UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCE

Ocean and Earth Science

Temporal Dynamics of Microplankton in the Sargasso Sea

by

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ABSTRACT

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TEMPORAL DYNAMICS OF MICROPLANKTON IN THE SARGASSO SEA

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The microplankton of the Sargasso Sea are a previously unstudied size fraction of the pelagic biological community, with only sporadic data available for the abundance of different microplankton groups in the literature. In this thesis, results from a decade-long series of microplankton net collected samples from the Bermuda Atlantic Time-series Study (BATS) site are presented and discussed with respect to different temporal and spatial scales. These different time scales range from monthly analysis, seasonal and 12-monthly, to long-term inter-annual analysis of the data. Of the seven microplankton groups analysed, only the radiolaria showed a significant difference in abundances between different months, in addition to a significant correlation with monthly averaged water column temperature. The monthly abundance patterns of the microplankton diatoms do not indicate the presence of a typical "spring bloom" scenario, although a small increase in abundance was observed in March or April samples from all of three twelve-month sampling periods analysed. Dinoflagellates were numerically dominant in ca. 90% of microplankton samples analysed, with a notable shift from a dinoflagellate-dominated microplankton sample in March 2000 to a diatom-dominated sample in April 2000.

The presence of mesoscale eddy features in the Sargasso Sea produces physical variability at the BATS site on a combined temporal-spatial scale that could influence the microplankton sampled community. Both radiolaria and diatoms abundances showed a significant positive correlation to mesoscale eddy presence indicated by variations in sea-level anomaly, however comparisons between total microplankton abundances and eddy types and ages proved inconclusive.

Two of the mineral-ballasted microplankton groups analysed, the diatoms and foraminifera, were subjected to geochemical analysis. Biogenic silica and particulate calcium were analysed on separate subsamples of microplankton and results compared to cell counts of diatoms and foraminifera respectively. A high variability in foraminifera volume was observed in March samples, with February samples indicative of abundant but small volume, lightly calcified foraminifera. Microplankton diatoms were found to contribute less than 8% to the total water-column biogenic silica concentrations, indicating that small size fraction diatoms are responsible for a large proportion of total biogenic silica in these waters.

A lack of significant seasonal changes in abundance of most of the seven microplankton groups investigated in this study suggest that the microplankton community remains relatively stable in terms of relative abundance (composition) at the BATS station in the Sargasso Sea.

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Academic Thesis: Declaration of Authorship

I, ……

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1 Introduction

The oligotrophic central gyre regions of the Atlantic, Pacific and Indian Oceans make up approximately 14 % by area of the marine environment, characterised by limited nutrient inputs with very low primary production. These areas are estimated to have increased by nearly 2 % per year since the late 1990's (Polovina *et al*., 2008). The majority of pelagic biological studies in these regions are focused mainly on small (<20µm) phytoplanktonic organisms, or larger (>200µm) metazoan zooplankton. The larger unicellular phytoplankton and the smaller unicellular zooplankton are often overlooked in these studies, resulting in a limited amount of detailed information reported in the scientific literature on the 'microplankton' size fraction (20 – 200 µm). The aim of the research presented in this thesis was to investigate the temporal variation in microplankton at the Bermuda Atlantic Time-series Study site in the western oligotrophic Atlantic. Detailed analysis of a unique archive of net collected microplankton samples from this oceanic time-series is presented with the aim of examining monthly, seasonal and interannual variability in the abundance of seven unicellular microplankton groups and their relative importance to the microplankton community. The variability of two mineral-ballasted microplankton groups (foraminifera and diatoms) is further analysed to determine variations in the levels of calcification and silicification, with regards to abundance and cell size. The data presented in this study represents the first long-term analysis of abundance and contribution of named unicellular microplankton groups to the overall microplankton community in the Sargasso Sea. The main objectives of this study were as follows;

- To document and analyse the variability of seven unicellular microplankton groups on monthly, seasonal and inter-annual timescales using a combination of traditional microscopy and the use of a FlowCAM.
- To identify any significant correlations between changes in the microplankton community and the presence of mesoscale eddy features at the BATS sampling site using a combination of satellite altimetry and microplankton abundance data.
- To determine pelagic foraminifera particulate calcium values and diatom biogenic silica cell values using a combination of microscopy and FlowCAM size and abundance data with geochemical analysis.

1.1 Long-term ocean observations

Long-term ocean observations or oceanic time-series are an important resource to help improve our understanding of global biogeochemical cycles, and in turn, the ability to predict how ecosystems will respond to climate change. Scales of variability in ocean biogeochemistry and their effects on the carbon cycle are measured most effectively through long-term time-series observations such as the Bermuda Atlantic Time-series Study (BATS) program (Steinberg *et al.*, 2001). Other ocean time-series currently in operation include the BATS 'sister station' off the islands of Hawaii; the Hawaii Ocean Time-series (HOT) station ALOHA, Ocean Weather Station 'P' and the Line P moorings in the North-east Pacific, the Porcupine Abyssal Plain (PAP) Observatory in the North Atlantic, and in more coastal waters, the L4 and E1 station positions off Plymouth. In addition to static time-series stations, detailed measurements are also being collected on much broader spatial scales, such as the Continuous Plankton Recorder (CPR) surveys and the worldwide network of Argo floats (~3,000 free-drifting profiling floats measuring temperature and salinity). Over 60 time-series stations around the world are now included in the OceanSITES network (http://www.oceansites.org/), as part of the Global Ocean Observing System, and are collecting data that can be used in conjunction with Argo float data as well as satellite altimetry.

Characteristics helping to define an ocean time-series site include long-term (continuous and sustained) in-situ oceanic sampling and observations in a specific region, or at a fixed geographical location. Observations can be made from autonomous moorings, automatically collecting data, or by shipboard sampling with regular occupation at a specific site e.g. the BATS data collection method. Continuous sampling from autonomous moorings has the added benefit of resolving high-frequency variability that may be overlooked when sampling on a less frequent timescale, such as monthly or bi-monthly shipboard measurements. Ducklow *et al*., (2009) have produced a description of some of the more "well known" oceanic time-series.

The location of time-series stations is usually determined by the oceanography of a particular area of chemical, physical or biological interest, or as a station considered to be representative of a specific body of water. For example, the Helgoland Roads time-series in the German Bight is a high frequency, fixed-position station specifically for meso- and macrozooplankton sampling (Greve *et al.*, 2004), whilst the initial research objective of the HOT site was to have a deep-ocean sampling site to act as a benchmark for a North Pacific oligotrophic system (Karl and Lukas 1996). Similarly, a number of different sampling programmes carried out at the Porcupine Abyssal Plain (NE Atlantic) as part of the PAP observatory over the years have acted to generate a decade-long time-series of both water column physical characteristics and benthic organisms, where such long-term studies of the deep sea benthos are rare (Billet *et al.*, 2001). The Continuous Plankton Recorder (CPR), currently run by the Sir Alistair Hardy Foundation for Ocean Science (SAHFOS) has been operating since 1931 to take monthly near-surface phytoplankton and zooplankton abundance measurements. CPR surveys are currently in operation in the North Atlantic, the North Pacific, and the Southern Ocean, cited as the "largest multi-decadal plankton monitoring programme in the world" (Richardson *et al.*, 2006). Geographically broad sampling areas mean that time-series such as the CPR surveys have a higher spatial resolution than those of a time-series located at a fixed sampling point; although the higher spatial resolution is linked with a lower temporal resolution (repeated surveys are rarely conducted at exactly the same location). Biological data collected by the CPR survey, such as the spatial distribution and abundance of phytoplankton, has been used to examine regime shifts and climatic anomalies in the North Sea in the early 1970s (Edwards *et al.*, 2002), and again between the years of 1982 and 1988 (Beaugrand 2004). Despite covering a large area of ocean with CPR tows, the area encompassing the Sargasso Sea (and therefore the BATS site), is not an area that the CPR routinely samples.

1.2 Microplankton

Plankton can be grouped by functionality (e.g. autotrophs, heterotrophs or mixotrophs) or more commonly, by size. The term 'Microplankton' generally refers to plankton included within the size range of 20-200µm (Sieburth *et al.,* 1978), a range that includes phytoplankton such as diatoms, some dinoflagellates and some coccolithophores, as well as protozoan microzooplankton such as tintinnids, foraminifera and radiolaria. In this study, the term 'Microplankton' is used only to describe single celled microplankton, rather than the multicellular metazoan organisms that occur in this size fraction. Due to the fact that plankton are free floating, they are subject to dramatic changes in distribution, with these distribution changes responding to variations in temperature and changing oceanic currents (Hays *et al.*, 2005). Obtaining information and knowledge of the variability (on a diel to weekly timescale) within the plankton is crucial to our understanding of the processes driving primary production and controlling the plankton biomass (Platt *et al.,* 1977). Plankton also have the potential for socioeconomic impacts involving bottom-up forcing of food webs and the subsequent effect this has on commercially exploited fish stocks (Hays *et al.*, 2005). When this is combined with the effect that plankton can have on the transport of carbon dioxide out of the surface waters, plankton have an important role in the ongoing 'health' of the marine ecosystem. Understanding this role can help scientists to accurately monitor and understand changes in the marine environment. The grazing organisms (microzooplankton) in this size fraction have been found to significantly impact primary producers, and are considered to be one of the main predators on smaller phytoplankton, particularly in tropical and subtropical oligotrophic waters (Calbet and Landry 2004; Calbet 2008).

Traditionally, a mixture of sampling techniques has been used for the collection of oceanic plankton. In the Sargasso Sea alone this includes the use of different mesh size net tows, bottle sampling of small or large water volumes, pumped water samples for filtering and even in some cases, diver collection of large plankton (Riley 1957; Swanberg and Caron 1991; Caron *et al.,* 1995b; Michaels *et al.,* 1995; Lessard and Murrell 1996; Nelson and Brzezinski 1997; Goldman and

McGillicuddy Jr 2003). More detail on the individual studies mentioned here can be found in Table 1 (Introduction 1.5.2). Whilst different sampling techniques have historically been used for different plankton groups (mainly dependent on plankton cell size), net sampling is still the most reliable and representative way to sample the entire microplankton (20 – 200 µm) community. This is especially true in areas of low plankton abundance such as the oligotrophic Sargasso Sea, where bottle collected samples run the risk of missing many of the low abundance cells.

1.3 Biological fluxes

The vertical flux of particulate carbon and other elements (e.g. silica) in the ocean, and how this varies over time is of major importance to ocean scientists, particularly those involved with time-series or monitoring programmes. Detailed measurements of the major sinks and sources of these elements are analysed and documented in order to help this understanding (Steinberg *et al.,* 2001). This vertical flux of carbon is commonly referred to as the global carbon pump, and can be described as comprising three different components; the carbonate pump, the solubility pump, and the biological (or soft-tissue) carbon pump (BCP) (Longhurst and Harrison 1989). Fluxes in the carbonate pump are maintained by the sinking of opal, calcite and aragonite from the surface waters of the ocean, whilst the solubility pump involves fluxes controlled by the differential solubility found along the vertical gradient of temperature in the oceans (Longhurst and Harrison 1989). The BCP, however, refers to the process of fixing inorganic carbon into organic matter in near-surface waters, and acts to mediate the flux of carbon into the deep ocean from the productive pelagic ecosystem of the euphotic zone (Longhurst and Harrison 1989; Ducklow *et al.,* 2001).

1.3.1 The Biological Carbon Pump (BCP)

The conversion of inorganic carbon into organic matter is mostly by the process of photosynthesis in the euphotic zone (Riebesell and Wolf-Gladrow 1992). However, other processes including chemoautotrophy are found to play a role in the production of organic matter, especially in suboxic or anoxic environments such as the Black Sea and the Baltic Sea (Yilmaz *et al.,* 2006; Grote *et al.,* 2008), chemical-rich environments such as hydrothermal vent sites (Jannasch and Wirsen 1979) and even in the interior of major oceans such as the North Atlantic (Varela *et al.,* 2011). The particular method used by cells to acquire carbon should affect the potential of CO_2 limitation for primary production, and how primary producers respond to changes in CO_2 levels (Tortell *et al.,* 2000). The processes involved (e.g. photosynthesis and cell decomposition) in the life cycle of phytoplankton act to drive the cycling of dissolved carbon, and other associated

chemicals such as nitrogen, oxygen and phosphorus in the ocean. The sinking of photosynthetically-derived particles acts to remove these chemicals from surface waters, with addition at depth (Archer 2004) (Figure 1).

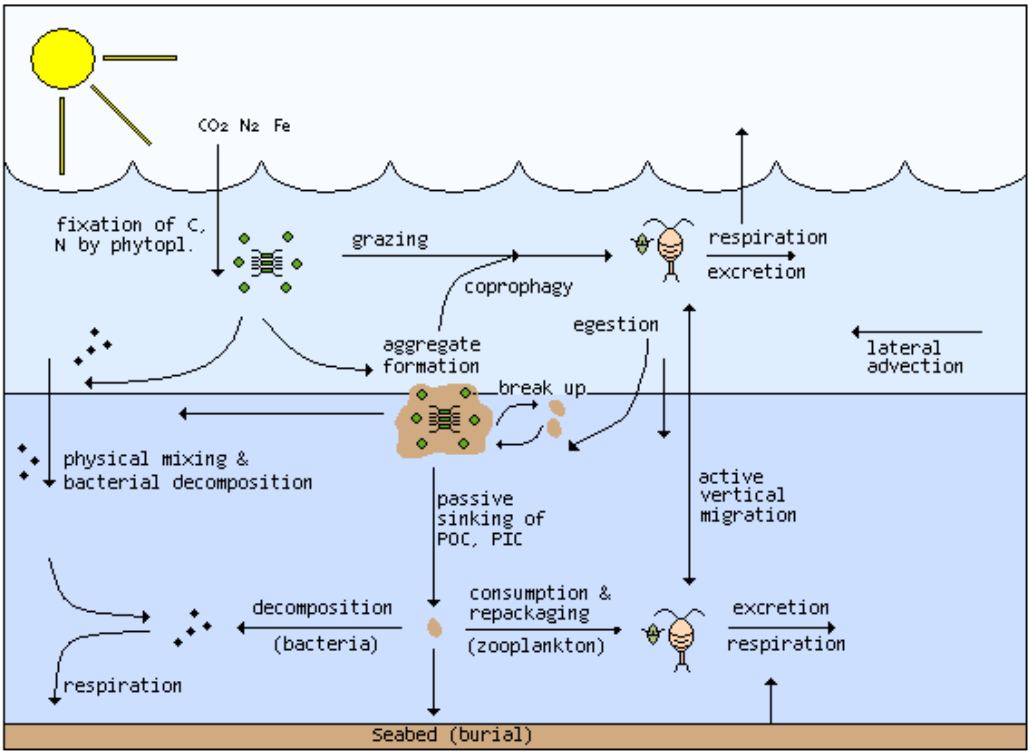


Figure 1 - Schematic diagram of the components and processes involved in the Biological Carbon Pump (BCP). Adapted from Ducklow *et al.*, (2001).

1.3.1.1 Understanding the BCP

Knowledge of the biological carbon pump, and the processes involved has improved dramatically since early reports e.g. Volk and Hoffert (1985) on the BCP. However there are still many details of the BCP that are "unknowns", such as how the efficiency of the BCP responds to variations in anthropogenic and natural CO_2 (Tortell *et al.,* 2000), with suggestions that the biological pump may increase in strength with increasing atmospheric CO_2 (Riebesell *et al.,* 2007). The differences and contradictions in the suggested responses of individual species or functional groups, such as the coccolithophorids (Iglesias-Rodriguez *et al.,* 2008; Riebesell *et al.,* 2008) to changes in CO_2 concentrations highlights the fact that

knowledge of the driving factors and principal components of the biological carbon pump is still far from complete. Improvements in the understanding of processes occurring throughout the euphotic zone are required in order to understand and predict the response of the biological carbon pump to future climate change, particularly in oligotrophic oceans which make up approximately 14% of the world's surface oceans by area.

1.3.1.2 Observational information on the BCP

Measurements such as biomass estimates and carbon content of various groups of marine plankton can be used in conjunction with models to help determine the overall efficiency of the biological carbon pump. Satellite data (e.g. ocean colour data) and sediment trap data (e.g. POC flux) are also important for the production of accurate export and process models and predictions. These global models can be used to predict how oceans and ecosystems might react to climatic change in the future. Combining carbon flux data from sediment traps and drifting moorings with euphotic zone data such as nutrient data, primary production rates and plankton taxonomy data helps to define the processes occurring in the upper water column (de La Rocha 2003). Each of the component processes occurring in oceanic cycles must be individually understood in order to fully understand the overall elemental cycle (Steinberg *et al.,* 2001). Measurements of primary production rates, plankton biomass and sinking rates enable an estimate to be made of the carbon flux from the euphotic zone to the deep interior ocean. Within these observations, there are still some aspects of the biological system that are overlooked. The majority of work carried out on primary production is focused on small size-fraction phytoplankton (<20 µm), whilst information on other size fractions of plankton, particularly the microplankton, is poorly documented. Although the smaller size fractions of plankton (picoplankton and nanoplankton) are often responsible for dominating phytoplankton communities and primary production in many of the world's oceans (Platt *et al.,* 1983; Agawin *et al.,* 2000; Steinberg *et al.,* 2001; Casey *et al.,* 2007; Richardson and Jackson 2007), other size fractions also contribute to primary production, carbon and mineral cycling, as well as providing key links in the oceanic food webs (Swanberg 1983; Lessard and Swift 1985; Goldman 1993; Caron *et al.,* 1995a; Caron *et al.,* 1995b).

Whilst the majority of information gathered on plankton is based on taxonomic or functional groups (Daniels *et al.,* 2006), examining the composition of plankton communities is still extremely important in order to understand group and/or species-specific interactions within the community. These interactions have a far-reaching impact on the carbon/mineral cycles, e.g. changes in cell composition, dominance by particular groups in certain oceanic regimes, etc. However, it still remains a technical challenge to elucidate the key processes (such as production rate and biomass) of major plankton species and groups (Daniels *et al.,* 2006). Challenges arise not only from selecting the most suitable sampling method; net tows, pumped samples, bottle samples, diver collection etc., but also from the difficulties associated with accurately determining abundances of various plankton groups (Caron *et al.,* 1995b).

1.3.2 Biogeochemical fluxes

Associated with (and analogous to) the production and export of organic matter from the euphotic zone are various chemicals and compounds utilised by phytoplankton, including calcium carbonate ($CaCO_3$), and biogenic silica (bSi) (Archer 2004). Due to the absolute silica requirement for the production of siliceous frustules, diatoms play a major role in the vertical distribution and cycling of biogenic silica (Paasche 1973; Nelson and Gordon 1981; Ragueneau *et al.,* 2006). When combining the export and nutrient cycling roles played by diatoms and other plankton in the carbon pump, it becomes clear that the biogeochemical cycles of silica and carbon are tightly linked (Ragueneau *et al.,* 2006). These biogeochemical cycles are not always "coupled" in their interactions and relative concentrations, which can be affected by both environmental and biological factors (Takeda 1998; Claquin *et al.,* 2002; Ragueneau *et al.,* 2002; Ragueneau *et al.,* 2006; Pondaven *et al.,* 2007).

In addition to the fluxes of carbon, silica and calcium, the major and minor elements that primary producers consist of are all included in the export flux of organic matter from the euphotic zone, including hydrogen, carbon, oxygen, phosphorus, nitrogen, sulphur, and more than 54 trace elements and metals (Schlesinger 1997; Falkowski 2004). Plankton can also play a role in effectively

removing unwanted elements from surface waters, including heavy metal ions such as lead, copper, cadmium, zinc and nickel, where these elements can cause toxin build-up in the food chain (Sheng *et al.,* 2004).

1.3.3 The role of plankton in biological fluxes

Both autotrophic and heterotrophic microplankton play a role in the BCP, not just as a food source for larger heterotrophs (e.g. diatoms), but as producers of small amounts of slow sinking, faecal pellets (Gowing and Silver 1985) and dead cells. Cells such as diatoms and foraminifera tend to sink more quickly from the euphotic zone due to the density and ballasting effects created by silica frustules and calcium carbonate skeletal material of these two groups respectively (Armstrong *et al.,* 2002; Sweeney *et al.,* 2003). Aggregations of plankton cells incorporated into large masses or flocs are also common, but depend on various factors including the stickiness of particles colliding, water properties such as turbulent shear, and the concentration of particles suspended in the water (Kiorboe *et al.,* 1994; Kahl *et al.,* 2008). This method of plankton vertical transport from the surface waters down to the seafloor has been implicated to be a mechanism of global significance in the cycling of organic carbon (Kiorboe *et al.,* 1990). Similar implications to the vertical transport of phytoplankton matter apply to aggregates of live phytoplankton cells (Smetacek 1985). Due to the lack of microplankton-specific studies, details of their contribution towards the individual biological fluxes are still unclear.

The Deep chlorophyll-a (CHLa) maximum (DCM) is an important feature of stratified oceanic water bodies (Figure 2). The DCM is usually closely linked to the mixing and stratification of the water column. During a phytoplankton bloom, the DCM is found nearer the surface, and in more stable oceanic environments such as the Sargasso Sea, the DCM is usually found close to the bottom of the euphotic zone (Longhurst and Harrison 1989) or below depths of ~80m at the BATS site (Steinberg *et al.,* 2001), and often referred to as the subsurface chlorophyll-a maximum (SCM). Although useful as an indication of the levels of phytoplankton present in the euphotic zone, information such as the depth and chlorophyll concentration of the DCM provide an incomplete description of

profiles of algal biomass and growth in the ocean (Longhurst and Harrison 1989). Identification of the cells present in the SCM and specific chlorophyll concentrations provide more information about the community structure present in the euphotic zone. Chlorophyll values can then be used to derive values for phytoplankton carbon per cell using a suitable Carbon to Chlorophyll a (C:CHLa) ratio that takes into account variables such as time of day, light levels, temperature and nutrient limitation etc. (Banse 1977; Longhurst and Harrison 1989; Riemann *et al.,* 1989; Sathyendranath *et al.,* 2009). However, this is usually a fairly generalized ratio devised for functional groups or genera, and the calculated carbon content of cells can therefore vary dramatically, depending on the method of volume calculation and the specific C:CHLa ratios used. Not only are plankton important when calculating C:CHLa ratios or cell carbon content etc., the structure of a plankton community can also provide invaluable details about delicate and sometimes highly specialised food-webs in different oceanic regimes such as oligotrophic regimes. This information can then be added to other information available for producing community, food-web, and even more general oceanic models.

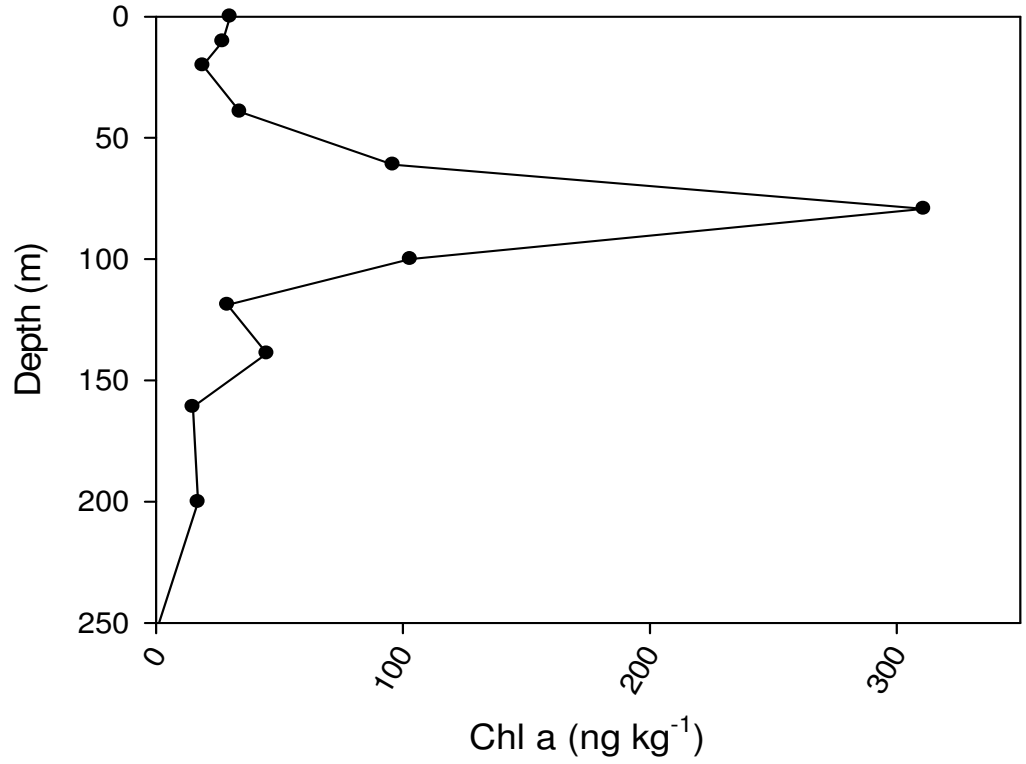


Figure 2 - A typical early summer (May 2000) depth profile of Chlorophyll a from the BATS site, showing a narrow single Chl a peak at a depth of ~80m.

1.4 The Sargasso Sea

1.4.1 Introduction to the Sargasso Sea

The Sargasso Sea (the western North Atlantic subtropical gyre) is an area in the North Atlantic (Figure 3) found to the north of the North Atlantic equatorial current, and bordered to the east and northeast by the Gulf Stream (represented by the most northerly black contour). A region of mode water formation is found between the Gulf Stream and 31°N latitude (Talley 1982) with deep mixed layers (~250-400m) being formed by convective mixing (Michaels and Knap 1996).

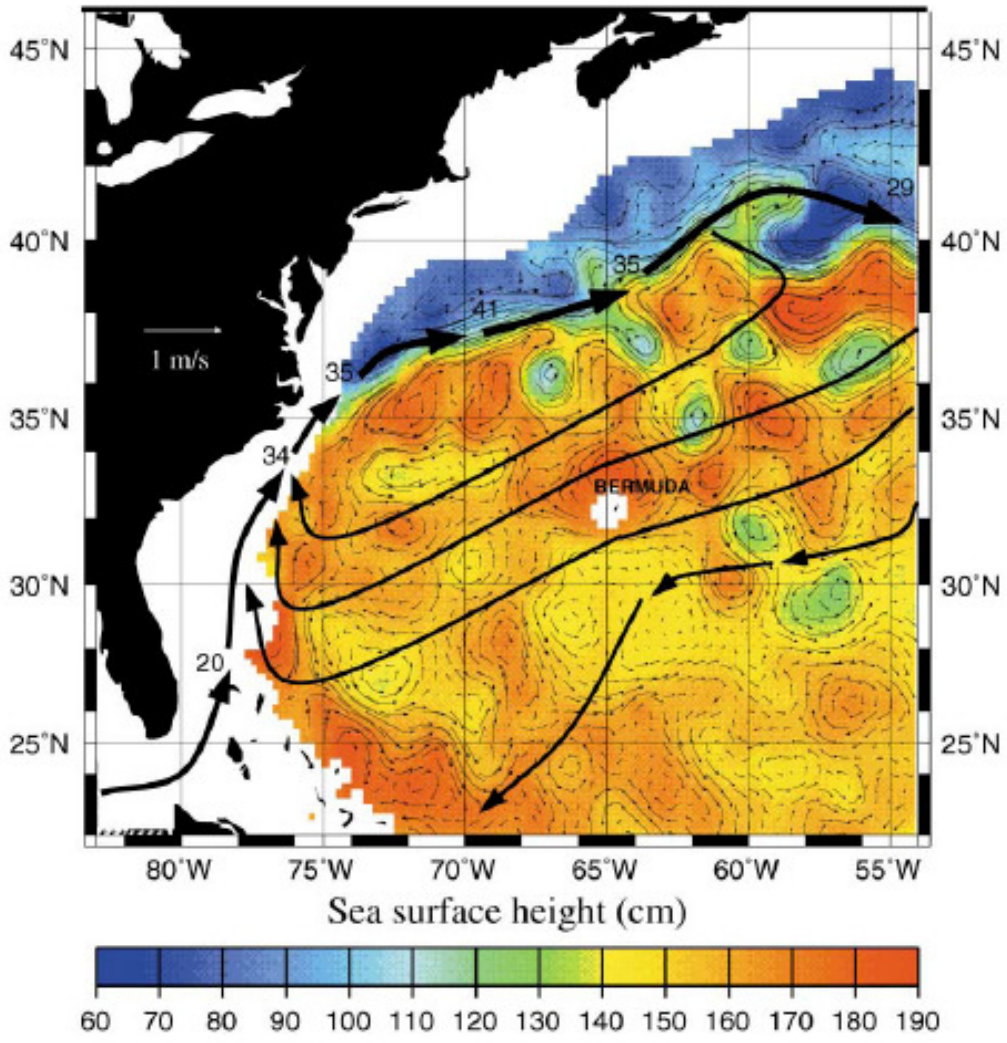


Figure 3 - Map of the Sargasso Sea showing surface height (SSH), geostrophic velocity and long-term mean circulation of the subtropical gyre including the Gulf Stream (thick black arrows). (Michaels and Knap 1996).

During the summer months, the Bermuda-Azores high pressure system influences most of the Sargasso Sea, preventing the movement of cold fronts and enabling a fresh, warm, shallow mixed layer to form, shoaling to depths less than 20m (Steinberg *et al.,* 2001). This high pressure system weakens throughout autumn and winter as cold, dry air and strong winds associated with storm fronts move down from North America and help to deepen the mixed layer by homogenizing and cooling the surface waters of the sea (Michaels and Knap 1996). This 18°C water (also known as subtropical mode water - STMW) separates the seasonal and permanent thermocline as it sinks and spreads southwards (Worthington 1976).

Levels of primary production in the Sargasso Sea are reliant on vertical mixing of the water column, and are highest in the deeply mixed winter and early spring waters (DuRand *et al.,* 2001) with chlorophyll a concentration peaks observed between 60-120m (Steinberg *et al.,* 2001). Due to the large seasonal variations seen in the structure of the water column, the Sargasso Sea is a prime sampling area for observing the effects of this seasonal physical forcing on phytoplankton physiology (Goericke and Welschmeyer 1998) and community composition.

For most of the year, the euphotic zone of the western Sargasso Sea has extremely low nutrient concentrations (Michaels *et al.,* 1994), creating an oligotrophic environment for the growth of marine organisms. Net vertical motions in the upper ocean driven by wind friction, and determined by divergence of surface currents, act to produce a net downwelling of nutrients in the subtropical gyres, resulting in a very small supply of nutrients available for photosynthesis (Peixoto and Oort 1992). The venting of the cold, nutrient rich 18°C water (STMW) to the surface in winter is quickly followed by the occurrence of the spring phytoplankton bloom (DuRand *et al.,* 2001). When the surface waters become stratified, this bloom is followed by a chlorophyll maximum close to the bottom of the euphotic zone, with production in these waters highly dependent on vertical mixing (DuRand *et al.,* 2001).

1.4.2 The Bermuda Atlantic Time-series Study (BATS)

1.4.2.1 History and importance of BATS

The Bermuda Atlantic Time-series Study commenced monthly sampling in October 1988 in the western North Atlantic subtropical gyre (Figure 4) as part of the US Joint Global Ocean Flux Study (JGOFS) program (Steinberg *et al.,* 2001), initiated with funding from the US National Science Foundation (NSF). One of the focuses of the US JGOFS program was "To determine and understand on a global scale the processes controlling the time-varying fluxes of carbon and associated biogenic elements in the ocean." (SCOR 1987). The BATS deployment area lies 82km southeast of the Bermuda islands (31°40'N, 64°10'W) in approximately 4680m of water (Steinberg *et al.,* 2001). There are other sampling programmes in operation in the area, including the Ocean Flux program (OFP) and the Bermuda Testbed Mooring site. Regular sampling in the Sargasso Sea grew into time-series sampling in 1954, when Henry Stommel and colleagues began the bi-weekly occupation of Hydrostation "S", 26km offshore from Bermuda (Michaels and Knap 1996); this time-series position is still regularly sampled to date. The regular 4-5 day long BATS research cruises are carried out at monthly intervals, with additional 'bloom' cruises occurring between January and April, and an annual 'validation' cruise to resolve spatial variability of biogeochemical parameters close to the BATS site (Steinberg *et al.,* 2001).

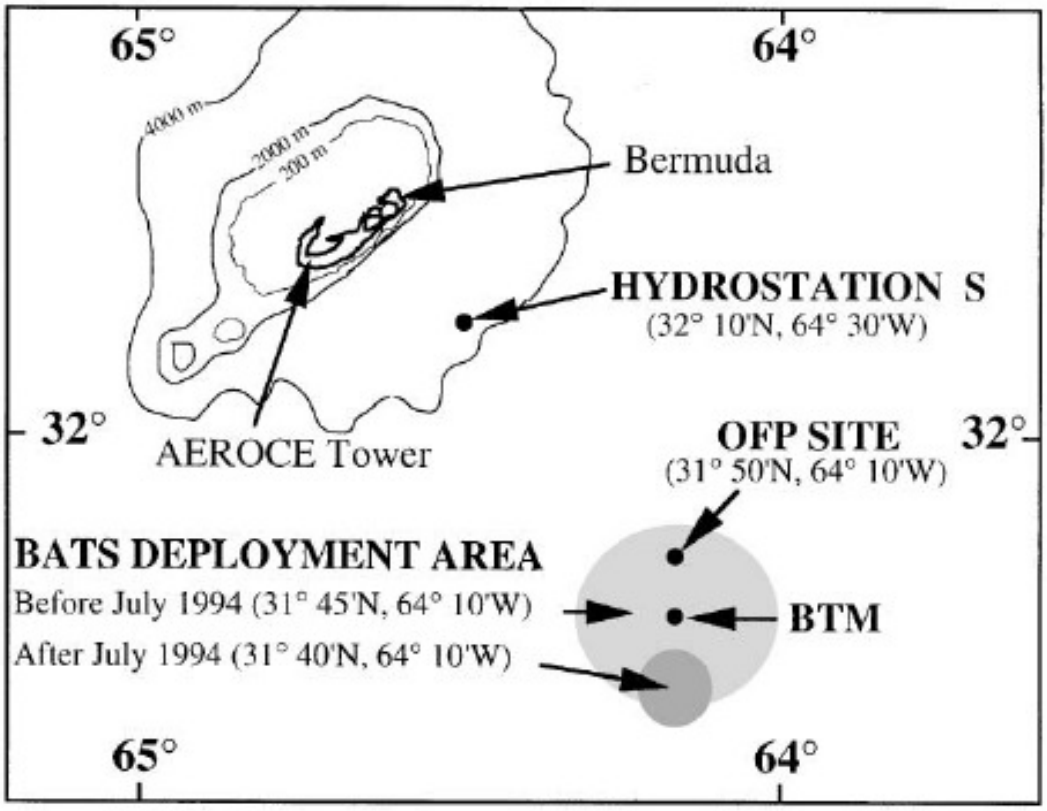


Figure 4 - Map showing location of Bermuda and the various time-series sampling sites. BTM; Bermuda Testbed Mooring. OFP; Ocean Flux Program, AEROCE; Air-Ocean Chemistry experiment (Steinberg *et al*., 2001).

1.4.2.2 Oceanic regime at the BATS site

The BATS sampling site is located in a region of the Sargasso Sea that shows net flow towards the southwest, with weak geostrophic recirculation (Siegel and Deuser 1997). This combination of geostrophic recirculation and net flow drives the Ekman transport, with a net downwelling rate of ~4cmd^{-1} (McClain and Firestone 1993). The BATS site has stronger seasonal forcing than its sister station HOT (Hawaii Ocean Time-series) in the Pacific, resulting in a higher input of new nutrients to surface waters, and export pathway characteristics in spring. The BATS site is dominated by a regeneration loop in summer and autumn (Brix *et al.,* 2006), which is consistent with low nutrient input resulting from summer stratification of the water column (the HOT site is dominated by the regeneration loop all year round) (Brix *et al.,* 2006). Variations in planktonic community composition can have a large impact on the carbon and nutrient reservoirs in the surface waters, and can act to alter air-sea surface exchanges of CO_2 and the amount of carbon stored in subsurface layers of the ocean (Brix *et al.,* 2006) and exported out of the euphotic zone (Cullen *et al.,* 2002).

Seasonal patterns in the biogeochemistry and physical regime of the BATS area have been described (Michaels *et al.,* 1994), as well as overviews of the U.S. JGOFS BATS site (Michaels and Knap 1996; Steinberg *et al.,* 2001). The biogeochemistry of the area is influenced by strong meridional gradients and the area of water south of Bermuda shows the characteristics of an oligotrophic ecosystem throughout the year, with a permanently stratified water column (Michaels and Knap 1996).

1.5 Plankton of the Sargasso Sea

The importance of phytoplankton to biogeochemical cycles has previously been discussed in section 1.3.3. The majority of marine plankton are also short-lived, highlighting a tight coupling between plankton dynamics and environmental change, as population size is only minimally influenced by community persistence from previous years (Hays *et al.,* 2005). In order to fully understand the processes controlling plankton biomass and driving cycles of primary production, a wide variety of key information is needed. This includes obtaining details of changes in community composition, cell size and abundance, along with the reactions of organisms to changing environmental parameters.

1.5.1 Plankton community structure

Significant seasonal and inter-annual variability in phytoplankton and bacterioplankton production, biomass, and community structure exist at the BATS site (Steinberg *et al.,* 2001). The cycle of phytoplankton production and abundance follows the seasonal patterns present in the water column structure, with the spring bloom occurring after the 18°C water becomes well mixed up to the surface of the water column (DuRand *et al.,* 2001). Figure 5 shows the depth profile of a typical summer water column, with Chl a representing phytoplankton biomass.

