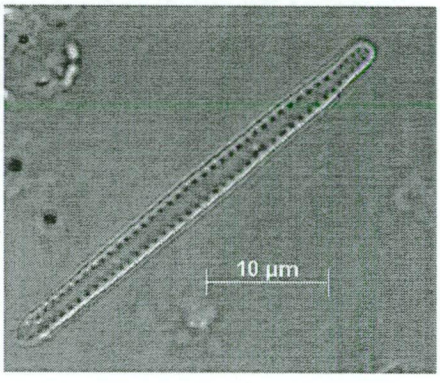
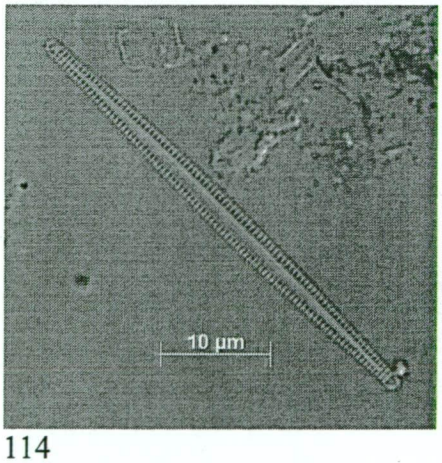


112



113



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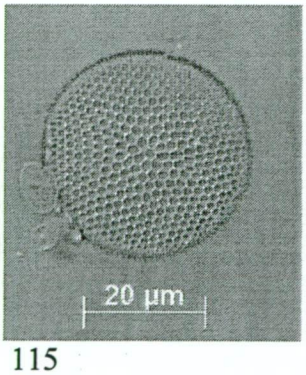

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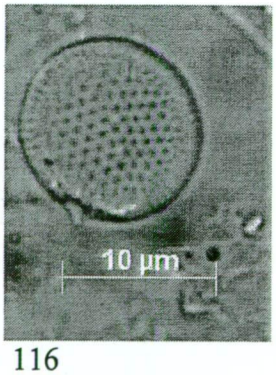
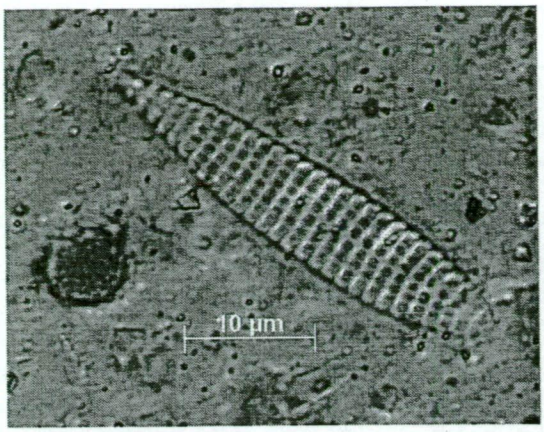


116

Plate 14: Diatom Photos

110. *Pseudonitzschia australis* Freng. 111. *Skeletonema costatum* (Grev.) Cl.
112. *Surirella fastuosa* (Ehr.) Kütz 113. *Synedra investiens* W. Smith
114. *Synedra tabulata* (Ag.) Kütz. var. *tabulata*
115. *Thalassiosira eccentrica* (Ehr.) Cl.
116. *Thalassiosira oestrupii* (Ostenfeld) Hasle



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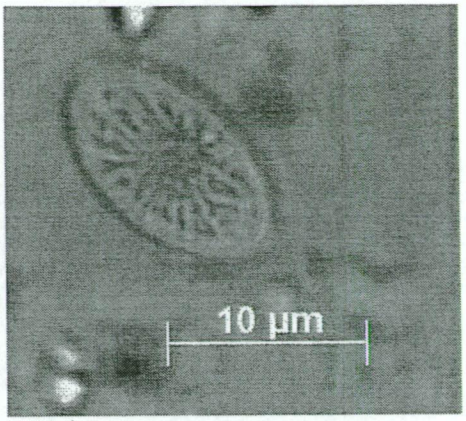

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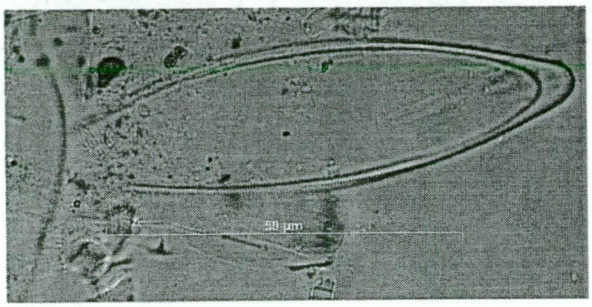

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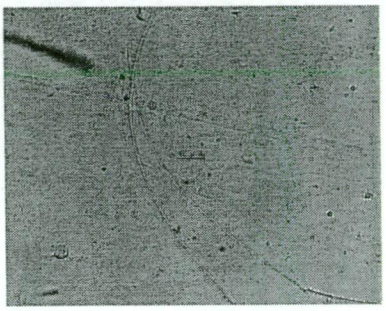

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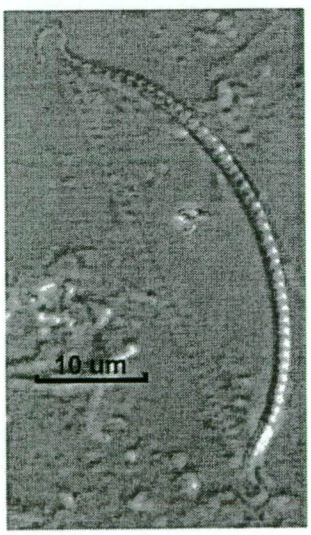

121

Plate 15: Diatom Photos

117. *Trachysphenia australis* Petit var. *australis* 118. Species 1

119. Species 2 120. Species 3 121. Species 4

Appendix 1: Spectrophotometry and Fluorometry Results Tables

Table 1.1: Chlorophyll *a* results for Tinderbox Marine Reserve and Conningham Beach using Spectrophotometry and Fluorometry

Date	Water Depth (m)	Tinderbox Mar. Res. Spectro chl *a* (mg/m2)	Tinderbox Mar. Res. Fluoro chl *a* (mg/m2)	Conningham Beach Spectro chl *a* (mg/m2)	Conningham Beach Fluoro chl *a* (mg/m2)
30/01/01	1	10.78	11.75	23.99	34.51
	1	19.69	16.24	24.53	33.73
	1	12.58	26.63	22.02	29.13
	2	32.27	27.62	31.64	29.09
	2	25.53	26.52	26.88	33.51
	2	25.53	32.04	31.64	34.25
	3	36.23	36.83	45.22	56.27
	3	32.09	44.27	40.00	56.57
	3	31.01	32.41	51.95	49.35
	4	29.67	22.69	27.42	24.42
	4	24.99	30.79	28.05	24.31
	4	21.94	20.70	26.25	23.39
	5	26.79	28.36	27.96	23.05
	5	24.99	22.72	27.96	23.20
	5	30.38	23.57	32.01	26.88
13/02/01	1	13.12	13.44	56.19	82.09
	1	14.30	15.65	36.58	51.82
	1	15.64	14.18	47.93	45.68
	2	30.84	30.93	30.39	35.35
	2	28.59	30.93	39.37	48.78
	2	28.75	33.14	40.00	48.06
	3	20.23	23.05	42.97	38.61
	3	21.40	18.08	34.06	50.27
	3	24.45	20.14	44.68	49.72
	4	33.35	29.46	41.09	49.05
	4	38.12	47.21	31.56	33.00
	4	30.84	32.78	48.99	46.40
	5	29.13	28.36	25.00	25.13
	5	39.46	25.41	28.05	25.85
	5	29.13	39.99	30.92	27.62
27/02/01	1	16.09	20.27	36.03	46.68
	1	17.98	23.04	41.97	52.98
	1	14.84	18.17	41.43	54.91
	2	40.55	40.49	66.51	79.82
	2	38.21	40.60	68.30	108.27
	2	29.85	29.09	59.94	81.98
	3	43.60	48.17	29.30	34.86
	3	44.76	50.49	34.69	44.63
	3	47.73	52.48	29.30	32.98
	4	29.22	30.88	72.90	97.66
	4	33.01	31.82	38.21	38.89
	4	28.60	32.04	47.19	46.40
	5	36.41	39.28	41.80	43.36
	5	33.90	36.96	34.61	37.18
	5	37.50	40.77	41.80	48.34

(*continued.....*)

Table 1.1 *(continued...)*

Date	Water Depth (m)	Tinderbox Mar. Res. Spectro chl *a* (mg/m2)	Tinderbox Mar. Res. Fluoro chl *a* (mg/m2)	Conningham Beach Spectro chl *a* (mg/m2)	Conningham Beach Fluoro chl *a* (mg/m2)
20/03/01	1	15.34	16.34	18.30	14.44
	1	8.71	9.51	no data	no data
	1	8.44	9.86	no data	no data
	2	8.83	9.27	7.94	8.13
	2	10.14	11.21	8.47	7.81
	2	9.40	9.94	5.51	5.41
	3	17.57	20.60	8.67	8.80
	3	13.76	16.34	8.13	7.46
	3	11.45	13.69	11.48	12.63
	4	12.22	13.34	15.06	17.64
	4	15.50	20.68	14.33	17.95
	4	13.99	15.43	19.38	21.86
	5	15.76	16.93	12.52	20.64
	5	12.76	13.57	15.06	18.11
	5	9.40	13.65	9.71	12.00
3/04/01	1	31.21	38.35	53.85	70.79
	1	29.67	37.33	53.38	69.44
	1	32.89	39.85	56.47	71.34
	2	35.90	43.88	34.28	42.06
	2	30.21	33.62	31.28	38.67
	2	39.52	48.61	31.28	35.83
	3	27.12	30.30	61.01	76.78
	3	27.20	30.54	36.91	41.82
	3	23.50	27.78	39.45	46.48
	4	41.99	46.32	44.54	38.98
	4	38.37	47.03	41.06	36.06
	4	45.07	49.08	37.91	39.46
	5	42.00	46.88	22.50	24.94
	5	39.91	49.32	30.21	32.43
	5	44.46	55.87	33.28	46.17
19/04/01	1	37.37	49.64	64.64	77.97
	1	31.20	40.64	51.09	66.29
	1	45.14	55.56	72.34	76.86
	2	40.45	46.56	60.01	46.56
	2	31.35	37.64	40.98	47.51
	2	40.52	49.72	39.44	57.61
	3	34.28	36.77	53.16	61.32
	3	35.83	42.30	49.62	49.87
	3	39.38	42.46	45.92	37.72
	4	55.79	54.53	36.22	67.08
	4	37.69	38.51	76.12	53.74
	4	38.30	38.12	49.62	37.72
	5	59.41	70.23	39.92	34.88
	5	54.71	60.84	38.84	38.27
	5	63.49	67.39	40.85	99.59

Appendix 2: Training Set Species No.s, Names, and Relative Abundance

Table 2.1: Species comprising > 2% relative abundance in Training Set (Chapter 2)

Species No.	Species Name
1	*Achnanthes brevipes* Agardh
2	*Achnanthes residensis* Foged
3	*Achnanthes oblongella* Østrup
4	*Amphora aequalis* Krammer
5	*Amphora decussata* Grunow
6	*Amphora exigua* Gregory
7	*Amphora laevissima* Gregory
8	*Amphora maletractata* var. *constricta* (Heiden) Simonsen
9	*Amphora* sp 1
10	*Amphora submontana* Hustedt
11	*Amphora subturgida* Hustedt
12	*Anaulus minutus* Grunow in Van Heurck
13	*Anorthoneis vortex* Sterrenburg
14	*Bacillaria paradoxa* Gmelin
15	*Cocconeis stauroneiformis* (Van Heurck) Okuno
16	*Catenula adhaerens* Mereschkowsky
17	*Chaetocerus* resting spores
18	*Cocconeis carminata* Cholnoky
19	*Cocconeis disculoides* Hustedt
20	*Cocconeis disrupta* Gregory
21	*Cocconeis molesta* var. *crucifera* Grunow in Van Heurck
22	*Cocconeis peltoides* Hustedt
23	*Cocconeis placentula* Ehrenberg
24	*Cocconeis placentula* Ehrenberg var. *euglypta* (Ehr.) Grunow
25	*Cocconeis scutellum* Ehrenberg
26	*Cocconeis scutellum* var. *parva* Grunow
27	*Cyclotella stelligera* Cleve & Grunow
28	*Cyclotella striata* (Kützing) Grunow
29	*Cymbella minuta* Hilse ex Rabenhorst
30	*Cymbella sumatrensis* Hustedt
31	*Delphineis surirella* (Ehrenberg) G. Andrews
32	*Dimerogramma minor* var. *nana* (Gregory) Van Heurck
33	*Diploneis notabilis* (Greville) Cleve
34	*Diploneis subovalis* Cleve
35	*Diploneis vacillans* (A. Schmidt) Cleve
36	*Ehrenbergia granulosa* (Grunow) Witkowski
37	*Fallacia litoricola* (Hustedt) D. G. Mann
38	*Fallacia subforcipata* (Hustedt) D.G. Mann
39	*Fragilaria atomus* Hustedt
40	*Fragilaria crotonensis* Kitton
41	*Fragilaria martyi* (Heribaud) Lange-Bertalot
42	*Fragilaria pinnata* Ehrenberg
43	*Fragilaria pinnata* Ehrenberg var. *pinnata*
44	*Fragilariopsis cylindrus* (Grunow) Krieger
45	*Gramatophora oceanica* Ehrenberg
46	*Gyrosigma fasciola* (Ehrenberg) Griffith & Henfrey
47	*Gyrosigma perthense* John
48	*Hyalodiscus scoticus* (Kützing) Grun.
49	*Lunella bisecta* Snoeijs
50	*Mastoglia smithii* Thwaites
51	*Mastogloia pusilla* (Grunow) Cleve var. *pusilla*
52	*Matsogloia* sp 1
53	*Melosira nummuloides* Agardh
54	*Navicula arenaria* Donken var. *rostellata* Lange-Bertalot

(*continued.....*)

Table 2.1 (*cont.*): Species comprising > 2% relative abundance in Training Set (Chapter 2)

Species No.	Species Name
55	*Navicula cancellata* Donkin
56	*Navicula cryptocephala* Kützing
57	*Navicula menisculus* Schumann
58	*Navicula monoculata* var. *omissa* (Hustedt) Lange-Bertalot
59	*Navicula nyella* Hustedt
60	*Navicula pygmaea* Kützing
61	*Navicula salinarum* Grunow var. *salinarum*
62	*Navicula* sp 1
63	*Navicula* sp 2
64	*Navicula tripunctata* (O. F. Muller) Borg.
65	*Nitzschia amphibia* Grunow
66	*Nitzschia dissipata* (Kützing) Grunow var. *dissipata*
67	*Nitzschia laevis* Hustedt
68	*Nitzschia longissima* (Brébisson) Ralfs
69	*Nitzschia lorenziana* Grunow var. *subtilis* Grunow
70	*Nitzschia ovalis* Arnott ex Grunow in Cleve & Grunow
71	*Nitzschia panduriformis* Gregory var. *minor* Gregory
72	*Nitzschia* sp 1
73	*Nitzschia* sp2
74	*Opephora martyi* Heribaud
75	*Opephora olsenii* Möller
76	*Paralia sulcata* (Ehrenberg) Cleve
77	*Parlibellus* cf. *plicatus* (Donkin) Cox
78	*Plagiogramma staurophorum* (Gregory) Heiberg
79	*Plagiotropis* sp 2
80	*Plagiotropis* sp1
81	*Planothidium delicatulum* (Kützing) Round & Buktiyarova
82	*Pseudonitzschia australis* Frenguelli
83	*Skeletonema costatum* (Greville) Cleve
84	*Surirella fastuosa* (Ehrenberg) Kützing
85	*Cocconeis* aff. *pinnata* Gregory ex. Greville
86	*Synedra investiens* W. Smith
87	*Synedra tabulata* (Agardh) Kützing var. *tabulata*
88	*Thalassionema nitzschoides* Hustedt
89	*Thalassiosira eccentrica* (Ehrenberg) Cleve
90	*Thalassiosira oestrupii* (Ostenfeld) Hasle
91	*Achnanthes* sp 1
92	Spp 1
93	*Navicula halophila* (Grunow) Cleve fo. *robusta* Hustedt
94	*Diploneis* sp 1
95	*Cocconeis* sp 1
96	*Navicula* sp 3
97	Spp 2
98	*Fragilaria* sp 1
99	*Cymatosira* aff. *belgica* Grunow
100	Spp 3
101	*Navicula cincta* (Ehrenberg) Ralfs in Pritchard
102	Spp 4
103	Spp 6
104	Spp 7
105	Species 5
106	Spp 8
107	Spp 9
108	Spp 10
109	Spp 11
110	*Diploneis* sp 2
111	*Fragilaria vaucheriae* Kützing Petersen

Appendix 2 (Cont...)

Table 2.2: Relative abundance (%) of Training Set Species

Species no...→	1	2	3	4	5	6	7	8
Site 1	0.00	4.87	0.00	0.00	0.00	0.00	0.00	0.00
Site 2	0.00	1.99	0.00	0.00	0.00	0.00	0.00	0.00
Site 3	0.00	0.00	0.00	0.00	0.00	0.00	0.47	0.00
Site 4	0.00	0.00	0.00	0.00	0.00	0.00	1.86	0.00
Site 5	0.00	2.73	0.91	0.00	0.00	0.00	0.00	0.00
Site 6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 8	0.00	2.43	0.00	0.00	0.00	0.00	0.00	0.00
Site 9	0.00	1.99	0.20	0.00	0.00	0.00	0.00	0.00
Site 10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.49
Site 11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 12	0.00	0.80	0.00	0.00	0.00	0.00	7.21	0.00
Site 13	0.00	2.08	0.00	0.00	0.69	0.00	0.00	0.00
Site 14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 15	0.00	0.00	0.00	0.00	4.09	10.82	43.75	0.00
Site 16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 17	0.00	2.99	0.00	0.00	0.00	0.00	0.00	0.00
Site 18	0.00	0.00	0.00	3.04	0.00	0.00	0.00	0.00
Site 19	0.00	0.00	0.00	0.94	0.00	0.00	0.75	0.00
Site 20	0.00	0.00	0.00	0.93	0.00	0.00	1.86	0.00
Site 21	0.00	0.00	0.00	2.95	0.00	0.00	0.00	0.00
Site 22	0.00	0.00	0.00	0.00	0.00	5.98	0.00	0.00
Site 23	0.00	2.22	0.00	0.00	0.00	1.33	0.00	0.00
Site 24	0.00	0.48	0.00	0.00	0.00	0.00	11.00	0.96
Site 25	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.00
Site 26	0.00	0.00	0.00	0.00	1.17	0.00	24.46	0.00
Site 27	0.00	0.19	0.00	0.00	0.00	0.00	0.37	2.59
Site 28	0.00	0.76	0.00	0.00	0.57	0.00	12.76	0.38
Site 29	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00
Site 30	5.25	0.25	2.00	0.00	0.00	0.00	0.00	5.00
Site 31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 32	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00
Site 33	0.00	0.74	0.00	0.00	0.00	0.00	0.50	5.46
Site 34	0.00	0.48	0.00	0.00	0.00	0.00	0.00	0.00
Site 35	0.00	0.25	0.00	0.00	0.00	0.00	0.50	1.50
Site 36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98
Site 38	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 39	0.00	0.00	0.00	1.73	0.00	0.00	0.43	1.73
Site 40	0.00	1.45	0.00	0.00	0.00	0.00	0.00	0.00
Site 41	0.00	3.64	0.00	0.00	0.00	0.00	0.00	0.00
Site 42	0.00	0.00	0.00	0.00	0.00	2.05	0.68	2.05
Site 43	0.00	1.03	0.00	0.00	0.00	0.00	2.06	7.56
Site 44	0.00	5.29	0.00	0.00	0.00	0.00	0.00	0.00
Site 45	0.00	1.23	0.00	0.00	0.00	0.00	1.48	3.69
Site 46	0.00	0.00	0.00	0.91	0.00	0.00	0.00	0.00
Site 47	0.00	0.46	0.00	8.35	0.00	0.00	0.23	0.00
Site 48	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00
Site 49	0.00	2.04	0.00	6.33	0.00	0.00	0.00	0.00
Site 50	0.00	0.48	0.00	2.40	0.00	0.00	0.48	0.00
Site 51	0.00	3.17	0.00	3.62	0.00	0.00	0.00	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...→	9	10	11	12	13	14	15	16
Site 1	0.00	0.00	4.64	0.00	0.00	0.93	0.00	3.25
Site 2	0.00	0.00	0.00	0.25	0.00	0.50	0.25	0.75
Site 3	0.00	0.00	2.33	0.00	0.00	0.00	0.00	0.23
Site 4	0.00	0.00	1.44	0.00	0.00	1.65	0.00	0.00
Site 5	0.00	0.00	0.00	0.00	0.00	1.82	0.68	0.91
Site 6	0.00	0.00	11.29	0.00	0.00	0.68	2.48	0.90
Site 7	0.00	0.00	10.20	0.00	0.00	0.00	0.00	0.00
Site 8	0.00	0.00	7.04	0.97	0.00	2.18	1.70	0.00
Site 9	0.00	0.00	5.77	0.99	0.00	0.80	4.97	1.59
Site 10	0.00	0.00	11.58	0.00	0.00	1.97	0.00	6.16
Site 11	0.00	0.00	3.20	1.97	0.00	1.48	0.00	0.00
Site 12	0.00	0.00	0.60	0.00	0.00	0.00	0.00	1.60
Site 13	0.00	0.00	4.17	0.00	0.00	2.31	0.46	2.31
Site 14	0.00	0.00	19.34	0.00	0.00	0.00	0.00	0.66
Site 15	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.48
Site 16	0.00	0.00	2.86	0.00	0.44	1.98	0.00	2.42
Site 17	0.00	0.00	6.79	5.99	0.20	1.40	0.00	3.59
Site 18	0.00	0.00	1.17	0.00	0.00	0.47	0.00	1.87
Site 19	12.38	0.00	2.44	1.50	0.00	2.25	0.00	1.50
Site 20	1.40	0.00	1.17	0.93	0.00	0.70	0.00	6.06
Site 21	0.00	0.00	0.84	0.63	0.00	0.42	0.00	16.46
Site 22	0.00	0.00	0.00	3.28	0.00	0.00	0.00	2.32
Site 23	0.00	0.00	0.67	2.44	0.89	0.00	0.00	1.33
Site 24	0.00	0.00	1.67	0.96	0.00	0.48	0.00	2.15
Site 25	0.00	0.00	6.82	0.00	0.00	2.05	0.91	1.82
Site 26	0.00	0.00	0.00	10.37	0.00	0.00	0.00	0.00
Site 27	0.00	0.00	4.63	0.00	0.00	0.37	0.37	0.00
Site 28	0.00	0.00	8.95	0.95	0.19	0.00	0.76	0.00
Site 29	0.00	0.00	0.00	0.00	0.00	0.00	1.42	0.94
Site 30	0.00	0.00	8.25	0.00	0.00	0.25	3.50	1.75
Site 31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.66
Site 32	0.00	0.00	7.34	0.00	0.00	9.40	0.92	0.92
Site 33	0.00	0.00	3.97	0.00	0.00	4.22	0.00	1.99
Site 34	0.00	0.00	23.68	0.00	0.00	3.83	0.00	0.24
Site 35	0.00	0.00	7.25	0.00	0.00	3.25	0.00	0.00
Site 36	0.00	0.00	16.82	0.00	0.00	2.13	0.00	0.00
Site 37	0.00	0.00	1.23	0.00	0.00	3.44	4.18	0.25
Site 38	0.00	0.00	6.73	0.00	0.00	3.99	0.00	1.00
Site 39	0.00	0.00	1.30	0.22	0.00	2.60	0.00	0.00
Site 40	0.00	0.00	0.00	5.30	2.65	0.00	0.00	3.61
Site 41	0.00	2.27	5.45	1.82	0.00	0.00	0.00	0.68
Site 42	0.00	0.00	6.16	0.00	0.00	1.83	0.00	0.00
Site 43	0.00	0.00	10.31	0.17	0.34	1.37	0.00	0.52
Site 44	0.00	0.00	5.92	0.00	0.42	0.21	0.00	0.21
Site 45	0.00	0.00	0.74	0.99	1.48	0.49	2.46	0.00
Site 46	0.00	0.00	0.00	1.59	0.00	0.00	0.00	2.05
Site 47	0.00	0.00	0.93	0.00	0.00	0.00	0.00	1.16
Site 48	0.00	0.00	3.47	4.40	1.39	0.00	0.00	1.62
Site 49	0.00	0.00	0.45	3.39	6.56	0.45	0.00	2.26
Site 50	0.00	0.00	4.80	0.72	0.00	0.00	0.00	3.84
Site 51	0.00	0.00	0.90	0.00	0.23	0.00	0.00	2.04