During "bloom" periods (periods showing rapid growth of phytoplankton populations), the increase in chlorophyll a concentration at BATS is reportedly not due to a significant increase by any one phytoplankton group, but instead the result of an increase in most of the picophytoplankton taxa found at BATS (Steinberg *et al.,* 2001). The prokaryotic picoplankton (prochlorophyte-like cells) regularly dominate the phytoplankton community at the BATS site, as suggested by the extracted Chl b values from HPLC pigment data (Steinberg *et al.,* 2001). Picoplankton such as *Prochlorococcus* are ubiquitous in tropical oceans, and are known to contribute to new production in oligotrophic areas such as the Sargasso Sea (Casey *et al.*, 2007). Although diatom blooms are rare, they do periodically occur at the BATS site, with a seasonal succession seen in the pattern of

phytoplankton (Riley 1957; Hulburt 1990; DuRand *et al.,* 2001; Steinberg *et al.,* 2001). Bidigare *et al*., (1990) documented an observed succession in the phytoplankton population during a spring bloom in the Sargasso Sea in 1985, starting with a diatom-dominated population, becoming more diverse with time to include prymnesiophytes, cyanobacteria, dinoflagellates, prasinophytes and diatoms. Analysis of the eukaryotic phytoplankton in the Sargasso Sea between the years of 1989 and 1994 found that the plankton was dominated by populations of small nanoplankton (2-4µm in diameter), with pennate diatom and coccolithophore populations also distinguishable (DuRand *et al.*, 2001). Including the coccolithophores, the eukaryotes showed a spring bloom and an autumn bloom, with picoplankton chlorophyll fluorescence and estimated cell size greater at depth (DuRand *et al.*, 2001).

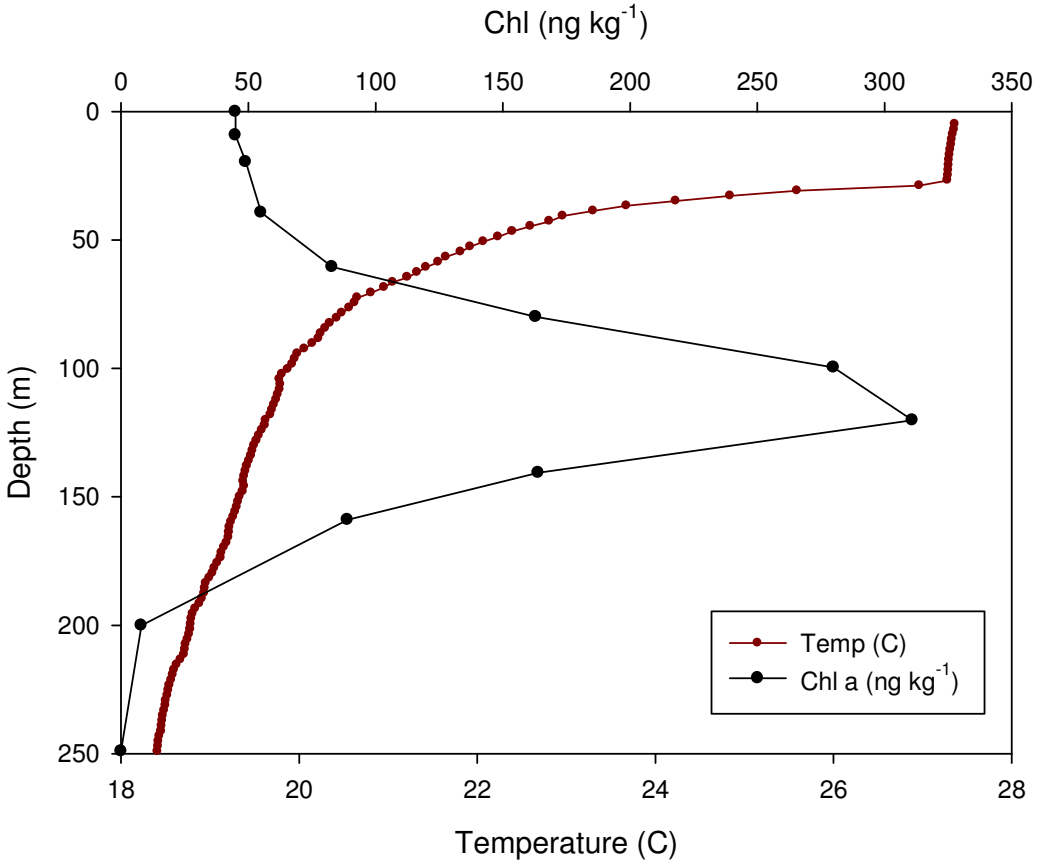


Figure 5 - Vertical profile of temperature and chlorophyll a at the BATS site; showing a typical autumn (August 1999) profile. A single, wide Chl a peak can be seen with a maximum at ~120m depth. Stratification of the upper water column is shown by a shallow mixed layer of ~ 28m depth.

The most abundant size group of the phytoplankton in the Sargasso Sea has previously been documented as the nanoplankton (2-15μm diameter) and the picoplankton (cells <2μm diameter) (Riley 1957; Goldman 1993; Lessard and Murrell 1996; DuRand *et al.,* 2001). However, it has also been reported that in the low-productivity, low-nutrient regimes associated with subtropical gyres (Sarmiento and Gruber 2006), small nano- and microplankton graze effectively on picophytoplankton (Brix *et al.*, 2006) and ultraplankton in the microbial loop, preventing large numbers of picophytoplankton accumulating in these regions. The ultraplankton (<5μm) do not make an appreciably large contribution to the biomass flux out of the euphotic zone, presumably due to their role in the microbial loop as prey (Glover *et al.*, 1988), and their extremely low sinking rates as individuals from the euphotic zone (Bienfang and Takahashi 1983). However, when considered as individual cells aggregating, even non-mineral ballasted cells that clump to a large size will still sink quickly according to the principles of Stokes' law (Orr 1966; Lamb and Lamb 1997).

Other shifts in phytoplankton communities have also been observed for example increased percentages of diatoms and dinoflagellates found to be present in mode-water eddies in the Sargasso Sea (Sweeney *et al.,* 2003) (see Introduction 1.6.1 for details on eddy types). It is suggested that although during the formation of eddies in subtropical gyres, the phytoplankton response comprises a shift in community structure towards a community dominated by larger individuals, it is only mode-water eddies that maintain this change in phytoplankton signal for time periods proportional to the eddy lifetime (Bibby *et al.,* 2008; Mouriño-Carballido 2009; Krause *et al.,* 2010). This build-up in diatom biomass in mode-water eddies is supported by further studies based on the impact of cyclonic and mode-water eddies on particle flux in the Sargasso Sea (Buesseler *et al.*, 2008). Zooplankton biomass was highly variable over an anticyclonic mode-water eddy, consistent with patchy diatom distribution in the region (Goldthwait and Steinberg 2008) observed alongside mesoscale eddy nutrient injection into the euphotic zone (Li and Hansell 2008).

The phytoplankton of the North Central Sargasso Sea were first described in 1957 from bottle-collected water samples, with the diatoms described as having a

spring increase in April, a seasonal minimum during the autumn months and a larger winter population than during the summer months (Riley 1957). This can be linked, in part, to their adaptations to turbulent, unstable conditions (Margalef 1978), although it has been noted that diatom blooms at the BATS site do occur, but are rare (Steinberg *et al.,* 2001). Other phytoplankton groups such as coccolithophores were also described as numerically important in later studies of the area (Marshall 1966). Processes such as coccolithophore calcification have been inferred from non-conservative decreases in alkalinity (Bates *et al.*, 1996), whilst additional studies have been carried out on the plankton at the BATS site and their oceanic controls (DuRand *et al.*, 2001). These studies include the diatoms, heterotrophic dinoflagellates and ciliates, the microbial size spectra and heterotrophic bacteria (Carlson *et al.*, 1996). Temporal and spatial variations in phytoplankton physiology have been confirmed by nutrient addition bioassays and other diagnostic experiments (Li and Hansell 2008), as well as different limiting factors being identified. These include P-limitation for bacterioplankton (Cotner *et al.*, 1997), P and Fe co-limitation of N-fixing organisms (Mills *et al.,* 2004), Si-limitation of marine diatoms (Brzezinski and Nelson 1996), as well as community-wide N-limitation (Graziano *et al.*, 1996).

1.5.2 Microplankton of the Sargasso Sea

Although a few descriptive reports exist of microplankton species found in the waters of the Sargasso Sea (Table 1), there is very little information available about the single-celled microplankton community as a whole, or indeed some of the other groups found in the microplankton, such as tintinnids and silicoflagellates. Species listings are generally from early studies published in the late 1950's and 1960's, and there is a distinct lack of recently published information available about the microplankton community composition at the BATS site. Table 1 documents the specific Sargasso Sea information available for each group being considered in this investigation, with lists of most common species/genera for each group (where available). Different sampling techniques are also mentioned, highlighting the wide variety of sampling historically used.

Reports of heterotrophic microplankton grazing effectively on the smaller picophytoplankton and preventing their numbers accumulating (Lessard and Murrell 1998; Worden and Binder 2003; Brix *et al.,* 2006) suggest that in some cases, microplankton in these areas act to exert a top-down feeding pressure on the smaller size classes. However, numerous studies reporting the picophytoplankton dominance of phytoplankton communities (Riley 1957; Platt *et al.,* 1983; Goldman 1993; Lessard and Murrell 1996; Caron *et al.,* 1999; DuRand *et al.,* 2001; Steinberg *et al.,* 2001; Casey *et al.,* 2007), particularly in the BATS area, suggest that microplankton abundance is too low to exert a sustained top-down pressure on the picophytoplankton. An argument to this view is presented in work by Worden and Binder (2003), Worden *et al*., (Worden *et al.,* 2004) and Strom *et al*., (Strom *et al.,* 2007) (amongst others), where microzooplankton grazing rates were recorded to be comparable in magnitude to phytoplankton physiological growth rates.

	Author and year	Key information	Methods	Dominant species	Notes
Diatoms	Riley, 1957	- Mainly subtropical, ubiquitous diatom spp., localised patches of temperate spp. - Diatom max. April, min. August (1950-1951). - Av. Daily rate of net phytoplankton primary production 0.15 g C m^{-2} d^{-1}. - Abundance ranged from 0-100,000 cells per litre (mostly 1-3,000 l^{-1}). - Little seasonal variation in the non-diatom fractions of the phytoplankton	400 ml sample from Nansen bottles, settled to 2.5 ml.	*Chaetoceros* spp., *Rhizosolenia* spp., *Corethron hystrix*, *Bacteriastrum delicatulum* *Stephanopyxis* spp., *Nitzschia* spp. (see Riley 1957 for list of diatoms contributing 5 % or more to total cell count on any one occasion)	- Samples taken at discrete depths of 0, 25, 50, 100 and 150 m. - Nanoplankton 5-15 µm included in counts (averaging ~100,000 cells per litre). - Patches of temperate water diatom spp. are suggested to indicate the presence of mesoscale eddies.
	Goldman, 1993	› Large, rare diatoms at the base of the euphotic zone contribute significantly to Sargasso Sea new production		*Stephanopyxis palmeriana*	
	Nelson & Brzezinski, 1997	· Small component of the Sargasso Sea phytoplankton at all times excl. winter/spring bloom · Actively growing even when abundance low	Rate of biogenic silica production measured using sediment traps.		· Silica uptake used to determine contribution of diatoms to new production
	Goldman & McGillicuddy, 2003	○ Diatoms able to utilize nutrients (input from eddies) at bottom of euphotic zone	Net tows at 100 m, laboratory culturing.		
Dinoflagellates	Riley, 1957	- Second in relative abundance to diatoms. - Several genera of dinoflagellate found thriving at 50 or 100 m	400ml Nansen bottle samples, settled to 2.5 ml	*Peridinium* spp., *Ceratium* spp., and *Dinophysis* spp.	- Less variable in abundance over the sampling period than diatoms.
	Hulburt, 1961	› Species recorded are characteristic of tropical open ocean, excluding a few.		*Oxytoxum variabile*, *Katodinium rotundatum*, *Ceratium* spp.	› Small range of values along the sampling transect, but slightly more diverse than predicted.

	Lessard & Murrell, 1996	· Average size of a >20 µm dinoflagellate cell was 4800 μm^3. · >20 µm cells dominated the integrated biomass, despite numerical dominance of cells <20 µm in size.	220 ml sample preserved with 1% glutaraldehyde, DAPI stained. 10-30 litres concentrated to 100 ml for rare cells.	*Gyrodinium* spp., *Protoperidinium* spp., *Diplopsalis* types., *Gymnodinium heterostriatum*,	· >92 % of dinoflagellate cells were <20 µm · Dinoflagellates contributed between 21-96 % of total biomass, abundance 2-28 ml^{-1}.
Tintinnids	Schnetzer & Steinberg 2002	- Loricae found in gut contents of 2 common copepods and 1 common euphausiid	500µm zoopl. net. Gut content analysis – dissection	*Cyttarocylis* spp., *Codonellopsis* spp., *Epiplocylis* spp., *Favella* spp., *Dictyocylis* spp., *Xystonellopsis* spp.	
Radiolaria	Swanberg & Caron, 1991	- Consume tintinnids, mollusc larvae & copepods as primary food source	10, 35 and 150 µm surface tows, preserved in buffered formalin for microscopy	*Styptosphaera* spp., *Physematium muelleri*, *Collosphaera* spp., *Collozoum serpentinum*	
	Caron *et al*., 1995	› Colonial and solitary Radiolaria showed highest rates of primary productivity from symbiotic algae › Typical abundances of ≤1 m^{-3} for large solitary and colonial Radiolaria.	Cells collected by divers. 20 L carboy samples also collected by divers.	*Collozoum* spp., *Physematium muelleri, Spongostaurus* spp., *Thalassicolla* spp., *Acrosphaera* spp.,	› Total symbiont production is generally <1 % of total production in surface waters due to low abundances of sarcodines. › Juveniles more abundant
	Michaels *et al*., 1995	Majority of cells counted were >330 µm size.			
Foraminifera	Swanberg & Caron, 1991	- Consume mostly copepods and ciliates.	10, 35 and 150 µm surface tows, preserved in buffered formalin for microscopy	*Globigerinella siphonifera, Hastigerina pelagic, Orbulina* spp., *Globigerinoides* spp.	

	Caron *et al*., 1995	› Typical abundance ≥10 L^{-1} (adult cells)	Cells collected by divers. 20 L carboy samples (diver collected).	*Globigerinella siphonifera, Globigerinoides* spp., *Orbulina universa*.	
	Michaels *et al*., 1995	· Abundance never exceeded 4 L^{-1}, mostly <1 L^{-1}. · Carbon content ave. 0.089 pg C μm^{-3} (higher in individuals without terminal spherical chamber) · Most spring cells were <83 µm, whilst autumn cells were mostly 83-330 µm.	Phyto. Tows, pumped and Niskin bottle samples. Biovolume estimated.		· Biovolume usually dominated by the smaller sized cells.
Silicoflagellates					No information available
Acantharia	Swanberg & Caron, 1991	- Consumed mostly tintinnids and 'other ciliates' as well as small acantharia and small radiolaria.	10, 35 and 150 µm surface tows; buffered formalin for microscopy	*Acanthometra* spp., *Amphilonche* spp., *Diploconus* spp., *Tetralonche* spp.	
	Michaels *et al*., 1995	› Low carbon: volume ratio (0.0026 pg C μm^{-3}) › Most cells in samples were <83 µm diameter. › Cells 83-330 µm usually <2 L^{-1}, cells >330 µm rarely >0.04 l^{-1}.			› Few cells >330 µm often contributed at least half of the biovolume (and biomass). › Abundances and biomass patterns similar to those for the Pacific.

Table 1 - Microplankton of the Sargasso Sea; overview of relevant journal articles and their key findings.

1.5.2.1 Microphytoplankton

Microphytoplankton include the photoautotrophic organisms within the microplankton size fraction, such as diatoms, silicoflagellates and some dinoflagellates. From early descriptions of the plankton in the Sargasso Sea, diatom concentrations were found to be highly variable, with cells per litre values ranging from ~0-100,000, although cell concentrations were mostly between 1,000-3,000 cells L^{-1} (Riley 1957). These samples were primarily bottle-collected samples containing phytoplankton of all sizes, rather than purely microphytoplankton samples as would be sampled by a 35µm mesh plankton net. The same study documented predominantly subtropical or ubiquitous diatom species, with the occasional localised patch of temperate-water diatoms suggesting the impact of small-scale eddy transfer from more northerly waters. Some of the diatoms identified to be dominant (accounting for >5% of sample on any one occasion) included *Rhizosolenia* spp., *Bacteriastrum* spp., *Coscinodiscus* spp., *Thalassiothrix* spp., *Chaetoceros* spp., and *Nitzschia* spp. (Riley 1957). A list of single celled microplankton genera and species identified from the current study is included in Appendix 10.2.

At Hydrostation "S", the late winter / spring diatom bloom has previously been reported to be responsible for 62% of the annual biogenic silica ($bSiO_2$) flux (Nelson and Brzezinski 1997; Scharek *et al.,* 1999b). Diatoms are also considered to be responsible for an estimated 30% of global primary production (Krause *et al.*, 2009), highlighting the importance that this phytoplankton group plays in oceanic cycles, as well as the source of most organic matter exported to depth in the Sargasso (Goldman 1993). Early studies reported microscopic identification and enumeration of diatoms (Riley 1957; Hulburt 1961), whereas the diatom pigment fucoxanthin is now used as a proxy measurement for estimating diatom biomass (Malone *et al.,* 1993; Krause *et al.,* 2009).

Diatoms have been reported to be the main phytoplankton source of particulate organic material (POM) production and sinking in the oceans (Michaels and Silver 1988). Despite their low cell abundances therefore in oligotrophic waters, diatoms may provide a disproportionately important role in the export of POM

from the euphotic zone down to depth. Scharek *et al*., (1999a) described the microplankton assemblages found in the DCM in the oligotrophic environment of the North Pacific Gyre (characterized by *Pseudonitzschia* spp. and *Thalassionema* cf. *bacillare*) as being different to those found in the mixed layer (characterized by the presence of *Mastogloia* spp., *Hemiaulus* spp. and *Guinardia cylindrus*). The requirement of silica for diatoms and the important role they play in the flux of biogenic silica (Scharek *et al.,* 1999a) means that particulate $bSiO_2$ can also be used as a proxy for estimating diatom biomass (Brzezinkski and Nelson 1995; Nelson and Brzezinski 1997). Silicoflagellates also have a silica requirement, as they posses an internal siliceous skeleton comprised of tubular silica elements arranged in a radial pattern (Sieburth 1979), however there are no reports in the literature detailing the silicoflagellate community in the Sargasso Sea.

Larger plankton species of diatom (>50µm diameter) are considered to have a disproportionately large contribution to carbon export production as they are ubiquitous in the world's oceans, despite normally being found in "background" numbers (Goldman 1993; Goldman and McGillicuddy Jr 2003). Counter to this suggestion are reports that although high in numbers, mats of large diatoms do not contribute significantly to carbon flux to the deep waters in the North Atlantic in the same way as in the North Pacific, even when they account for the majority of plankton flux from the euphotic zone (Sancetta *et al.,* 1991; Pilskaln *et al.,* 2005). Even when present only in low 'background' abundances, models show that these larger phytoplankton species are also responsible for a large fraction of the biomass flux out of the euphotic zone (Michaels and Silver 1988). Background level abundances of large, rare, fast-growing diatoms respond quickly to elevated nutrient availability, and subsequently produce the coupled export out of the euphotic zone (Goldman and McGillicuddy Jr 2003). The levels of biomass flux are enhanced by the occurrence of episodic blooms, either found in oceanic surface waters e.g. *Stephanopyxis* and *Pseudoguinardia* (Alldredge and Silver 1982), or in distinct layers near the base of the euphotic zone (Goldman 1993). Diatom blooms at this depth can easily go unnoticed especially if they are of a short duration or occur just above the nutracline, hence the slightly distorted view on ocean production by this size group (Goldman 1988). The problem of

"overlooking" these occurrences is compounded by the fact that most sampling carried out is not targeted specifically to the bloom-forming diatoms.

Diatoms are generally considered more important as planktonic primary producers than dinoflagellates (Guillard and Kilham 1977; Sieburth 1979), although in some areas, dinoflagellates are found to dominate over the diatoms. This is often the case in the subtropics and tropics, where the warmer stratified waters are dominated by small dinoflagellate populations, with high species diversity (Sieburth 1979). Dinoflagellate abundances in the Sargasso Sea are reported to be less variable than diatom numbers, with *Peridinium, Ceratium* and *Dinophysis* species all found at depths of 50-100m (Riley 1957). A study by Lessard and Murrell (1996) reported that large dinoflagellates (>20μm, predominantly *Gyrodinium* and *Protoperidinium* spp.) dominated the integrated biomass, with the majority of the dinoflagellate biomass found below the mixed layer. In an early description of phytoplankton within the Sargasso Sea, dinoflagellates were found to be less abundant than other groups of phytoplankton (e.g. diatoms), with abundances averaging around 1000 cells L^{-1} when combined with the coccolithophores and silicoflagellates (Riley 1957). In addition to the autotrophic species within the dinoflagellates, heterotrophic and mixotrophic species of dinoflagellate are also commonly found (Stoecker 1999).

1.5.2.2 Microzooplankton

There has been little attention given to the heterotrophic role of dinoflagellates as microzooplankton in the world's oceans, despite the fact that the non-photosynthetic nature of many species has been documented since early taxonomic studies of this functional group (Lessard and Swift 1985; Jacobson 1999) and references therein. This may be due to a number of factors, including the inability to distinguish between heterotrophic and photoautotrophic species of dinoflagellates in preserved samples using traditional light microscopy methods, and without the use of epifluorescence (Lessard and Swift 1985). Whilst there are many species of dinoflagellate that are photoautotrophs, there are also some heterotrophic species which exhibit a phagotrophic nutrition mode as well as mixotrophic species; some acting as obligate heterotrophs, some with the ability

to pursue opportunistic mixotrophy (Gaines and Taylor 1984; Hansen and Caladao 1999; Jacobson 1999; Jeong 1999; Stoecker 1999).

As awareness and knowledge of the microbial loop has improved and progressed over the years, more importance and ecological significance has been given to groups such as the dinoflagellates (Jacobson 1999), not only as prey for planktonic predators such as zooplankton (Schnetzer and Steinberg 2002) but also as grazers on smaller phytoplankton and juvenile zooplankton (Lessard and Swift 1985; Jacobson 1999; Jeong 1999; Stoecker 1999). Observations such as these have led to the conclusion that the heterotrophic and mixotrophic dinoflagellate community play an important role as microheterotrophic grazers. This role is thought to be equally as important (and larger than previously thought), as the role played by typical microzooplankton such as ciliates (Lessard and Swift 1985; Calbet 2008). This is an important conclusion, due to the fact that these organisms can be present as a significant portion of the net microzooplankton assemblage (Lessard 1984; Jeong 1999). An average ratio of 0.4 heterotrophic dinoflagellates to total dinoflagellates found in Atlantic plankton (Lessard 1984) can be used to provide an estimate of heterotrophic vs. autotrophic fractions of the dinoflagellate population.

In addition to mixotrophic and heterotrophic dinoflagellates, radiolaria, foraminifera, acantharia and tintinnids are included in the single-celled microzooplankton. Radiolaria are included in the group of 'planktonic sarcodines' along with the foraminifera and acantharia and, like the diatoms, also have a silica requirement for their siliceous skeletons. Radiolaria are mostly restricted to tropical and subtropical waters, and are almost exclusively found in oceanic regions, with characteristically low abundances of <1-10 per m^3 in oligotrophic environments (Michaels *et al.*, 1995). The patchy distribution and the delicate structural nature of these organisms, combined with their low abundances, make the radiolaria (as with the other planktonic sarcodines), difficult to study especially when all planktonic sarcodines are represented in one sample (due to preservation complications) (Swanberg and Caron 1991; Michaels *et al.,* 1995). Previous studies have deemed radiolaria in the Sargasso Sea to be too sparse to allow abundances to be estimated accurately, although sizes of individuals

sampled were mostly >330µm diameter (Michaels *et al.*, 1995). Swanberg and Caron (1991) documented radiolaria consuming other zooplankton such as tintinnids and copepod nauplii, as well as mollusc larvae, and it is suggested (contrary to previous thinking) that there is substantial overlap between the diets of radiolaria, foraminifera and the acantharia (Swanberg and Caron 1991).

Planktonic foraminifera also have a cosmopolitan distribution in the world's oceans, and are of significant biological and geological importance, especially in oligotrophic oceans, with their $CaCO_3$ skeletons used in paleo-climatological reconstructions, and the analysis of marine sediments (Bolli *et al.,* 1985; Gast and Caron 1996). It is suggested that forams (and indeed other planktonic sarcodines) may be important in the vertical flux of material out of the euphotic zone, due to their high metabolic activities, their large size and the high specific gravity of their calcite skeletal material, allowing relatively rapid sinking (Michaels *et al.*, 1995). Carbon content can vary by as much as an order of magnitude between individuals of different species (Michaels *et al.*, 1995). They are active grazers, are known to consume a range of phytoplankton, as well as smaller zooplankton such as copepods and copepod nauplii (Swanberg and Caron 1991). In the waters around Bermuda, forams have varying vertical patterns and abundances mostly <1 L^{-1}, never exceeding 4 L^{-1} with spring sizes usually <83µm and autumn sizes 83-330µm, recorded from pumped water samples and Niskin bottle samples (Michaels *et al.*, 1995). Abundances of adult specimens in these waters are reported to be ≥ 10 Cells L^{-1} estimated from diver-collected 20 L water samples (Caron *et al.,* 1995b).

Less is known about the acantharia, primarily due to the problems of storage and preservation (Massera-Bottazzi *et al.,* 1971). Their strontium sulphate ($SrSO_4$) spines dissolve in most common preservatives (Massera-Bottazzi *et al.,* 1971; Swanberg and Caron 1991), unless a buffered preservative with added strontium (e.g. borate buffered formalin with added strontium chloride) is used (Beers and Stewart 1970). When compared with other planktonic sarcodine abundances (radiolaria and foraminifera) in the Sargasso Sea, the acantharia were on average the most abundant with a maxima near the surface, and a low carbon: volume ratio (Michaels *et al.*, 1995). As almost all acantharia species possess algal

symbionts at some point in their life cycle, this euphotic zone maxima may represent the need of the symbionts for light to support photosynthesis (Michaels 1991). In general, sarcodine fluxes in the Sargasso sea show a maximum during winter, spring and early summer, followed by low sarcodine fluxes in summer and early autumn (Michaels *et al.*, 1995). Despite what is already known about the acantharia and other members of the planktonic sarcodines, the overall importance of sarcodines on the euphotic zone carbon flux is still somewhat of an unknown quantity, and is poorly documented (Michaels *et al.*, 1995). Long-term seasonal and annual data on Acantharia abundances and their contribution to the total microplankton population is presented in this study for the first time.

1.6 Temporal variability

Temporal variability within the world's oceans can occur on a number of different time scales, from the daily (diel) variations of migrating zooplankton, to seasonal, annual, and even long timescales such as decadal. Recognisable variations occur on the mesoscale (both temporally and spatially), such as the passage of an eddy through a particular area, although diel and seasonal changes are most frequently reported (Côté and Platt 1983). All scales of variability are important when considering the biological response of communities to changes in biogeochemical and physical parameters in their environment.

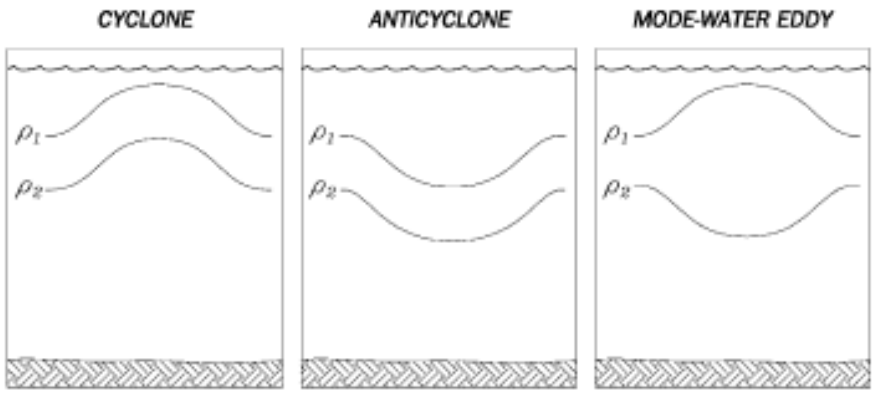
1.6.1 Scales of variability and their driving forces in the Sargasso Sea

Seasonal patterns are seen not only in the physical structure of the water column at the BATS site, but also within the nutrient cycling and geochemistry of the area, including alkalinity (Bates *et al.*, 1996), the export of particulate silica (Brzezinkski and Nelson 1995) and phosphorus availability (Lomas *et al.*, 2004). As described in section 1.4.2.2, there is strong seasonal variability at the BATS site, evident in many sampled parameters from temperature, chlorophyll a concentrations and dissolved organic and inorganic carbon (DOC / DIC). These seasonal changes are driven by deep winter mixing in the water column as storm fronts pass through the area, and summer stratification as a result of the Bermuda-Azores high pressure system (Michaels and Knap 1996; Steinberg *et al.,* 2001). Seasonal variations in physical parameters such as temperature and mixed layer depth prompt the input of nutrients into the euphotic zone in winter, followed by the spring seasonal variation in biological activity, most clearly observed in the pigment concentrations such as chlorophyll a. It is possible that seasonal changes may be masked by one-off events such as the passage of eddies or hurricanes through the sampling area, or that biological processes in the Sargasso Sea are linked more closely than previously thought with events such as the passage of eddies.

Inter-annual variability at BATS is driven mainly by the seasonal variability seen in the surface hydrography due to wind stress and heat flux (Steinberg *et al.,* 2001). Annual cycles are closely linked to the physical forcing in the area, due to the importance of the Bermuda-Azores high pressure system, and storm fronts acting to stabilize and destabilise respectively the oceanic regime at BATS (Michaels and Knap 1996; Steinberg *et al.,* 2001). Large-scale climate variability such as the North Atlantic Oscillation (NAO) and the El Niño Southern Oscillation (ENSO) have been identified as a partial link and driving force behind the inter-annual anomalies seen within the biogeochemistry and hydrography of the BATS area (Bates 2001). Anomalies such as alkalinity and salinity have been correlated to the Southern oscillation index (SOI) and lag ENSO events by approximately 6-12 months, whilst primary production, mixed-layer depth and temperature anomalies have been shown to correlate to the NAO variability (Bates 2001).

Mesoscale variability is also seen regularly at the BATS site, as there are at least three recognised types of mesoscale eddies which commonly occur in the Sargasso Sea; cyclonic, anti-cyclonic and mode-water eddies (McGillicuddy Jr *et al.*, 1999) (Figure 6). Cyclonic eddies are characterised by a depression of the sea surface, elevation of the seasonal and permanent thermocline, and cold-water cores (McGillicuddy Jr *et al.*, 1999). Anticyclonic eddies show the opposite characteristics; sea surface elevation, depression of the seasonal and permanent thermocline, and a warm-water core (Sweeney *et al.*, 2003). Mode-water eddies also have a positive sea level anomaly associated with them, although the seasonal thermocline is elevated whilst the permanent thermocline is depressed (Sweeney *et al.*, 2003). Both cyclonic and mode-water eddies cause elevations in the upper ocean isopycnals, allowing the upwelling of nutrient-rich waters into the euphotic zone and surface waters (McGillicuddy Jr *et al.*, 1998). Eddies occurring in the region of the Sargasso sea are found to have a lifespan of between several months and up to a year or longer, and have a general east to west propagation at a mean speed of 3-5km d^{-1} (Siegel *et al.*, 1999). Eddies passing through the Sargasso Sea and BATS area can be of local origin, but are also known to form as a result of baroclinic instability in the eastern Atlantic, often being a definable feature for ~6 months before they reach the BATS area (Bibby *et al.*, 2008). Bibby *et al.* (2008),

observed mode water eddies in the Sargasso sea as showing elevated chlorophyll concentrations, and a domination of the phytoplankton by large diatoms (Bibby *et al.*, 2008). All of the eddies sampled showed increased chlorophyll concentrations and an elevated deep chlorophyll maxima (DCM) at the eddy centre, exposing phytoplankton communities affected by these upwelling-induced changes to a very different environment (Murphy and Haugen 1985) from the background oligotrophic system of the Sargasso Sea. Eddy-induced variability of major nutrients such as N, Si and P is linked tightly to the deep chlorophyll maxima of eddies and the chlorophyll biomass, with the suggestion that eddies impact ocean biology primarily through control of nutrient availability (Li and Hansell 2008).



	Cyclone	Anticyclone	Mode-water Eddy
T_{surf}	Decreased	Increased	Decreased
SLA	Depressed	Elevated	Elevated
Rotation	CCW	CW	CW
Isopycnal displacements	Upwelling	Downwelling	Upwelling

Figure 6 - Depiction of major water column characteristics of three eddy types found in the Sargasso Sea. ρ_1 and ρ_2 refer to the seasonal and permanent thermoclines respectively. T_{surf} refers to the upper ocean temperature, SLA to sea level anomaly, CCW and CW to counter-clockwise and clockwise respectively. (Sweeney *et al.*, 2003)

2 Methods

2.1 BATS microplankton collection

Plankton net tows are typically performed on every BATS core (routine, monthly cruises to the BATS station) and bloom (additional, bi-weekly cruises during January - April) cruise. When sample analysis for this thesis started, microplankton samples from January 1997 through to December 2008 were available for analysis. There are samples present from 127 BATS cruises (Table 2), with the largest gap in sampling being a 9-month period between August 1998 and April 1999. There is no record of why microplankton samples were not collected during this period, although it is thought that a combination of poor weather and broken/missing nets are to blame. Missing January tows may also be due to the ship remaining in the shipyard for longer than expected over its annual winter re-fit period.

Year	**Tow samples available**	**Tow samples counted**	**Missing samples**
1997	13 (15)	6	Nov, Dec
1998	9 (15)	4	Apr (b), Aug, Sep, Oct, Nov, Dec
1999	8 (15)	5	Jan, Jan (b), Feb, Feb (b), Mar (b), Mar, Apr
2000	14 (14)	11	
2001	14 (14)	9	
2002	15 (15)	12	
2003	12 (14)	5	May, Oct
2004	11 (15)	7	Jan, Mar (b), Mar, Jul,
2005	10 (15)	10	Jan, Feb (b), Sep, Nov, Dec
2006	8 (15)	4	Jan, Apr, May, Sep (b), Sep (b2), Oct, Nov (b)
2007	7 (15)	5	Feb (b), Mar (b), Apr, Jun, Jul, Sep, Nov, Dec
2008	6 (14)	2	Jan, Feb, Feb (b), Mar, Mar (b), Apr, Nov, Dec

Table 2 - Table showing number of BATS cruises per year from which microplankton tows were conducted and the total number of cruises in parentheses. Missing cruises are identified by month. Bloom cruises are denoted by (b).

The number of samples analysed was determined initially by the availability of spring (February, March and April) and autumn (August and September) samples. Where available, all samples from these months were analysed to provide a representative spring and autumn comparison in the data. Due to this higher frequency of sample analysis, it is considered that the spring and autumn samples show more 'reliable' results than the remaining two seasons which both have fewer tow samples analysed. Three twelve-month periods were also selected (August to July) based on the availability of samples in a twelve-month period.

Prior to March 2010, one microplankton tow per cruise was carried out, usually after the night-time zooplankton tows. From March 2010 onwards, three microplankton tows are carried out on each cruise to the BATS station; back-to-back tows done after the zooplankton tows, enabling intra-cruise variability to be statistically evaluated. Due to the nature of the net used, these collections are very dependent upon the weather out at the sampling site. A database of available samples was created in Excel (see Appendix 10.1) to allow easy recognition of whether a sample from a specific cruise had been collected and stored. All of the tow metadata available from the core and bloom cruises such as time and length of deployment, maximum distance of wire out, wind speed, flowmeter reading was collated to provide a clearer view of which cruises had microplankton samples available, and if not, whether there was any pattern or reason behind this. It also enabled samples to be selected on the basis of regularity, or using variables such as wind speed or maximum depth etc.

A 0.25m radius, circular-mouth plankton net is used to collect microplankton, with a 35µm mesh size and a plastic, non-filtering, screw-top cod-end. This is towed to a maximum depth of 150m for 20 minutes. A flow-meter is secured across the mouth of the net to take a measurement of the volume of water passing through the net during the tow. A temperature and depth recorder (TDR) is also attached when available (Figure 7), to provide a more accurate measurement of maximum depth sampled, duration and profile of the tow. Maximum depths are recorded on the tow spreadsheets, although individual tow profiles are rarely saved for microplankton tows. Generally the maximum tow depth is around 150m, so that the net will sample mainly at 150m depth, but still samples upon the

deployment and retrieval through the euphotic zone. The deeper depths attained for samples collected in early 2000 (260m and 275m respectively) were recorded when the meter wheel onboard the ship was not working, and therefore more wire than usual was let out. Once recovered, the net is rinsed with seawater into the cod end, before the sample is split in two using a Folsom plankton-splitter and decanted into 250ml glass jars. A volume of ~17.5ml (7.5% of relative sample volume) 4% borate-buffered formalin is added to each sample, with an additional 2.5ml (1% by volume) of strontium chloride ($SrCl_2$) added to one of the jars before labelling.

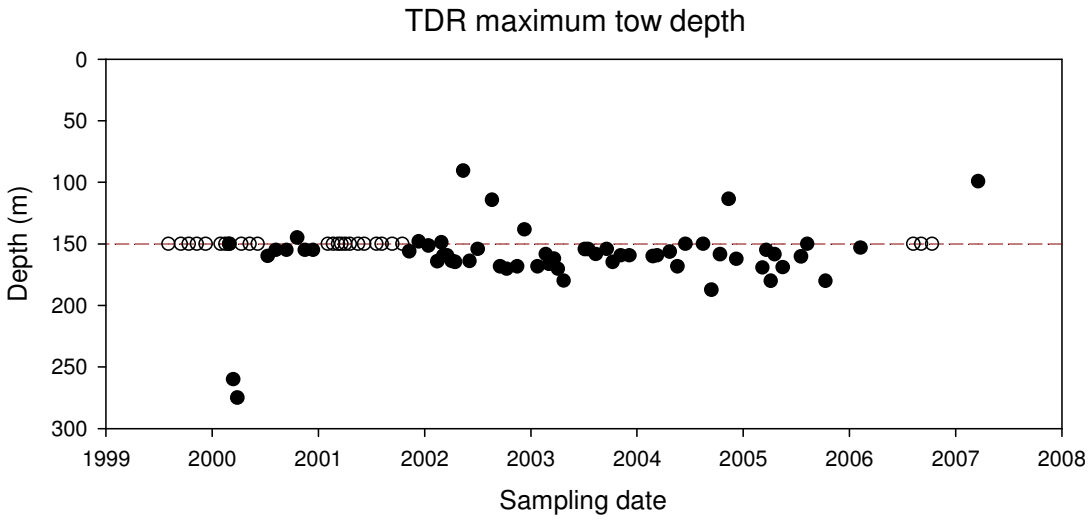


Figure 7 - Maximum tow depth data for cruises where the TDR was attached to the net. Open circles represent data points with uncertain levels of accuracy, dotted line represents 150m depth horizon.

This represents the standard BATS microplankton collection methodology that is followed by the BATS technicians for the routine microplankton tows. The methodology was updated in March 2009, after it was discovered that although both halves of the samples had been kept, no $SrCl_2$ had been added to samples since February 2006. A new flowmeter was also brought into use at the same time, after it was noticed that one of the three blades on the old flowmeter was damaged. A comparison was made over several tows where both old and new flowmeters were attached, to try and ascertain a margin of error or a transformation to be applied to earlier tows. As the date on which the flowmeter

was damaged was not known, it was suspected that the flowmeter had been damaged for quite some time before it had been reported to the PI of the BATS programme. It was therefore difficult to work out which samples to apply a margin of error or transformation to, in order to compensate for the difference in flow-meter readings. As the relationship between the new and old flowmeter readings appeared to be fairly constant, it was decided to apply a transformation to all tows sampled between February 2006 and April 2009. These dates were chosen on the basis of the accuracy with which tow data was recorded over the previous decade. Very complete records exist for tows up to and including February 2006, whereas the records become less detailed after that, coinciding with a change of personnel on the BATS programme. Due to the large volumes of water passing through the net during the tows, this discrepancy in flow measurements was not as large as was initially feared, with the old flowmeter recording counts approximately 13% lower than the new flowmeter. Flowmeter counts were used to plot a comparison graph between readings from both the old and new flowmeters (Figure 8).

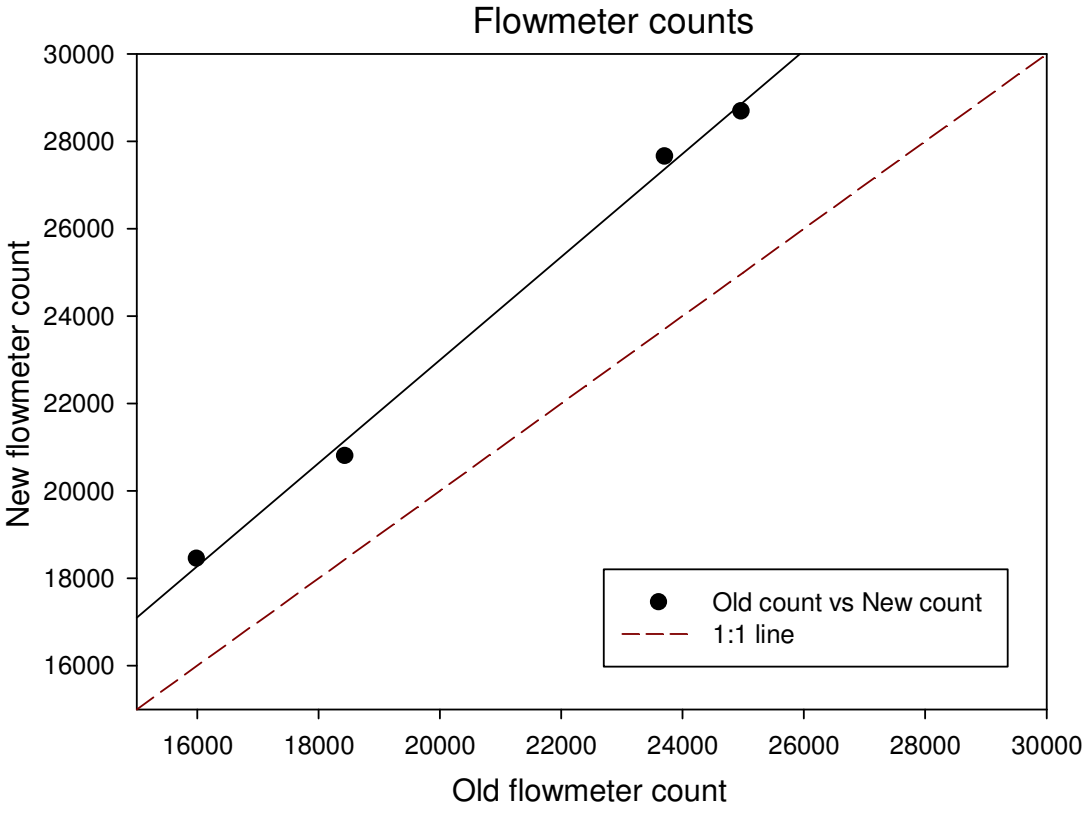


Figure 8 - Relationship between old flowmeter and new flowmeter revolutions. 1:1 ratio line also plotted for comparison. Model 1 linear regression, $r^2 = 0.99$

A linear regression line ($r^2 = 0.99$) was applied to compare the relationship of data points to the ideal 1:1 ratio line. This graph shows that as expected, the disparity between old and new flowmeter readings gets wider as the counts increase, creating a linear relationship between the two sets of flowmeter readings with an increasing offset ($y = 1.18x - 612.5$). The old flowmeter recorded readings were on average 13% lower than the readings generated by the new flowmeter. When this conversion was applied to cell abundance calculations, it translated to a 15% reduction in cell abundance counts, corroborating the 13% underestimation of volume filtered by the broken flowmeter. This conversion was applied to five microplankton tows carried out between February 2006 and January 2009 (Figure 9), to check the impact of the flowmeter readings on the scaled cell abundances. A model 1 linear regression ($r^2 = 0.99$) is used to show a strong linear relationship between the two sets of counts, again with an increasing offset.

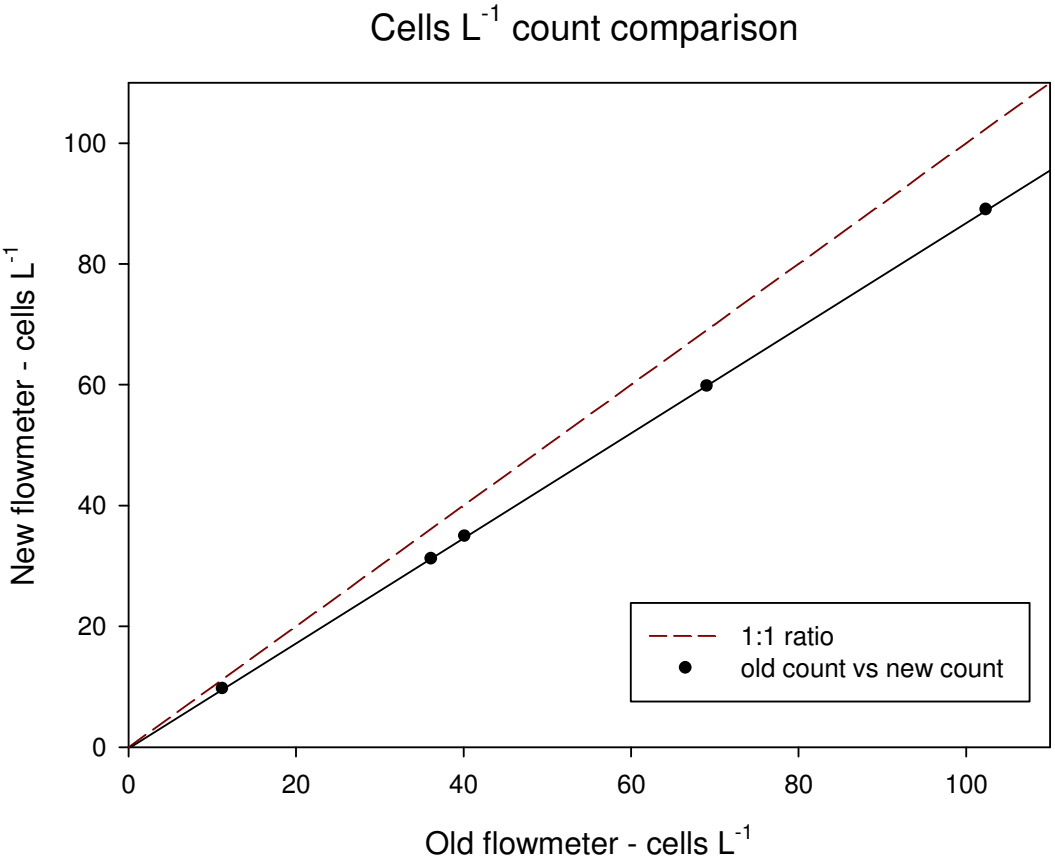


Figure 9 - Cell abundances for cruises since February 2006, using old and new flowmeter values in quantification calculations. 1:1 ratio line plotted for ease of comparison. Model 1 linear regression, $r^2 = 0.99$

2.2 Microplankton microscopy

The microplankton samples were examined in the lab at both BIOS and at NOCS using inverted light microscopy. Whilst in Bermuda, a Lumenera Infinity 2 digital camera attached to a Wild M4 inverted light microscope (40x objective) was used in conjunction with the Infinity Analyze and Infinity Capture software to count and collect images of the samples. All microscopy was carried out using the Utermöhl counting technique (Utermöhl 1958), with 100ml HydroBIOS settling chambers. A Stempel pipette was used to subsample 1ml of the preserved microplankton sample, which was then diluted with 99ml of low-nutrient, filtered seawater (LNSW) obtained from the BATS station. This represents approximately $1/500^{th}$ of the concentrated net tow sample. 1ml was determined to be an appropriate aliquot volume to use for analysis after examination of several different aliquot volumes; 0.5, 1, 2 and 4ml. Volumes greater than 1ml proved to be too concentrated for accurate cell counts to be performed and volumes less than 1ml too dilute. Samples were left to settle for 24 hours (minimum) before examination under an inverted light microscope. Samples were counted at NOCS on a Brunel SP200 inverted light microscope (40x objective).

For the microscopic analysis performed at the NOCS, 114 sub-samples of 30ml each, from 63 cruises was selected to examine, after mixing of the original sample. It was not feasible to count all microplankton samples available from the time series study. To investigate long-term changes, all August and September samples were selected to act as summer/autumn representative samples, with late February, March and early April samples selected for spring representative samples. Three sets of samples spanning a complete year (August 1999 – July 2000, August 2001 – July 2002 and August 2004 – July 2005) were also sub-sampled in the same way, and examined to investigate the temporal patterns seen over the course of three different years. These three sampling periods were chosen for analysis as they contained the most complete set of samples from the BATS cruises. Samples from 80 different cruises have been analysed and counted, with a total of 154 microscope counts performed. This represents analysis of over 62% of available microplankton samples.

2.2.1 Qualitative microscopy – Identification and Preservation

Initial microscopic analysis was carried out during the first visit to BIOS in January 2009; during this visit this analysis was used to become familiar with the microplankton found in the Sargasso Sea and to support initial attempts at identification of organisms present. "Identifying Marine Phytoplankton" (Tomas 1997), "Identifying Marine Diatoms and Dinoflagellates" (Tomas 1996) and the "Plankton Net" website (http://www.planktonnet.eu/) hosted by the Alfred Wegner Institute were used for general microplankton taxonomy and identification, as no specific North Atlantic/Sargasso Sea plankton identification guide was available.

From 25 different cruises, both the formalin only, and formalin + strontium chloride preserved samples were analysed, to determine possible changes in the condition of acantharia cells between samples. Whilst the addition of strontium chloride to the borate-buffered formalin made very little difference to the total number of microplankton cells observed in a 1ml concentrated sample, much more variation was seen in the relationship between the two different preservation techniques in the number of acantharia present (Figure 10). A model 1 linear regression shows an r^2 value of 0.76 for total cell counts. The acantharia data points (r^2= 0.13) are observed mostly to the right of the 1:1 ratio line showing a large variation in the pattern of acantharia abundance, consistent with the poor preservation of acantharia observed without the addition of $SrCl_2$.

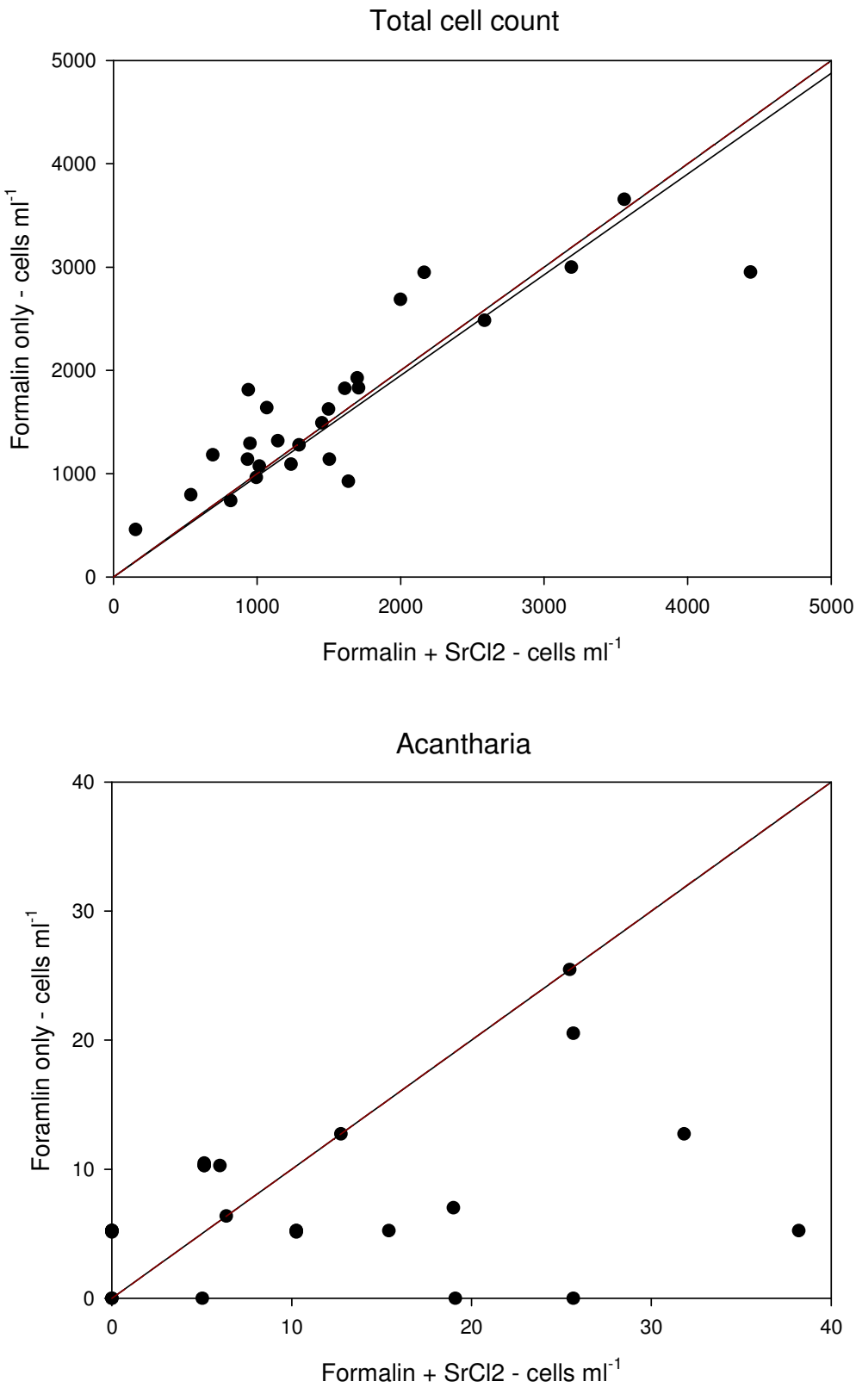


Figure 10 - Total cell abundance (top) and acantharia abundance (bottom) under the two different preservation techniques (Formalin only, and Formalin + Strontium Chloride) in plankton net tow concentrate.

The difference in preservation (lack of additional $SrCl_2$) was evident in the physical structure of some acantharia cells (Figure 11), corresponding with observations documenting the very rapid (12-24 hour) dissolution of spicules in borate-buffered 5% formalin (Beers and Stewart 1970).

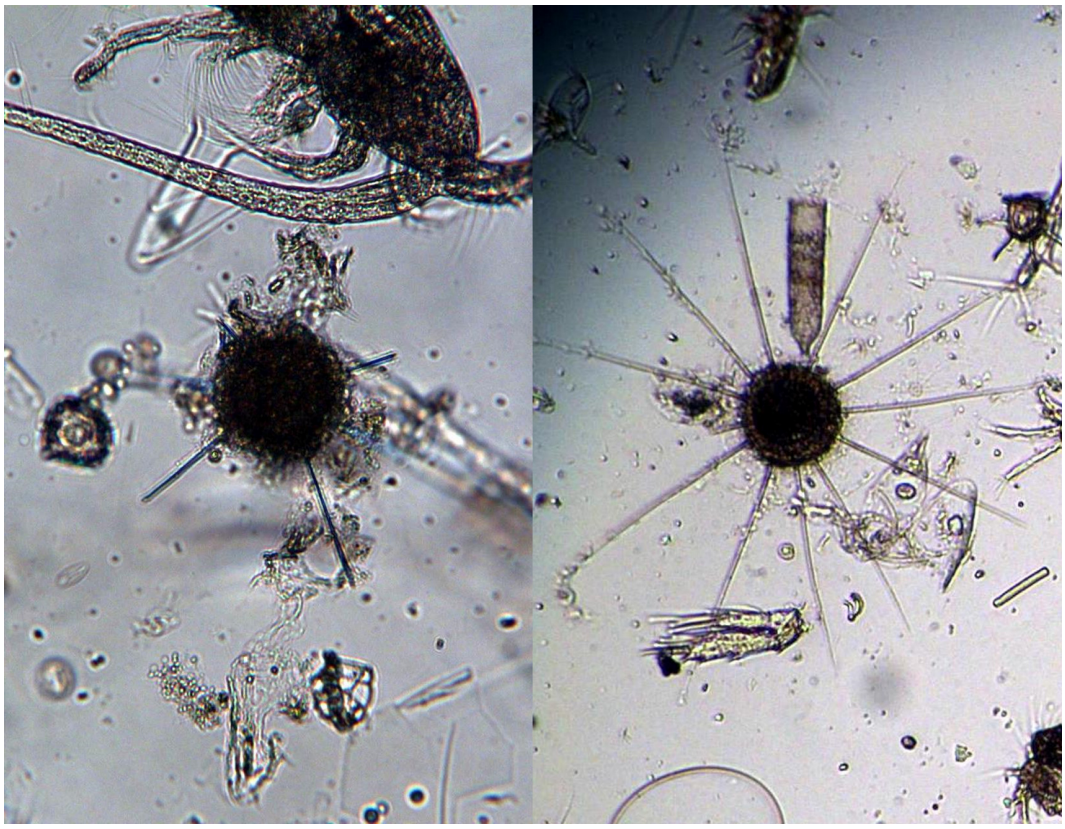


Figure 11 - Acantharia preservation in solution without (l) and with (r) additional Strontium Chloride.

The formalin used for preservation is borate-buffered, although there were initially some concerns about how stable the buffering would be in samples stored for an extended length of time. To check the reliability of the buffering in the preservation, the pH of samples was also tested using Whatman narrow-range pH paper. All 15 samples tested showed pH values in the range of 7.5-8 on the narrow-range pH paper. These values fall within the pH range accepted for seawater (Marion *et al.,* 2011), consistent with the addition of borate as a buffer to the formalin. pH values in this range should not have caused any cell preservation issues. Samples were analysed in reverse order, starting with most recent samples

to check whether increased degradation and dissolution of cells was observed. No obvious dissolution of diatom frustules was recorded, or increased damage of cells in older preserved samples.

2.2.2 Quantitative microscopy – Transect counts

After a familiarisation period, quantitative microscopic analysis was performed to count numbers of organisms within various 'microplankton groups'. The following groups of single-celled organisms were counted; Diatoms, Dinoflagellates, Tintinnids, Radiolaria, Foraminifera, Silicoflagellates and Acantharia. Each time a sample was settled, both vertical and horizontal transects across the slide were examined, and counts recorded. The area covered by these transects was calculated, and counts scaled up to an estimate of total slide count. The total transect area of a slide examined using the microscope at BIOS accounted for 15.71% of the total slide area, whilst the total transect area of a slide examined at NOCS was calculated as 19.48% of the total slide area. The groupings of organisms allow percentage composition of the samples to be examined, as well as calculation of abundances of cells per litre of seawater, using the data from the flow meter. To determine whether counting a vertical and horizontal transect for each sample provided a representative count for the entire sample, total slide area counts were performed regularly in addition to transect counts. Of the 154 microscopic counts completed, 12 of them were counts of the entire microscope slide area, rather than transects. Figure 12 shows the comparison between the predicted count (scaled up from transect counts) plotted against the actual number of organisms counted on an entire slide. Tintinnids and a commonly found genus of dinoflagellate (*Ceratium* species) were used in this case to highlight differences in the two estimates and error in the method. A model 1 linear regression was applied to the data ($r^2 = 0.80$ *Ceratium*, $r^2 = 0.91$ Tintinnids). *Ceratium* sp. data ($y = 1.06x + 8.9$) do not show any significant difference between predicted and counted abundances (t-test, $p = 0.625$). Although Tintinnid data ($y = 1.35x - 15.5$) appear to show more variation away from the 1:1 ratio line, there was no significant different between predicited and counted abundances (Tintinnid $p = 0.485$). A similar pattern was observed when predicted and counted values for total microplankton abundance were plotted (y =

1.35x -71.4, $r^2 = 0.96$), again with no significant different observed between predicted and counted abundances ($p = 0.415$).

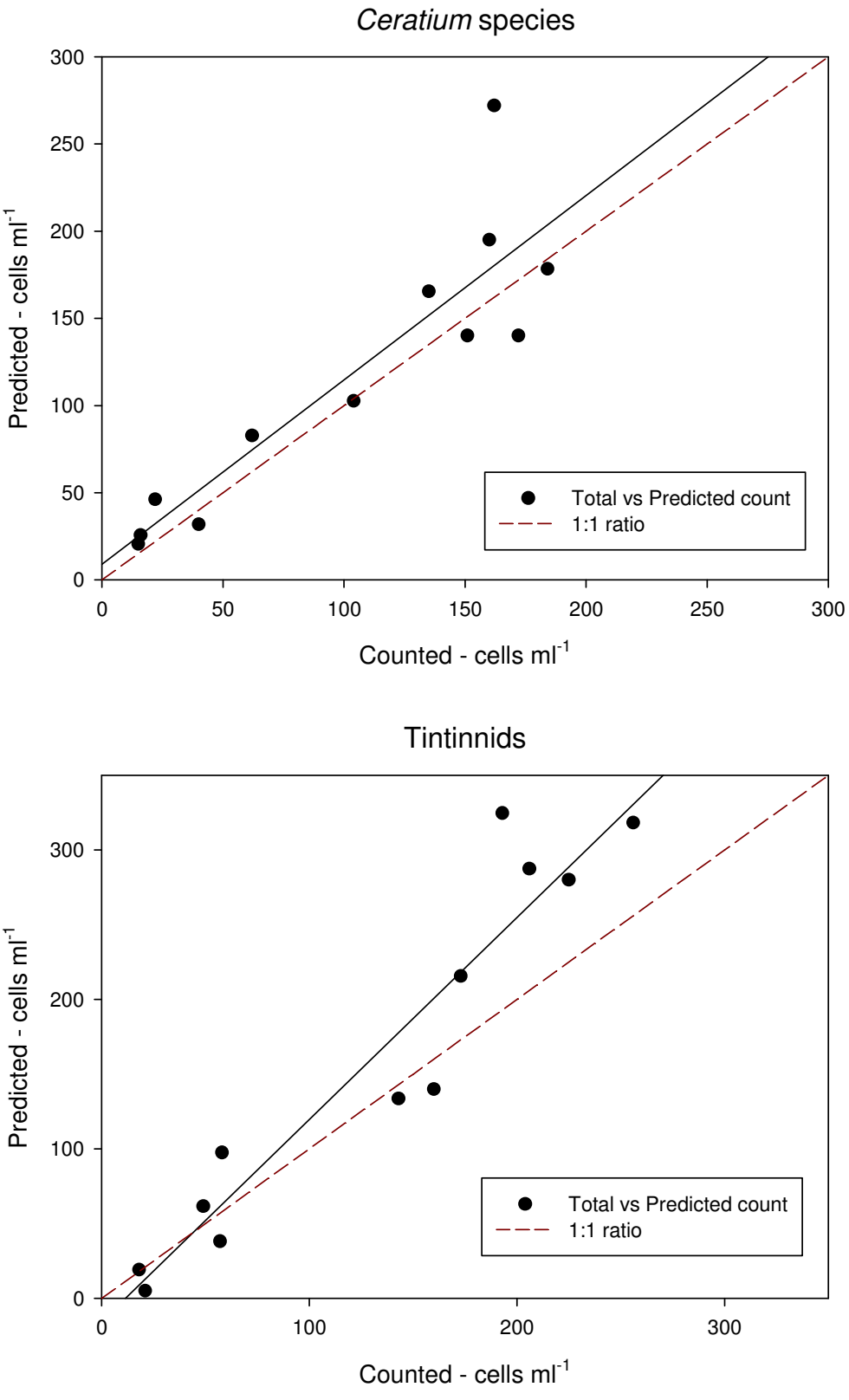


Figure 12 - Predicted counts (scaled from transect counts) and total counts of Ceratium sp. ($r^2 = 0.80$) and tintinnid numbers ($r^2 = 0.91$) per 1ml of settled sample. 1:1 ratio line plotted for comparison. R^2 values from Model 1 linear regressions.

2.3 FlowCAM microscopy

A FlowCAM instrument at NOCS was made available towards the end of the project; 101 samples from 63 cruises were analysed using version 1.8.66 software. All samples were pre-filtered using a 100µm mesh to remove the large copepods and other metazoan zooplankton from the samples. This filter size was found to allow through >97% of the single-celled microplankton, whilst removing the majority of large copepods. Before filtering, 4ml (2 x 2ml Stempel pipette) of a plankton tow sample was diluted to a total of 52ml using low nutrient filtered seawater. Using the software in Image Acquisition mode, a sample rate of 7 frames per second was selected, with a particle filter size bracket of 30-300µm. A syringe pump was found to produce the smoothest and most reliable flow of sample, set at a pump rate of 7ml per minute. The total volume of sample represented by the images collected was also recorded (average of 1.87ml), allowing for FlowCAM counts to be scaled and compared directly with microscope counts (Figure 13).

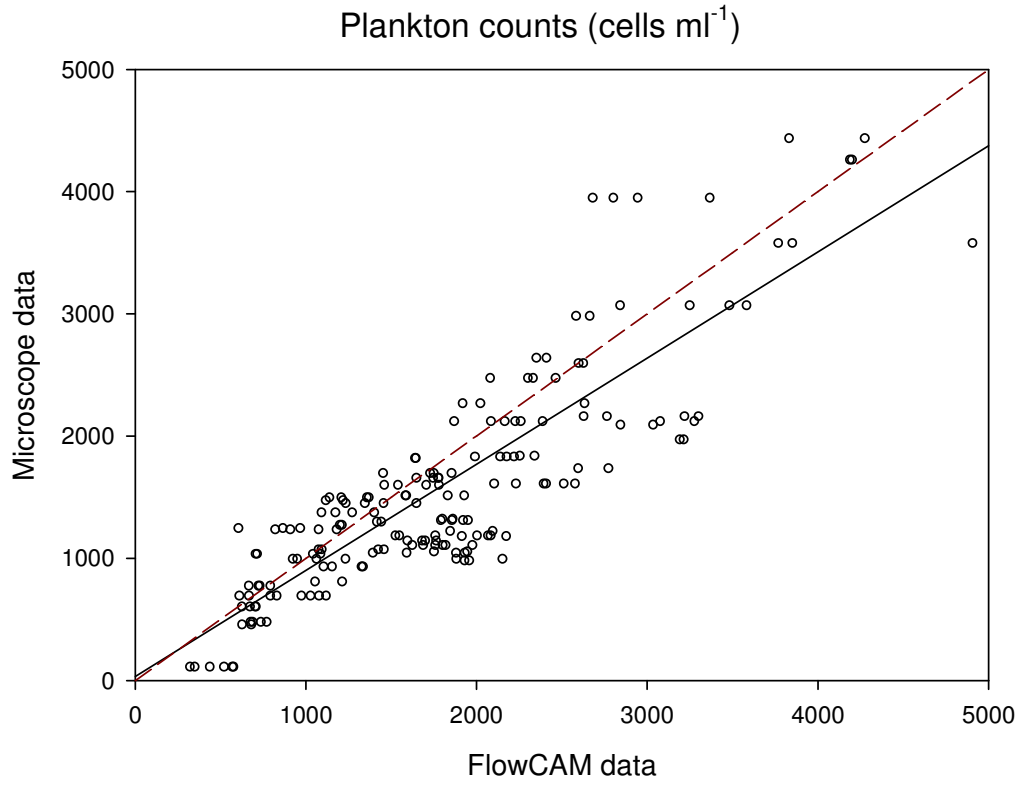


Figure 13 - Relationship between FlowCAM microplankton cells counts and microscope microplankton cell counts per 1ml of microplankton sample. Dashed red line indicates a 1:1 ratio. Black line shows a Model 1 linear regression ($y = 0.87x + 34.6$, $r^2=0.67$).

The FlowCAM was also used to examine intra-cruise variability from multiple net tow samples collected on four cruises between March 2010 and May 2010. Three microplankton tows were performed back-to-back, to determine the reproducibility of a single net tow. Total cell counts were compared between the three microplankton tows on each of the four cruises B256, B256a, B258 and B259 with coefficients of variation of 4.9%, 8.51%, 6.31% and 9.18% respectively (Figure 14).

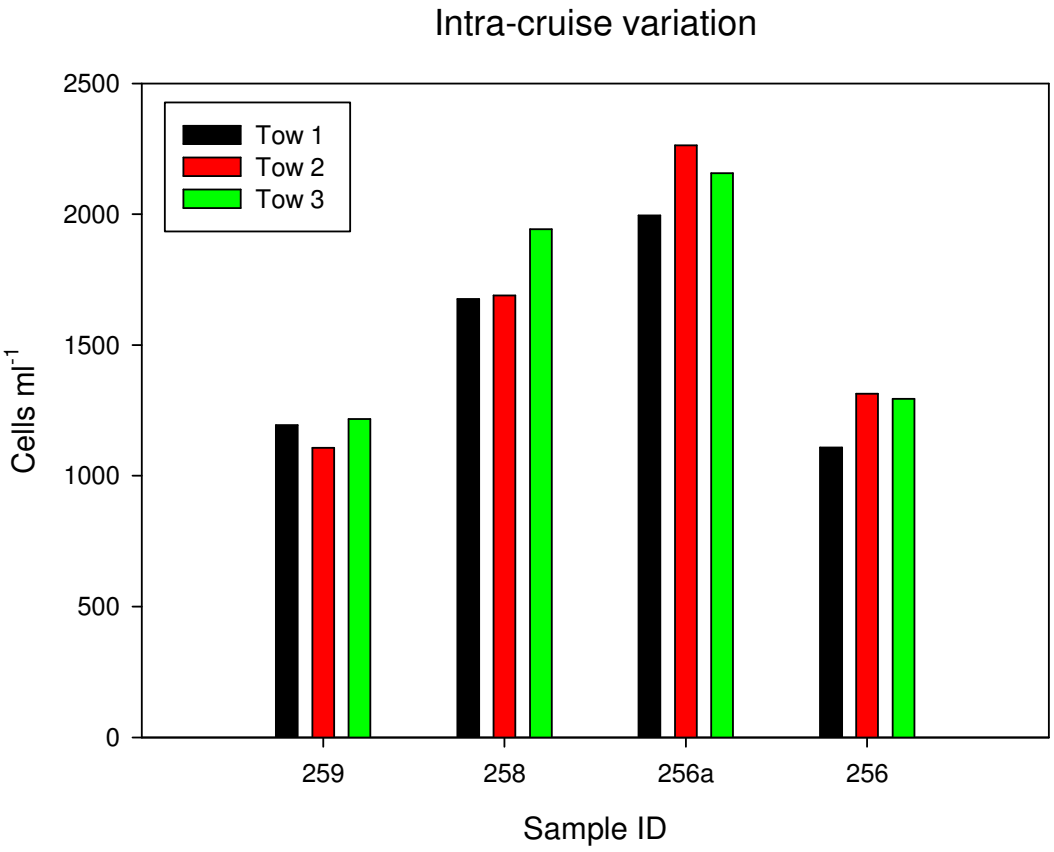


Figure 14 – Intra-cruise variability of total microplankton cell counts (per 1ml of tow sample), performed on four different cruises (BATS 259, 258, 256a and 256).

The use of the FlowCAM allowed relatively rapid sample analysis repetitions to be carried out, in addition to acquiring cell sizes for groups such as the foraminifera and centric diatoms. An example of FlowCAM-generated images can be seen in Appendix 10.3.

2.4 Biogenic Silica Analysis

95 sub-samples from 63 cruises were also subjected to analysis for particulate biogenic silica, using a modified version of the method of Brzezinski & Nelson (1989), which uses the sodium hydroxide (NaOH) digestion of Paasche (1973). A 2ml Stempel pipette aliquot of plankton tow sample was filtered through a 100µm plankton mesh onto a 5µm polycarbonate membrane filter. The sample was filtered under vacuum <25cm Hg, using an all-plastic filter apparatus. The filter was then rinsed with ~2ml of pre-filtered LNSW before being folded in half and placed in a plastic petri-dish and dried overnight. Filters were then transferred to a test tube for overnight digestion in 2ml 0.2N NaOH, before being neutralized with 0.8ml 0.5N Hydrochloric acid (HCl). A 1ml aliquot of the neutralized sample was then analysed for reactive silicate using the method of Strickland and Parsons (1968). Ammonium molybdate reagent was added to the neutralized sample and left for 10 minutes before the Mixed Reduction Reagent (MRR) was added. Samples were then left for a minimum of two hours (maximum of 4 hours) for the reduced silicomolybdate complex to form, and full colour to develop before colourimetric analysis. Using a Unicam 8625 UV/Vis spectrophotometer with 1cm cuvette, the absorbance of the reduced silicomolybdate complex was measured at 810nm. A blank of MilliQ water plus reagents (Ammonium molybdate plus MRR) were used, with prepared silica standards of 50, 25, 10, 5 and 1µmol/l (Figure 15). In addition to using all-plastic filtering apparatus, it was found to be essential to prepare the NaOH using analysis-grade NaOH pellets (VWR Anal-R) with a maximum silica concentration of ten parts per million Si, made up in a plastic bottle.