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...→	17	18	19	20	21	22	23	24
Site 1	0.00	10.90	5.57	0.46	0.00	2.32	0.93	8.58
Site 2	0.00	0.00	10.95	0.25	0.00	0.00	0.75	2.99
Site 3	0.00	0.00	2.33	0.00	0.00	0.00	0.23	0.70
Site 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 5	0.45	0.00	0.00	0.00	0.00	0.00	0.00	1.82
Site 6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90
Site 7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 8	1.70	0.00	2.67	0.00	0.00	0.00	0.00	0.00
Site 9	8.95	0.00	0.20	0.00	0.00	0.00	0.00	0.00
Site 10	0.25	0.00	2.22	0.00	0.00	0.00	0.00	1.97
Site 11	0.00	0.00	0.49	0.00	0.00	0.00	0.00	0.99
Site 12	0.00	0.00	3.21	2.81	0.00	2.81	0.00	0.00
Site 13	0.00	0.00	0.46	0.00	0.00	1.62	0.00	0.46
Site 14	0.00	0.00	1.98	0.00	0.00	0.00	0.00	0.00
Site 15	0.00	0.00	1.20	0.00	0.00	0.00	0.72	0.00
Site 16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 17	0.00	0.00	0.00	1.00	0.00	1.20	0.60	0.00
Site 18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 21	0.00	0.00	0.00	0.00	0.00	0.00	0.84	0.00
Site 22	0.00	0.00	0.00	0.19	0.00	0.00	3.47	2.70
Site 23	0.00	0.00	0.00	0.00	0.00	2.66	0.00	0.00
Site 24	0.96	0.00	2.39	0.00	0.00	1.67	0.96	0.00
Site 25	2.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 26	0.00	0.00	0.39	0.00	0.00	0.00	0.00	0.00
Site 27	0.00	0.00	0.19	0.19	0.00	4.81	0.37	0.00
Site 28	0.00	0.00	0.19	0.38	0.00	0.76	0.00	0.00
Site 29	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00
Site 30	0.00	0.00	0.00	0.00	1.25	0.00	0.00	0.50
Site 31	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00
Site 32	1.38	0.00	0.00	0.92	0.92	0.23	0.00	0.00
Site 33	0.00	0.00	1.24	0.00	1.99	0.00	0.74	4.96
Site 34	0.00	0.00	2.39	0.00	1.44	0.00	0.00	0.00
Site 35	0.00	0.00	0.75	0.00	2.00	0.00	0.50	2.25
Site 36	0.00	0.00	9.95	0.00	0.00	0.00	0.00	0.00
Site 37	1.72	0.00	0.00	0.00	0.00	0.74	0.25	0.00
Site 38	0.00	0.00	2.24	0.00	2.00	0.00	1.00	1.00
Site 39	0.43	0.00	4.11	0.00	1.08	4.55	0.22	0.87
Site 40	0.00	0.00	6.02	0.00	0.00	0.00	1.20	0.00
Site 41	0.00	0.00	6.36	0.68	0.00	4.32	0.00	0.00
Site 42	0.00	0.00	2.28	0.00	0.00	1.14	0.00	0.00
Site 43	0.00	0.00	1.72	0.86	0.00	0.52	0.00	0.00
Site 44	0.00	0.00	3.38	3.38	0.00	1.27	0.00	0.00
Site 45	0.00	0.00	4.93	0.00	0.00	2.46	0.00	0.00
Site 46	0.00	0.00	6.38	0.23	0.00	0.00	0.00	0.00
Site 47	0.00	0.00	7.19	0.00	0.00	0.23	0.00	0.46
Site 48	0.00	0.00	3.70	0.23	0.00	0.46	0.00	1.85
Site 49	0.00	0.00	4.07	0.23	0.00	1.36	0.00	0.90
Site 50	0.00	0.00	7.19	0.00	0.00	0.48	0.00	2.40
Site 51	0.00	0.00	6.33	0.00	0.00	0.68	0.00	5.20