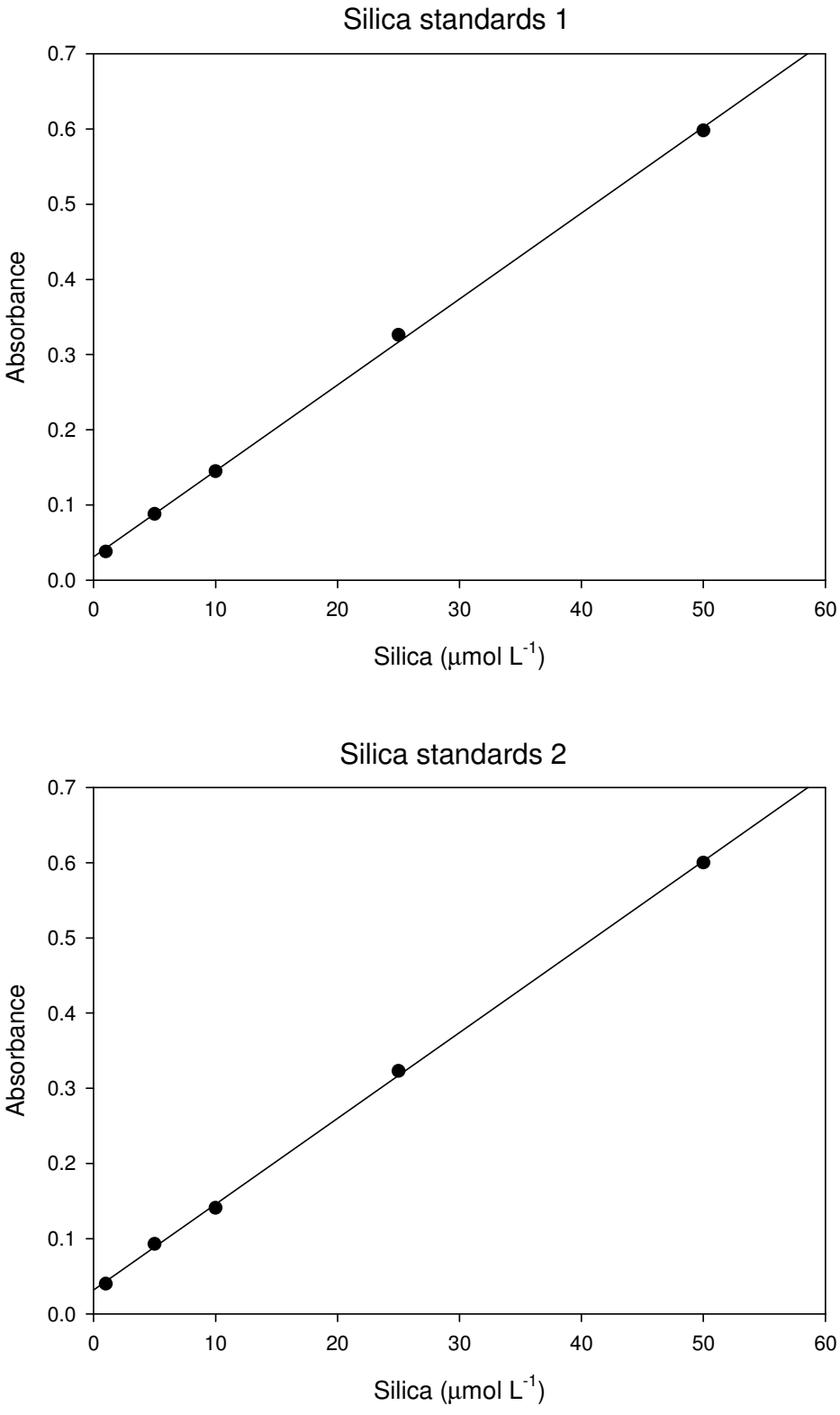


Figure 15 - Silica standards (1, 5, 10, 25 and 50µmol L^{-1}) for run 1 (top) and run 2 (bottom) of biogenic silica analysis.

2.5 Particulate Calcium Analysis

77 sub-samples from 64 cruises were also subjected to analysis for particulate calcium concentration. Due to the size range of organisms targeted by the microplankton net, it is likely that the only calcium-carbonate-depositing organisms found in the samples are foraminifera. All glassware and test tubes were washed in a 10% HCl acid bath prior to use. 0.4µm pore size, 25mm diameter Polycarbonate filters were stored in a solution of 5mM ammonium bicarbonate. 2.5ml aliquots of plankton sample were pre-filtered through a 100µm mesh and rinsed with LNSW onto 25mm 0.4µm polycarbonate filters. Samples were filtered under <5mg vacuum pressure, and then rinsed with 5ml of ammonium bicarbonate under <5mg vacuum pressure. Filters were then placed in acid-washed, pre-weighted test tubes using acid washed plastic tweezers. Test tubes were then weighed again to determine the weight of the filter and organic matter. 10ml of 0.4M Nitric acid was then added to each sample test tube, weighed again to determine exact volume of acid added, and left overnight. Approximately 6ml of each sample was then syringed into nitric acid-washed glass test-tubes. Samples were then analyzed for calcium using Inductively Coupled Plasma source Atomic Emission Spectra (ICP-AES). Sodium was measured at 590 nm, Calcium at 423, 318 and 316 nm, and Strontium was measured at 4.8 and 422 nm. A multi-element stock was made up from Nitric acid, spiked with sodium, strontium and calcium standards. Four experimental standards were then made from the multi-element stock, with varying concentrations of the three elements (Table 3).

	STD 1	**STD 2**	**STD 3**	**STD 4**
Sodium (µg/g)	1.896	3.850	7.523	11.480
Calcium (µg/g)	0.471	0.957	1.871	2.855
Strontium (µg/g)	0.142	0.288	0.563	0.859

Table 3 - Elemental concentrations of 4 multi-element standards used for calibrating ICP-AES calcium detection analysis.

2.6 Derived variables and statistics

Unless otherwise stated, non-parametric statistics are used due to the non-normal distribution of data. Where regressions or correlations are calculated, a strong correlation is classed as a coefficient >0.5, a weak correlation is classed as a coefficient <0.5. Statistical significance is taken as the p = 0.05 limit.

With the exception of Figure 57, all graphs and statistical tests were carried out using SigmaPlot version 11.0. Figure 57 was produced using PRIMER version 6. Where correlations and regressions are calculated, a weak correlation / trend is defined as less than 0.5, a strong correlation / trend as greater than 0.5.

FlowCAM and microscope cell counts were tested to ensure statistical similarity using a Mann Whitney U test on 58 samples (all samples where flowmeter data was available). No significant differences between these two sets of data were recorded (p = 0.6), allowing for the treatment of both FlowCAM data and microscope data as the same data set.

Similarity of sample replicates was also tested for each cruise sample where flowmeter data and replicate samples were available, to ensure reproducibility of abundance data. For all 56 cruise samples tested, none returned a statistically significant difference between abundances on sample replicates.

A monthly abundance anomaly was calculated for each month of the year using the following formula;

$$A'_m = (\bar{A}_m - \bar{A}_{ts}) / \sigma (A_{ts})$$

Where:

A'_m is the abundance anomaly for month m

$\bar{A}_m$ is the average abundance for month m

$\bar{A}_{ts}$ is the average abundance for the whole time-series ts

σ (A_{ts}) is the standard deviation of abundance for the whole time-series ts
Adapted from (Widdicombe *et al.,* 2010)

A sample abundance anomaly was calculated for each BATS cruise of the time-series using a similar formula;

$$A'_s = (\bar{A}_s - \bar{A}_m) / \sigma (A_{ts})$$

Where:
A'_s is the abundance anomaly for sample s
$\bar{A}_s$ is the average abundance for sample s
$\bar{A}_m$ is the average abundance for the month m
σ (A_m) is the standard deviation of abundance for the month m

3 Observational data

The hydrographic regime in the Sargasso Sea has been well documented and summarised previously (see section 1.4). Observational data is shown here to provide a more detailed background of the physical and biogeochemical data available from the BATS site. This includes routine measurements from CTD data such as temperature, salinity, fluorescence, as well as bottle data such as nutrient (phosphate, silicate, nitrate etc.) and pigment concentrations (chlorophyll and accessory pigments e.g. Fucoxanthin and Peridinin). All contour plots are produced with the Ocean Data View package, with open-access BATS archive data.

3.1 Physical data

The physical characteristics of the water column at the BATS site are summarized by Steinberg *et al*. (2001) and Michaels and Knap (1996); shallow mixed layers in the summer/autumn, well mixed, homogenized waters in the winter/spring. These characteristics can be seen in depth profiles taken from CTD measurements of temperature and salinity (Figure 16), using a typical spring profile (BATS 197a – March 2005) and a typical summer profile (BATS 131 – August 1999). The difference in the mixed layer and stratification depths between the two seasons is clearly visible; in spring the top 200m of water is homogenized, whilst in summer there is a warm, shallow mixed layer at approximated 20m depth. Differences in the fluorescence profile are also visible (used as a proxy for chlorophyll, therefore phytoplankton abundance), with a defined fluorescence peak at around 120m seen in the summer profile, whilst the spring profile shows a fluorescence peak value that is lower but is present over a larger depth in the water column.

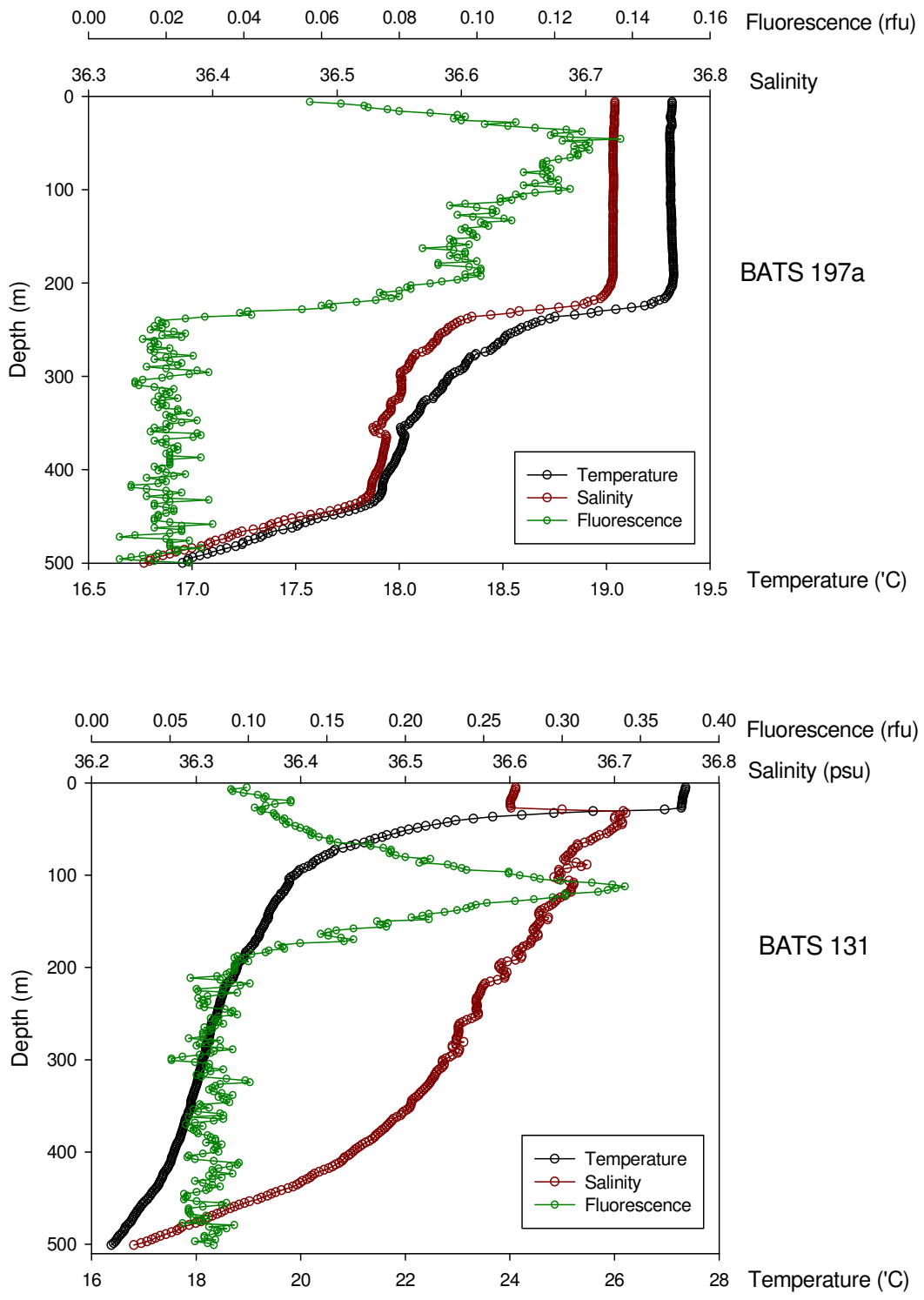


Figure 16 - CTD depth profiles of Temperature, Salinity and Chlorophyll fluorescence, showing a typical spring profile; BATS 137a (top), and a typical summer profile; BATS 131 (bottom).

These stratification and mixing differences between summer and winter are also very apparent when the CTD data is examined for the whole time-series (Figure 17), particularly in the water temperature data. The seasonal signal is not seen as strongly in the salinity data, presumably due to the contrasting effects that rainwater and evaporation exert on the surface waters. Dissolved Inorganic Carbon (DIC) and Particulate Organic Carbon (POC) are also measured on BATS cruises (Figure 18). DIC levels throughout the water column again show a seasonal pattern, but also show a steady increase both in surface waters, and at depths from 100 – 250m. POC concentrations also show a pronounced seasonal pattern, with variations in the depth that POC levels exceed 10 $\mu g\ kg^{-1}$.

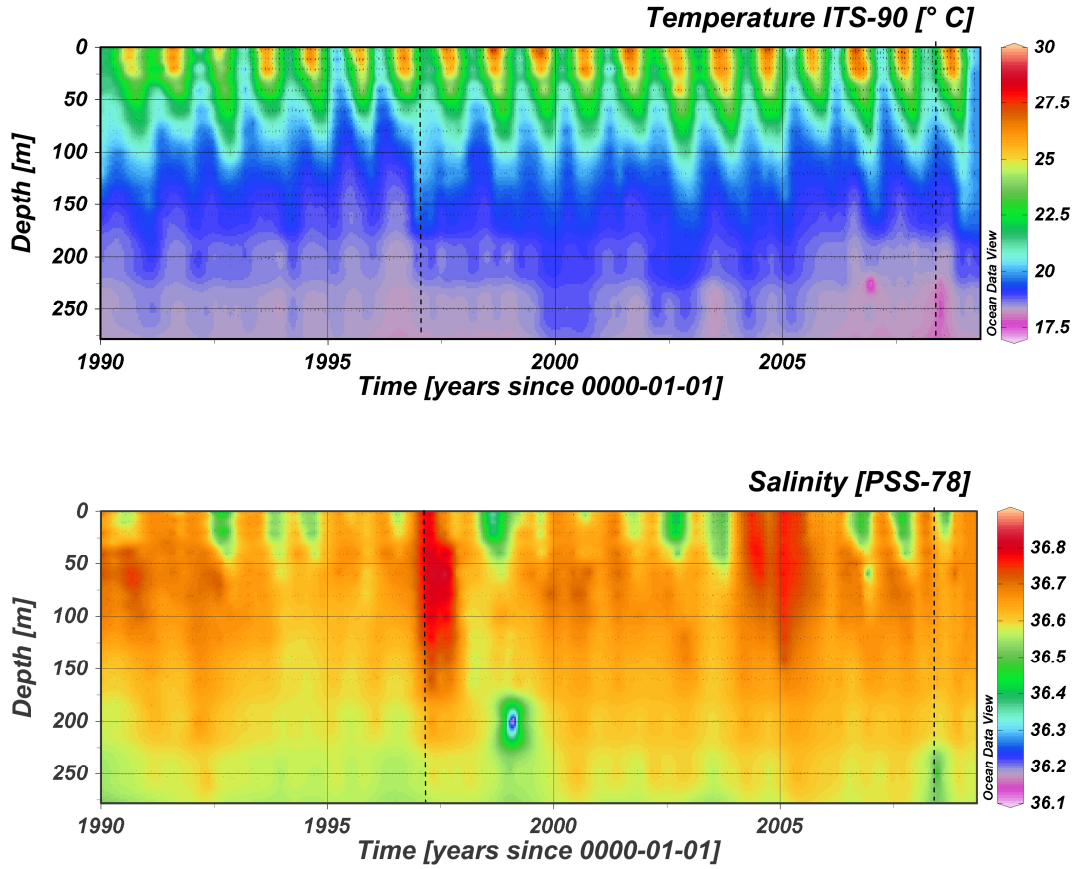


Figure 17 - Time-series contour plots of Temperature and, Salinity with depth, 1990-2009. Dashed lines enclose sampling period of microplankton analysis.

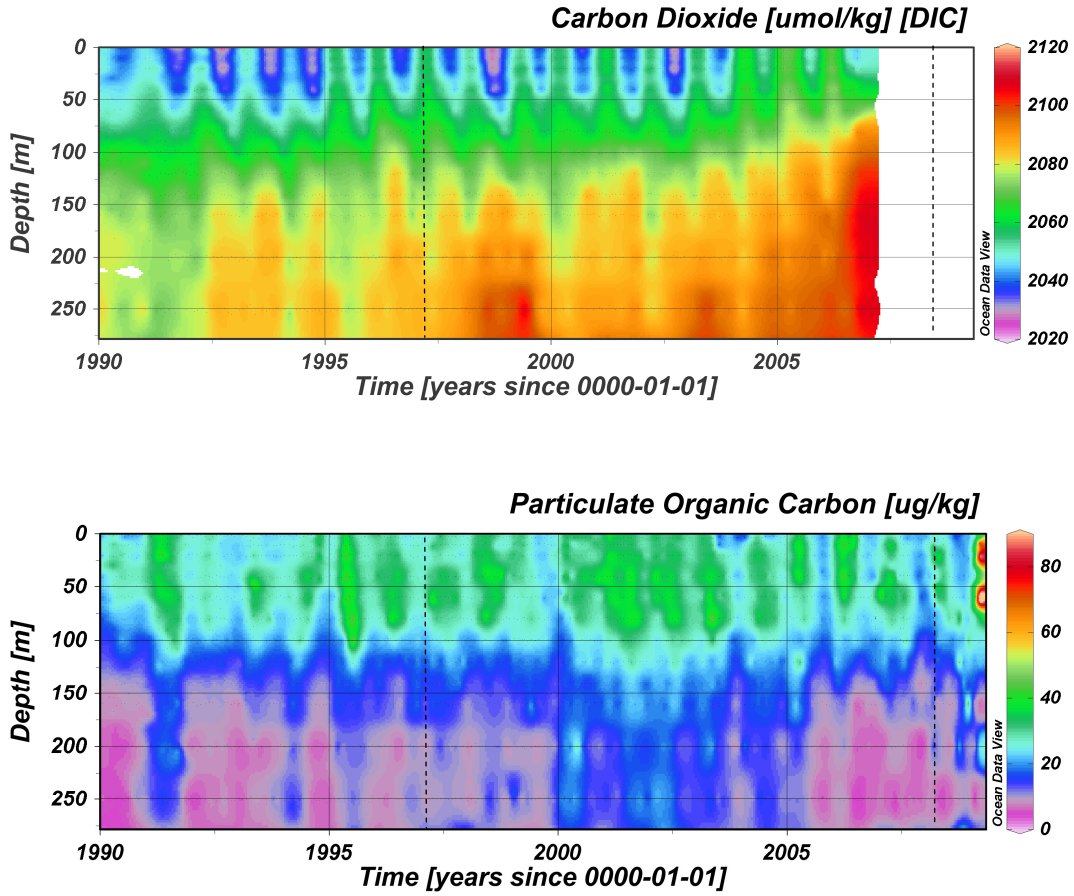


Figure 18 - Time-series contour plots of Dissolved Inorganic Carbon (DIC) and Particulate Organic Carbon (POC) with depth, 1990-2009. Dashed lines enclose sampling period of microplankton analysis.

3.2 Nutrient data

The Sargasso Sea surface waters around the BATS site have very low inorganic nutrient concentrations, as summarized by Michaels *et al.* (1994), and despite primary production in oceanic gyres commonly considered to be nitrogen limited (Cotner *et al.,* 1997), primary production in the Sargasso Sea is suggested to be controlled by phosphorus availability (Lomas and Bates 2004). Very low concentrations of phosphate, nitrate and nitrite are recorded in the euphotic zone at BATS (Figures 19 and 20), with slightly higher levels of silicate in the upper water column.

There is little seasonality seen in the upper water column for phosphate and combined nitrate and nitrite, although individually measured nitrite shows a maximum at around 120m depth, with a higher level of seasonality than other nutrients. Seasonality and total concentration of nitrite measurements is weak in the top 100m of the water column, and below 150m depth. Phosphate shows some seasonality at depths below ~150m, although very little of this appears to extend upwards into the euphotic zone. Large mixing events such as the passage of a storm or hurricane act to bring these nutrients up from depths to the nutrient-depleted surface waters.

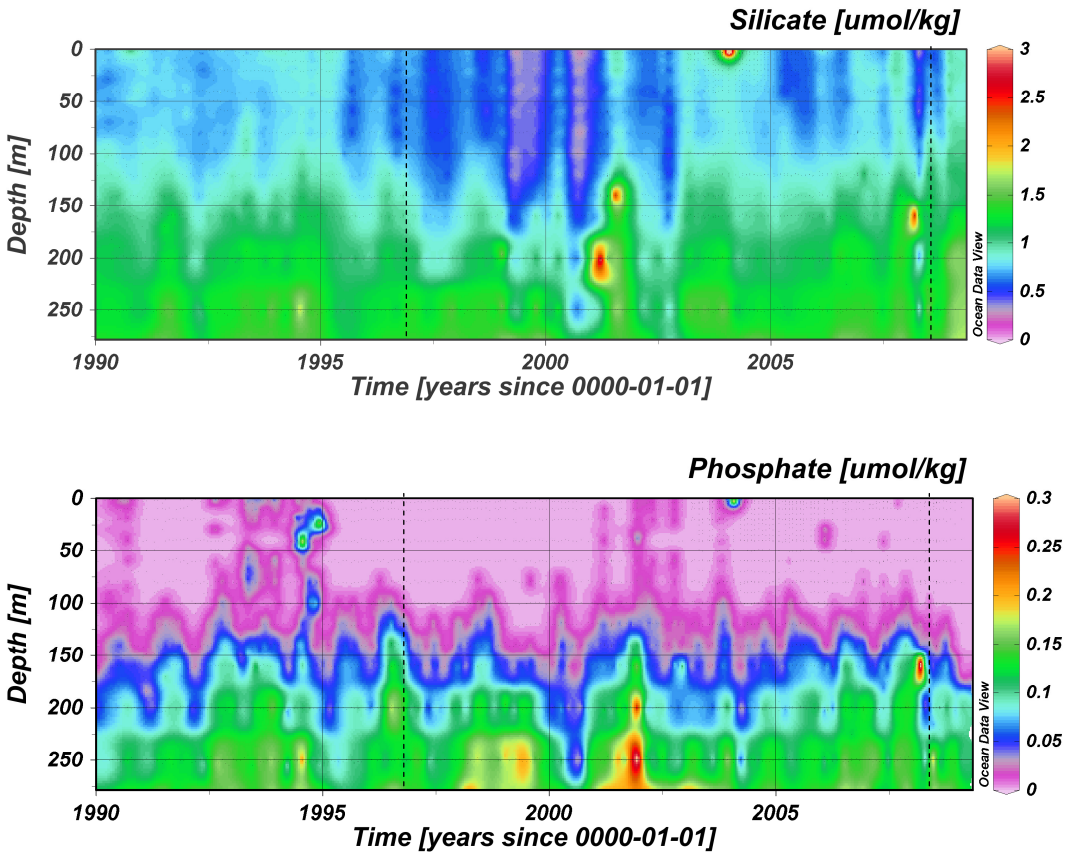


Figure 19 - Time-series contour plots of major nutrients at the BATS site 1990-2009. Top - bottom; Silicate and Phosphate. Dashed lines enclose sampling period of microplankton analysis.

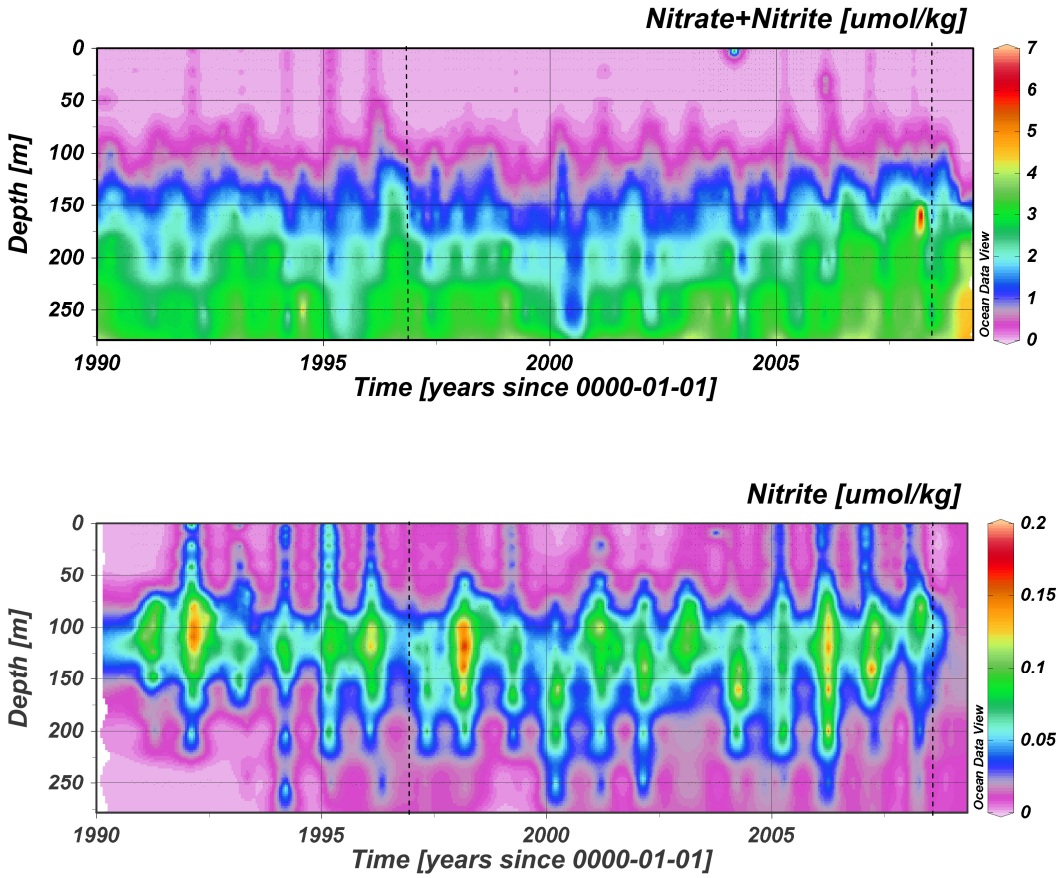


Figure 20 - Time-series contour plots of major nutrients at the BATS site 1990-2009. Top - bottom; Combined Nitrate and Nitrite, and Nitrite. Dashed lines enclose sampling period of microplankton analysis.

3.3 Pigment data

Pigment data taken from the CTD bottle casts can also be used to examine depth profiles, highlighting the importance of the subsurface chlorophyll maximum (SCM) (Figure 21). In oceanic environments such as the Sargasso Sea, this SCM is usually well below the surface, towards the bottom of the euphotic zone (Longhurst and Harrison 1989) as seen below, using data from a summer cruise (August 1999). Potential density is used to illustrate the structure of the water column, seen here with a shallow stratified layer in the surface waters, with more stable density below ~80m. A strong seasonality in primary production has been shown at the BATS site (Michaels *et al.,* 1994; Steinberg *et al.,* 2001) and this is reflected in the time-series of pigment data including chlorophyll a, Fucoxanthin and Peridinin (Figure 22).

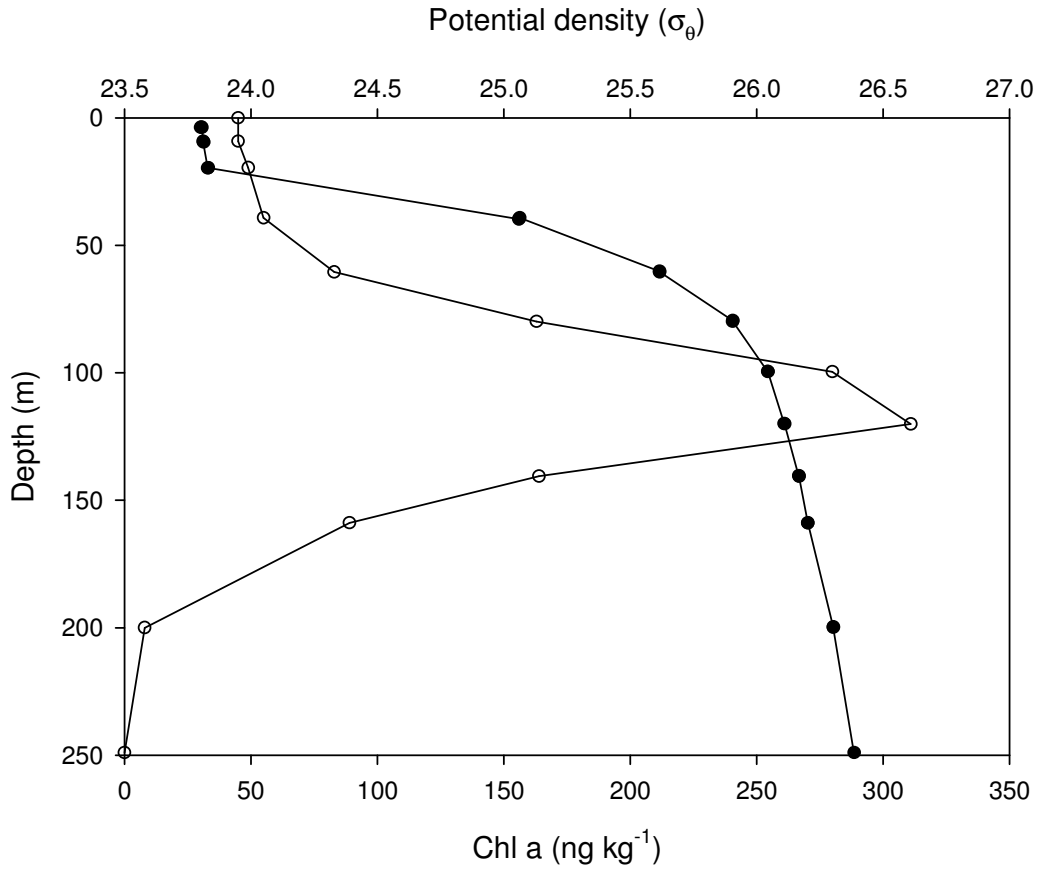


Figure 21 - Depth profile of chlorophyll a (open circles) and potential density (filled circles) showing the subsurface chlorophyll maximum (SCM). Data from cruise BATS 131 (August 1999).

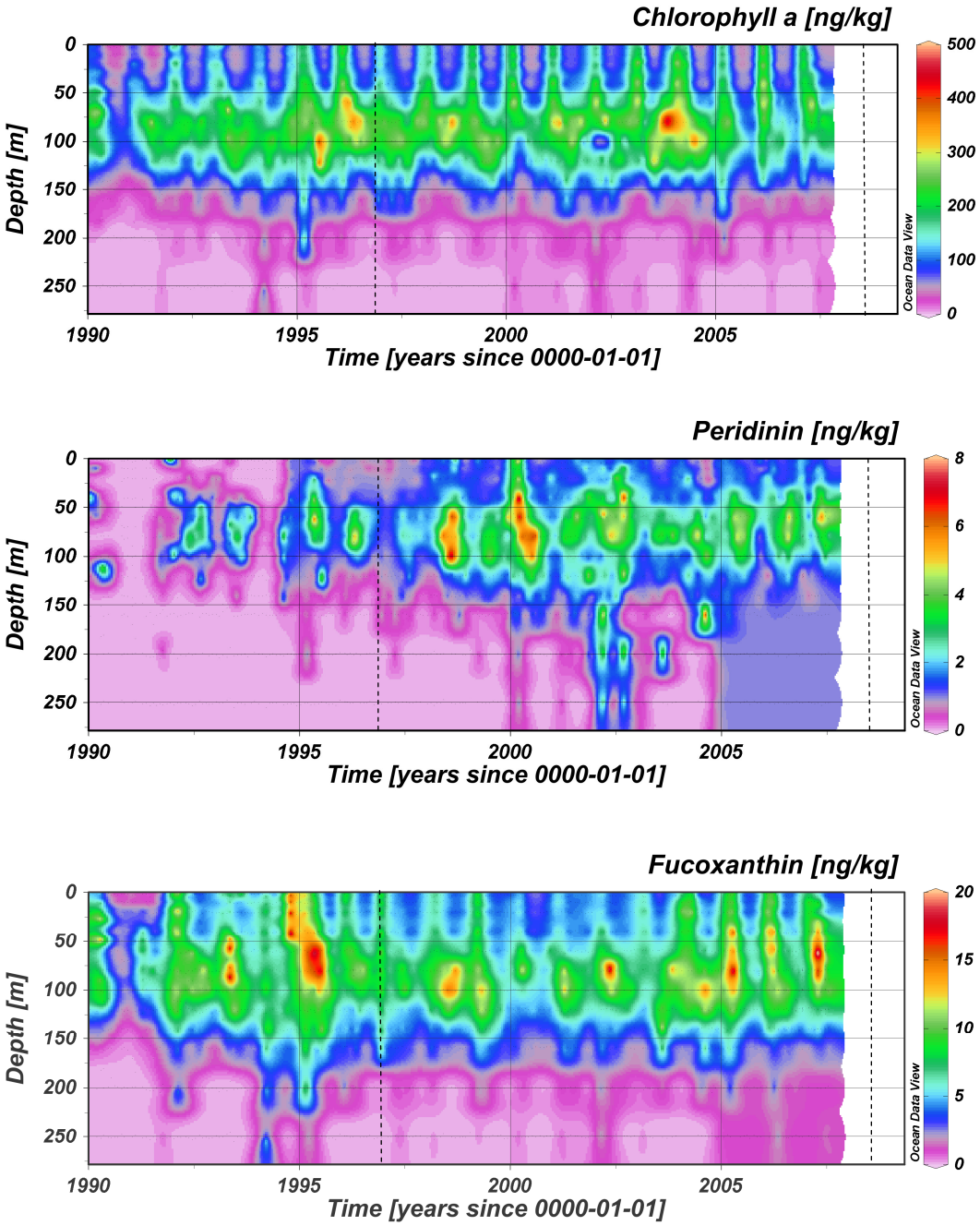


Figure 22 - Time-series contour plots of major pigments at the BATS site 1990-2009. Top - bottom; Chlorophyll a, Peridinin and Fucoxanthin. Dashed lines enclose sampling period of microplankton analysis.

Using Chl a as a proxy measurement for phytoplankton biomass, in the surface waters low Chl a values occur in the summer months (June – August) when the water column is stratified and nutrient levels are very low. Peaks in Chl a values occur in the spring months (February - April) coinciding with increased mixing of the water column due to winter/spring storms and signifying the spring bloom (DuRand *et al.,* 2001). High Chl a levels are seen down to depths of ~150m, representing the bottom of the euphotic zone, with peak concentrations around 100m.

Peridinin is a marker pigment for the presence of dinoflagellates (Wright *et al.,* 1991), whilst Fucoxanthin is a marker pigment for diatoms. There is less seasonality seen in the Peridinin data set, although highest concentrations are recorded at similar depths to that of the DCM. Peridinin concentrations below the DCM have also increased slightly since 2005, although the maximum concentration at the DCM does not show a similar increase over the same time period. Fucoxanthin shows a more consistent seasonal signal than Peridinin, indicating the presence of diatoms year round, with a spring increase above 150m as reported by Michaels *et al.*, (1994); DuRand *et al.,* (2001) and Steinberg *et al.,* (2001). Maximum concentrations of Fucoxanthin is seen at the same depth as the DCM, with notably lower concentrations observed in spring 2000 when compared to other years.

4 Results I – Monthly and Seasonal variability

In the following chapter, microplankton abundance data and relative abundance (composition) data are presented and analysed on monthly (Results 4.1) and seasonal (Results 4.2) timescales. Physical and chemical data for the sampling area are also presented to elucidate the forcing factors of any variability observed. Starting with small-scale variability, it has been well documented that diel variations occur in phytoplankton photosynthesis (and therefore primary production) (Yentsch and Ryther 1957; Ryther *et al.,* 1961; Sournia 1974; MacCaull and Platt 1977), and zooplankton migration (Ryther *et al.,* 1961; Lo and Biggs 1996), combining to cause small scale variations in the export of organic matter from the surface waters of the ocean (Madin *et al.,* 2001). This within day variability is driven primarily by three factors; the availability of optimal light for phytoplankton photosynthesis (Doty and Oguri 1957; Yentsch and Ryther 1957), the movement of zooplankton within the water column for enhanced grazing opportunities, and the movement of zooplankton within the water column for predator avoidance (Zaret and Suffern 1976; Haney 1988; Lampert 1989; Dodson 1990; Madin *et al.,* 2001). Elucidating patterns on the diel-scale of variability is only possible however with an intensive sampling regime; sampling the euphotic zone every day over a period of time. Replicate day and night microplankton tows would enable the error of diel data to be calculated. This study however, focuses on the timescale variations greater than two weeks (the minimum time between sampling at the BATS station), rather than data analysed at the shorter (diel) timescales. As described previously (Methods 2.2), the bulk of samples analysed consist of spring (February – April) and autumn (August and September) samples. Summer and winter samples are included in analysis for years 1999-2000, 2001-2002 and 2004-2005. When seasons are referred to they are defined as such; *Spring*: February, March and April. *Summer*: May, June and July. *Autumn*: August, September and October. *Winter*: November, December and January.

4.1 Monthly variability

Investigating microplankton variability at the BATS station on a monthly timescale provides information about patterns occurring on the smallest timescale available, with the ability to observe patterns and trends in the data which may be overlooked when long-term analysis is carried out.

4.1.1 Microplankton abundance

Open circular data points in Figures 23 and 24 show cell abundances of the seven main single-celled microplankton groups enumerated in microplankton net tow samples, collected at BATS between 1997 and 2008. Data are plotted on a decimal year scale (January 1st = 0, December 31st = 1), to enable visualisation of data spread throughout the year. The density of data points also show which months have been intensively analysed e.g. February and March, compared with those from which few samples have been analysed e.g. January. This also reflects the increased sampling effort with BATS core cruises in February, March and early April. The magnitude spread of data points within each month may also be examined using box and whisker plots. Month-by-month comparisons can be made more easily when decimal year data are collated into monthly bins as shown on the box and whisker plots, allowing a clearer view of abundance changes throughout the year, in addition to calculating monthly averaged data. Non-parametric statistical analysis was carried out using the Kruskal-Wallis one-way Analysis of Variance on ranks to identify significant differences between monthly median abundances (Table 4). In addition, a monthly abundance anomaly was calculated for each month of the year using the method described in Methods 2.6. This anomaly allows identification of those months with abundance averages that lie above or below the long-term time-series abundance average (Figures 25 and 26). A list of common microplankton genera and species is shown in Appendix 10.2.

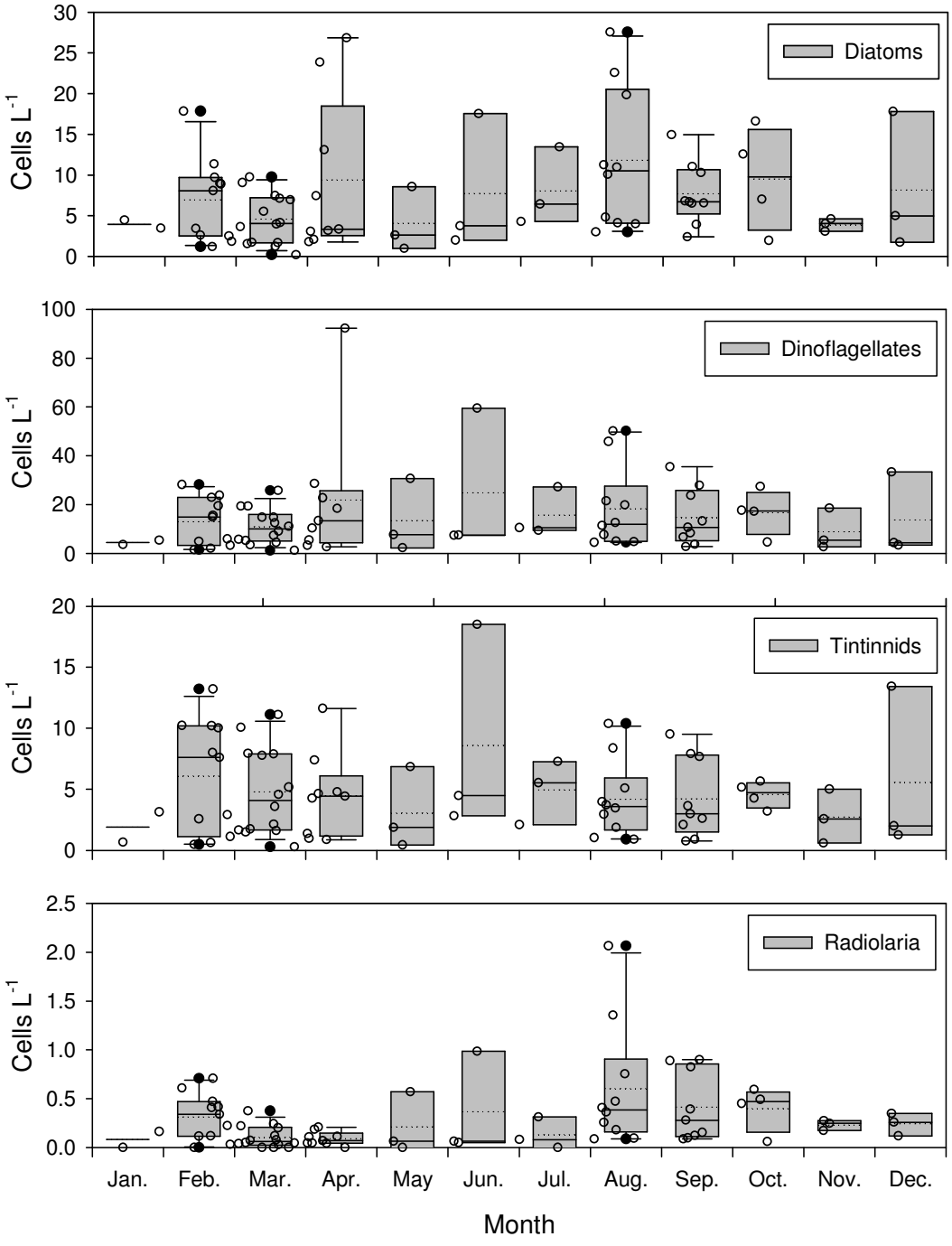


Figure 23 - Monthly box and whisker plots of microplankton abundance by group. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines show median abundance; dotted lines show mean abundance (circular data points show outliers). Open circular points show average abundance from each BATS cruise sampled.

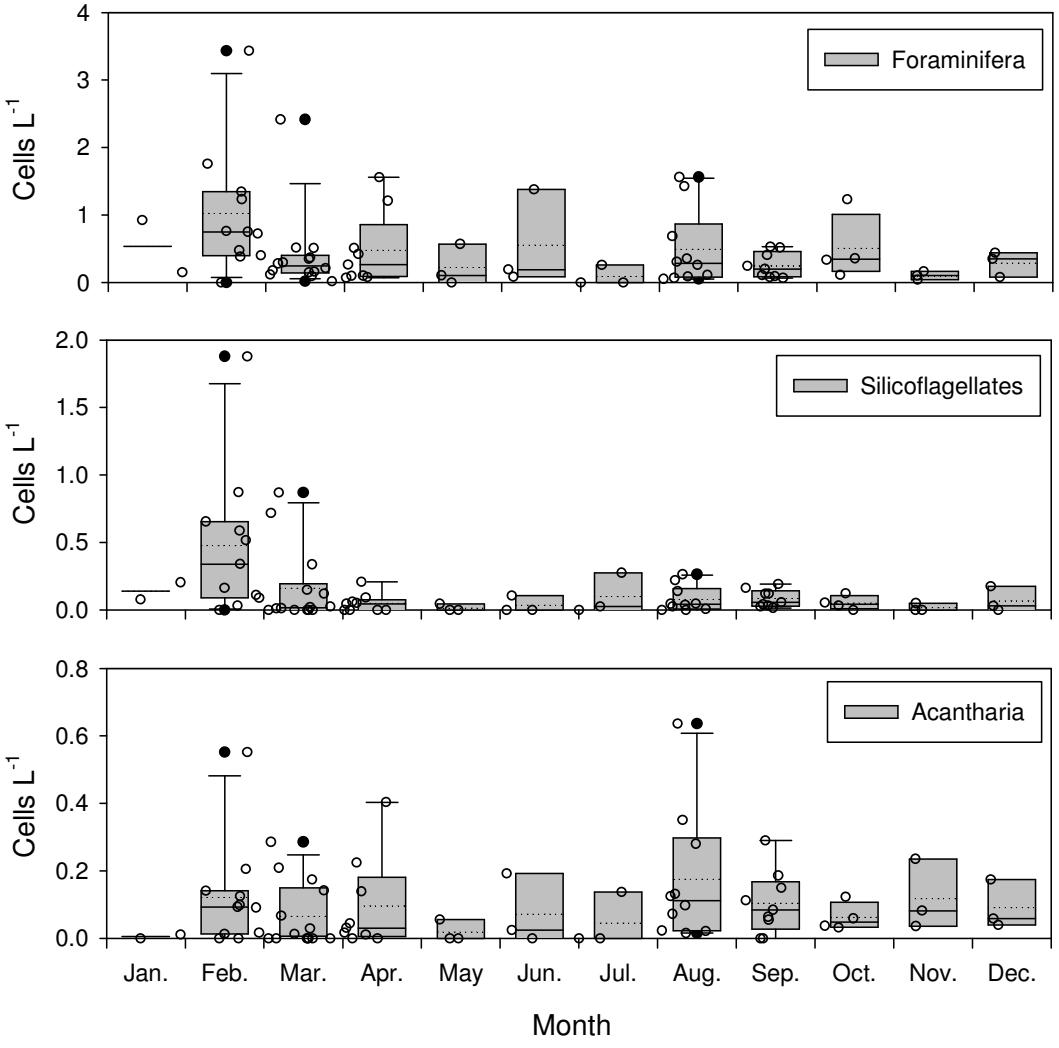


Figure 24 - Monthly box and whisker plots of microplankton abundance by group. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines show median abundance; dotted lines show mean abundance (circular data points show outliers). Open circular points show average abundance from each BATS cruise sampled.

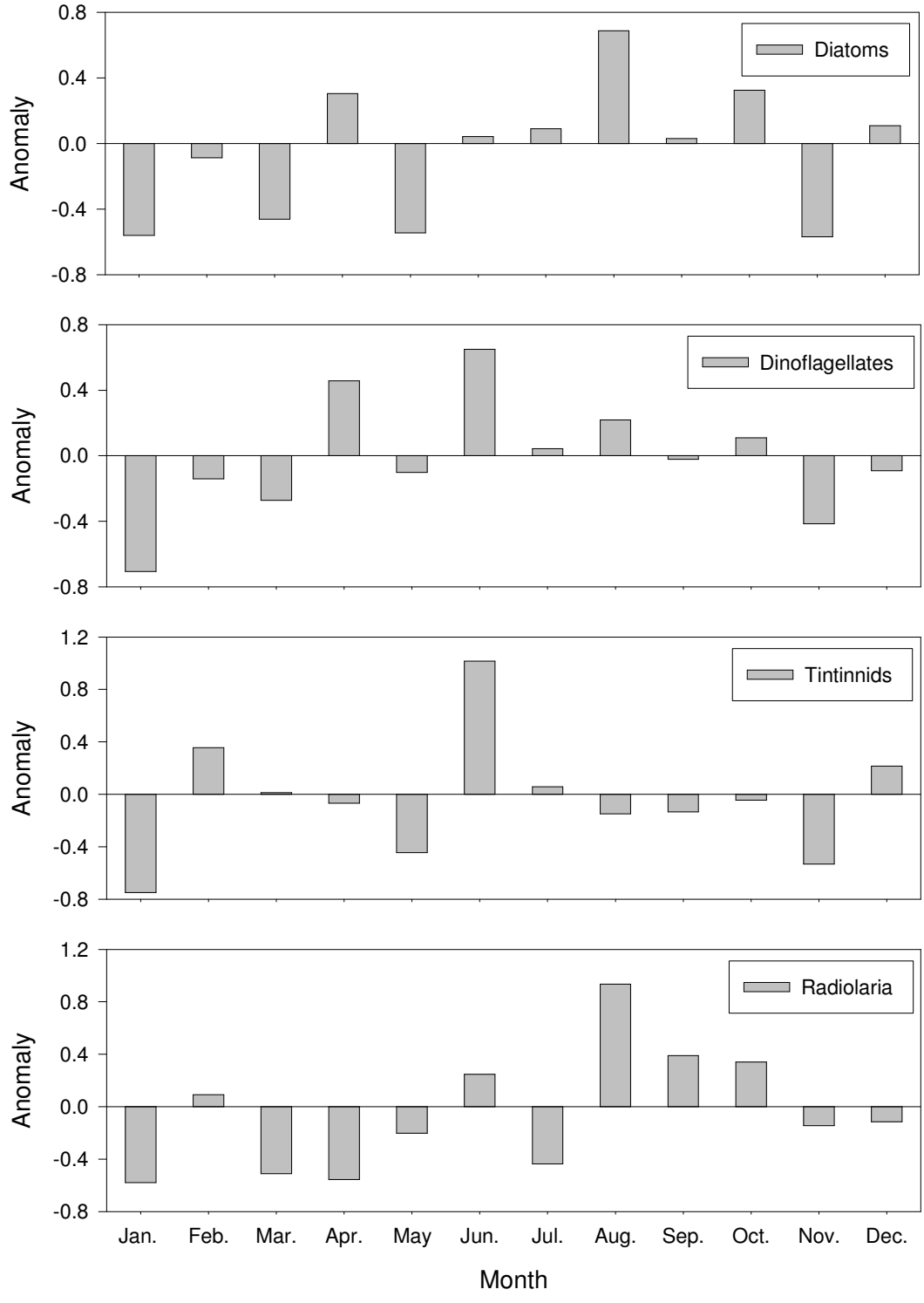


Figure 25 - Monthly anomaly data of microplankton abundance by group.

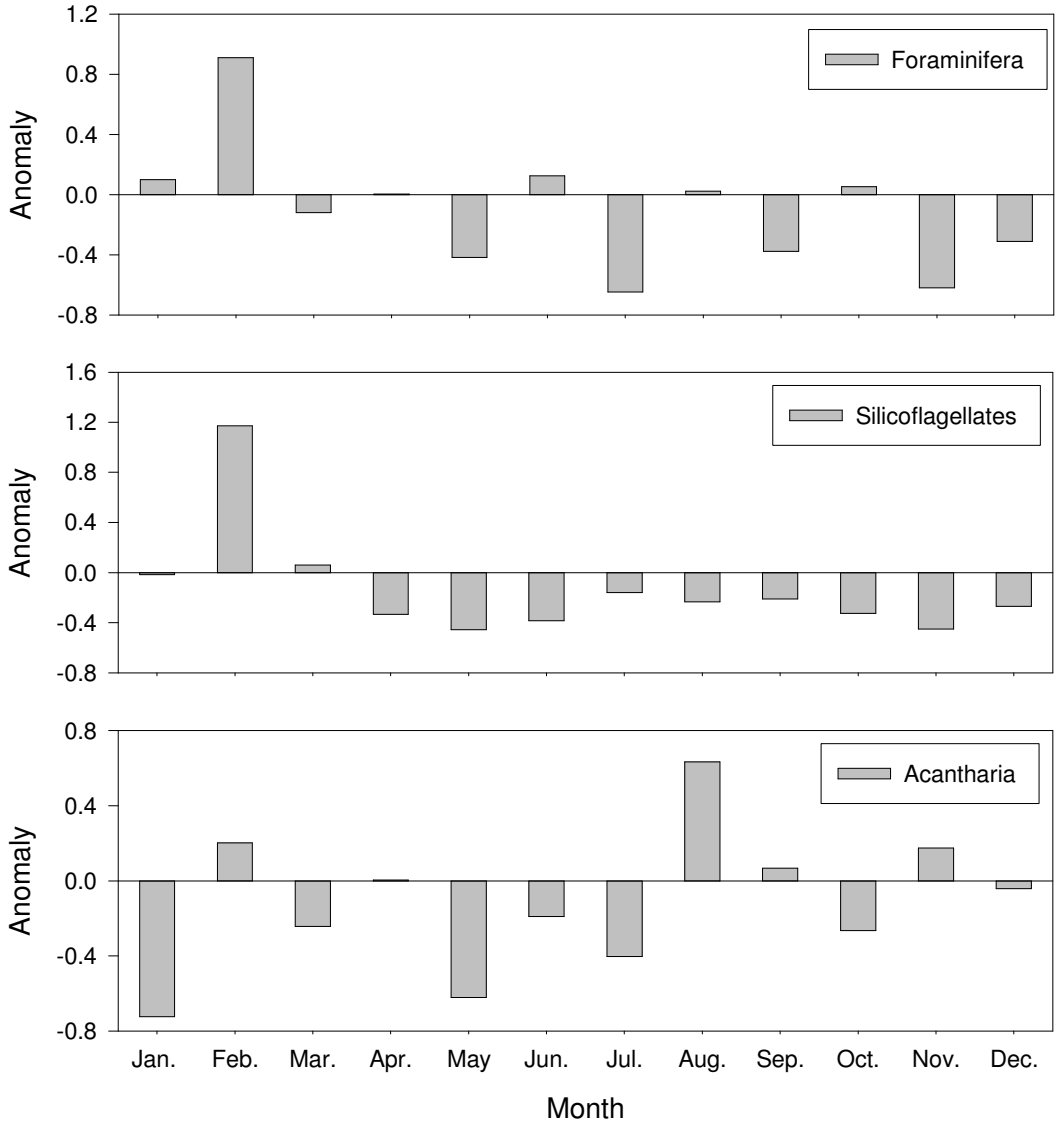


Figure 26 – Monthly anomaly data of microplankton abundance by group.

Diatom abundances are observed to be most variable during the months of April and August, with ranges of 2 - 27 cells L^{-1} and 3 - 28 cells L^{-1} respectively. The lowest (highest) single abundance value was observed in a March (August) sample at 0.2 (27.5) cells L^{-1}. Whilst the range of diatom cell abundance varies from month to month, there is little variation in the average monthly abundance, with all months within the range of 3.9 - 12.0 Cells L^{-1}. Average diatom abundance was highest in August samples (12.0 cells L^{-1}), closely followed by April and October averages (9.5 cells L^{1}). The difference in median abundances between months was not found to be statistically significant ($p = 0.51$), suggesting the presence of a diatom population that remains relatively stable in abundance over the course of a year. With the exception of April, the first five months of the year are represented by negative abundance anomalies, with the majority of summer and autumn samples (June – October) represented by positive abundance anomalies (Figure 25). This suggests slightly elevated summer/autumn abundances compared with winter and spring abundances. Although caution must be employed when interpreting these anomaly results alongside an overall 'non-significant' return from sample medians, monthly anomaly data can still help to highlight general trends between months (even when non-significant).

The abundance of dinoflagellates in the microplankton net samples follows a more uniform pattern than that of the diatoms when observing the scatter of abundance data plotted on a decimal year basis (Figure 23), although less variation within each month is observed during February and March samples compared to diatoms. Dinoflagellate abundances are approximately two times greater than diatom abundances however, with the highest (lowest) abundance recorded in an April (March) sample at 92.2 (1.2) cells L^{-1}. Due to this high value, the largest variation in dinoflagellate abundances is observed in April samples. The majority of all other samples fall within the abundance range of 1 - 60 cells L^{-1}. Highest monthly-averaged dinoflagellate abundances were recorded in June (25.3 cells L^{-1}), with the lowest average recorded in January (4.5 cells L^{-1}). The differences between median abundance values for each month were not found to be significant ($p = 0.92$), suggesting a relatively stable dinoflagellate population. The monthly anomaly calculated for dinoflagellate abundance suggests slightly elevated summer/autumn values with a mainly positive anomaly

from June – October. A negative anomaly calculated from November – March suggests a lower abundance population during winter and spring months (Figure 25).

Tintinnid cell abundances are roughly 50% of diatom abundances, with decimal day data scattered fairly evenly between abundances of 0 - ~15 cells L^{-1} in most months (Figure 23). The largest variation in tintinnid abundance and the highest single abundance is seen during June, with abundances ranging from 2.8 – 18.5 cells L^{-1}. February and December also show large ranges of abundances, with 0.5 – 13.2 cells L^{-1} and 1.3 – 13.4 cells L^{-1} respectively. June also shows the highest monthly averaged abundance at 8.9 cells L^{-1}, driven by the highest single tintinnid abundance. Average abundances for the remaining months are generally between 2-6 cells L^{-1}. The differences in median abundances between months was not found to be statistically significant ($p = 0.92$), highlighting the lack of a clear seasonal trend within this group. Monthly anomaly data (Figure 25) confirm this, with low anomalies recorded for March, April, July – October. June and January show the largest anomalies (1 and -0.7 respectively), suggesting that what little difference there is in abundance can be found mainly between these two months.

Decimal day abundance data for the radiolaria show a tight clustering of spring data points (Figure 23), with a larger spread of data points during the summer/autumn months. Higher summer/autumn variability is confirmed, with August having both the largest variation in abundance (0.1 - 2.1 cells L^{-1}), and the single highest radiolaria abundance (2.1 cells L^{-1}). November – January samples show low values and little variation within each month, with February – April abundances more variable in range, but still low in abundance. The highest monthly averaged radiolaria abundance is observed in August (0.60), immediately following one of the lowest average abundances in July (0.10 cells L^{-1}). Monthly averaged abundances peak in July, and appear to decline through the winter, reaching a minimum in January (0.08 cells L^{-1}) until a small peak in February (0.32 cells L^{-1}). Median abundances were found to be statistically different between months, ($p = 0.015$), with a difference identified between low January abundances and high August abundances. The differences between these two months can clearly be seen in Figure 25, with January having the largest negative

monthly anomaly and August the largest positive anomaly. Anomalies are generally negative from November through to May, with June and August – October returning positive abundance anomalies.

Foraminifera abundances show less variation within each month during September, November and December, whilst February, March and April show a wider variation of abundances. Figure 24 shows the highest abundance variation within a month (0 – 3.4 cells L^{-1}) to be in February, with 3.4 cells L^{-1} also recorded as the highest single abundance. Variation within March samples is also large (0.1 – 2.4 cells L^{-1}). An absence of foraminifera was noted in some samples from February, May and July with the notable latter having a monthly average of just 0.02 cells L^{-1}. The highest monthly averaged foraminifera abundance was recorded in February at 1.1 cells L^{-1}, with most other months between 0.4 – 0.5 cells L^{-1} on average. Despite observing apparent differences between high February and low July abundances, these proved to be non-significant when tested ($p = 0.15$). When comparing monthly anomalies, the majority are either negative anomalies or positive anomalies less than or equal to 0.1. A positive anomaly of 0.9 is calculated for February samples, highlighting the difference between this month and the rest of the year (Figure 26).

Silicoflagellate abundance data follow similar trends to the foraminifera data; decimal day data are tightly clustered from April – December, with an increased spread of abundances in February and March samples (Figure 24). These two months show a spread of abundances from 0 – 1.9 cells L^{-1} and 0 - 0.9 cells L^{-1} respectively. Abundances less than 0.3 are recorded for all other months, with little variability within months. When combined with the monthly averaged silicoflagellate abundance, a February abundance peak is observed, with an average of 0.5 cells L^{-1}. No significant difference was found between the mean abundance from each month ($p = 0.111$).

Decimal day abundance data for acantharia do not show the tight clustering (low variation) seen in silicoflagellate data, although overall abundance values are the lowest of all seven microplankton groups analysed (Figure 24). Without the tight clustering of data points, zero abundance data points are more clearly visible. The absence of acantharia was noted in samples from January – July and September. The largest range of abundances recorded in one month was 0.02 - 0.64 cells L^{-1} in August. A similar range of 0 – 0.55 cells L^{-1} was recorded for the month of February. Monthly averaged acantharia abundances are variable but low with all being 0.18 cells L^{-1} or less. The highest (lowest) average value is seen in August (January) at 0.18 cells L^{-1} (<0.01 cells L^{-1}). Statistical testing revealed no significant differences in the median abundance between each month ($p = 0.35$) suggesting a stable, but low, abundance population of acantharia.

Group	*Test*	*Significance (p-value)*	*Pairwise comparison*
Diatoms	Kruskal-Wallis	Non-sig. p=0.513	
Dinoflagellates	Kruskal-Wallis	Non-sig. p=0.920	
Tintinnids	Kruskal-Wallis	Non-sig. p=0.917	
Radiolaria	Kruskal-Wallis	Sig. p=0.015	Aug. vs. Jan.
Foraminifera	Kruskal-Wallis	Non-sig. p=0.149	
Silicoflagellates	Kruskal-Wallis	Non-sig. p=0.111	
Acantharia	Kruskal-Wallis	Non-sig. p=0.347	
Total	Kruskal-Wallis	Non-sig. p=0.927	

Table 4 - Summary of statistical testing on month-by-month abundance data, including statistical test used, significance of result, and pair of months responsible for difference (where applicable).

Total microplankton abundance plotted as decimal day abundance points (Figure 27 A) show an apparent increase in the range of abundances within a month in April, May and August samples, with tighter clustering observed in all other months. Both the highest single abundance (125 cells L^{-1}) and the largest range of abundances (7 – 125 cells L^{-1}) are recorded for the month of April (Figure 27 B). The highest (lowest) monthly averaged abundance however is recorded for June (January) at 43 cells L^{-1} (11 cells L^{-1}). Monthly anomalies highlight this, with predominantly positive anomalies from April to October, and negative anomalies from November to March (Figure 27 C). This suggests a slight increase in total microplankton abundance during the summer months (May - July), with a slight fall in abundance during winter months. Differences between median abundances for each month were not statistically significant ($p = 0.927$), representing a microplankton community that is overall relatively stable, with no sudden large increases or decreases in abundance month-by-month.

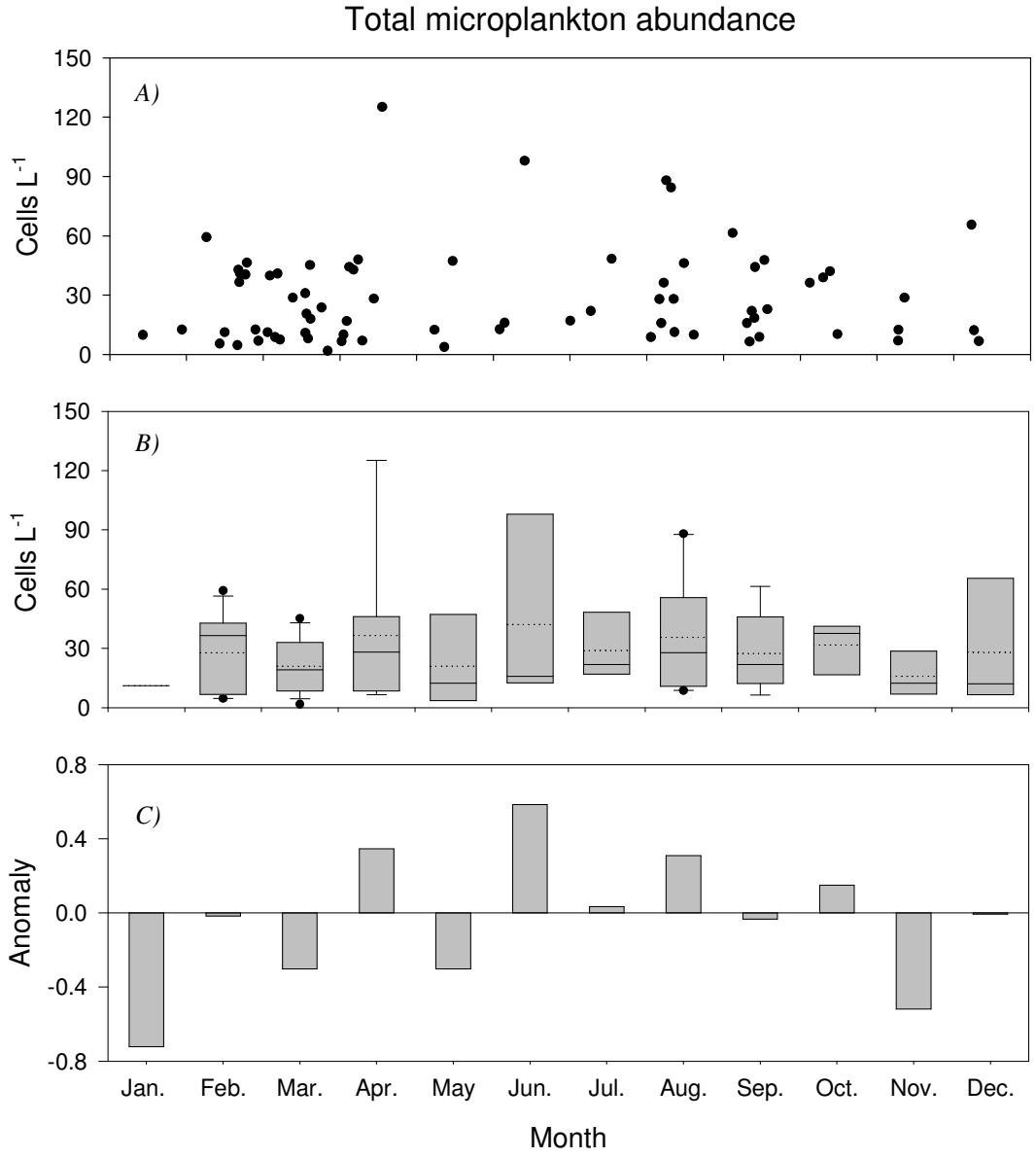


Figure 27 - Total microplankton abundance data. From top to bottom: A = decimal day abundance data, B = monthly box and whisker data (boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines show median abundance; dotted lines show mean abundance, circular data points show outliers), C = monthly abundance anomaly

When monthly averaged physical and chemical data averaged from the top 150m (Figures 28 and 29) is compared with monthly averaged microplankton abundance, there are very few significant correlations seen in the data. Non-parametric statistical testing was performed using Spearman Rank Order Correlations, with a sample number of 12. The results of these statistical tests are summarized in Appendix 10.4. Significant correlations between microplankton groups and various physical/chemical parameters are described below and illustrated in Figures 30 and 31. For the purpose of this analysis, any correlation coefficient >0.5 is considered a strong correlation; a coefficient <0.5 as a weak correlation. Abundance data are monthly averaged, whilst physical/chemical data is a water-column average value (top 150m), again on a monthly-averaged basis.

Radiolaria were the only group to show a significant correlation with temperature, with a strong positive correlation coefficient of 0.601 ($p = 0.036$). Highest total abundance, average abundance and largest within-month variation were recorded for August samples, confirming that the radiolaria population prefer the warm, stratified conditions found at the BATS site during the summer months. This confirms previous observations of high sarcodine abundances during summer months in oligotrophic areas (Swanberg and Caron 1991). Radiolaria also showed strong negative correlations with average nitrite and silicate concentrations in the top 150m (nitrite correlation coefficient -0.601, $p = 0.036$, silicate correlation coefficient -0.587, $p = 0.042$). Combining this negative correlation with nutrients and a positive correlation with temperature, it is therefore suggested that the radiolaria are increasing as nutrients are drawn down by smaller phytoplankton during periods of rapid spring growth. Diatoms and dinoflagellates are both included in the list of radiolaria prey (Swanberg and Caron 1991); as microzooplankton, the radiolaria respond to increases in spring prey, resulting in a net negative correlation between radiolaria abundances and nutrients. This is reflected in increasing abundances during the summer months, with lower winter and spring monthly averaged abundances (i.e., a simple time lag is seen between the increase in nutrients and therefore radiolaria prey, and the increase in radiolaria abundances).

Silicoflagellates displayed a strong negative correlation with monthly water-column averaged phosphate concentrations; correlation coefficient -0.580, $p = 0.045$. Silicoflagellate abundances appear to reach maximum values in early spring samples (February averages are highest), earlier than the other microplankton groups. It is suggested that they are able to utilise nutrients present at lower concentrations than other phytoplankton during early spring months. Once winter and spring mixing act to raise nutrient concentrations sufficiently to support the spring bloom scenario seen in other phytoplankton groups, silicoflagellates appear to be outcompeted for nutrients and a decrease in abundance is observed (Figure 24).

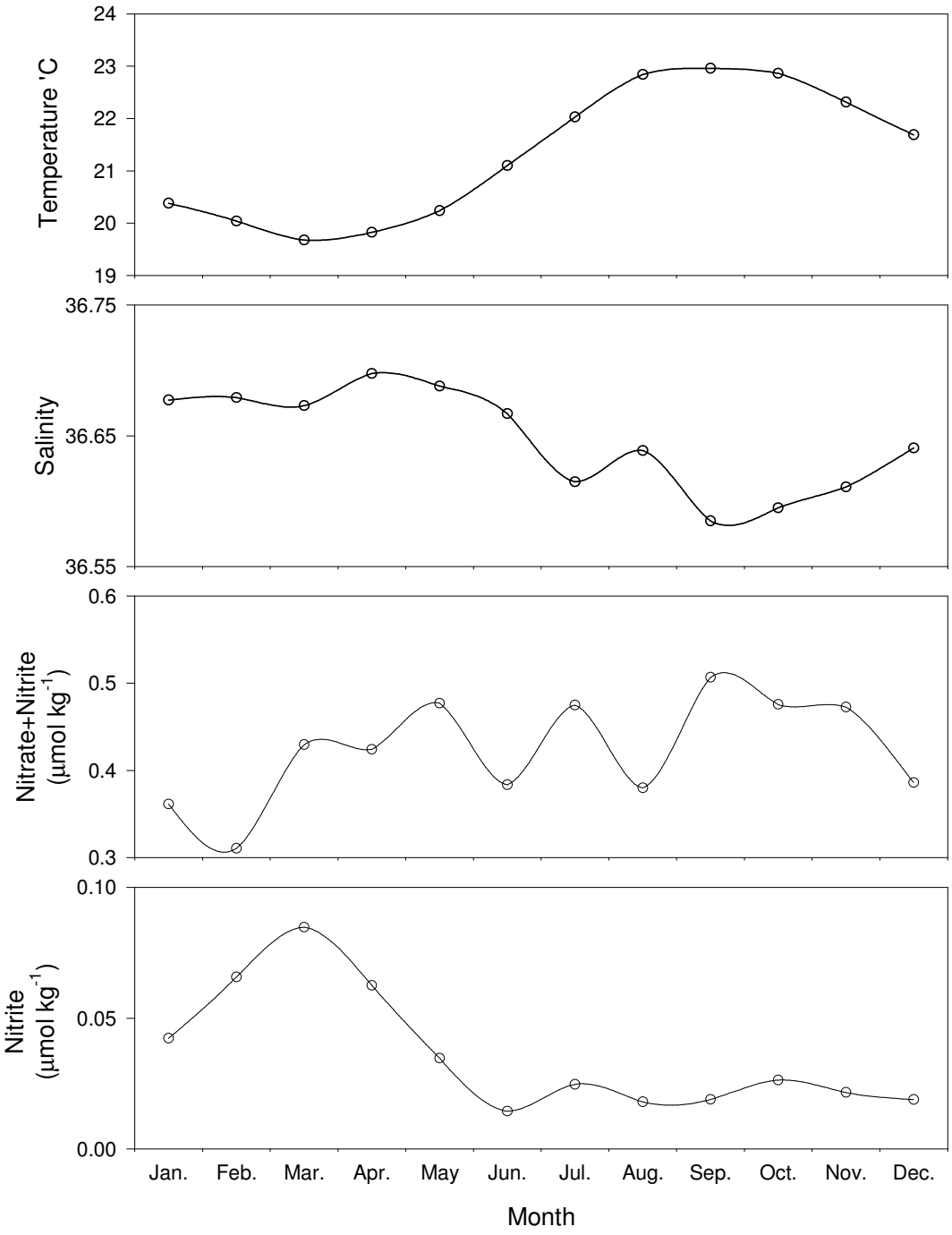


Figure 28 - Monthly averaged CTD physical and chemical data for the BATS sampling site, averaged over the top 150m of the water column. Top to bottom; Temperature, Salinity, Nitrate+Nitrite and Nitrite.

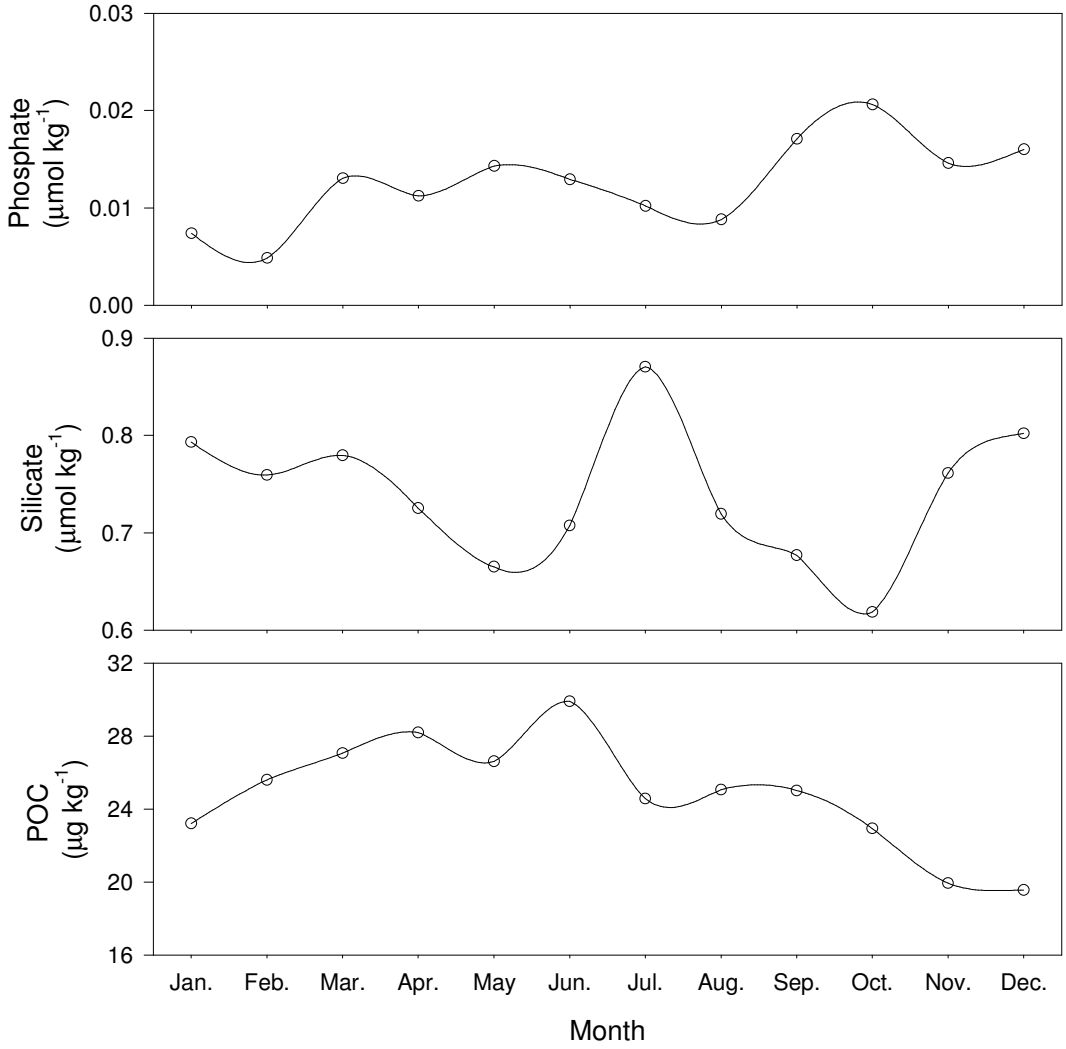


Figure 29 - Monthly averaged physical and chemical data for the BATS sampling site, averaged over the top 150m of the water column. Data taken from CTD casts for every cruise available. From top to bottom; Phosphate, Silicate and POC (Particulate Organic Carbon).