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	25	26	27	28	29	30	31	32
Site 1	0.00	5.80	2.32	1.86	0.23	0.00	0.00	0.00
Site 2	1.49	0.00	6.22	22.89	1.74	0.00	0.00	0.00
Site 3	0.00	0.00	2.33	1.16	0.00	0.00	0.23	0.00
Site 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 5	0.91	2.27	1.14	22.50	0.45	0.68	0.00	2.27
Site 6	4.06	0.00	0.00	3.84	0.00	0.00	0.00	0.00
Site 7	0.00	1.22	0.61	0.41	0.00	0.00	0.00	0.00
Site 8	0.24	0.00	0.00	0.24	0.00	0.00	0.00	0.00
Site 9	4.77	0.00	0.40	0.60	0.00	0.20	0.00	0.00
Site 10	2.22	0.00	0.74	0.00	0.49	0.00	0.00	0.00
Site 11	1.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 13	0.00	0.00	0.46	0.69	0.00	0.23	0.00	0.00
Site 14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 15	0.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 18	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00
Site 19	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00
Site 20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 22	1.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 23	1.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 24	0.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 25	3.41	0.00	0.00	0.45	0.00	0.00	0.00	0.00
Site 26	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00
Site 27	1.30	0.00	0.00	0.00	1.67	0.74	0.00	0.00
Site 28	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 30	2.25	0.00	0.00	0.00	2.50	2.00	0.00	0.00
Site 31	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.22
Site 32	3.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 33	6.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 34	3.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 35	6.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 36	1.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 37	4.67	0.00	0.00	0.00	0.00	0.00	0.00	0.74
Site 38	2.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 39	11.90	0.00	0.00	0.00	0.00	0.00	0.00	0.22
Site 40	7.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 41	0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 42	20.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 43	3.09	0.00	0.00	0.00	0.34	0.00	5.15	0.17
Site 44	1.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 45	2.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 46	11.16	0.00	0.00	0.00	0.00	0.00	6.83	0.00
Site 47	0.46	0.00	0.00	0.00	0.00	0.00	0.70	0.00
Site 48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23
Site 49	0.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	33	34	35	36	37	38	39	40
Site 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 3	0.00	0.93	0.00	0.00	3.26	0.00	0.00	0.47
Site 4	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 5	0.00	5.68	0.00	0.00	11.14	0.00	0.00	0.00
Site 6	0.00	0.00	0.00	0.00	19.19	0.00	0.00	1.13
Site 7	0.61	0.00	0.00	0.00	0.41	0.00	0.00	0.00
Site 8	0.00	3.88	0.00	0.00	6.07	0.97	0.00	1.70
Site 9	0.00	0.00	0.00	0.00	6.96	0.00	0.00	0.80
Site 10	0.00	0.00	0.00	0.00	4.68	0.00	0.00	0.00
Site 11	0.00	0.00	1.23	0.00	0.74	0.00	0.00	0.00
Site 12	6.61	0.00	4.01	0.00	9.22	6.61	0.00	0.00
Site 13	0.00	0.00	1.39	0.00	0.00	0.00	0.00	0.00
Site 14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 16	0.00	0.00	0.00	0.00	0.00	0.66	0.00	1.54
Site 17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 21	0.00	0.00	0.00	0.00	0.21	0.42	0.00	0.00
Site 22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 23	0.00	0.00	0.00	0.00	0.89	0.00	0.00	0.00
Site 24	0.48	0.00	3.35	0.00	4.31	2.63	0.24	0.00
Site 25	0.00	0.00	0.45	0.68	4.55	0.00	0.00	1.59
Site 26	0.00	0.39	0.00	0.00	0.00	0.00	0.00	0.00
Site 27	0.00	0.00	1.11	0.74	0.37	0.74	0.00	0.00
Site 28	0.38	0.95	1.33	0.00	0.19	0.00	0.00	0.00
Site 29	0.00	0.00	0.71	0.47	0.00	0.00	0.00	0.00
Site 30	0.00	0.00	0.50	0.00	6.50	1.00	0.00	0.00
Site 31	0.00	0.00	0.00	21.71	0.00	4.61	0.00	0.00
Site 32	0.00	0.00	0.00	0.69	0.46	0.00	0.00	3.67
Site 33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 35	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00
Site 36	0.00	0.00	1.18	0.00	0.00	0.00	0.00	0.00
Site 37	0.49	0.98	10.32	2.70	1.47	1.23	0.00	0.00
Site 38	0.00	0.75	0.00	0.50	0.00	0.00	0.00	0.00
Site 39	0.00	0.00	0.43	0.00	1.73	0.87	0.00	0.00
Site 40	0.00	0.00	0.00	0.72	0.00	2.89	0.00	0.00
Site 41	0.00	0.00	0.68	1.82	1.59	0.00	2.50	0.00
Site 42	0.00	0.00	0.00	0.00	1.14	0.00	0.00	0.00
Site 43	0.00	0.00	3.95	20.96	0.00	0.34	0.00	0.00
Site 44	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00
Site 45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.49
Site 46	0.00	0.00	0.00	0.91	0.00	0.00	0.00	0.00
Site 47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 50	0.00	0.00	0.00	0.00	0.00	0.48	0.00	0.00
Site 51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	41	42	43	44	45	46	47	48
Site 1	0.00	0.00	0.00	0.70	0.93	0.00	0.23	0.00
Site 2	0.00	0.00	0.75	0.50	0.00	0.00	0.00	0.00
Site 3	0.00	0.00	16.74	0.70	0.47	0.00	0.00	0.00
Site 4	0.00	0.00	0.41	0.00	0.00	0.00	0.00	0.00
Site 5	0.00	0.00	0.00	1.59	0.68	0.00	0.00	0.23
Site 6	0.00	0.68	0.00	0.68	0.00	0.00	0.00	0.00
Site 7	0.00	0.00	0.00	0.41	0.00	0.00	0.00	0.00
Site 8	0.00	0.00	0.24	0.00	0.49	0.00	0.00	1.21
Site 9	0.00	0.00	0.00	0.00	1.99	0.00	0.00	1.99
Site 10	0.00	0.00	0.00	0.00	3.94	0.00	0.00	0.74
Site 11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 13	0.00	1.62	0.00	0.00	0.23	0.00	0.00	0.46
Site 14	1.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 15	0.00	2.88	0.00	0.72	0.00	1.92	0.00	0.00
Site 16	2.42	3.08	0.44	0.00	0.00	0.00	0.00	0.00
Site 17	0.00	3.79	0.00	0.80	0.00	0.00	0.00	0.00
Site 18	0.00	3.74	0.00	1.64	0.00	0.00	0.00	0.00
Site 19	2.81	6.94	0.56	0.00	0.00	0.00	0.00	0.00
Site 20	0.00	4.20	0.47	1.86	0.00	0.00	0.00	0.00
Site 21	0.00	0.84	0.00	9.92	1.48	0.00	0.00	0.00
Site 22	0.00	1.54	0.00	0.00	0.00	0.19	0.00	0.00
Site 23	2.66	4.88	0.00	0.00	0.00	0.00	0.00	0.00
Site 24	0.00	0.00	0.00	0.00	0.96	0.00	0.00	0.00
Site 25	0.00	0.45	0.00	0.45	2.73	0.00	0.00	3.18
Site 26	4.31	4.31	0.00	0.00	0.00	5.87	0.00	0.00
Site 27	3.52	2.59	0.00	0.56	0.19	0.00	0.00	0.00
Site 28	0.00	1.90	0.00	0.00	0.00	0.19	0.00	0.00
Site 29	0.94	24.06	1.65	0.00	0.00	0.00	0.00	0.00
Site 30	0.00	0.00	0.00	1.25	1.25	0.00	0.00	0.00
Site 31	0.00	12.94	1.75	0.00	0.00	0.00	0.00	0.00
Site 32	0.00	0.46	0.00	0.00	0.92	0.92	2.06	0.00
Site 33	0.00	0.00	0.00	0.50	0.25	0.00	0.00	0.00
Site 34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 35	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00
Site 36	0.00	0.00	0.00	0.00	3.32	0.00	0.00	0.00
Site 37	0.00	0.00	0.00	0.00	6.63	0.00	0.00	0.00
Site 38	0.00	0.00	0.00	0.00	1.25	0.25	1.25	0.00
Site 39	0.87	2.81	0.00	0.00	1.95	0.00	0.00	0.00
Site 40	0.00	0.00	0.00	0.00	0.72	0.00	0.00	0.48
Site 41	0.00	3.18	0.00	0.23	0.00	0.00	0.00	0.45
Site 42	0.00	4.57	0.00	0.00	5.02	0.00	0.00	2.97
Site 43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 44	0.00	0.85	0.00	0.00	1.69	0.21	0.42	0.00
Site 45	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00
Site 46	1.37	12.30	0.00	3.64	0.23	0.00	0.00	0.00
Site 47	5.34	13.92	0.23	0.46	0.00	0.00	0.00	0.00
Site 48	0.00	6.71	0.00	0.00	0.00	0.00	0.00	0.00
Site 49	0.00	2.26	0.00	0.00	0.00	0.00	0.00	0.00
Site 50	0.00	3.36	1.20	0.00	0.24	0.00	0.00	0.00
Site 51	0.00	0.23	0.00	0.23	0.00	0.00	0.00	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	49	50	51	52	53	54	55	56
Site 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 3	0.00	0.00	0.00	0.00	0.00	0.47	0.00	0.00
Site 4	0.00	0.00	0.00	0.00	3.71	1.44	0.00	0.00
Site 5	0.00	0.00	0.00	0.00	0.68	0.00	0.00	0.00
Site 6	0.00	0.00	0.00	0.00	2.71	0.00	0.00	0.00
Site 7	0.00	0.00	0.00	0.00	2.04	0.00	0.00	0.00
Site 8	0.00	0.00	0.00	4.85	0.24	0.97	0.00	0.00
Site 9	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00
Site 10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 11	0.00	0.00	0.25	0.00	0.00	0.25	0.00	0.00
Site 12	0.00	0.00	0.00	5.41	0.00	0.00	0.00	0.00
Site 13	0.46	0.00	0.00	0.00	0.00	12.50	6.94	0.00
Site 14	0.00	0.00	0.00	0.00	0.00	0.00	0.88	0.00
Site 15	0.00	0.00	0.00	0.00	0.00	0.72	1.20	0.00
Site 16	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.00
Site 17	0.00	0.00	0.00	0.40	0.00	0.80	0.00	0.00
Site 18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 20	0.00	0.00	0.00	0.70	0.00	2.80	0.00	0.00
Site 21	0.00	0.00	0.63	0.00	0.00	0.00	2.53	0.00
Site 22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 23	11.09	0.00	0.89	1.33	0.00	0.67	0.00	0.00
Site 24	2.63	0.00	0.00	0.00	0.00	0.00	6.46	3.83
Site 25	0.00	0.00	0.00	0.00	0.00	1.82	0.23	0.00
Site 26	7.83	0.00	0.00	0.00	0.00	0.00	5.48	0.00
Site 27	0.00	0.00	1.11	0.00	0.00	1.11	0.00	0.00
Site 28	0.57	0.00	2.10	1.14	0.00	0.00	3.43	0.00
Site 29	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00
Site 30	0.00	0.00	3.50	0.00	0.25	0.00	0.00	0.00
Site 31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 32	0.00	0.00	0.46	0.69	0.00	4.13	0.00	0.00
Site 33	0.00	0.00	0.00	0.00	0.50	2.73	1.74	0.00
Site 34	0.00	0.00	1.91	0.00	0.00	1.20	1.44	0.00
Site 35	0.00	0.00	1.25	0.50	1.00	4.50	0.00	0.00
Site 36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 37	0.00	2.95	0.00	4.91	0.00	0.00	0.00	0.00
Site 38	0.00	0.00	0.00	2.00	0.00	0.25	1.25	0.00
Site 39	0.00	0.00	0.00	1.08	0.00	0.43	2.81	0.00
Site 40	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00
Site 41	0.00	0.00	0.68	0.68	0.00	1.82	0.00	0.00
Site 42	0.00	0.00	0.00	0.23	0.00	0.91	7.08	0.23
Site 43	0.00	0.00	0.00	1.89	0.00	0.00	0.69	0.00
Site 44	0.00	0.00	0.85	0.00	0.00	0.21	3.59	0.00
Site 45	0.00	0.00	0.74	0.74	0.00	0.00	3.69	0.00
Site 46	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00
Site 47	0.00	0.00	0.00	0.00	0.00	0.00	0.70	0.00
Site 48	0.00	0.00	0.00	0.69	0.00	0.23	0.69	0.00
Site 49	0.00	0.00	0.00	4.07	0.00	0.00	0.45	0.00
Site 50	0.00	0.00	0.00	1.20	0.00	0.00	1.20	0.00
Site 51	0.00	0.00	0.00	0.00	0.00	1.36	2.04	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	57	58	59	60	61	62	63	64
Site 1	0.00	2.78	0.00	0.00	0.00	0.23	0.00	0.00
Site 2	0.00	3.23	0.00	0.00	0.00	0.00	0.00	0.00
Site 3	0.00	1.40	0.00	0.00	0.00	0.00	0.00	0.00
Site 4	0.00	2.68	0.00	0.00	0.00	0.62	0.00	0.00
Site 5	0.00	0.91	1.36	0.00	0.91	0.00	0.00	0.00
Site 6	0.00	7.45	0.00	0.90	0.00	0.00	0.00	0.00
Site 7	0.00	0.82	0.00	0.00	0.00	0.00	0.00	0.00
Site 8	0.00	2.43	0.00	0.00	0.00	0.00	0.00	0.00
Site 9	0.00	1.59	0.00	0.00	0.00	0.00	0.00	0.00
Site 10	0.00	3.69	6.16	3.69	0.00	0.00	0.00	0.00
Site 11	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00
Site 12	0.00	23.05	0.00	0.00	0.20	0.00	0.00	0.00
Site 13	0.00	12.04	0.00	0.00	2.31	0.00	0.00	1.39
Site 14	0.00	5.93	0.00	0.00	0.00	0.00	1.10	0.22
Site 15	0.00	9.62	0.00	0.00	0.00	2.64	0.00	0.00
Site 16	0.00	3.52	0.00	0.00	0.00	1.10	2.64	0.00
Site 17	0.00	23.15	0.00	0.00	0.00	3.19	0.00	0.00
Site 18	0.00	1.40	0.00	0.00	0.00	1.40	0.00	0.00
Site 19	0.00	7.32	0.00	0.00	0.00	0.75	0.00	0.00
Site 20	0.00	11.89	0.00	0.00	0.00	1.63	0.00	0.00
Site 21	0.00	6.96	0.00	0.00	0.00	0.00	0.00	0.00
Site 22	0.00	34.36	0.00	0.00	5.79	12.36	0.00	0.00
Site 23	0.00	38.58	0.00	0.00	0.00	0.00	0.00	0.00
Site 24	0.00	27.75	0.48	0.00	2.63	0.00	0.00	0.72
Site 25	0.00	7.95	0.00	0.00	0.00	0.00	0.00	0.45
Site 26	0.00	11.35	0.00	0.00	0.00	0.00	0.00	0.00
Site 27	0.00	15.19	0.00	0.00	0.00	0.00	0.00	0.00
Site 28	0.00	24.19	0.00	0.19	0.00	0.38	0.00	0.76
Site 29	0.00	16.75	2.36	0.00	0.00	0.00	0.00	0.00
Site 30	7.75	5.00	0.00	0.00	0.25	0.50	0.00	3.25
Site 31	0.00	14.47	0.00	0.00	0.00	0.00	0.00	0.00
Site 32	0.00	10.78	0.00	2.29	0.00	0.00	0.00	6.65
Site 33	0.25	15.14	0.00	0.50	0.00	0.00	0.00	0.50
Site 34	2.15	7.89	0.48	1.44	0.48	0.00	0.00	0.00
Site 35	0.25	2.50	0.00	0.00	0.00	0.00	0.00	0.00
Site 36	0.00	7.58	0.00	0.00	0.00	1.66	0.00	0.00
Site 37	5.65	3.44	0.00	0.00	0.49	2.70	0.00	0.00
Site 38	0.75	14.96	0.00	0.00	0.50	0.00	0.00	6.23
Site 39	0.00	17.32	0.00	0.00	0.00	0.43	0.00	0.00
Site 40	0.00	27.71	0.00	0.00	0.48	0.00	0.00	0.00
Site 41	0.00	32.73	0.00	0.00	0.00	0.00	0.00	0.91
Site 42	0.00	21.00	0.00	0.00	5.02	0.91	0.00	0.23
Site 43	0.00	9.79	0.00	0.00	0.52	0.00	0.00	3.61
Site 44	0.00	31.08	0.00	0.00	0.00	0.00	0.00	1.69
Site 45	0.00	56.16	4.43	0.00	0.00	0.00	0.00	0.25
Site 46	0.00	10.02	0.00	0.00	0.00	0.68	0.00	0.00
Site 47	0.00	14.62	0.00	0.00	0.00	1.39	0.00	0.46
Site 48	0.00	52.55	0.00	0.00	0.46	0.00	0.00	0.00
Site 49	0.00	14.48	0.00	0.00	0.00	0.00	0.00	0.00
Site 50	0.00	7.19	0.00	0.00	0.00	0.00	0.00	0.00
Site 51	0.00	30.32	0.00	0.00	0.00	0.68	0.00	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	65	66	67	68	69	70	71	72
Site 1	2.32	0.00	0.00	0.00	0.93	0.00	0.70	0.00
Site 2	3.48	0.00	0.00	0.00	0.00	0.00	1.74	0.00
Site 3	6.74	0.00	0.00	0.00	0.00	0.70	0.70	0.00
Site 4	2.27	0.62	0.00	0.00	0.00	0.00	0.00	6.19
Site 5	5.23	0.00	0.00	0.00	0.00	0.00	3.64	0.00