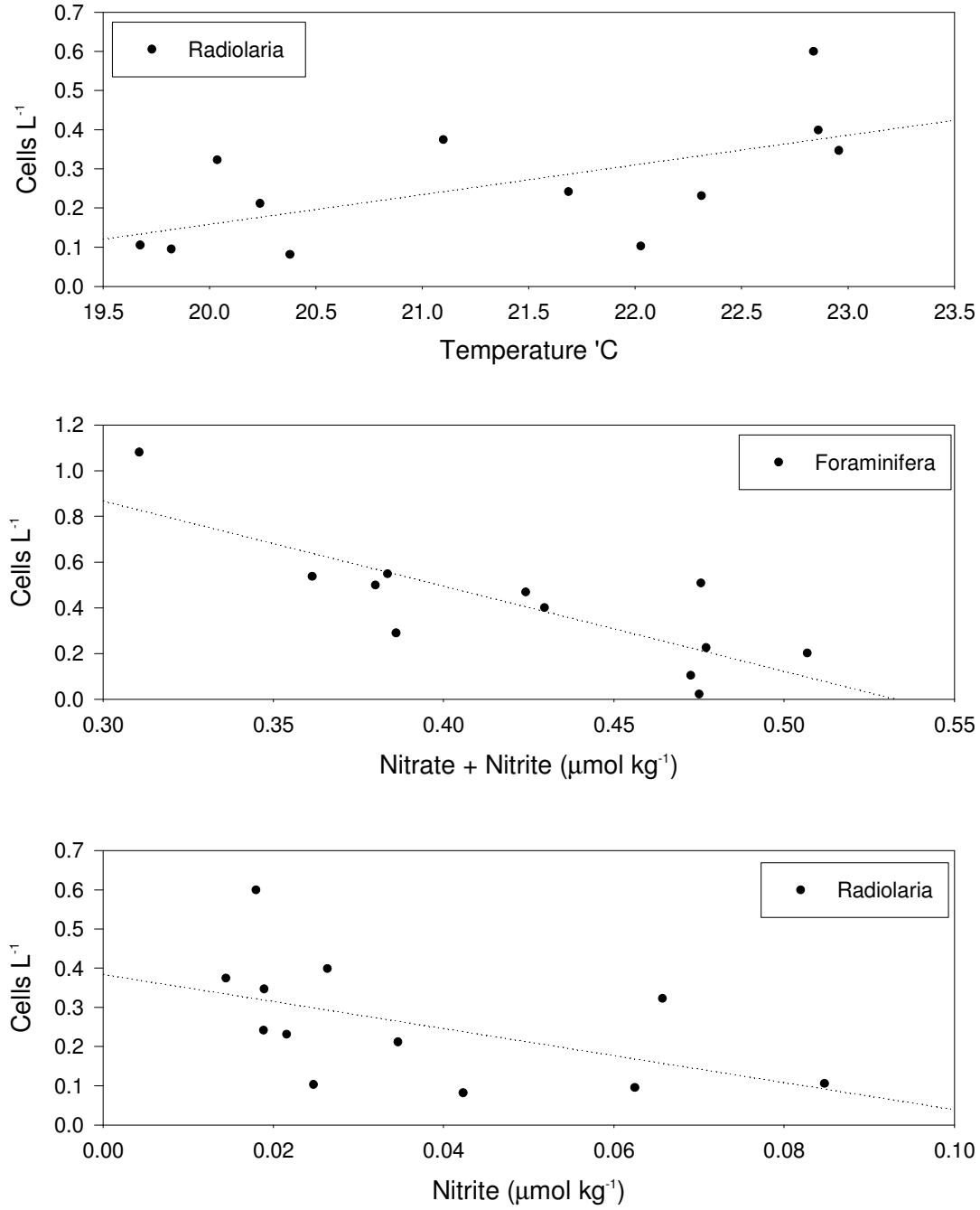


Figure 30 - Model 1 linear regressions applied to microplankton groups with significant correlations to various physical/chemical water column parameters. All data points represent monthly-averaged microplankton abundance data, and physical/ chemical data monthly and water column (top 150m) averaged. From top to bottom; Radiolaria and Temperature, Foraminifera and Nitrate+Nitrite, Radiolaria and Nitrite.

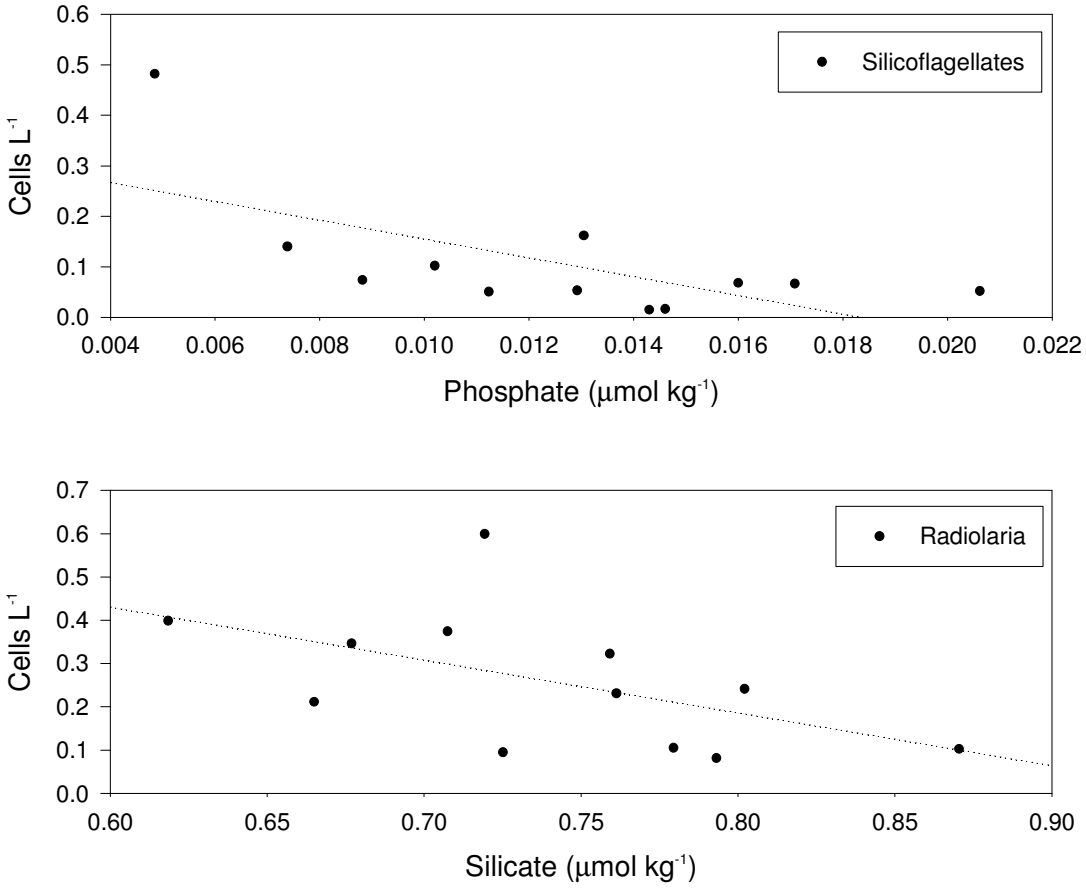


Figure 31 - Model 1 linear regressions applied to microplankton groups with significant correlations to various physical/chemical water column parameters. All data points represent monthly-averaged microplankton abundance data, and physical/ chemical data monthly and water column (top 150m) averaged. From top to bottom; Silicoflagellates and Phosphate, and Radiolaria and Silicate.

4.1.2 Microplankton relative abundance

In addition to abundance data, community composition was also analysed as a percentage contribution (or relative abundance) of each group to the total microplankton abundance. The resulting percentage composition is shown below on a cruise-by-cruise basis, and on a monthly averaged basis (Figure 32). It can clearly be seen that dinoflagellates numerically dominate the microplankton community, followed by diatoms and tintinnids. A summary of statistical testing is provided in Table 5, with more detail on the contribution of each of these microplankton groups given below (Table 6).

In the case of most of the microplankton groups, relative abundances were observed to be most variable during spring months. The exceptions to this pattern are the radiolaria and acantharia, with most variation in autumn and winter months respectively. Monthly medians, means and outlying data points for each microplankton group can be seen in Figures 33 and 34.

Diatoms were recorded to be the dominant group in terms of relative abundance in samples from only 8 of the 80 cruises examined. These diatom dominated samples were recorded in samples from January, February, April (x2), September (x2), November and December. These samples were characterised by low abundances of dinoflagellates (<50 % of time-series dinoflagellate average), with the exception of one April sample. The diatom-dominated composition of this sample (BATS 115, April 1998) was driven instead by very high diatom abundances (>300 % of time-series diatom average). In monthly averaged samples, January and June were identified as the source of a significant difference between medians of monthly composition ($p = 0.002$).

The relative abundance of the remaining 72 samples (90 % of total samples) was numerically dominated by the dinoflagellates. An apparent increase in monthly averaged dinoflagellate contribution is noted from January through to May, before contribution declines through to December (Figure 33). May and January were identified as the months driving a significant difference in monthly medians ($p = 0.031$) (Table 5).

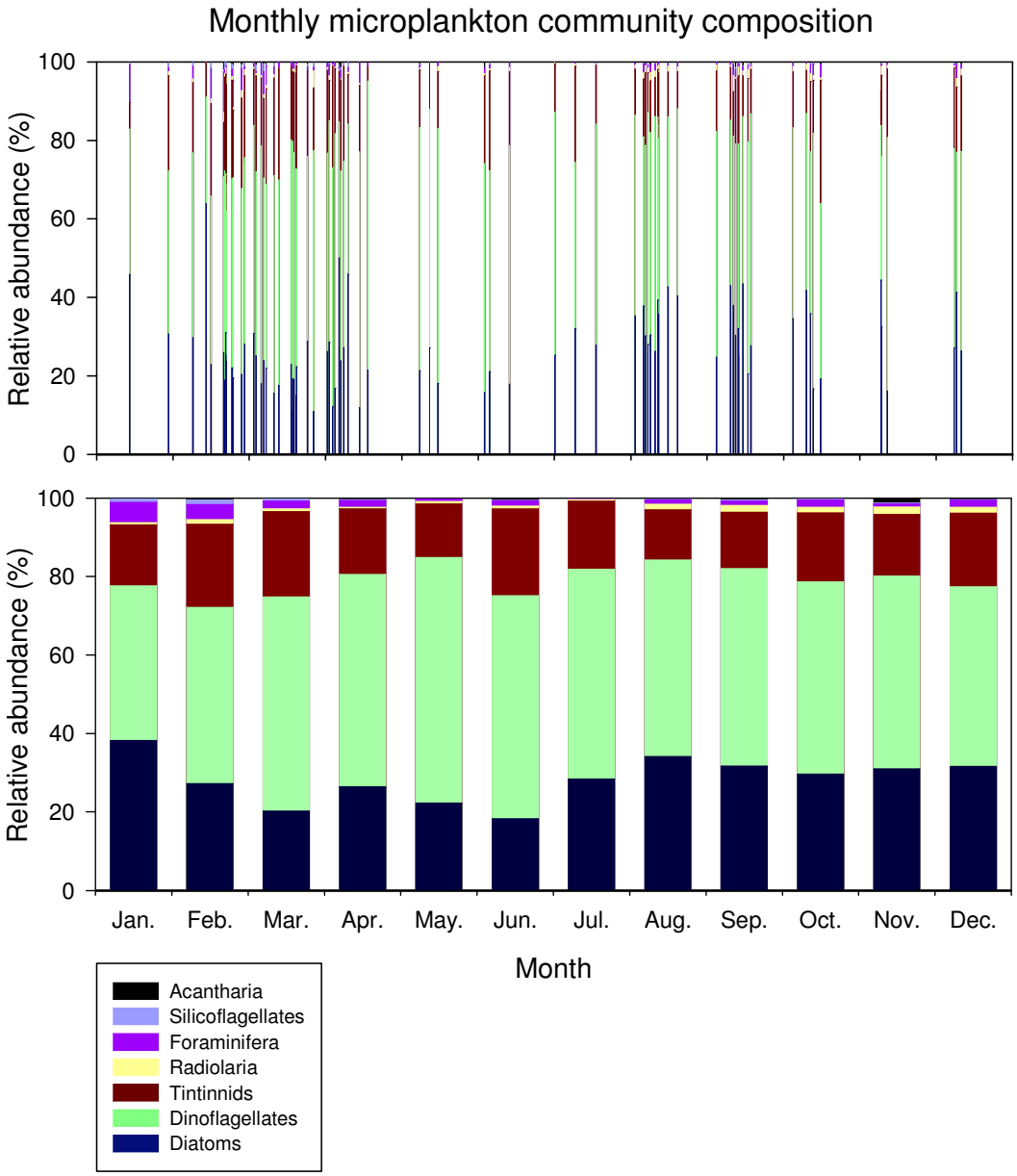


Figure 32 – Relative abundance of microplankton groups; cruise - by - cruise (top) and monthly-averaged data (bottom).

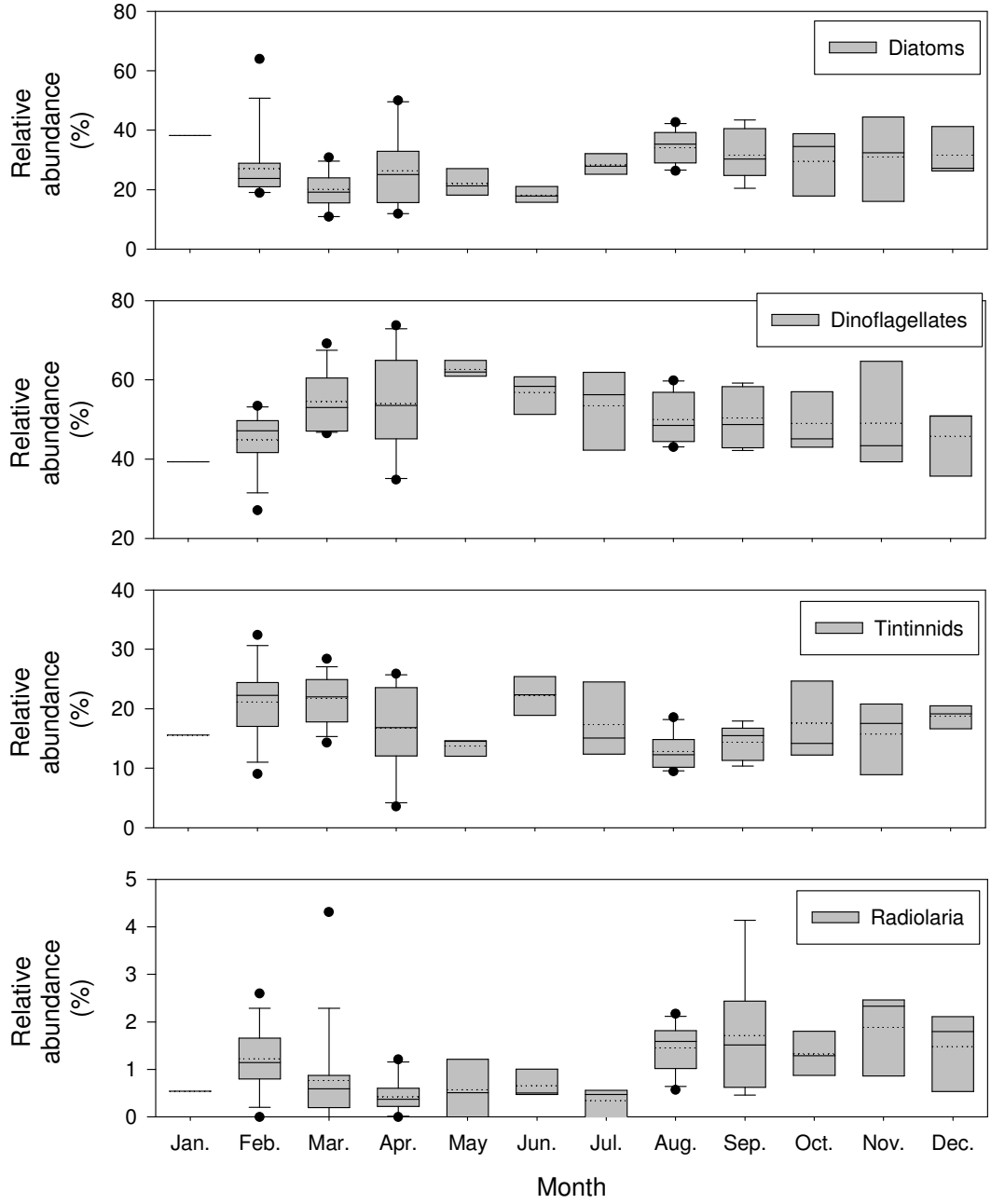


Figure 33 - Monthly box and whisker plots of relative abundance by group. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines show median abundance; dotted lines show mean abundance (circular data points show outliers).

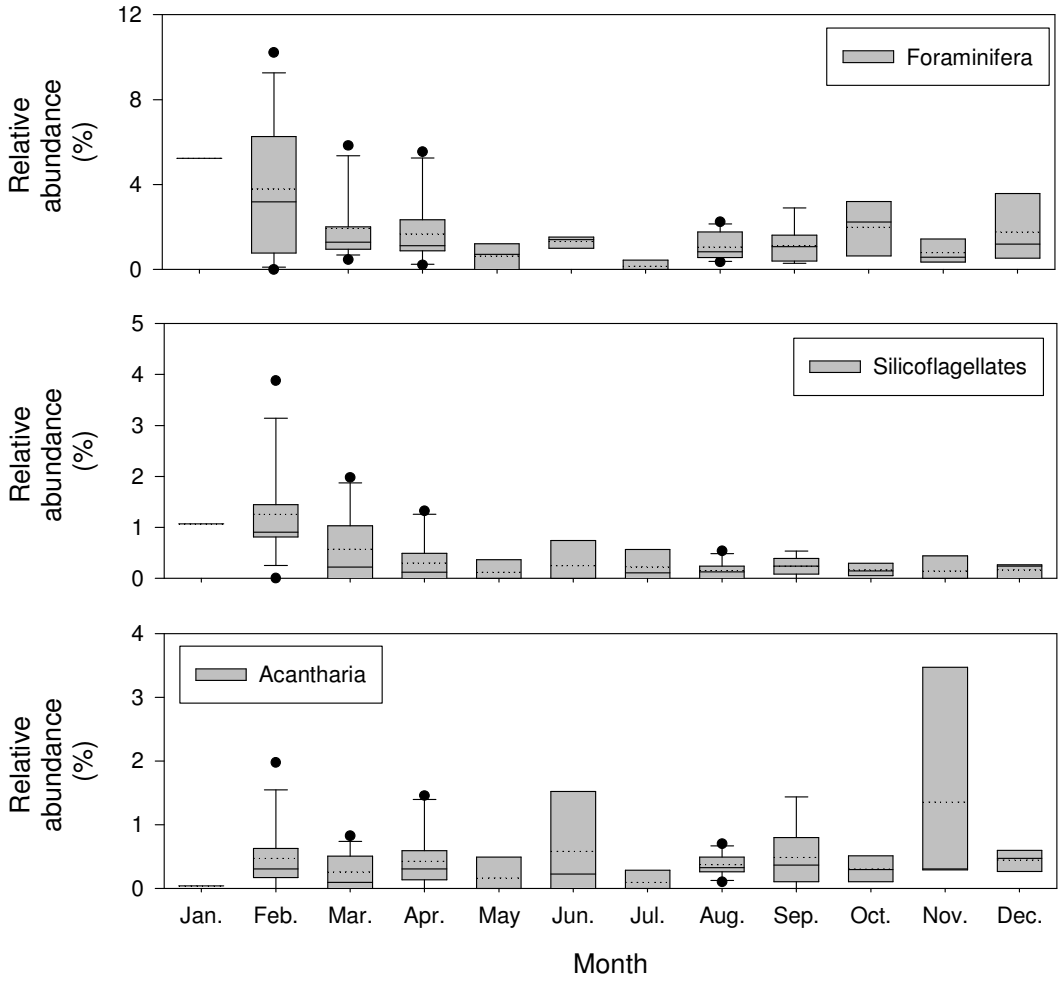


Figure 34 - Monthly box and whisker plots of relative abundance by group. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines show median abundance; dotted lines show mean abundance (circular data points show outliers).

Tintinnids were also large contributors to the total microplankton community, with relative abundances of a similar magnitude to that of the diatoms. A large variation of tintinnid contribution within months is observed, although little apparent variation between months. Despite this low range in monthly abundance (10 %) when compared to diatoms and dinoflagellates (ca 20 %), the months of June and August were identified as the drivers of significant difference between the monthly medians ($p = 0.003$) (Table 5). Although third in overall contribution to the microplankton community, monthly averaged contributions for March and June were larger for the tintinnids than for diatoms. Further analysis showed samples from 23 of the 80 cruises to have higher tintinnid contributions than diatoms (Figure 35). These data points were mainly during February (7 cruises), March (9 cruises) and June (3 cruises).

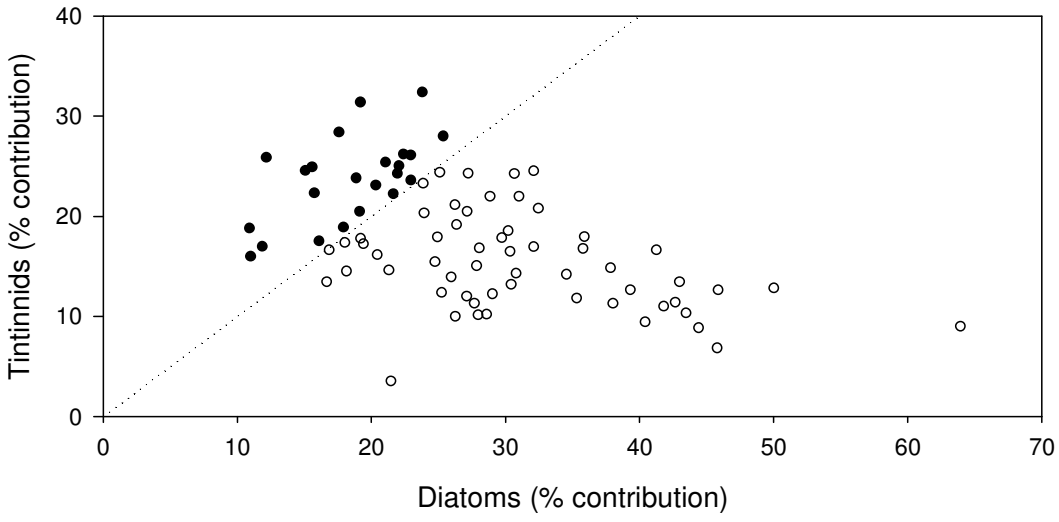


Figure 35 – Relative abundance of diatoms and tintinnids to total microplankton community. Dotted line represents 1:1 ratio, where tintinnid contribution equals diatom contribution. Filled circles represent those samples above the 1:1 line, where tintinnid contribution exceeds that of diatom contribution.

Although the relative abundance of radiolaria varied most during the month of September, a single high contribution in March is noted as an outlier in Figure 35. The remaining March samples show much less variation in relative abundance. This particular sample (BATS 138a, March 2000) was characterised by very low diatom, dinoflagellate and tintinnid abundances rather than above-average radiolaria abundance. April – July radiolaria contributions to total microplankton are lower (and also less variable) than August – December samples, suggesting an increase in importance of radiolaria in autumn and winter samples. This is confirmed by the monthly averaged data, with August – September samples all > 1.2 %, and April – July samples all < 0.7% relative abundance. This increase in relative abundance is not due solely to an increase in total abundance of radiolaria, but also to lower abundances of other groups in autumn and winter months, relative to other months. See Results I - 4.2 for more details on seasonal variation. Statistical analysis of the median composition for each month identified November and July as responsible for a significant difference found between months ($p < 0.001$) (Table 5).

The contribution of foraminifera to total microplankton abundance follows a similar pattern to that of foraminifera abundance; higher variability and individual values during February, with a stable population (less variability) observed during May – December (Figure 35). All months excluding January (5.2 %) and February (3.8 %) averaged < 2 % contribution. This increase in relative abundance during these two months is driven by the increase in absolute abundance over the same period. Despite the observed pattern in relative abundance for the foraminifera, there were no significant differences found between median contributions of different months ($p = 0.081$).

As with the foraminifera, the relative abundance of the silicoflagellates is driven directly by increases and decreases in absolute abundance of this group, rather than apparent abundance changes in other groups. This is most likely due to the fact that these organisms are present in such low numbers when compared to the larger groups of diatoms, dinoflagellates and tintinnids, they are less influenced by any changing relationships between relative abundances of other groups. January and May were identified as the months driving a significant difference

between monthly medians (Kruskal-Wallis, p = 0.006) (Table 5). A stable population of silicoflagellates from April – December contributed, on average, less than 0.4% of the total sample, compared to January and February samples where average relative abundances of 1.1 and 1.3 % respectively were recorded.

Lastly, the contribution of acantharia to the total microplankton community is the smallest of all groups, with little variation suggesting that the acantharia provide a relatively stable contribution to the community. A single relatively large contribution in November (3.5 %) can be attributed to a sample (BATS 158, November 2001) where abundances of all other groups are below average, whilst acantharia abundance is above average.

When considering patterns within and between months, it is extremely useful to examine both abundance and relative abundance data. As previously mentioned, there are cases where a high percentage contribution, for instance, may actually reflect on abundance changes in all other groups, rather than the specific group being examined. For this reason, it is important to consider both abundance and composition data together when drawing conclusions on any time-scale. The data presented in this section highlight a diatom community with a relatively stable base abundance throughout the year, showing slight (but non-significant) elevation in abundances during summer and autumn months.

Due to the diatoms, dinoflagellates and tintinnids having the largest relative abundances in the total microplankton samples, these groups do not show independent behaviour when relative abundances are looked at, i.e. a decreased relative abundance of diatoms can also be observed as an increased relative abundance of tintinnids etc. The overall lack of seasonal signal observed in the major microplankton groups (diatoms, dinoflagellates and tintinnids) is reflected in the analysis of the total microplankton community abundance. Whilst there are no significant differences observed between months, the overall trend appears to be a slight increase in summer abundances, with a slight decrease in winter abundances. This is somewhat in contrast to the pattern reported for the smaller phytoplankton size-classes; a picoplankton based community showing a defined response to winter mixing; following a “spring-bloom” scenario (DuRand *et al.,*

2001; Steinberg *et al.,* 2001). From the monthly data discussed in this chapter, it can be concluded that the microplankton community appears to exist at a relatively stable abundance and composition year-round, with only minor variations observed in a few microplankton groups.

Group	*Test*	*Significance (p-value)*	*Pairs responsible for differences*
Diatoms	Kruskal-Wallis	Sig. p=0.0.002	Jan. vs. Jun.
Dinoflagellates	Kruskal-Wallis	Sig. p=0.031	May vs. Jan.
Tintinnids	Kruskal-Wallis	Sig. p=0.003	Jun. vs. Aug.
Radiolaria	Kruskal-Wallis	Sig. p<0.001	Nov. vs. Jul.
Foraminifera	Kruskal-Wallis	Non-sig. p=0.081	
Silicoflagellates	Kruskal-Wallis	Sig. p=0.006	Jan. vs. May
Acantharia	Kruskal-Wallis	Non-sig. p=0.452	

Table 5 - Summary of statistical testing on month-by-month relative abundance data, including statistical test used, significance of result, and the pairs of months responsible for significant differences (where applicable).

Group	Lowest contribution	Highest contribution	Most variable months	Highest monthly average	Lowest monthly average	Kruskal-Wallis differences between months
Diatoms	10.9 % (March)	63.9 % (February)	February (18.9 – 63.9 %) April (11.9 – 50.0 %)	38.2 % (January)	18.2 % (June)	January vs. June ($p = 0.002$)
Dinoflagellates	27.0 % (February)	73.7 % (April)	April (34.7 – 73.7 %)	62.6 % (May)	39.3 % (January)	January vs. May ($p = 0.031$)
Tintinnids	3.5 % (April)	32.4 % (February)	February (9.0 – 32.4 %) April (3.5 - 25.9 %)	22.2 % (June)	12.8 % (August)	June vs. August ($p = 0.003$)
Radiolaria	0 % (Jan. – May, July)	4.3 % (March)	September (0.5 – 4.1 %)	1.9 % (November)	0.3% (July)	July vs. November ($p < 0.001$)
Foraminifera	0 % (Feb., May – July)	10.2 % (February)	February (0 – 10.2 %)	5.2 % (January)	0.1 % (July)	Non-significant
Silicoflagellates	0 % (all exc. Jan., Jul. and Sep.)	3.9 % (February)	February (0 – 3.9 %)	1.3 % (February)	0.1 % (May)	January vs. May ($p = 0.006$)
Acantharia	0 % (all exc. Aug., Oct. – Dec.)	3.5 % (November)	November (0- 3.5 %)	1.4 % (November)	<0.1 % (January)	Non-significant

Table 6 - Summary of relative abundance (% contribution) of different microplankton groups to the community. Highest and lowest individual and monthly-averaged contributions are listed, as well as any statistically significant differences recorded between months (where applicable)

4.2 Seasonal variability

In addition to presenting data on a monthly time-scale, it can also be useful to analyse the data in larger groupings, to determine any changes present on a slightly longer time-scale. In the case of this study, the seasons are defined as such; *Spring*: February, March and April. *Summer*: May, June and July. *Autumn*: August, September and October. *Winter*: November, December and January.

Box and whisker plots of each microplankton group are used to highlight variability, median abundance, mean abundance and outlying (anomalous) abundances within a season (Figures 36 and 37). Non-parametric statistical analysis was performed using Mann-Whitney U-tests, to identify significant differences in seasonal abundances for each microplankton group (analysis performed on one pair of seasons at a time). Significant differences are reported below; full statistical results can be seen in Appendix 10.5. Any month-by-month patterns in the dinoflagellates, tintinnids, foraminifera or silicoflagellates are cancelled out when looking at seasonal variability, with no significant differences seen between seasons for these four microplankton groups. Diatoms show a significant difference between spring and autumn abundances ($p = 0.028$), with autumn samples recorded as having higher abundances. Although winter abundances appear to be low (Figure 36), there were no significant differences found between winter and the other seasons. This is a somewhat unexpected result, as other phytoplankton size-groups such as the picoplankton at the BATS site have maximum abundances during the 'spring bloom', coincident with winter and spring mixing of the water column (Steinberg *et al.,* 2001). Highest abundances in autumn samples suggest the presence of large diatoms that are able to successfully utilize low nutrient concentrations, where a bloom of smaller phytoplankton may not be supported.

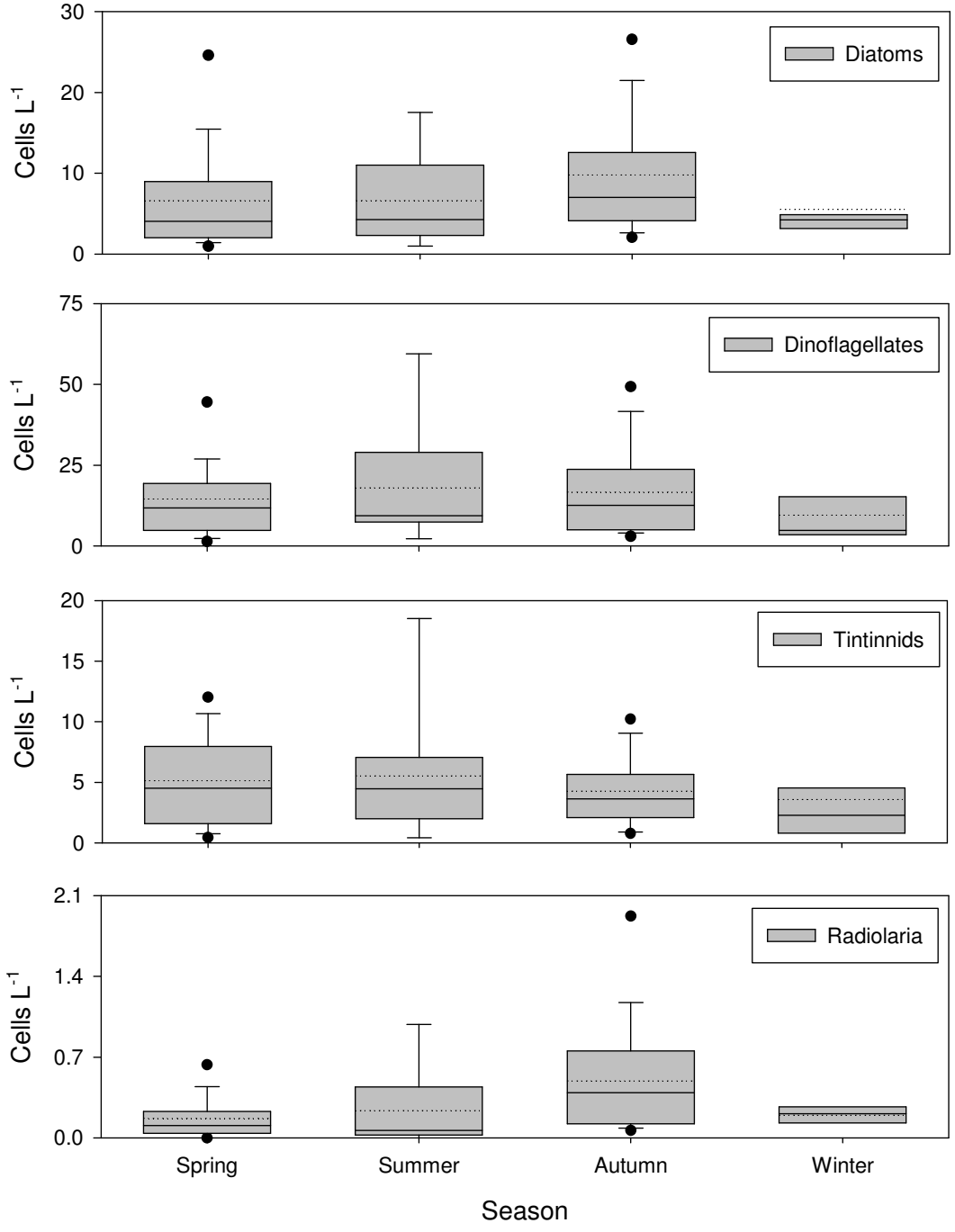


Figure 36 - Seasonal box and whisker plot of microplankton abundance by group. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines show median abundance; dotted lines show mean abundance (circular data points show outliers).

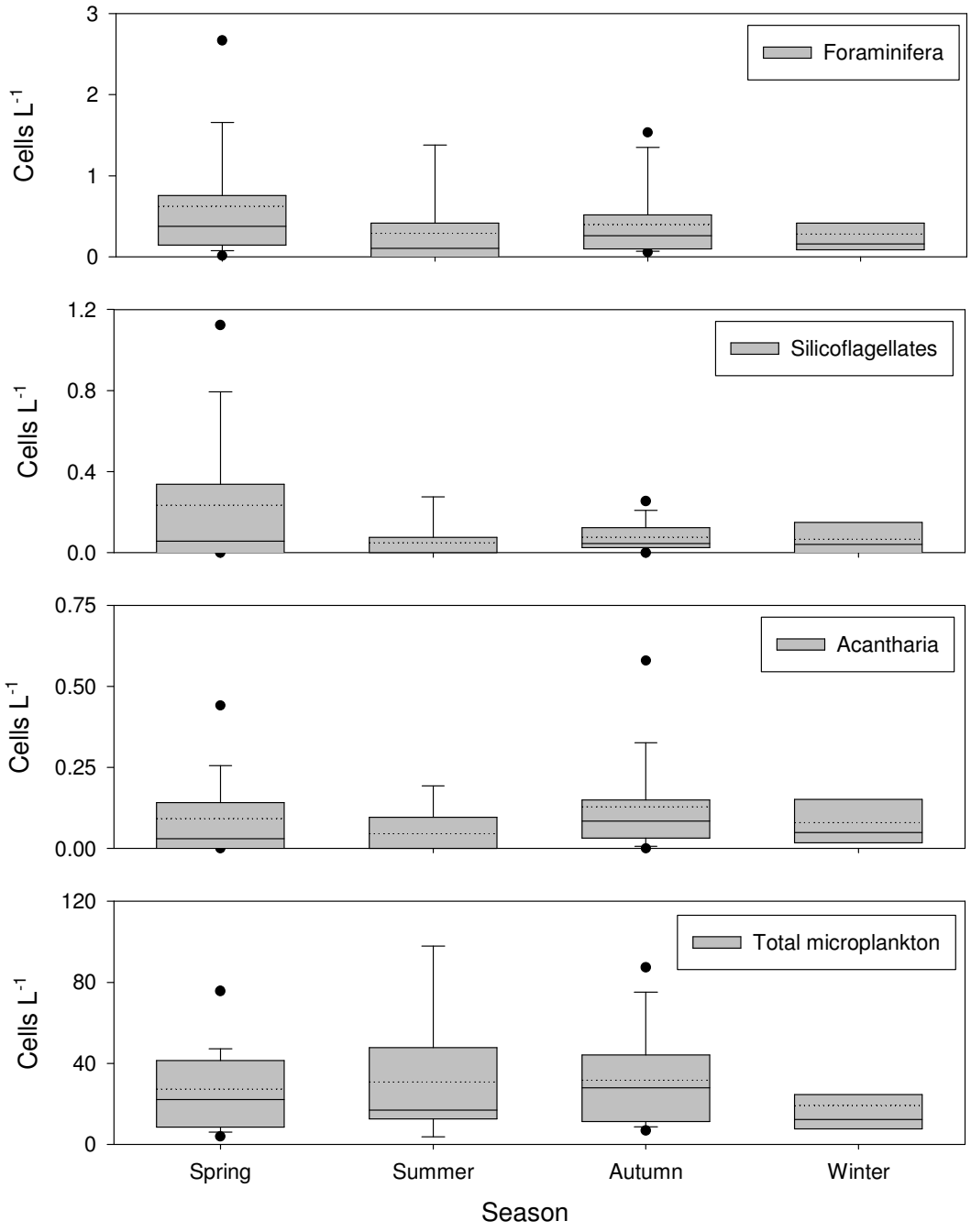


Figure 37 - Seasonal box and whisker plot of microplankton abundances by group. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines show median abundance; dotted lines show mean abundance (circular data points show outliers).

Significant differences were observed in radiolaria abundances between autumn and spring ($p < 0.001$), and between autumn and summer ($p = 0.026$). In both cases, autumn samples were recorded to have higher abundances. Similarly, autumn acantharia abundances were found to be significantly higher than summer acantharia abundances ($p = 0.039$). Although average total abundances are lowest during winter, and highest in autumn (increasing from spring to autumn) there was no statistical significance to this pattern.

4.3 Discussion

High levels of variability are observed in the single-celled euphotic-zone microplankton abundance data, on a monthly timescale from the BATS site. Absolute abundance values for each group were recorded, in addition to calculating a relative abundance (the percentage contribution of each measured group to the whole single-celled microplankton sample). Of the seven microplankton groups analysed, only the Radiolaria showed a significant difference between abundances in different months with low abundance January samples and high abundance August samples driving this difference.

Diatom contribution is more variable during spring months and stable during autumn and winter months. Abundance of diatoms is second in magnitude only to dinoflagellates; with 10% of samples numerically dominated by diatoms. Although there was no significant difference found when comparing diatom abundances by month, spring samples (February – April) were found to be significantly lower than autumn diatom abundances. This pattern is contrary to that seen in early studies of the Sargasso Sea plankton by Riley (1957) who documented a diatom increase each April and minimum in August (from bottle-collected plankton samples), Menzel and Ryther (1960) described a spring increase in primary productivity and of phytoplankton standing stocks (based on chlorophyll a concentrations and bottle-collected plankton samples), also contrary to more recent studies concerning other plankton size-groups analysed at BATS e.g. eukaryotic phytoplankton showing highest concentrations during spring blooms, using pigment data and flow cytometry (Steinberg *et al.,* 2001). The abundance patterns of the microplankton diatoms do not indicate the presence of a typical 'spring bloom' scenario, with no large increase in cell abundance, however it is worth noting that although diatom blooms have been recorded at BATS, generally they are considered to be a rare occurrence (Steinberg *et al.,* 2001). When the spring mixing of nutrients into the euphotic zone is considered, it is perhaps expected to record an observable response from the microplankton diatoms to these nutrient increases. Despite this, no significant correlations were observed between monthly averaged diatom abundances and biogeochemical factors such as nutrient availability, temperature, salinity, or indeed measured

pigments including chlorophyll a and the diatom marker pigment, Fucoxanthin. The lack of any significant correlation particularly between the microplankton-sized diatoms and the Fucoxanthin concentrations suggests that smaller diatoms are contributing more to the measured Fucoxanthin than the larger microplankton diatoms, particularly when the abundances of smaller phytoplankton in the Sargasso Sea are much higher than the values recorded for microphytoplankton in this study (Riley 1957; Hulburt 1961). Highest diatom abundances during autumn samples would suggest that the microplankton sized diatoms are more able to successfully utilize low nutrient concentrations found in the summer/autumn stratified water column, than other microplankton groups, or indeed other sized diatoms are able to. This lack of a 'driving factor' behind variable diatom abundances and relative abundances suggests the presence of a more complicated relationship between the microplankton sized diatoms and the biogeochemical parameters measured at the BATS site than initially suggested (Riley 1957; Menzel and Ryther 1960; Hulburt 1961). Diatoms were numerically dominant only in ten percent of samples, mostly due to low abundances of dinoflagellates, rather than particularly high diatom abundances. Although appearing relatively stable in relative abundance throughout the year, a significant difference between high January contributions and low June contributions to the total microplankton samples was recorded.

A relatively stable base abundance of dinoflagellates throughout the year is seen with a slight (but non significant) summer and autumn abundance elevation and depressed abundances during winter and spring months. This is reflected in an increase in percentage contribution (relative abundance) from January to May, before a gradual decrease towards December. When analysing month-by-month, no significant differences in absolute dinoflagellate abundance were recorded, although a significant difference between high May and low January relative abundances was noted. The existence of both heterotrophic and autotrophic species of dinoflagellate has traditionally been recognized, in addition to mixotrophic species (Stoecker *et al.,* 1997; Stoecker 1999). Identifying autotrophic species from heterotrophic species in preserved samples is however extremely difficult. The most commonly used method is using the presence of Chl a as an identifying feature for labelling a dinoflagellate as an autotroph, or the

absence of Chl a in heterotrophs (Stoecker 1999). The loss of chlorophyll autofluorescence is unavoidable when long-term storage of samples is necessary (Bloem *et al.,* 1986; Sherr and Sherr 1993; Sherr *et al.,* 1997), resulting in the need for microscopic analysis and taxonomic identification of autotrophic versus heterotrophic species. Dinoflagellates dominate 90% of samples; monthly averaged contribution ranges from 40 - 60 % of the total microplankton community. As with the diatoms, there were no significant correlations recorded between monthly averaged dinoflagellate abundance and biogeochemical parameters, including for the dinoflagellate marker pigment Peridinin. The presence of autotrophic, heterotrophic and mixotrophic dinoflagellates could account for the lack of significant correlation between dinoflagellate abundances and Peridinin concentrations, with heterotrophic dinoflagellates not possessing Chl a and accessory pigments (Stoecker *et al.,* 1997; Stoecker 1999). Further detailed taxonomic analysis of the dinoflagellate community at BATS would be needed in order to determine the ratio of heterotrophic dinoflagellates to autotrophic dinoflagellates.

Tintinnids show high levels of variability both within and between months, with an average June abundance maximum driving a relative abundance maximum in the same month. There is little apparent seasonal cycle in the relative abundance of Tintinnids, showing that they are a stable component of the microplankton population in the Sargasso Sea. From reports of tintinnid feeding, it is known that they consume not only much smaller organisms such as picoflagellates (Bernard and Rassoulzadegan 1993), but also other microplankton such as dinoflagellates and smaller tintinnids (Beers and Stewart 1967; Capriulo 1982; Verity 1985). Abundances were on average approximately 50% of dinoflagellate abundances, with tintinnids occasionally being numerically dominant over the diatoms. In these cases, it is suggested that although there is a significant negative correlation between the tintinnid and diatom relative abundances, this is driven purely by (positively correlated) changes in absolute abundances of each group, rather than the idea that the two groups may be competing with each other for the same ecosystem/environmental ‘niche’ (due to their different feeding strategies; autotrophic diatoms versus heterotrophic tintinnids). Relative abundance is also very variable, even within a season; a significant difference was recorded between

high June and low August relative abundances. Any monthly variability is cancelled out when seasonal abundances are considered, with no significant differences in tintinnid abundances.

Radiolaria are found to have a small autumn increase in abundance, with a lower, but stable population over the winter and spring. This is reflected in the return of a significant difference between low spring abundances and high autumn abundances. High August abundances and low January abundances drive a significant difference between months. The increase in autumn abundances is not masked by the contributions of the three numerically dominant microplankton groups (dinoflagellates, diatoms and tintinnids) and is observed as an increase in percentage contribution of the radiolaria during August – November. This is due to a combination of increases in radiolaria abundances during these months, and a decrease in abundance of other larger groups such as the dinoflagellates. High relative abundances in November samples and low relative abundances in July samples were responsible for driving a significant difference between months. A single high radiolaria relative abundance signal (March 2000), was determined to be caused primarily due to low diatom, dinoflagellate and tintinnid abundances, rather than above-average radiolaria abundance. The radiolaria were also one of the few microplankton groups to show any significant correlations to the biogeochemical data collected from the BATS station. A strong positive correlation to average water temperature in the top 150m, and strong negative correlations to average Nitrite and average Silicate concentrations in the top 150m were returned. This correlation with water temperature agrees with earlier observations by Swanberg and Caron (1991), who documented highest radiolaria abundances during warm, stratified summer conditions. Highest radiolaria abundances, highest monthly averaged radiolaria abundance and the largest monthly variability in radiolaria abundances were all recorded for the month of August. The negative correlation to nitrite and silicate is suggested to be indicative of a simple time-lag between the availability of nutrients (and therefore the increase and availability of radiolaria prey including small diatoms, dinoflagellates and ciliates (Swanberg and Caron 1991), and the increase in abundance of radiolaria.

Differences in monthly foraminifera abundances were not observed to be statistically significant although a combination of high average foraminifera abundances and positive monthly anomalies highlight a spring bloom trend in the foraminifera. Despite the increase in both absolute and relative abundance of foraminifera in January and February, differences in abundance between seasons were also non-significant. Although foraminifera are heterotrophic microzooplankton known to feed on a range of plankton including diatoms and ciliates (Swanberg and Caron 1991), roughly half of open-water foraminifera species are reported to be host to symbiotic algae at some stage in life (Caron *et al.,* 1995b; Gast and Caron 2001). The combination of having small phytoplankton as prey (Swanberg and Caron 1991) and the presence of symbiotic algae in the foraminifera (Caron *et al.,* 1995b; Gast and Caron 1996; Gast and Caron 2001) can help to explain the increase in variability of foraminifera abundances in early spring. As early winter mixing of the euphotic zone brings not only the foraminifera themselves (potentially with symbiotic photosynthetic algae), but also their photosynthetic prey organisms into the surface waters, foraminifera are able to respond and an increase in abundance is observed.

Silicoflagellates followed a pattern similar to that of the foraminifera, with minimal variability in abundance throughout the majority of the year, with higher values recorded during February samples. Although the average monthly abundance for February is more than twice as large as for other months, there was no significant difference between months or seasons for absolute abundance. This spring increase trend observed in the silicoflagellates highlights a mismatch in the responses of the two solely photoautotrophic microplankton groups; the diatoms and the silicoflagellates. Increased abundances of silicoflagellates during the winter months is in agreement with previous studies performed in the Mediterranean Sea, where silicoflagellates were detected only under the thermocline in summer and autumn months (Gómez and Gorsky 2003). Although the scenario reported by Gómez and Gorsky (2003) would explain the lack of silicoflagellates in summer/autumn surface waters, it does not fit well in the context of the hydrographic regime at BATS. If a similar situation was occurring with the Sargasso Sea silicoflagellates, the 150m oblique phytoplankton tow samples the majority of the euphotic zone, providing an integrated euphotic zone

phytoplankton sample. As obligate autotrophs, any silicoflagellate cells must be within the euphotic zone to photosynthesize and would therefore still be sampled by the BATS phytoplankton net tow.

Acantharia are present in the lowest abundances of all seven microplankton groups, with the highest monthly average in August more than 2 orders of magnitude less than that of the dinoflagellates. Although no significant differences were observed on the monthly timescale, higher autumn abundances and lower summer abundances were responsible for a significant difference in acantharia abundance between seasons. This pattern in seasonal abundance agrees with observations of acantharia from the oligotrophic North Pacific Central Gyre, with studies by Michaels (1991) documenting low and uniform acantharia cell abundances during winter and spring seasons. Highest abundances of acantharia in the North Pacific gyre (3-4 L^{-1}) (Michaels 1991) were still an order of magnitude larger than abundances recorded in this study. This may be partially due to problems associated with sampling larger, delicate organisms with the use of a towed phytoplankton net. Michaels *et al*., (1995) report observing damaged acantharia cells from plankton tow samples, suggesting that abundance may well be underestimated when acantharia are net-collected. When these net-tow abundance figures are compared with pump-collected acantharia abundance figures, there is a large difference; estimates of up to 18 cells L^{-1} were recorded in autumn samples in 1991 from pump profiles, whilst the maximum abundance recorded from BATS microplankton net tows is just 0.6 cells L^{-1} in an autumn 2005 sample.

The overall lack of seasonal signal that is observed in the major microplankton groups (diatoms, dinoflagellates and tintinnids) is reflected in the analysis of the total microplankton community abundance. Whilst there is no significant difference observed between months or seasons, the overall trend in total microplankton abundance at BATS is an increase in summer/autumn abundances, with a decrease in winter/spring abundances. This is in contrast to the pattern reported for the smaller phytoplankton size-classes; a picoplankton based community showing a defined response to winter mixing; following a "spring-bloom" scenario (Steinberg *et al.,* 2001). From the monthly data discussed in

Results I - 4.1, it can be concluded that the microplankton community appears to exist at a relatively stable abundance and composition year-round, with only minor variations seen in some (but not all) microplankton groups. With so few significant correlations to the biogeochemical data collected from the BATS sampling station, it is difficult to identify any driving factors behind variability in the microplankton. Due to the low levels of variability observed, it may simply be that the microplankton are less responsive to both positive and negative changes in the biogeochemistry and structure of the water column than smaller plankton are. It is also possible that the ability to detect seasonality throughout this long-term time series may be dampened when data are grouped by season, due to the fact that samples have been collected over a time period of several years.

5 Results II - Mesoscale variability

5.1 The impact of mesoscale eddy features on the BATS microplankton

In contrast to the net Ekman downwelling (~4 cm day^{-1}) observed in the oligotrophic waters around Bermuda (McClain and Firestone 1993), the presence of mesoscale eddies is suggested to be as important a mechanism of nutrient transport into the euphotic zone as convective mixing in oligotrophic ocean gyres (McGillicuddy Jr and Robinson 1997), although not all eddies carry nutrients vertically into the euphotic zone (Archer 2004). It is therefore of extreme importance in the Sargasso Sea (an area where the passage of eddies is common) (Michaels *et al.,* 1996) primarily because annual new production in the Sargasso sea cannot be explained purely by the process of winter mixing (Michaels and Knap 1996; Buesseler *et al.,* 2008). Direct observations of the impact eddies have on the biological community are difficult to observe, due to the mismatch in physical and biological timescales (McGillicuddy Jr *et al.,* 1999), even though eddies can be 'sampled' with a sea-level anomaly proxy every few days, with a combination of data from different satellites providing a daily eddy field (Sweeney *et al.,* 2003). Due to a lack of collected observations and samples, expectations of how communities will respond to the influence of eddies are largely still hypotheses. Whereas eddies can influence the physical and chemical structure of the water column (uplifting isopycnals, supplying nutrients to the euphotic zone etc.) for weeks or even months, the biological community is capable of nutrient utilization on a far shorter timescale of days with rapid growth rates, easily missed by monthly ship-board sampling (McGillicuddy Jr *et al.,* 1999). Whilst isopycnals remain uplifted, nutrient influx to an eddy system will continue; in a mature eddy system where upwelling has ceased, nutrients will be entirely depleted (McGillicuddy Jr *et al.,* 1999). Measured phytoplankton production would cease at this time, although a perceived drop in productivity or Chl a values could also be due to increased grazing from higher trophic levels e.g. migration of zooplankton (Eden *et al.,* 2009). During sampling of a late-stage cyclonic eddy, increased zooplankton biomass was observed on the edges of the eddy, whilst a local Chl a minimum was observed in the eddy centre

(McGillicuddy Jr *et al.,* 2007). As discussed in Introduction 1.6.1, there are three types of eddy observed in the waters around Bermuda: cyclonic, anti-cyclonic and mode-water eddies. Each of these three eddy types results in a characteristic change in the water column as previously detailed.

To identify eddies passing through the sampling area, sea-level anomaly (SLA) data from Archiving, Validation, and Interpretation of Satellite Data in Oceanography (AVISO, 1997) altimetry was used (Figure 38). This incorporates TOPEX/Poseidon (T/P), European Remote Sensing (ERS), Jason (JSN), GEOSAT Follow-On (GFO) and Envisat (ENV) satellite altimetry data. Sea-level anomaly data may be used to detect mesoscale (eddy) features due to the lower or higher than average sea height caused by changes in water density inside eddies. Inside a cyclonic eddy, denser than normal water present in the water column results in a depression of the sea surface, showing up on satellite altimetry as a negative SLA. Anticyclonic eddies produce the opposite effect on sea height, due to depressed isopycnal surfaces and a less dense water column.

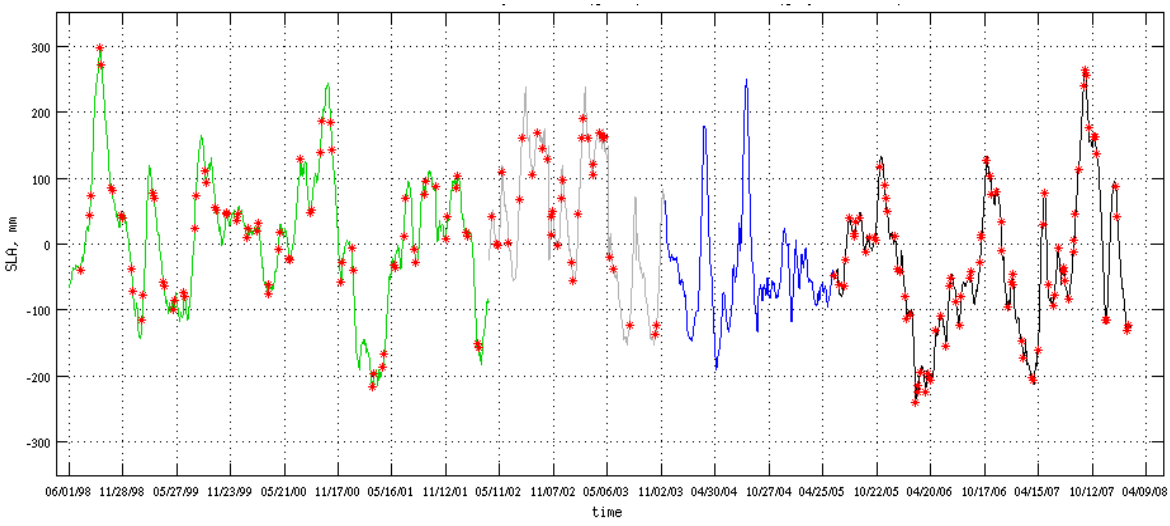


Figure 38 - Sea Level Anomaly (SLA) from AVISO altimetry; June 1st 1998 through to April 9th 2008. Red asterisks mark direct overflight of the altimeter. Gaps between are interpolations based on objective analysis. Different coloured lines represent AVISO altimetry data from different satellites; T/P, ERS (green), JSN (gray), GFO (blue) and ENV (black). (McGillicuddy, 2011 *pers. comm.*). SLA data to end 2009 can be seen in Figure 40.

T/P and ERD data are combined to ensure adequate resolution of eddy features in the area; T/P has a repeat cycle of 10 days, with ground tracks at a distance of ~250km apart, whilst ERS (1 and 2) have longer repeat cycles of 35 days, but a closer spacing of 70km (Sweeney *et al.,* 2003). Measurements between direct overhead passes of the altimeter were interpolated using objective analysis. ERS and T/P data were merged by AVISO staff using previously defined algorithms of Le Traon (1995) and Le Traon and Ogor (1998). SLA data was kindly provided by Dennis McGillicuddy. Further details of SLA altimetry analysis can be found in Sweeney *et al*., (2003).

As described in Sweeney *et al*. (2003), only features with an SLA greater than 10cm were selected, due to an along-track SLA error of ~3-5 cm (AVISO 1997) (greater where data is interpolated (Sweeney *et al.,* 2003). Hydrographic profiles from BATS were also examined to confirm the presence of an eddy feature, i.e. identifying the vertical displacement of the thermocline, the potential density isopycnal and general structure of the measured parameters in the upper water column, indicative of the presence of an eddy feature. Animations of the TOPEX/Poseidon data were also used to estimate an eddy 'age', and to determine the location of an eddy centre in relation to the BATS site (http://science.whoi.edu/users/mcgillic/tpd/anim.html). The eddy 'age' was estimated as described in Sweeney *et al,* (2003), by tracking the path of an identified eddy as far back in time as possible using the data animations, to the point at which the SLA exceeded +/- 10cm in magnitude. These animations were also used to determine whether the eddy centre passed directly over the BATS station or only the eddy edges. Where possible, eddy types were also determined (Figure 39) for eddies when microplankton samples were available.

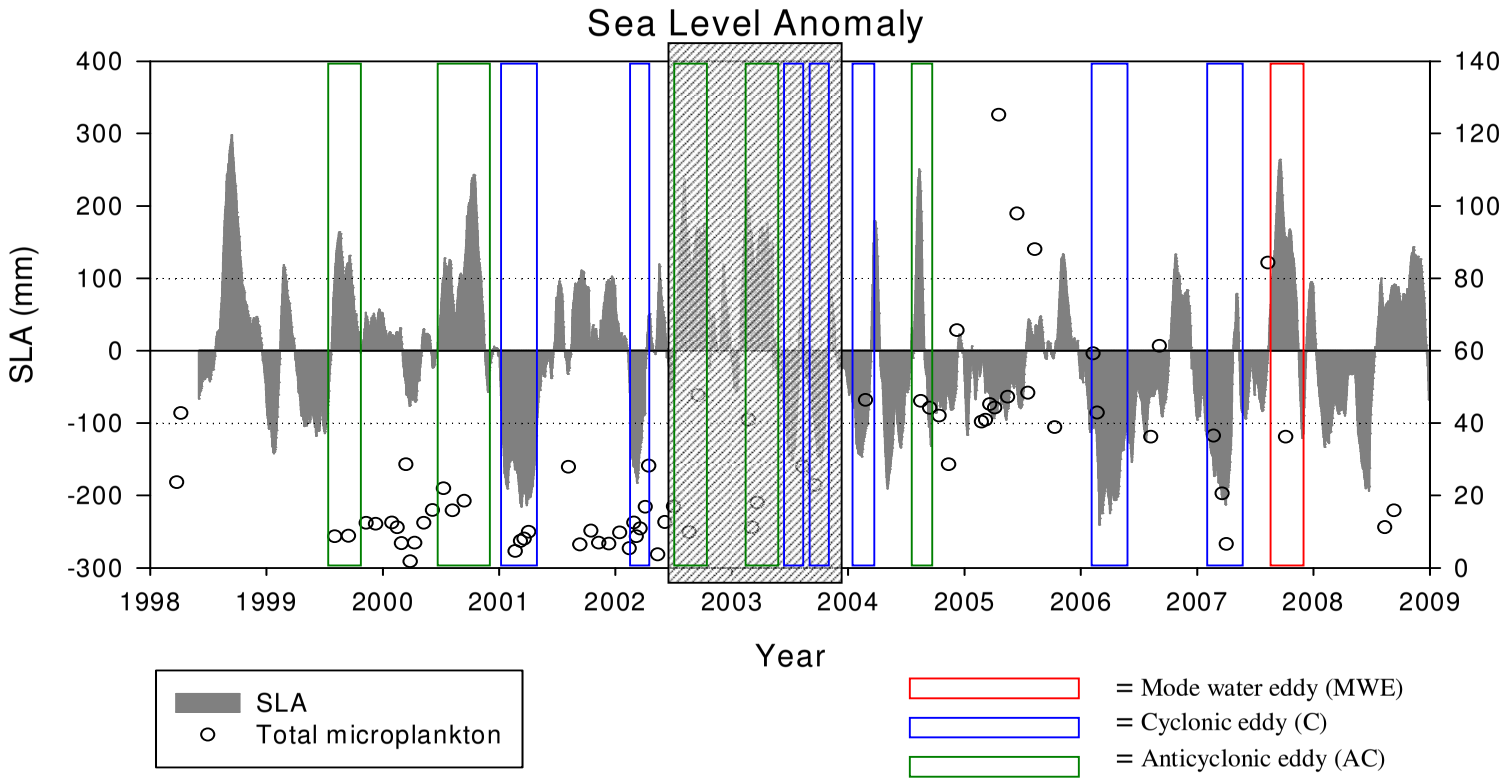


Figure 39 - Modified Sea Level Anomaly (SLA) graph, depicting eddy-identified features. Only features with an accompanying microplankton sample were identified and examined. Dotted horizontal lines represent the ±100mm displacement boundaries, in between which values are not considered significantly different to background levels. The hatched area represents area of SLA uncertainty, due to apparent replication of data. Open circles represent total microplankton abundance (Cells L^{-1}).

During the course of sampling, several eddy features were identified, as shown above. Five cyclonic eddy features were identified in 2001, 2002, 2004, 2006 and 2007, three Anticyclonic eddy features in 1999, 2000 and 2004, and one mode water eddy in 2007. Although both cyclonic and mode water eddies are associated with the upwelling of nutrients into the euphotic zone, it has been observed that eddies >4 months old do not show a biological response (Sweeney *et al.,* 2003).

Microplankton abundance data from these nine cruises was examined and tested for significant correlations with the Sea Level Anomaly data (as a proxy for eddy presence). Results from the seven microplankton groups and total microplankton data are presented in Table 7, with a model 1 linear regression applied in Figures 40 and 41.

Microplankton group	**Correlation coefficient**	**p - value**
Diatoms	0.603	0.005*
Dinoflagellates	0.417	0.067
Tintinnids	0.286	0.218
Radiolaria	0.589	0.006*
Foraminifera	-0.123	0.599
Silicoflagellates	0.294	0.203
Acantharia	0.285	0.220
Total Microplankton	0.520	0.019*

Table 7 - Correlation coefficients for microplankton groups and Sea Level Anomaly data. Correlations analysed using Spearman Rank Order testing, n = 20. * represent statistically significant p values ($p < 0.05$).

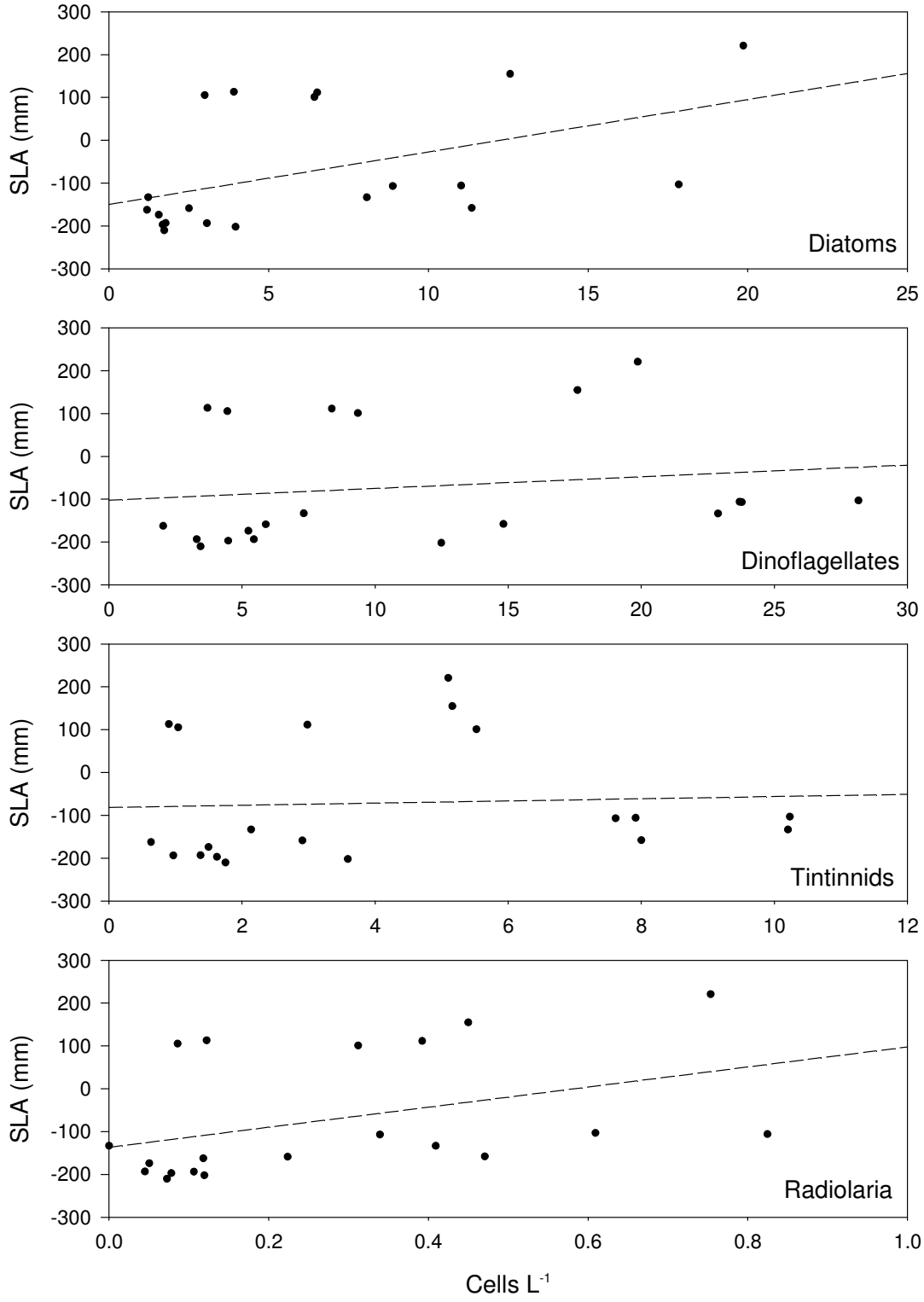


Figure 40 - Model 1 linear regression applied to show correlation between Sea Level Anomaly (as a proxy for eddy presence) magnitude +/- 100mm, with microplankton abundance for each analysed microplankton group.

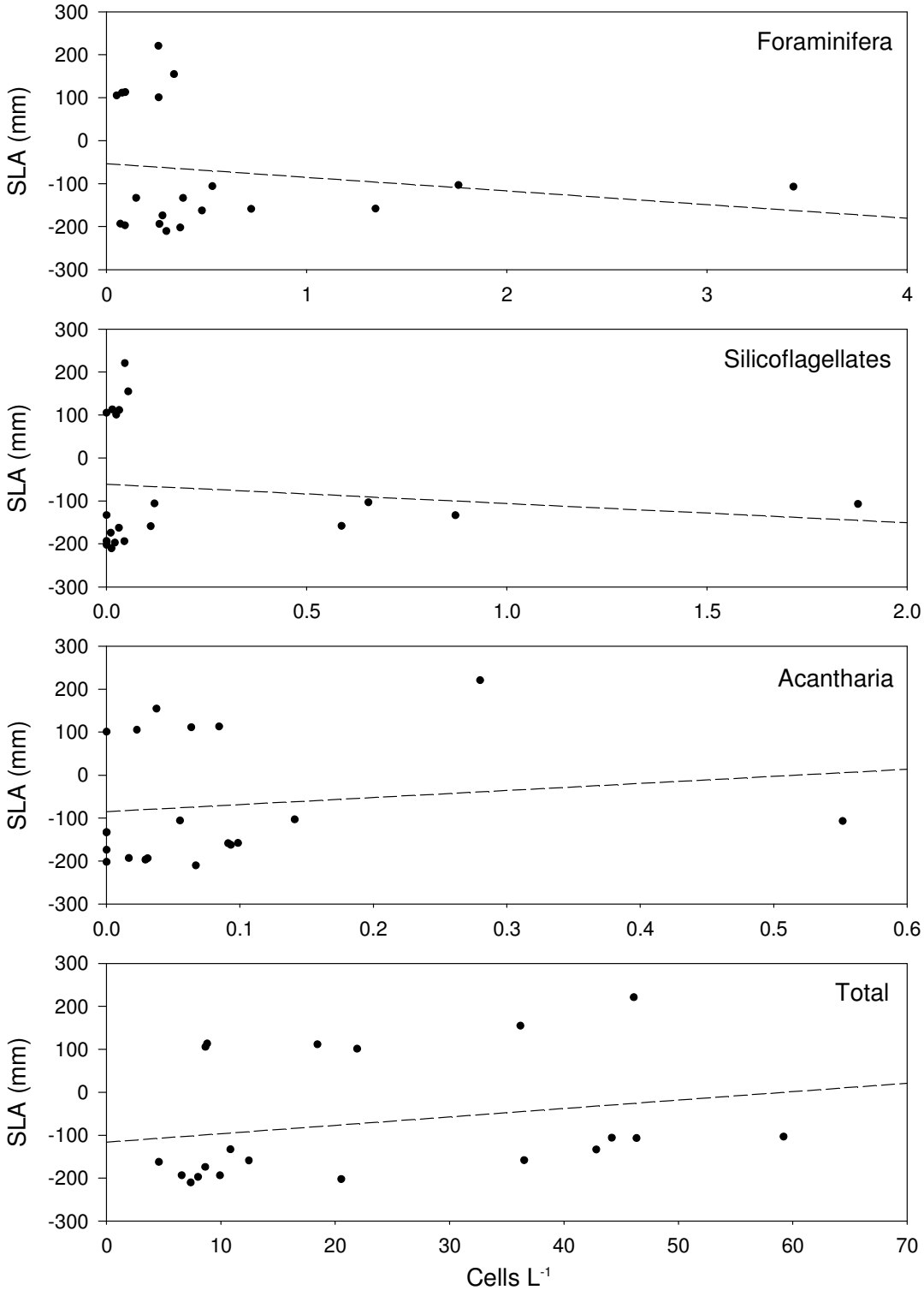


Figure 41 - Model 1 linear regression applied to show correlation between Sea Level Anomaly (as a proxy for eddy presence) magnitude +/- 100mm, with microplankton abundance for each analysed microplankton group.

Within the microplankton groups, only diatoms (correlation coefficient 0.603, p = 0.005) and radiolaria (correlation coefficient 0.589, p = 0.006) show a significant correlation between abundance and the Sea Level Anomaly (magnitude and sign) using Spearman Rank Order testing (non-parametric) (Table 7). Both these groups show an increase in abundance with increasing SLA.

This may initially appear as a slightly unexpected result, taking into consideration how different eddy types affect the sea level anomaly, and the effects that these types of eddies have on the water column biogeochemistry. Cyclonic eddies are responsible for depressed SLA, but due to an upward displacement of isopycnals, upwelling into the euphotic zone is usually associated with cyclonic eddies when forming or intensifying. Of the eddies that cause an elevation of sea levels, only mode-water eddies have a similar effect. This is characterised by elevation of seasonal isopycnals (with upwelling at the base of the euphotic zone) whilst anticyclonic eddies cause net downwelling in the euphotic zone. However, out of all eddies identified, only one positive SLA was associated with a mode water eddy, versus three anticyclonic eddies. The mode water eddy identified in 2007 did have a large positive SLA, and was present at the BATS station for longer than the other eddy (2004) with a similar magnitude positive SLA.

With the exception of the 2006 and 2004 cyclonic eddies, all other eddies were identified using animated SLA data as being older than ~4 months, or in the process of decaying (SLA returning to 0), suggesting a lack of biological response as previously reported (Sweeney *et al.,* 2003). The cyclonic eddy identified in 2004 was newly formed, but short lived (~ 1 month duration of SLA >100mm). When comparing animated SLA files (eddy intensity and duration) to the timing of BATS cruises, it is very apparent to see the mismatch in sampling time-scales, with BATS cruises rarely coinciding with the passage of an eddy directly over the BATS site. This is a factor frequently commented on in other studies, with the acknowledgement that in order to fully understand the biological response to eddies, sampling through the complete life-span of an eddy is needed. Without microplankton samples taken directly from an intensifying/forming eddy, or from transects through an eddy, it is difficult to determine exactly how the microplankton are responding to the presence of eddies, both in terms of temporal

variation as well as spatial variation through an eddy. SLA animations were not available for 2007 – 2009.

5.2 Discussion

When analysing trends within eddies, there are several factors and previous findings that should not be overlooked. A number of eddy features in the Sargasso Sea have been studied previously; see (McGillicuddy Jr *et al.,* 2007; Bibby *et al.,* 2008; Ewart *et al.,* 2008; Li and Hansell 2008) with varying results in terms of primary production, water column structure and community composition. In a cyclonic eddy, increased (bacterial) production was measured at the eddy edges (Ewart *et al.,* 2008), due to the downwelling of previously upwelled water acting to split the deep chlorophyll maximum (Li and Hansell 2008). Contrary to this, in mode water eddies higher production and total Chl a were observed at the eddy centres relative to edges (Ewart *et al.,* 2008; Li and Hansell 2008), although a high level of variability in zooplankton and bacterioplankton biomass was also recorded.

Within the different groups of single-celled microplankton examined, only the diatoms and the radiolaria showed any correlations to the Sea Level Anomaly (as an indicator of the presence of an eddy). Both groups show significant positive correlations to increasing sea level anomalies, from large negative anomalies through to large positive anomalies. The strong significant correlations of these two groups also drive a significant positive correlation for the total microplankton abundance and SLA. Although a positive correlation does not fit the hypothesis that cyclonic eddies (negative SLA) promote plankton growth by inputting new nutrients and anticyclonic eddies (positive SLA) generally suppress plankton growth by depressing nutriclines, when spatial and temporal variability of the eddy features are taken into account, this result is more easily understood.