Site 6	4.97	0.00	0.00	0.00	0.00	0.00	1.58	0.00
Site 7	3.67	0.00	0.00	0.00	0.00	0.00	0.00	3.88
Site 8	21.60	0.00	0.00	0.00	0.00	0.00	1.70	0.00
Site 9	16.30	0.00	0.00	0.00	0.00	0.00	0.40	0.00
Site 10	23.89	0.00	0.00	0.00	0.00	0.00	2.96	0.00
Site 11	7.64	0.00	0.00	0.00	0.49	0.00	0.25	0.49
Site 12	0.40	0.80	1.00	0.00	0.00	0.00	0.80	0.00
Site 13	7.87	0.00	0.93	0.00	1.85	0.46	2.31	0.00
Site 14	14.73	0.00	0.44	0.00	0.00	0.00	0.88	1.32
Site 15	0.72	0.00	0.24	0.00	0.24	0.00	0.48	0.00
Site 16	3.30	0.00	0.66	0.00	0.44	0.00	0.44	3.08
Site 17	15.97	0.00	0.00	0.00	0.40	0.00	1.40	1.20
Site 18	6.07	0.00	4.44	0.00	1.17	0.00	0.47	0.00
Site 19	11.44	0.00	0.00	0.00	3.75	0.00	0.38	1.31
Site 20	6.99	0.00	0.23	0.00	0.00	0.00	1.17	0.00
Site 21	11.39	0.00	0.00	0.00	0.42	0.00	2.95	0.00
Site 22	1.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 23	1.77	0.00	1.77	0.00	0.00	0.00	1.11	0.00
Site 24	0.00	0.00	1.44	0.00	0.48	0.00	0.48	0.00
Site 25	6.82	0.00	0.00	0.00	0.00	0.00	2.73	0.00
Site 26	6.85	0.00	0.00	0.00	1.76	0.00	2.15	0.00
Site 27	26.30	0.00	0.74	0.00	0.00	0.00	0.00	0.56
Site 28	0.00	1.33	0.00	0.00	8.00	0.00	1.52	0.00
Site 29	2.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 30	2.50	0.00	0.25	0.00	0.25	0.00	2.00	1.00
Site 31	3.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 32	7.80	0.00	0.00	0.00	1.15	0.00	0.92	2.06
Site 33	7.69	0.00	0.00	0.00	0.00	2.23	0.50	1.24
Site 34	24.88	0.00	0.96	0.00	0.00	0.00	0.96	0.00
Site 35	2.75	0.00	0.00	0.00	0.00	1.75	3.75	0.00
Site 36	32.23	0.00	1.42	0.00	0.71	0.00	0.47	0.00
Site 37	1.47	0.00	0.00	0.00	1.72	0.00	1.97	0.25
Site 38	13.22	0.00	0.00	0.00	0.75	0.00	0.00	1.25
Site 39	8.87	0.00	0.00	0.00	0.00	0.00	0.87	0.00
Site 40	5.30	0.00	0.00	0.00	0.00	0.00	0.48	0.00
Site 41	12.05	0.00	0.00	0.00	0.00	0.00	0.68	0.68
Site 42	0.46	0.00	0.00	0.00	0.00	0.00	0.23	0.00
Site 43	4.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 44	15.22	0.42	0.00	0.00	0.00	0.00	0.00	1.69
Site 45	0.25	0.00	0.00	0.00	0.00	0.00	0.49	0.25
Site 46	3.19	0.00	0.00	6.83	0.00	0.00	1.59	0.00
Site 47	6.73	0.00	0.00	0.00	0.23	0.00	0.23	0.00
Site 48	4.40	0.00	0.00	0.00	0.00	0.69	0.00	0.00
Site 49	7.24	0.00	0.00	0.00	2.94	0.00	0.45	0.00
Site 50	1.44	0.00	0.96	0.00	1.92	0.00	0.24	0.96
Site 51	1.81	0.00	0.00	0.00	0.90	0.00	0.00	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	73	74	75	76	77	78	79	80
Site 1	0.00	0.00	4.87	2.32	0.00	0.00	0.00	0.00
Site 2	1.00	0.00	3.23	1.49	0.00	1.00	0.00	0.00
Site 3	0.23	3.49	21.86	0.00	0.00	0.00	0.00	0.00
Site 4	0.00	0.00	0.82	0.00	0.00	0.00	0.00	0.00
Site 5	0.00	0.00	3.86	3.64	0.00	2.27	0.00	0.00
Site 6	0.00	0.00	0.90	0.00	0.00	0.00	0.00	0.00
Site 7	0.00	0.00	3.88	0.00	1.43	0.00	0.00	0.00
Site 8	0.49	0.00	11.65	0.00	0.00	0.49	0.00	0.00
Site 9	0.00	0.00	0.40	1.59	0.00	0.80	0.00	0.00
Site 10	0.00	0.00	2.96	0.74	0.00	0.00	0.00	0.00
Site 11	1.97	0.00	0.00	0.00	0.00	0.74	0.00	0.00
Site 12	0.00	0.00	0.40	0.00	0.00	1.60	0.00	0.00
Site 13	0.46	0.00	2.08	0.00	0.00	0.00	0.00	0.00
Site 14	0.44	0.00	2.20	0.00	0.44	0.00	0.00	0.00
Site 15	0.00	0.00	0.96	0.00	0.00	1.92	0.00	0.00
Site 16	0.66	0.00	2.86	0.88	0.00	3.52	0.00	0.00
Site 17	0.20	0.00	5.79	0.00	0.00	0.80	0.00	0.00
Site 18	1.40	0.00	18.69	0.00	0.00	32.71	0.00	0.00
Site 19	0.75	0.00	1.88	0.00	0.00	2.06	0.00	0.00
Site 20	0.70	0.00	1.63	2.56	0.00	2.80	0.00	0.00
Site 21	1.90	0.00	4.43	0.00	0.00	2.53	0.00	0.00
Site 22	0.00	0.00	8.69	0.00	0.00	0.39	0.00	0.00
Site 23	0.22	0.00	9.31	0.00	0.00	0.00	0.00	0.00
Site 24	0.00	0.00	1.44	0.00	0.00	0.96	0.00	0.00
Site 25	1.59	0.00	2.73	1.36	0.00	0.45	0.00	0.00
Site 26	1.37	0.00	0.78	0.00	0.00	0.00	0.20	0.00
Site 27	0.00	0.00	20.56	0.00	0.00	0.00	0.00	0.00
Site 28	0.00	0.00	1.33	0.00	0.00	0.00	2.10	0.00
Site 29	0.00	0.00	16.27	0.00	0.00	0.00	0.00	0.00
Site 30	0.00	0.00	2.50	1.50	0.50	0.00	0.00	0.00
Site 31	0.00	0.00	13.16	0.00	1.32	0.00	0.00	0.00
Site 32	0.00	0.00	9.40	0.00	0.00	0.00	0.00	0.00
Site 33	0.00	0.00	0.50	0.25	0.00	0.00	0.00	0.00
Site 34	0.00	0.00	3.83	0.00	5.02	0.00	0.00	0.00
Site 35	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00
Site 36	0.00	0.00	2.37	0.00	0.95	0.47	0.00	0.00
Site 37	0.00	0.00	1.97	1.47	0.00	0.00	0.00	0.00
Site 38	0.00	0.00	10.22	0.00	1.00	0.00	0.00	0.00
Site 39	0.00	0.00	8.23	0.00	0.00	0.00	0.00	0.00
Site 40	0.00	0.00	16.63	0.00	0.00	0.00	0.00	0.00
Site 41	0.00	0.00	5.91	0.00	0.00	0.23	0.00	0.00
Site 42	0.00	0.00	0.68	0.00	0.00	1.14	0.00	0.00
Site 43	0.00	0.00	5.50	0.00	0.52	0.00	0.00	0.00
Site 44	0.00	0.00	0.42	0.00	1.69	0.00	4.02	2.54
Site 45	0.00	0.00	1.97	0.00	0.00	0.00	0.00	0.00
Site 46	0.00	0.00	9.11	2.28	0.00	0.00	0.00	0.00
Site 47	0.00	0.00	28.07	0.00	0.00	0.00	0.46	0.00
Site 48	0.00	0.00	3.70	0.00	0.00	0.00	0.00	0.00
Site 49	0.00	0.00	7.92	0.00	0.00	0.00	0.00	0.00
Site 50	0.00	0.00	18.23	5.04	0.00	0.00	0.00	0.00
Site 51	0.00	0.00	4.30	0.00	0.00	3.39	0.00	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	81	82	83	84	85	86	87	88
Site 1	8.58	0.00	0.00	0.00	0.00	0.00	3.94	0.00
Site 2	5.97	0.00	0.00	0.00	0.25	0.00	0.00	4.98
Site 3	3.02	0.00	4.65	0.00	0.00	0.00	0.47	1.40
Site 4	0.62	0.62	70.10	0.00	0.00	0.00	0.00	0.00
Site 5	0.91	0.00	0.00	0.00	0.00	0.00	1.14	0.00
Site 6	0.90	0.00	19.64	0.00	0.00	0.00	1.58	1.81
Site 7	0.00	0.00	65.51	0.00	0.00	0.41	0.82	0.00
Site 8	0.24	0.00	1.46	0.00	0.24	0.00	2.18	0.49
Site 9	0.80	0.00	2.98	0.20	0.00	0.40	14.12	1.19
Site 10	3.20	0.00	2.22	0.00	0.00	1.23	4.19	0.00
Site 11	0.00	8.37	46.31	0.00	0.00	0.00	0.99	0.00
Site 12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 13	0.00	0.00	1.39	0.00	0.00	0.00	2.31	1.39
Site 14	1.10	0.00	4.40	0.00	0.00	0.00	1.54	0.00
Site 15	0.48	0.00	0.72	0.00	0.00	0.00	2.16	0.00
Site 16	0.66	2.42	43.96	0.00	0.00	1.54	1.32	0.00
Site 17	7.58	0.80	1.40	0.00	0.60	0.00	0.00	0.00
Site 18	0.00	0.23	8.41	0.00	0.00	0.00	0.93	0.00
Site 19	0.38	0.94	32.65	0.00	0.00	0.00	0.19	0.00
Site 20	3.26	0.00	3.96	0.00	0.00	0.00	0.00	0.00
Site 21	0.84	0.21	2.53	0.00	0.00	0.00	0.00	0.21
Site 22	4.44	0.00	0.00	0.00	0.00	0.00	0.19	0.00
Site 23	0.00	0.00	0.00	0.00	0.22	0.00	1.33	0.67
Site 24	0.48	0.00	0.00	0.00	0.24	0.00	1.44	0.00
Site 25	0.00	0.00	7.73	0.00	0.00	0.00	15.45	2.05
Site 26	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00
Site 27	0.19	0.00	0.00	0.00	0.00	0.00	0.56	0.00
Site 28	0.00	0.00	6.10	0.00	0.00	0.00	0.00	0.00
Site 29	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00
Site 30	2.25	0.00	1.00	0.00	0.25	0.00	0.00	0.00
Site 31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 32	0.00	3.90	0.92	6.88	0.00	0.00	0.00	0.00
Site 33	0.99	0.00	1.24	0.00	0.25	2.73	2.98	0.00
Site 34	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.00
Site 35	0.25	4.25	25.50	0.00	0.00	9.50	2.00	0.00
Site 36	3.79	0.00	2.13	0.00	0.00	0.95	0.71	0.00
Site 37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 38	1.75	0.00	0.00	0.00	0.00	1.25	1.75	0.00
Site 39	0.22	0.00	0.87	0.00	0.00	0.00	1.73	0.87
Site 40	6.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 41	0.23	0.00	0.00	0.68	0.23	0.00	0.45	0.00
Site 42	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.00
Site 43	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 45	0.49	0.00	0.00	0.00	0.00	0.25	0.49	0.00
Site 46	1.37	0.00	4.10	0.00	1.82	0.00	2.51	0.00
Site 47	1.62	0.00	1.86	0.00	0.46	0.00	0.00	0.00
Site 48	2.31	0.00	0.00	0.00	1.39	0.00	0.00	0.46
Site 49	15.16	0.00	0.90	0.00	4.30	0.00	0.68	0.00
Site 50	8.39	0.00	0.00	0.00	7.43	0.00	0.00	0.00
Site 51	14.03	0.00	1.58	0.00	9.73	0.45	0.90	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	89	90	91	92	93	94	95	96
Site 1	1.86	0.00	0.00	0.46	0.00	0.00	0.00	0.00
Site 2	0.00	5.72	0.00	7.21	0.00	3.48	0.00	0.00
Site 3	0.23	0.00	0.00	0.47	0.00	14.19	0.00	0.00
Site 4	0.00	0.00	0.00	0.00	0.00	0.62	0.00	0.00
Site 5	10.23	0.00	0.00	1.36	0.00	0.00	0.00	0.00
Site 6	4.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 8	0.97	0.00	0.00	3.40	0.00	1.21	0.00	0.00
Site 9	5.96	0.00	0.00	3.18	0.60	0.00	0.00	0.00
Site 10	0.00	0.00	0.00	1.48	0.00	0.00	0.00	0.00
Site 11	0.00	0.00	0.00	6.65	0.00	1.23	0.00	0.00
Site 12	0.00	0.00	0.00	0.00	0.00	0.00	3.21	0.00
Site 13	0.23	3.24	2.55	3.01	0.00	0.00	0.00	0.00
Site 14	0.00	0.00	0.66	1.10	0.00	0.00	1.32	0.00
Site 15	0.00	0.00	0.00	0.00	2.40	0.00	0.72	0.00
Site 16	1.10	0.00	0.00	0.88	0.00	0.66	0.00	0.00
Site 17	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
Site 18	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 19	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00
Site 20	0.00	1.17	0.00	0.00	0.00	0.00	0.00	2.33
Site 21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 22	0.00	0.00	0.00	1.16	0.00	0.00	0.19	0.00
Site 23	0.00	0.00	0.00	0.89	0.00	0.00	0.00	0.00
Site 24	0.48	0.00	0.00	0.00	0.96	0.00	0.00	0.00
Site 25	0.45	0.00	0.00	2.27	0.00	0.00	0.00	0.00
Site 26	0.00	0.00	0.00	0.00	0.39	0.00	0.00	0.00
Site 27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 28	0.00	0.00	2.48	0.00	0.00	0.00	0.19	0.00
Site 29	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.00
Site 30	0.00	0.00	0.75	1.25	0.00	0.00	0.00	0.50
Site 31	0.00	0.00	0.00	0.00	0.00	2.19	0.00	0.00
Site 32	0.00	0.00	0.00	0.46	0.00	0.00	0.00	0.00
Site 33	0.00	0.00	0.00	16.63	0.25	0.00	0.00	0.00
Site 34	0.00	0.00	0.00	4.55	0.00	0.00	0.00	0.00
Site 35	0.00	2.75	0.25	0.00	1.00	0.00	0.00	0.00
Site 36	0.00	0.00	0.00	1.66	0.00	0.00	0.00	0.00
Site 37	0.00	0.00	0.00	20.88	0.00	0.00	0.00	0.00
Site 38	0.00	0.00	0.00	4.99	3.49	0.00	1.00	0.00
Site 39	0.00	0.00	0.00	11.90	0.00	0.00	0.00	0.00
Site 40	0.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 41	0.00	0.00	0.68	0.68	0.00	0.00	0.23	0.00
Site 42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 43	0.00	0.00	0.00	0.52	1.03	0.00	0.00	0.00
Site 44	0.00	1.27	0.00	0.42	0.00	0.00	0.00	0.00
Site 45	0.00	0.25	0.00	0.00	1.97	0.00	0.00	0.00
Site 46	0.00	0.46	0.00	0.00	0.00	0.00	2.28	0.00
Site 47	0.00	0.00	0.00	0.00	0.00	0.00	1.16	0.00
Site 48	0.00	0.00	0.00	0.00	0.69	0.00	0.00	0.00
Site 49	0.00	0.45	0.00	0.00	0.00	0.00	0.00	0.00
Site 50	0.00	0.00	0.00	0.00	0.00	0.48	0.24	0.48
Site 51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	97	98	99	100	101	102	103	104
Site 1	0.00	0.00	0.00	6.96	0.00	0.93	0.00	0.00
Site 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 3	0.00	0.00	0.00	0.00	0.47	3.72	0.00	0.00
Site 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 5	0.00	0.00	0.68	0.00	0.23	0.00	0.00	0.00
Site 6	0.00	0.00	0.00	0.00	0.00	3.39	0.00	0.00
Site 7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 8	0.00	0.00	0.00	0.00	1.94	2.91	0.00	0.00
Site 9	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00
Site 10	0.00	0.99	0.74	0.00	0.00	0.00	0.00	0.00
Site 11	0.00	0.00	0.00	0.00	0.00	7.64	0.00	0.00
Site 12	0.00	0.00	0.00	0.00	1.60	0.00	0.00	0.00
Site 13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 14	0.66	0.00	0.00	0.00	0.00	34.95	0.00	0.00
Site 15	0.00	0.00	0.00	0.00	0.00	0.00	2.16	0.00
Site 16	0.00	0.00	5.93	0.00	0.00	1.98	0.00	0.00
Site 17	0.00	0.00	5.39	0.00	0.00	0.00	0.00	0.00
Site 18	0.00	0.70	6.31	0.00	0.00	0.93	0.00	0.00
Site 19	0.00	0.00	0.75	0.00	0.00	0.56	0.00	0.00
Site 20	0.00	0.00	31.93	0.00	0.00	0.70	0.00	0.00
Site 21	0.00	0.00	25.95	0.00	0.00	0.00	0.00	0.00
Site 22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 23	3.33	0.00	0.00	0.00	0.00	0.44	0.00	0.00
Site 24	0.00	0.00	0.00	0.00	3.83	0.48	0.00	0.00
Site 25	0.91	0.00	0.00	0.00	0.45	7.05	0.00	0.00
Site 26	0.00	0.00	0.00	0.00	0.00	1.57	0.00	0.00
Site 27	1.11	0.00	0.00	0.00	1.67	0.00	0.00	0.00
Site 28	2.67	0.00	0.00	0.00	4.57	1.14	0.00	0.00
Site 29	0.00	0.00	0.00	0.00	4.72	0.71	0.00	0.00
Site 30	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00
Site 31	0.00	3.73	0.00	0.00	0.00	0.00	0.00	0.00
Site 32	0.00	0.00	0.00	0.00	0.00	1.38	0.00	0.00
Site 33	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00
Site 34	0.00	0.96	0.00	0.00	0.00	0.00	0.00	0.00
Site 35	0.00	0.00	0.00	0.00	0.00	0.25	0.50	0.00
Site 36	0.00	0.00	0.00	0.00	3.79	0.24	0.00	0.00
Site 37	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00
Site 38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 39	0.00	0.22	0.00	0.00	0.87	0.00	0.00	0.00
Site 40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.78
Site 41	0.00	0.23	0.00	0.00	0.00	0.45	0.00	0.00
Site 42	1.83	0.68	0.00	0.00	1.37	0.00	0.00	0.00
Site 43	0.00	0.00	0.00	0.00	0.00	5.84	0.00	0.00
Site 44	3.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Site 45	0.00	0.74	0.00	0.00	0.00	0.00	0.00	0.00
Site 46	0.00	0.00	2.73	0.00	0.00	0.00	0.00	0.00
Site 47	0.46	0.70	0.23	0.00	0.00	0.00	0.00	0.00
Site 48	0.00	0.00	0.00	0.00	0.00	0.00	3.24	0.00
Site 49	0.00	1.58	6.56	0.00	0.00	0.90	0.00	0.00
Site 50	0.00	0.00	12.95	0.00	0.00	0.00	0.00	0.00
Site 51	0.00	0.00	3.85	0.00	0.00	0.00	0.00	0.00

Table 2.2 (*continued...*): Relative abundance (%) of Training Set Species

Species no...	105	106	107	108	109	110	111	Other sp
Site 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.58
Site 2	0.00	0.00	0.00	0.50	0.00	0.00	0.00	4.48
Site 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.26
Site 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.27
Site 5	0.00	0.00	0.00	0.00	0.00	0.45	0.00	3.64
Site 6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.39
Site 7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.67
Site 8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.80
Site 9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.77
Site 10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.43
Site 11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.94
Site 12	0.00	0.00	0.00	9.62	0.00	0.00	0.00	6.41
Site 13	0.00	0.00	0.00	0.00	5.32	0.00	0.00	9.26
Site 14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.98
Site 15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.05
Site 16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22
Site 17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.60
Site 18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.34
Site 19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.44
Site 20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.96
Site 21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.48
Site 22	0.00	0.00	6.37	0.00	0.00	0.00	0.00	3.67
Site 23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.32
Site 24	0.00	0.00	1.20	0.00	0.00	0.00	0.00	5.26
Site 25	0.00	0.00	0.00	0.23	0.00	0.00	0.00	5.23
Site 26	0.00	5.68	0.00	0.00	0.00	0.00	0.00	2.94
Site 27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.33
Site 28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.86
Site 29	0.00	0.00	0.00	0.00	0.00	3.54	16.98	1.42
Site 30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.75
Site 31	12.94	0.00	0.00	0.00	0.00	0.00	1.10	5.26
Site 32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.05
Site 33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.94
Site 34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.50
Site 35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.75
Site 36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.79
Site 37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.86
Site 38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.98
Site 39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.25
Site 40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.37
Site 41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.41
Site 42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.53
Site 43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.98
Site 44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.71
Site 45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.69
Site 46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.19
Site 47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46
Site 48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.17
Site 49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.68
Site 50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.60
Site 51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.81

Appendix 3: ^{210Pb} Dating Data, Pittwater Core Nutrients, Species No.s, Names & Relative Abundance

Table 3.1: ^{210}Pb Dating Data

Mid section depth (cm)	+ /- cm	Po - 210	Ra -226	Excess Pb-210
0.7	0.5	2.43 +/- 0.12	0.29 +/- 0.02	2.14 +/- 0.12
4.3	0.5	2.78 +/- 0.12	0.23 +/- 0.01	2.55 +/- 0.12
9.1	0.5	2.15 +/- 0.06	0.26 +/- 0.01	1.89 +/- 0.06
16.3	0.5	1.73 +/- 0.05	0.22 +/- 0.01	1.50 +/- 0.05
25.9	0.5	1.07 +/- 0.04	0.26 +/- 0.02	0.81 +/- 0.05
36.7	0.5	0.64 +/- 0.02	0.26 +/- 0.02	0.38 +/- 0.03
42.7	0.5	0.77 +/- 0.03	0.24 +/- 0.01	0.52 +/- 0.03
48.7	0.5	1.22 +/- .0.35	0.27 +/- 0.02	0.95 +/- 0.04
57.1	0.5	0.51 +/- 0.02	0.27 +/- 0.02	0.24 +/- 0.03
66.7	0.5	0.40 +/- 0.02	0.27 +/- 0.02	0.13 +/- 0.03

NB: All sample analyses performed by Environmental Division Laboratories, ANSTO, Lucas Heights, Sydney

Dating data provided forms part of a larger project:

Project Title: Impact of catchment changes on the ecology of the Pitt Water estuary.

AINSE Grant No. 01/106

Project Leader: Iona Mitchell, TAFI Marine Research Laboratories, Taroona

Table 3.2: Number and effective number of diatom species in the fossil data set, and number of species in the fossil data present in the training set (see key below table)

Core Depth (cm)	N_Fossil	N2_Fossil	N_In_Modern
1.2	21	13.6	21
2.4	20	11.3	20
3.6	18	12.2	18
4.8	24	14.0	24
6	23	14.2	23
7.2	20	11.9	20
8.4	25	14.7	25
9.6	19	12.4	19
10.8	21	10.2	21
12	25	15.3	25
14.4	21	11.8	21
16.8	25	16.7	25
18	25	15.9	25
19.2	23	16.1	23
21.6	20	13.5	20
24	21	14.3	21
26.4	24	16.0	24
28.8	22	15.3	22
31.2	19	13.6	19
33.6	18	13.0	18
36	19	12.9	19
38.4	19	11.3	19
40.8	22	12.3	22
43.2	16	8.9	16
45.6	19	12.8	19
48	17	9.7	17
50.4	13	7.2	13
52.8	16	9.0	16
55.2	15	8.5	15
57.6	15	9.6	15
60	15	9.9	15
62.4	13	8.0	13
64.8	14	8.6	14
67.2	15	8.3	15
68.4	17	9.3	17

Key:

N_Fossil	Number of species in fossil data
N2_Fossil	Effective number of species in fossil data - (N2 – (Hill 1973))
N_In_Modern	Number of species in fossil data present in training set

Table 3.3: Reconstructed NO_{2-3} and SiO_2 concentrations for Pittwater core

Depth (cm)	Spring NO_{2-3} (µmol/L)	Spring SiO_2 (µmol/L)
1.2	0.95	17.56
2.4	1.03	12.96
3.6	0.83	15.98
4.8	1.00	16.11
6	0.89	16.48
7.2	0.76	9.47
8.4	1.30	17.31
9.6	0.56	11.68
10.8	0.46	7.92
12	0.59	11.78
14.4	0.83	12.93
16.8	0.73	12.67
18	0.79	14.36
19.2	0.90	12.31
21.6	0.88	12.84
24	0.97	13.40
26.4	0.69	11.15
28.8	0.70	12.22
31.2	0.75	15.64
33.6	0.87	15.84
36	0.73	15.04
38.4	0.53	15.78
40.8	0.65	16.34
43.2	0.89	24.76
45.6	0.89	19.69
48	0.59	18.43
50.4	0.28	18.82
52.8	0.51	19.68
55.2	0.47	19.16
57.6	0.74	21.66
60	0.73	24.65
62	0.53	20.65
64.8	0.68	22.73
67.2	0.31	13.52
68.4	0.64	20.24

Appendix 3 (cont...)

Table 3.4: Diatom species No. and name from the Pittwater core

Species No.	Species Name
19	*Cocconeis disculoides* Hustedt
24	*Cocconeis placentula* Ehrenberg var. *euglypta* (Ehr.) Grunow
32	*Dimerogramma minor* var.*nana* (Gregory) Van Heurck
36	*Ehrenbergia granulosa* (Grunow) Witkowski
42	*Fragilaria pinnata* Ehrenberg
43	*Fragilaria pinnata* Ehrenberg var. *pinnata*
45	*Gramatophora oceanica* Ehrenberg
54	*Navicula arenaria* Donken var. *rostellata* Lange-Bertalot
58	*Navicula monoculata* var. *omissa* (Hustedt) Lange-Bertalot
69	*Nitzschia lorenziana* Grunow var. *subtilis* Grunow
70	*Nitzschia ovalis* Arnott ex Grunow in Cleve & Grunow
71	*Nitzschia panduriformis* Gregory var. *minor* Gregory
74	*Opephora martyi* Heribaud
75	*Opephora olsenii* Möller
76	*Paralia sulcata* (Ehrenberg) Cleve
78	*Plagiogramma staurophorum* (Gregory) Heiberg
81	*Planothidium delicatulum* (Kützing) Round & Buktiyarova
85	*Cocconeis* aff. *pinnata* Gregory ex Greville
86	*Synedra investiens* W. Smith
98	*Fragilaria* sp 1
99	*Cymatosira* aff. *belgica* Grunow
100	Spp 3
112	*Aulocoseira ambigua* (Grunow) Simonsen
113	*Campylodiscus daemelianus* Grunow
114	*Catenula adhaerens* Mereschkowsky
115	*Cyclotella atomus* Hustedt
116	*Glyphodesmis distans* (Gregory) Grunow
117	*Lyrella david-mannii* Witkowski
118	*Nitzschia punctata* var. *coarctata* (Grunow) Hustedt
119	*Odentella aurita* (Lyngbye) Agardh
120	*Plagiogramma appendiculatum* Giffen.
121	*Planothidium quarnerensis* (Grunow)
122	*Petrodictyon gemma* (Ehrenberg) D. G. Mann
123	*Trachysphenia australis* Petit var. *australis*
124	*Dactyliosolen* spp.
125	*Fragilaria* sp 2

Appendix 3 (cont...)

Table 3.5: Relative abundance (%) of diatoms in the Pittwater Core

	Species Nos.																		
Depth (cm)	19	24	32	36	42	43	45	54	58	69	70	71	74	75	76	78	81	85	86
0-1.2	1.22	0.73	0.49	0.00	0.98	1.47	0.24	2.44	0.00	1.47	0.00	0.00	0.00	7.33	0.49	1.22	2.69	11.49	1.47
1.2-2.4	1.20	0.24	0.00	0.00	0.00	2.64	0.72	0.96	0.48	1.20	0.00	0.00	0.00	3.85	0.00	0.96	0.00	11.54	0.48
2.4-3.6	1.97	2.21	0.00	0.00	3.93	0.98	0.00	0.74	1.47	2.21	0.25	0.00	0.00	9.09	0.25	0.00	2.21	7.37	0.74
3.6-4.8	0.97	0.24	0.00	0.49	0.24	3.16	1.46	1.46	1.22	0.97	0.00	0.00	0.49	6.57	0.24	0.97	1.95	13.87	0.00
4.8-6.0	1.68	0.00	0.24	0.00	1.20	1.44	0.00	0.48	1.92	0.96	2.40	0.00	0.00	7.93	0.48	1.20	2.64	9.13	1.20
6.0-7.2	1.24	0.00	0.00	0.00	0.00	0.74	0.50	0.00	1.49	1.74	0.74	0.50	0.00	7.94	0.74	1.24	0.74	9.93	0.25
7.2-8.4	0.72	0.24	0.24	0.00	0.48	1.92	0.96	0.00	1.20	2.64	0.00	0.00	0.24	5.53	0.96	1.68	1.20	17.07	0.00
8.4-9.6	1.71	0.49	0.00	0.00	4.63	2.68	0.49	0.00	0.73	0.24	0.00	0.00	0.98	5.37	0.00	1.46	3.90	10.24	0.00
9.6-10.8	0.73	0.24	0.00	0.98	1.96	0.98	1.96	0.00	0.00	0.00	0.00	0.24	0.00	4.89	0.24	2.20	0.24	14.18	0.00
10.8-12	0.48	1.20	0.24	1.20	7.91	2.16	1.92	0.00	1.92	0.00	0.00	0.00	0.00	6.95	0.24	2.16	0.24	8.39	0.48
13.2-14.4	0.25	0.49	0.25	0.00	4.69	6.67	1.23	0.00	0.49	0.00	0.00	0.00	0.49	4.20	0.49	3.46	0.99	8.40	10.86
15.6-16.8	1.46	0.24	0.97	3.89	3.65	0.24	6.08	0.00	0.00	0.49	0.00	0.49	2.19	1.95	6.33	9.73	0.73	22.14	0.00
16.8-18	0.74	1.96	0.74	2.45	10.29	1.72	3.68	0.25	0.49	0.00	0.00	0.74	0.00	5.64	1.23	7.60	1.72	10.29	0.00
18-19.2	0.70	0.23	2.79	13.95	0.93	0.47	20.23	0.00	0.00	0.00	0.00	2.09	0.47	0.23	5.81	9.30	0.00	6.05	0.00
20.4-21.6	0.48	0.48	2.88	1.92	1.92	0.72	14.90	0.00	0.00	0.48	0.00	0.96	1.20	1.20	2.16	8.65	0.00	17.79	0.00
22.8-24	0.74	1.23	3.93	3.69	0.98	1.47	16.95	0.00	0.00	0.00	0.00	0.49	5.65	0.49	2.70	6.88	0.49	17.69	0.00
25.2-26.4	0.94	0.70	1.64	3.29	1.88	0.94	14.32	0.00	0.00	0.00	0.00	5.40	1.41	0.47	3.99	4.93	0.94	17.14	0.00
27.6-28.8	0.00	0.00	3.81	15.48	0.00	0.24	13.81	0.00	0.00	0.48	0.00	0.24	5.00	0.24	15.95	2.38	0.24	5.48	0.00
30-31.2	0.66	0.00	3.06	34.35	0.00	0.00	9.19	0.00	0.00	0.00	0.00	0.00	1.97	0.00	20.35	1.75	0.22	2.19	0.00
32.4-33.6	0.22	0.00	2.88	35.03	0.00	0.00	7.76	0.00	0.00	0.00	0.00	0.00	3.10	0.00	19.73	3.10	0.00	2.22	0.00
34.8-36	0.93	0.00	2.10	34.11	0.00	0.00	8.64	0.00	0.00	0.00	0.00	0.00	2.57	0.00	18.22	1.87	0.00	3.27	0.00
37.2-38.4	0.00	0.00	2.37	50.00	0.24	0.24	1.66	0.00	0.00	0.00	0.00	0.00	3.55	0.00	18.25	0.47	0.00	1.18	0.00
39.6-40.8	0.24	0.24	3.37	42.79	0.00	0.24	3.85	0.00	0.00	0.00	0.00	0.00	3.61	0.00	20.67	1.44	0.00	1.92	0.00
42-43.2	0.00	0.00	3.29	46.12	0.00	0.00	2.59	0.00	0.00	0.00	0.00	0.00	0.71	0.00	24.00	0.24	0.00	0.24	0.00
44.4-45.6	0.00	0.00	2.87	36.60	0.00	0.00	3.11	0.00	0.00	0.96	0.00	0.00	2.39	0.00	21.77	0.96	0.00	1.67	0.00
46.8-48	0.49	0.00	1.95	45.50	0.00	0.00	3.16	0.00	0.00	0.24	0.00	0.00	2.43	0.00	23.60	0.73	0.00	0.49	0.00
49.2-50.4	0.00	0.00	2.63	57.04	0.00	0.00	0.95	0.00	0.00	0.00	0.00	0.00	1.19	0.00	20.76	0.00	0.00	0.00	0.00
51.6-52.8	0.00	0.00	6.96	34.11	0.23	0.00	2.09	0.00	0.00	0.00	0.00	0.00	2.55	0.00	33.18	0.00	0.00	0.46	0.00
54-55.2	0.00	0.00	4.18	41.52	0.25	0.00	2.46	0.00	0.00	0.00	0.00	0.00	1.47	0.00	28.99	0.00	0.00	0.25	0.00
56.4-57.6	0.00	0.00	3.87	33.94	0.00	0.00	2.51	0.00	0.00	0.00	0.00	0.00	2.73	0.00	30.30	0.00	0.00	0.46	0.00
58.8-60	0.48	0.00	5.04	30.46	1.20	0.00	5.52	0.00	0.00	0.00	0.00	0.00	1.92	0.00	32.61	0.00	0.00	0.00	0.00
61.2-62.4	0.00	0.00	3.77	35.61	0.00	0.24	3.77	0.00	0.00	0.00	0.00	0.00	1.42	0.00	35.38	0.00	0.00	0.00	0.00
63.6-64.8	0.00	0.00	1.66	36.02	0.00	0.00	4.74	0.00	0.00	0.00	0.00	0.00	0.71	0.00	31.04	0.00	0.00	0.71	0.00
66-67.2	0.25	0.00	1.49	39.80	0.00	0.50	2.49	0.00	0.00	0.00	0.00	0.00	1.24	0.00	28.11	0.00	0.00	0.00	0.00
67.2-68.4	0.25	0.00	1.50	35.75	0.00	0.50	4.25	0.00	0.00	0.00	0.00	0.00	1.75	0.00	32.00	0.00	0.00	0.00	0.00

(continued....)

Table 3.5 (*continued...*): Relative abundance (%) of diatoms in the Pittwater Core

Depth (cm)	Species No.s 98	99	100	112	113	114	115	116	117	118	119	120	121	122	123	124	125	Other spp
0-1.2	1.47	46.45	0.00	0.00	0.00	5.38	0.00	1.22	0.24	2.69	0.00	0.00	0.00	2.69	0.00	0.00	0.00	6.11
1.2-2.4	0.72	56.25	0.00	0.24	0.00	7.69	1.44	1.92	0.00	0.96	0.00	0.00	0.24	2.16	0.00	0.00	0.00	4.09
2.4-3.6	1.47	44.23	0.00	0.00	0.00	7.86	0.00	2.95	0.00	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.09
3.6-4.8	0.49	48.18	0.00	1.70	0.00	2.68	0.00	2.19	0.00	1.95	0.00	0.00	0.49	2.19	0.24	0.00	0.00	5.60
4.8-6.0	1.20	48.32	0.00	0.00	0.00	6.73	0.72	1.68	0.00	1.20	0.00	0.00	0.24	1.20	0.24	0.00	0.00	5.53
6.0-7.2	1.99	51.61	0.00	0.00	0.00	5.71	0.00	1.49	0.00	0.99	0.00	0.00	0.50	1.99	0.00	0.00	0.00	7.94
7.2-8.4	0.72	36.06	0.00	1.20	0.00	7.69	0.96	2.40	0.00	1.92	0.24	0.00	0.48	3.13	0.24	0.00	0.00	9.86
8.4-9.6	3.90	40.98	0.00	0.00	0.00	7.07	0.24	1.22	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00	3.41	10.00
9.6-10.8	0.73	55.99	0.49	0.00	0.00	9.78	0.24	0.49	0.00	0.00	0.00	0.00	0.24	1.22	0.00	0.00	0.49	1.47
10.8-12	1.92	35.97	2.16	0.48	0.00	9.11	2.88	1.44	0.00	0.00	0.24	0.00	0.24	0.24	0.00	0.00	1.92	7.91
13.2-14.4	0.74	50.12	0.25	0.49	0.00	0.00	0.25	2.96	0.00	0.00	0.00	0.00	0.00	1.23	0.00	0.00	0.00	0.99
15.6-16.8	0.49	8.27	1.46	1.70	0.00	12.17	0.00	5.35	0.00	0.00	0.24	0.73	0.97	0.00	0.00	0.73	0.00	7.30
16.8-18	0.49	15.44	1.47	0.00	0.00	18.14	0.00	7.35	0.00	0.00	0.25	0.74	0.25	0.00	0.00	0.25	0.49	5.39
18-19.2	0.00	0.00	2.79	1.86	0.00	3.49	0.00	9.77	0.47	0.00	0.70	1.86	2.33	0.00	0.93	3.72	0.00	8.84
20.4-21.6	0.00	0.00	6.25	0.00	0.00	24.52	0.00	6.73	0.00	0.00	0.00	0.00	1.68	0.00	0.48	0.72	0.00	3.85
22.8-24	0.00	0.00	0.98	0.25	0.00	15.48	0.00	10.32	0.00	0.00	0.00	0.98	0.00	0.00	1.23	1.23	0.00	6.14
25.2-26.4	0.23	0.47	4.46	0.00	0.00	14.79	0.00	6.57	0.00	0.00	0.23	0.94	1.17	0.00	0.23	0.94	0.00	11.97
27.6-28.8	0.00	0.00	4.05	1.90	0.71	1.90	0.00	6.43	0.00	0.00	1.90	1.90	0.95	0.00	1.19	3.33	0.00	11.90
30-31.2	0.00	0.00	3.28	1.97	1.09	0.00	0.00	1.53	1.09	0.00	1.53	0.88	2.63	0.00	2.41	2.84	0.00	6.56
32.4-33.6	0.00	0.00	1.77	2.66	0.44	0.00	0.00	3.33	0.89	0.00	0.89	1.55	1.77	0.00	2.00	2.44	0.00	7.76
34.8-36	0.00	0.00	1.87	4.44	0.47	0.93	0.00	3.74	0.47	0.00	0.93	0.47	1.17	0.00	0.47	2.80	0.00	10.05
37.2-38.4	0.00	0.00	1.42	3.32	3.08	0.00	0.00	0.47	1.18	0.00	0.47	0.24	2.13	0.00	1.66	3.55	0.00	3.79
39.6-40.8	0.00	0.00	1.44	4.33	0.24	0.24	0.24	1.68	0.48	0.00	0.96	0.96	0.96	0.00	1.20	1.44	0.00	6.97
42-43.2	0.00	0.00	1.18	5.65	1.41	0.00	0.47	1.18	3.53	0.00	0.00	0.47	0.00	0.00	0.47	0.94	0.00	6.35
44.4-45.6	0.00	0.00	1.20	4.07	3.59	0.00	1.20	1.67	1.91	0.00	0.24	0.72	0.72	0.00	0.72	2.39	0.00	9.81
46.8-48	0.00	0.00	0.24	4.62	1.95	0.00	0.73	0.00	1.95	0.00	0.00	0.24	0.00	0.00	1.70	1.70	0.00	7.06
49.2-50.4	0.00	0.00	0.72	4.30	1.91	0.00	0.72	0.00	0.72	0.00	0.00	0.00	0.24	0.00	0.48	1.67	0.00	5.97
51.6-52.8	0.23	0.00	0.00	2.78	0.70	0.00	1.39	0.23	0.70	0.00	0.00	0.00	0.23	0.00	3.71	3.94	0.00	5.80
54-55.2	0.00	0.00	0.00	7.13	0.98	0.00	1.23	0.00	1.23	0.00	0.00	0.25	0.74	0.00	0.98	1.47	0.00	6.14
56.4-57.6	0.00	0.00	0.23	5.92	1.59	0.00	1.37	0.23	2.05	0.00	0.00	0.23	0.00	0.00	2.96	3.64	0.00	7.29
58.8-60	0.00	0.00	0.48	2.88	0.48	0.00	2.16	0.72	1.92	0.00	0.00	0.00	0.00	0.00	1.44	3.12	0.00	8.87
61.2-62.4	0.00	0.00	0.00	6.13	0.47	0.00	2.12	0.00	2.12	0.00	0.00	0.00	0.24	0.00	0.24	4.01	0.00	3.77
63.6-64.8	0.00	0.00	0.00	6.16	1.18	0.00	4.27	0.00	0.71	0.24	0.00	0.00	0.24	0.00	1.42	3.55	0.00	6.87
66-67.2	0.50	0.00	0.00	9.45	2.24	0.00	0.50	1.00	0.25	0.00	0.00	0.00	0.50	0.00	0.00	8.46	0.00	3.23
67.2-68.4	0.00	0.00	0.25	7.25	1.00	0.00	2.25	1.00	0.50	0.00	0.25	0.25	0.00	0.00	0.75	5.25	0.00	4.75