Physical interactions such as eddy/eddy interactions, or eddy/wind interactions can also have a large effect on the characteristics of an eddy, and the potential biological response (McGillicuddy Jr *et al.,* 2007). Eddy/eddy interactions can act

to increase the concentration or flux of organic/inorganic nutrients at the eddy edge, prompting increases in production and Chl a similar to those observed at the edge of cyclonic eddies (Ewart *et al.,* 2008). Eddy/wind interactions act to suppress eddy-induced upwelling in cyclonic eddies, whilst enhancing upwelling in regular anticyclonic eddies in addition to mode water eddies (McGillicuddy Jr *et al.,* 2007). The effect of eddy/eddy and eddy/wind interactions helps explain the large variability that can be observed between different eddies of the same type.

This combination of variables makes it extremely difficult to determine what effect if any, is caused by the presence of mesoscale features in the Sargasso Sea. The data used to identify the presence of eddies (SLA, animated movie files) certainly highlight the presence of eddies at the BATS site on a frequent basis. However it was also clear that the regular BATS cruises very rarely coincided with the direct passage of an eddy feature over the intended sampling area. Of the eddy features present in the BATS area during microplankton sampling, all but 2 were greater than four months old (the age at which it is determined they no longer have an impact on the biology (Sweeney *et al.,* 2003), or in the process of decaying. To enable an accurate determination of changes in the biological community within an eddy system, specific cruises are needed to sample along transects through an eddy feature. The biological community within an eddy and at eddy edges can then accurately be compared to the biological community present in the surrounding waters outside the eddy.

Previous findings of different eddy features in the Sargasso Sea report a large variation in results with regards to primary production, water column structure and the community composition. These results vary not only between different types of eddies, but also within eddies of the same type, and also depending on where in the eddy samples were collected. The difficulty of accurately determining the effects of mesoscale variability on the biological communities in open water is well summarized by Garçon *et al.* (2001), who states "How to measure and quantify the role of the mesoscale variability on the biology of pelagic ecosystems constitutes a formidable challenge". Both the temporal and spatial variability of eddies result in sampling problems, in terms of measuring the

intended part of an eddy (i.e. the edges vs. the centre) and at the intended time (i.e. a developing, reasonably steady, or even a decaying eddy). The importance of eddies as significant contributors to nutrient transport into the euphotic zone has been well documented (Michaels and Knap 1996; McGillicuddy Jr and Robinson 1997; McGillicuddy Jr *et al.,* 1999; McGillicuddy Jr *et al.,* 2007), although difficult to establish correlations with biological communities due to the sampling difficulty mentioned above.

It is suggested therefore that the presence of these eddies in the BATS area is having an impact on microplankton abundance, although elucidating a pattern of response has proven to be extremely difficult when dealing with eddies of different ages, upwelling characteristics, and with different physical (eddy/eddy, eddy/wind) interactions. In order to successfully identify the mesoscale variability in the microplankton community driven by eddies, specific ship-based sampling through an eddy feature is recommended as a far more preferential method of sampling rather than time-series samples from one station.

6 Results III - 12-month data

6.1 12-month data; 1999 – 2000, 2001 – 2002 and 2004 - 2005

Whilst looking at a long term data set on a monthly averaged basis can highlight general patterns over an annual period (see Results I - 4.1), patterns from specific years may be overlooked by averaging monthly data. In this section, microplankton abundance and relative composition data from three separate years are examined. Samples analysed are from August – July, in order to maintain a follow through of winter/spring data from the same seasonal cycle, in addition to the availability of microplankton samples. Microplankton abundances from 1999-2000, 2001-2002 and 2004-2005 are reported here.

During the 1999-2000 sampling period, total microplankton abundances appear to follow a fairly typical "spring bloom" scenario, with an increase in early March samples, followed by a decrease or 'crash' in microplankton abundance in late March (Figure 42). When individual microplankton groups are examined, however, it is seen that although abundance increases are observed in the dinoflagellates, diatoms and tintinnids, it is the dinoflagellates which contribute the highest relative abundance (66.4 %) to the sample. During April, this changes to the diatoms having the highest relative abundance (45.9 %), driven mainly by a reduction in dinoflagellate abundance and tintinnid abundance rather than a large increase in diatom abundance. This suggests that the dinoflagellate and tintinnid populations are not able to recover as quickly as diatoms after the microplankton minimum recorded in late March samples. Despite this early March increase in microplankton abundance appearing to fit well with the "spring bloom" scenario, the magnitude of abundance increase is not as large as reported in the literature for this region from other earlier studies (Riley 1957; Ryther 1958; Menzel and Ryther 1960; Nelson *et al.,* 2004; Irigoien *et al.,* 2005). Consequently, when samples were analysed using a non-parametric Kruskal-Wallis One-way ANOVA, there was found to be no significant difference in microplankton abundances between different samples ($p = 0.935$) during the 1999 – 2000 period.

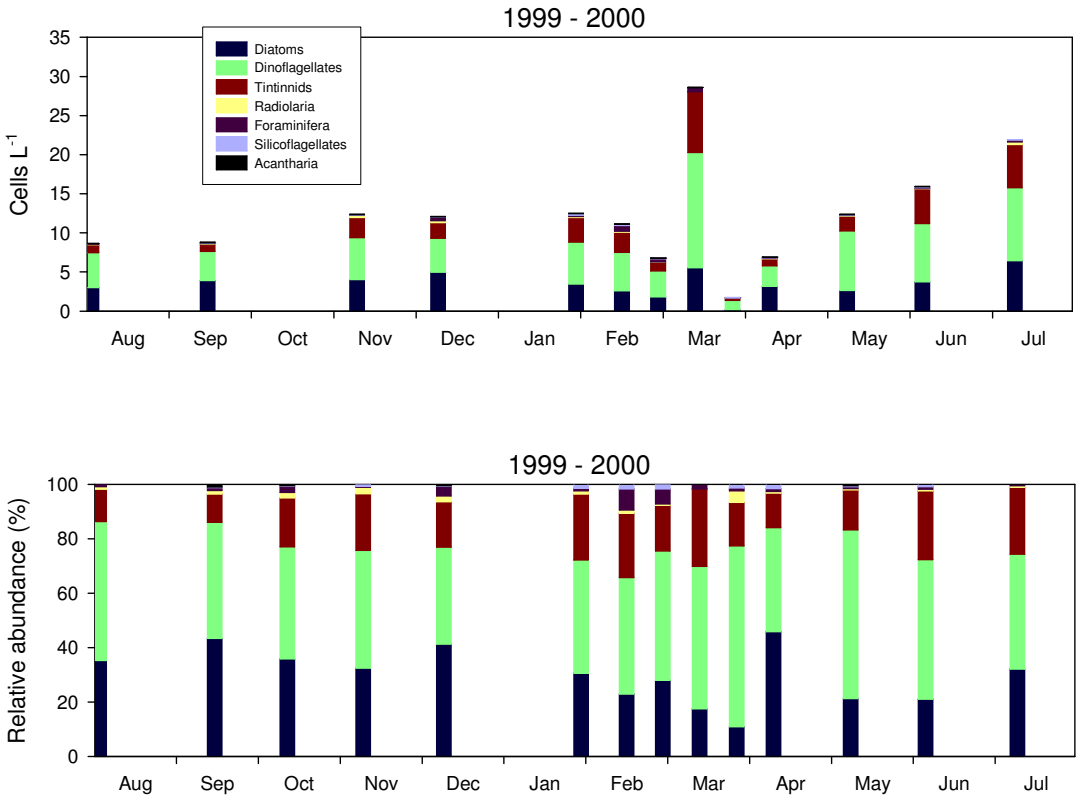


Figure 42 - Microplankton abundance (top) and relative abundance (bottom) by groups for all samples analysed between August 1999 and July 2000.

Although diatoms increased in abundance during early March, their highest abundance (6.4 cells L^{-1}) was recorded in July 2000. Previous observations at BATS recorded the presence of the diatom indicator pigment Fucoxanthin to be generally present at all depths sampled during spring blooms (4L bottle samples), but also to occasionally show dramatic increases in concentration during other periods of the year (Steinberg *et al.,* 2001). There are advantages and disadvantages to both sampling methods; discrete bottle samples and net tows. Data from discrete samples such as those mentioned above provide a useful snapshot of a particular depth in the water column at the moment of sampling, but can lead to under or over-estimation of whatever is being measured due to localised variability. Water column-averaged samples such as the BATS microplankton net samples cover the possibility of localised variations, but effectively provides data for a homogenised water column – a situation we know to be unlikely, especially during summer months with increased stratification of

the water column structure (Steinberg *et al.,* 2001). When diatom abundances were compared with 0 - 150m Fucoxanthin concentrations (Spearman Rank Order Correlation, n=12), there was no significant correlation (p = 0.206) (Figure 43).

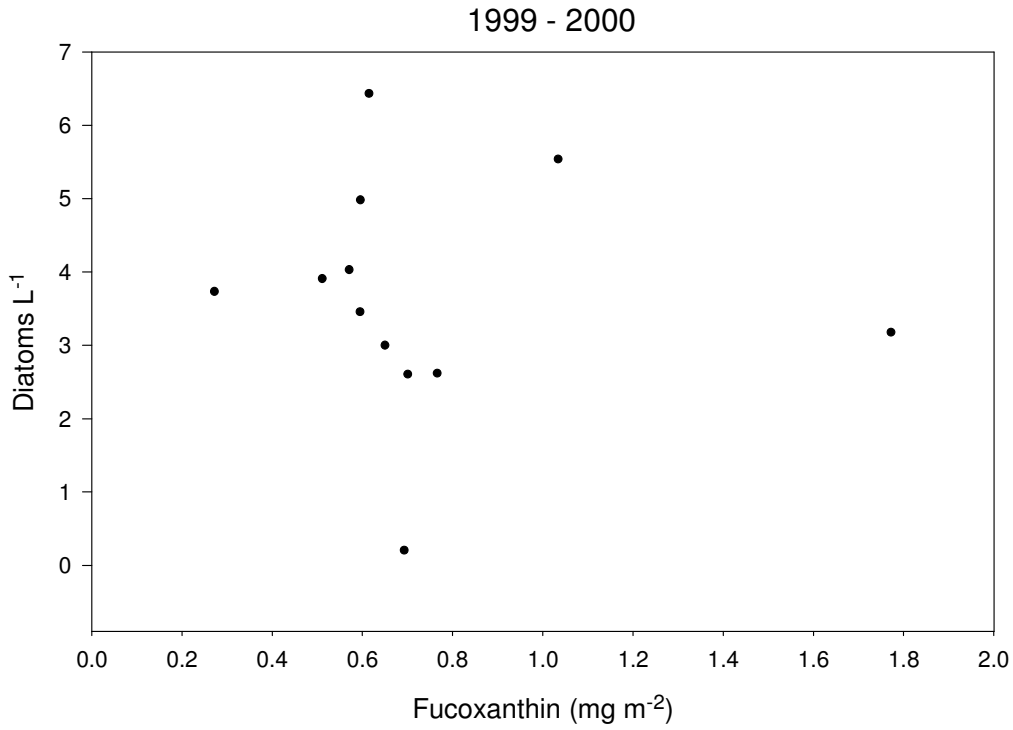


Figure 43 - Scatter plot of Diatom abundance (cells L^{-1}) plotted against measured Fucoxanthin concentrations 0 – 150m (mg m^{-2})

A similar pattern in total microplankton abundance to 1999 – 2000 is observed in samples taken from August 2001 through to July 2002 (Figure 44). Total microplankton abundance in August 1999 was almost as high (27.9 cells L^{-1}) as the mid-April maximum (28.2 cells L^{-1}). This high August abundance is caused by a combination of increased abundances of both diatoms and dinoflagellates, resulting in a similar relative composition to the September sample. A spring maximum in total microplankton abundance is again observed during this 12-month period, although approximately one month later than in 2000. In both 2000 and 2002, the maximum abundance of microplankton cells was similar, with 28.6 and 28.2 cells L^{-1} respectively. Although mid-April 2002 and August 2001 samples have higher recorded total abundances, there were no significant

differences in total microplankton abundances recorded between different samples (Kruskal-Wallis One way ANOVA, p = 0.903). Diatoms are seen to have a large relative abundance in early February samples, due to a combination of very low dinoflagellate and tintinnid abundance (1.4 and 0.5 cells L^{-1} respectively), combined with higher than average diatom abundance (3.4 cells L^{-1}). As with the 2000 spring maximum, the spring increase in relative abundance is due mainly to the increase of dinoflagellate and tintinnid absolute abundances, rather than diatom abundances. However, the decrease in dinoflagellate abundances relative to diatom abundances recorded in April 2000 following the March maximum was not observed in spring 2002.

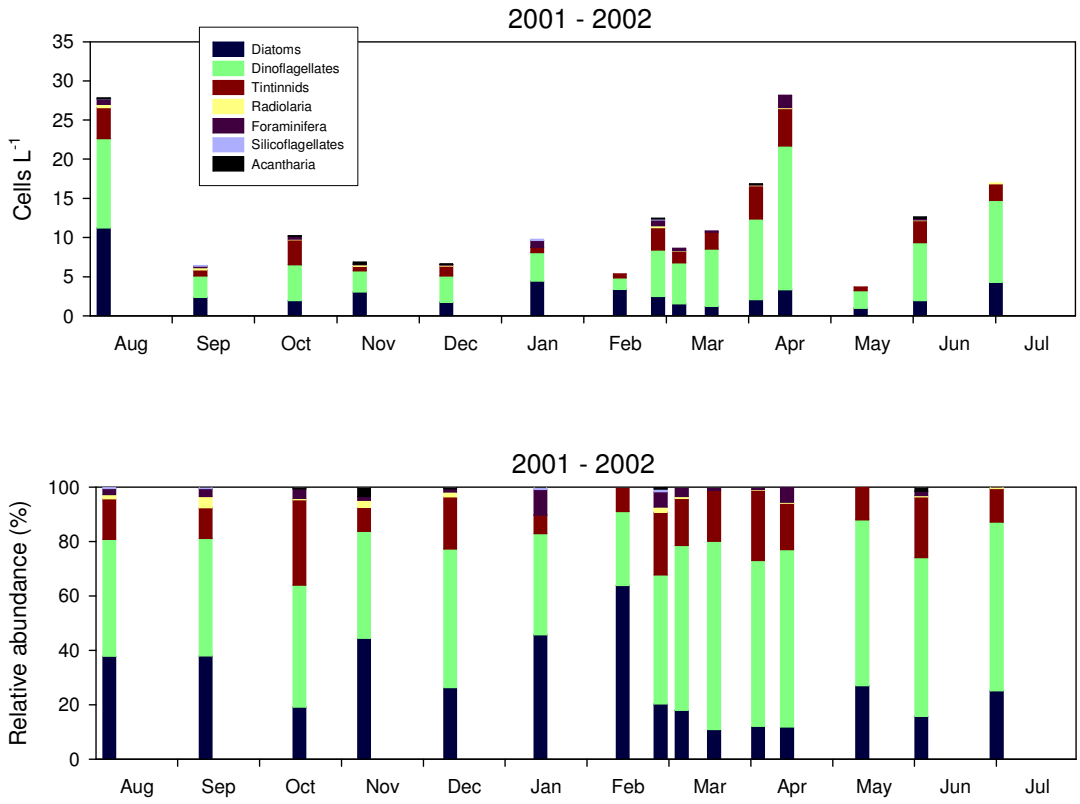


Figure 44 - Microplankton abundance (top) and relative abundance (bottom) by groups for all samples analysed between August 2001 and July 2002.

Relative abundance of the microplankton community remained fairly stable throughout March and April, with a slight increase in diatom relative abundance (May 2002) caused by a decrease in tintinnid abundance. Foraminifera also reached a maximum abundance (1.6 cells L^{-1}) during April, whilst the maximum radiolaria abundance (0.4 cells L^{-1}) was recorded during August 2001.

Total microplankton abundances were much higher during the 2004 – 2005 sampling period compared to 1999 – 2000 and 2001 - 2002, with minimum abundances of 28.6 cells L^{-1} (November 2004), and maximum abundances of 125 cells L^{-1} (April 2005) (Figure 45). Although higher values were recorded in April, again, there is not the expected increase in magnitude normally associated with a 'spring bloom' scenario, with April abundances only double the value of December abundances. The timing for this abundance increase was very similar to 2002, with maximum abundances recorded in mid-April. Samples collected during February, March and early April all showed similar total abundances (40 - 45 cells L^{-1}), with April increases due mainly to the increase in both diatoms and dinoflagellates, with no increase in tintinnid abundances. As with the previous two year-groups, there was no statistically significant difference in total microplankton abundances between samples when tested (Kruskal-Wallis One way ANOVA, $p = 0.999$). Diatoms had their highest relative abundance in August 2004, caused both by a high diatom abundance and a low dinoflagellate abundance (both at 19.9 cells L^{-1}). Diatoms were also recorded to have an increase in abundance in December 2004, a pattern also observed in tintinnid abundances. Both tintinnids and radiolaria were recorded as having maximum abundances in June 2005, of 18.5 and 1.0 cells L^{-1} respectively. Foraminifera and silicoflagellates however, had maximum abundances in early March samples (2.4 and 0.9 cells L^{-1} respectively).

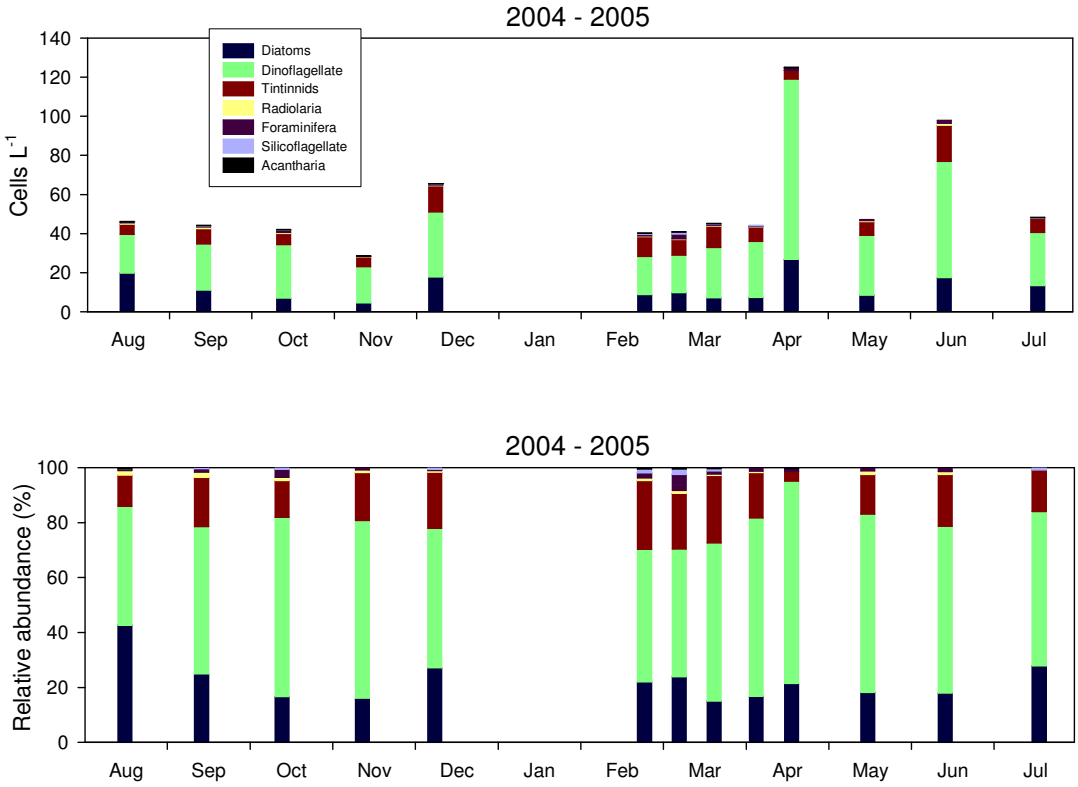


Figure 45 - Microplankton abundance (top) and relative abundance (bottom) by groups for all samples analysed between August 2004 and July 2005.

Total single-celled microplankton abundances during the twelve month sampling period covering 2004 – 2005 were higher than previous years, with minimum abundances in 2004 – 2005 matching the maximum abundances from the other two twelve-month periods (Figure 46). The timing of the spring increase in 2005 was similar to that in 2002 (late April), again approximately one month after the spring increase in 2000. In 2005 the water column remained well mixed until mid-April, in comparison to 2000 where similar (cooler) water column temperatures were recorded only until mid-March. Peak microplankton abundance was once again numerically dominated by the dinoflagellates, although an increase in diatoms and decrease in tintinnid abundance was observed. Total microplankton abundances were lower in May, but did not show the same abundance minimum as in the previous two sampling periods, with the relative abundance of the three main groups remaining similar to that in March and early April samples. When microplankton abundances are considered for all three 12-

month data sets, it is possible to see the changes in the timing of the spring abundance increase in addition to the different magnitude of abundances in different years. Despite 2004 – 2005 having much higher cell abundances, when these are examined in the context of the long-term data set (see Results IV - 7.1), the abundances do not stand out as particularly anomalous. Although it is visible that there are increases in the total abundance of microplankton during the typical spring bloom period (March to April), the variability in the timing of this abundance increase between years may dampen this spring signal when examined across the whole data set, particularly on a monthly-averaged basis (see Results I - 4.1).

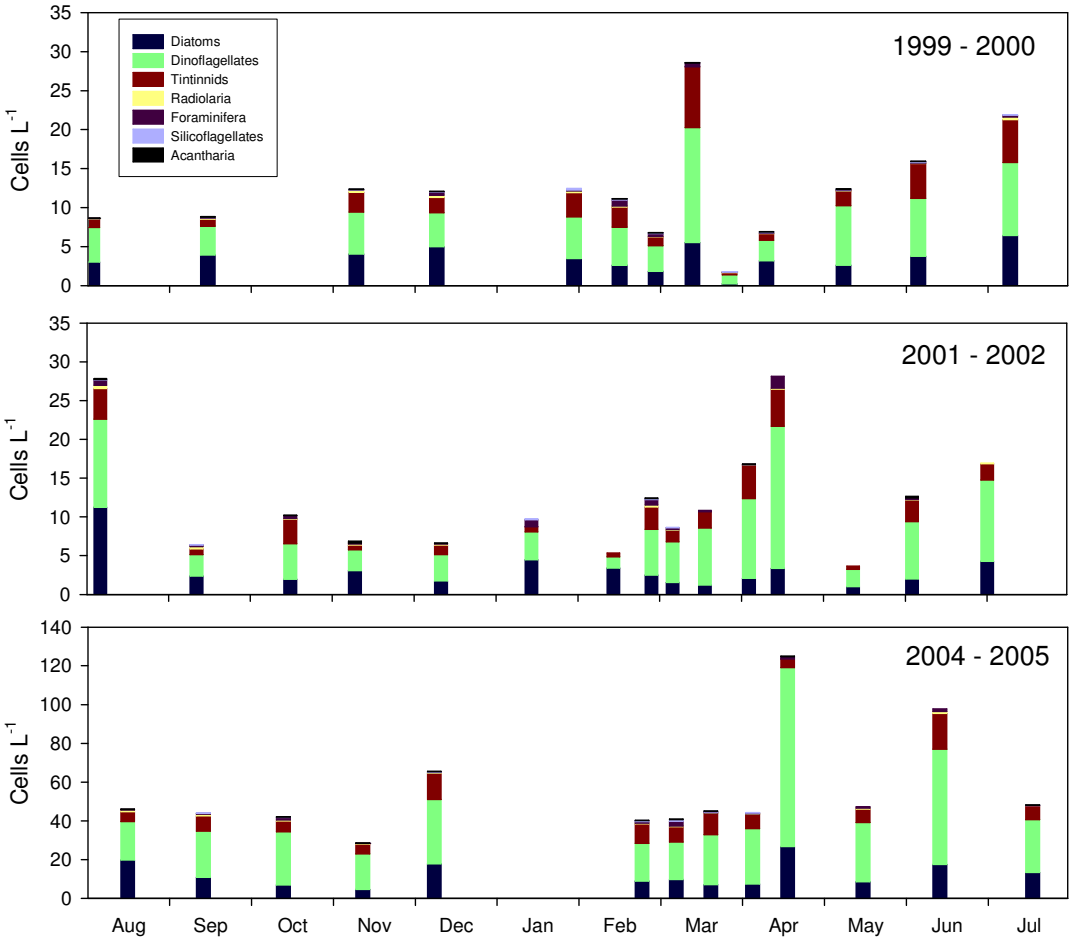


Figure 46 - Microplankton abundance by group for all samples analysed between August and July 1999 - 2000 (top), 2001 - 2002 (middle) and 2004 - 2005 (bottom).

The microzooplankton groups included in this study show a large amount of variation in abundance as shown by the tintinnids in Figure 47. As with the diatoms, there are samples throughout the year with high abundances, not just spring samples. In 2000 and 2002 however, the maximum abundances coincide with maximum total microplankton abundances. Since they are microzooplankton grazers, this is likely to be a response to food availability, and the increase in abundance of smaller phytoplankton.

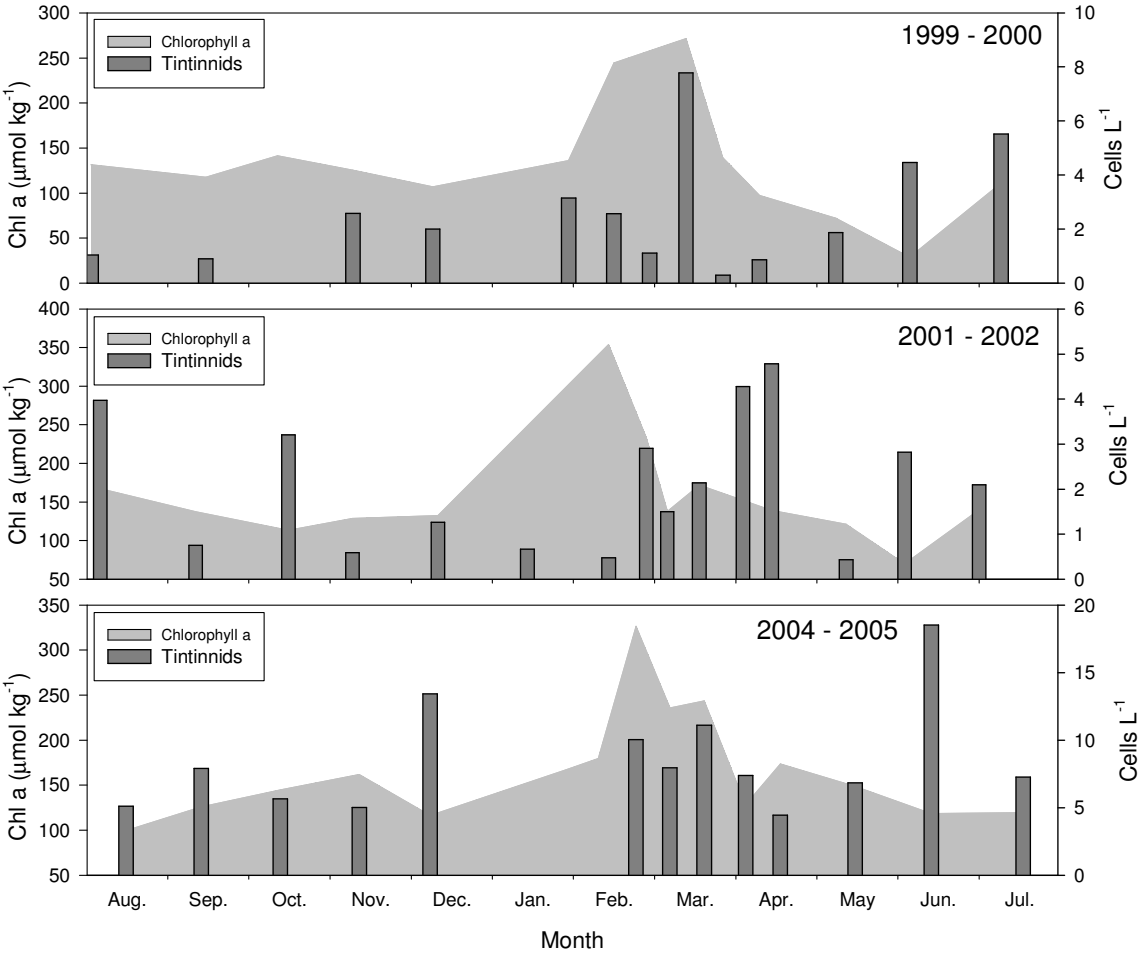


Figure 47 - Microplankton tintinnid abundance and water column 150m-averaged Chl a for all samples analysed between August and July 1999 - 2000 (top), 2001 - 2002 (middle) and 2004 - 2005 (bottom). Shaded area represents average Chl a values (0 – 150m), bars represent Tintinnid abundances for each sample.

When diatom abundances are considered separately from the other microplankton groups (Figure 48), the variability in diatoms between samples and years can be more clearly seen. Whilst in 2000 and 2005, the diatoms show a high abundance at the same time as the dinoflagellates, there are also other samples within the three data sets with high diatom abundances at various points in the year. Within the diatoms, any abundance increases observed are still on a small scale when compared to other phytoplankton bloom scenarios reported in the literature, with abundances of up to 136,000 phytoplankton cells L^{-1} reported for the Sargasso Sea (Riley 1957) (bottle sampled whole phytoplankton community).

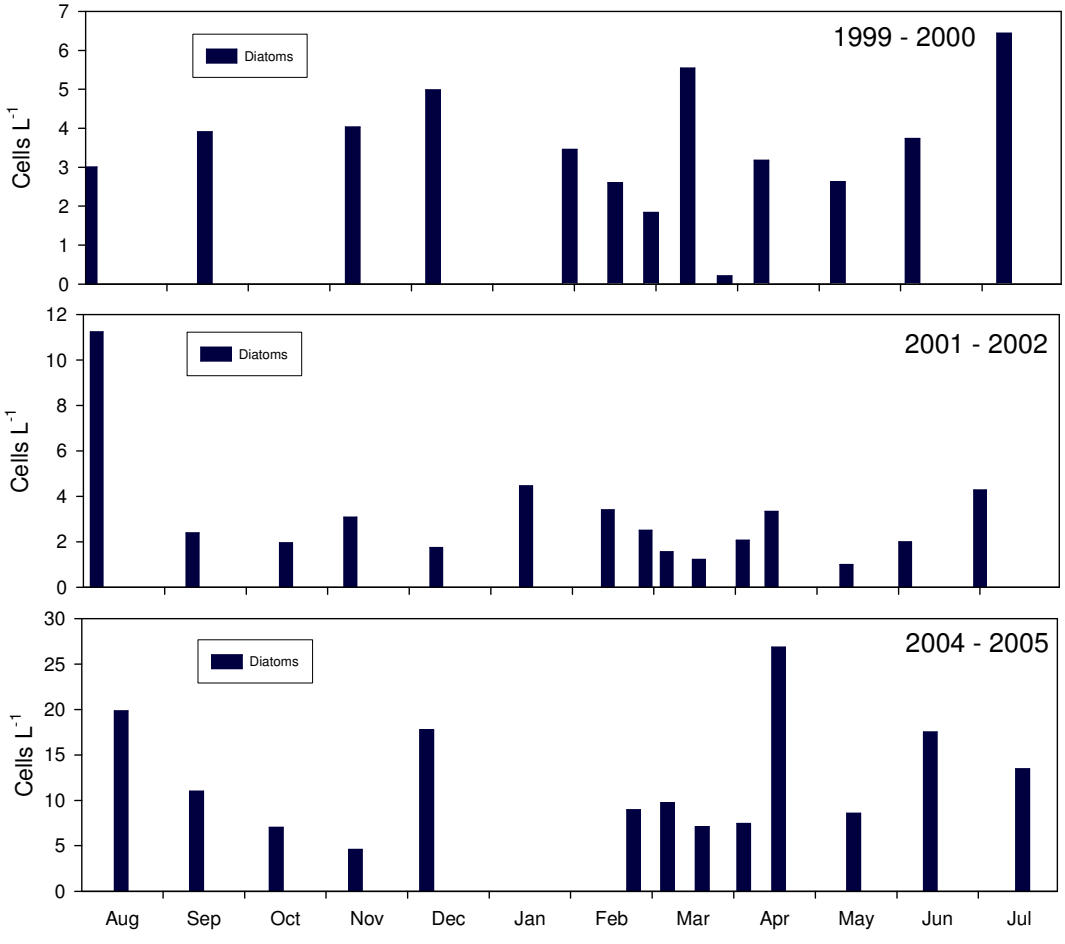


Figure 48 - Microplankton diatom abundance for all samples analysed between August and July 1999 - 2000 (top), 2001 - 2002 (middle) and 2004 - 2005 (bottom).

The small abundance increases (in comparison to smaller phytoplankton groups) suggests that although the microphytoplankton are utilizing nutrients brought into the euphotic zone by winter mixing, they do not appear to be responding in the typical "bloom" pattern. Although there were no significant correlations between the estimated nutracline depth and diatom abundances for 1999/2000 and 2004/2005, during the 2001/2002 sampling period, diatom abundances showed a very strong positive correlation with increasing nutracline depth (Spearman Rank Correlation coefficient = 0.82, $p < 0.001$) (Figure 49). The maximum depth of the nutracline was estimated from measured nutrient data in February 2002, with deeper depths (and associated higher diatom abundances) also observed in August 2001 and July 2002.

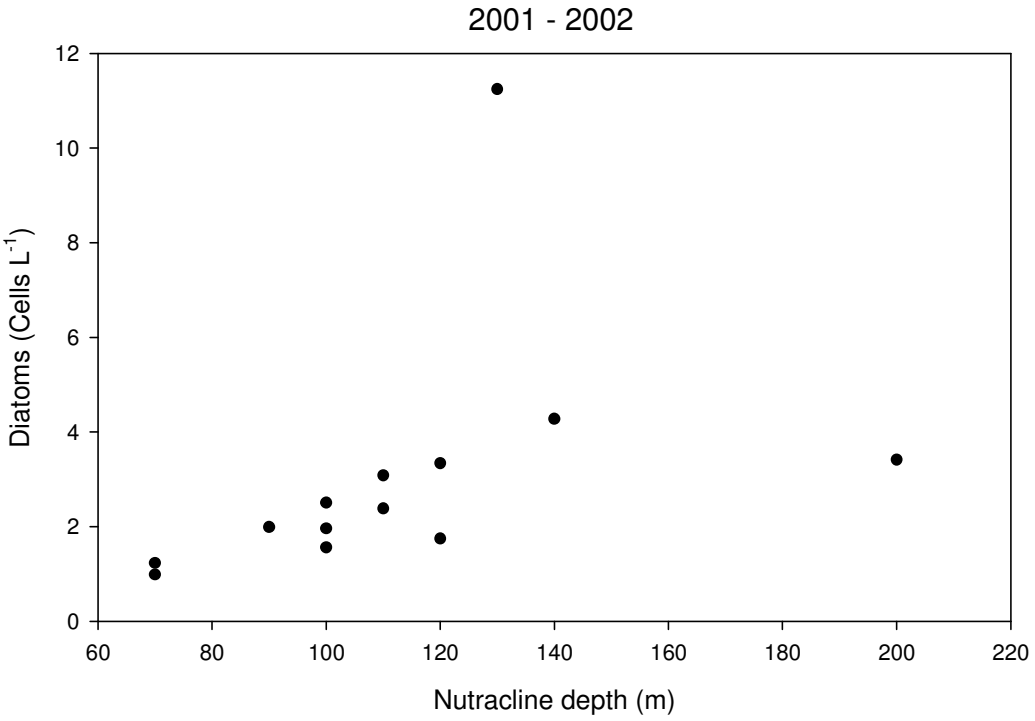


Figure 49 - Scatter plot showing relationship between diatom abundance (Cells L^{-1}) and estimated nutracline depth (m).

The presence (particularly of the microplankton diatoms) at the base of the euphotic zone increases the likelihood of these cells being missed in a typical 150m net haul, or that very localised high-abundance patches are being overlooked as the sample becomes a water-column integrated sample (as the net samples all the way through the euphotic zone). It has been recorded in laboratory studies that microplankton diatoms of the size and species found to be present in the Sargasso Sea such as *Thalassiosira* sp. and *Planktionella* sp. are capable of fast growth and rapid utilisation of nutrients when they become available (Goldman 1988; Goldman 1993; Goldman and McGillicuddy Jr 2003) for instance after winter mixing, in the presence of upwelling eddies, or simply at other times of the year when nutriclines may be elevated into the bottom of the euphotic zone where these rare cells are found. Despite these laboratory studies, there is no evidence that the microplankton diatoms are responding in this way at BATS.

6.2 Discussion

From the three twelve-month data sets analysed, a spring increase in abundance was observed in March or April, although this varied in both timing and magnitude. This increase in abundance was not as large as expected, when compared to descriptions of the "spring bloom" in the literature for the oligotrophic regime at BATS, with large increases of up to 3 orders of magnitude (Riley 1957; Ryther 1958; Menzel and Ryther 1960; Nelson *et al.,* 2004; Irigoien *et al.,* 2005). Despite these spring increases in total microplankton abundance, there was no significant difference found between samples for each of the three 12-month sampling periods.

During the 1999 – 2000 sampling period, dinoflagellates generally contributed the highest relative abundance to the total microplankton samples, with April samples numerically dominated by diatoms due to low dinoflagellate and tintinnid abundances. The dinoflagellate and tintinnid populations are seemingly slow to recover in abundance after a late March total microplankton 'abundance minimum'. This slow recovery may be due to a lack of food sources for

heterotrophic dinoflagellates and tintinnids; the abundance of grazing microplankton would depend on the availability of smaller sized plankton as prey. If these smaller-size plankton populations are crashing in abundance at the end of a bloom (by grazing or viral lysis) then a time-lag response from the heterotrophic microplankton would be expected. Highest diatom abundances were observed during July, although they showed no significant correlation to the concentration of Fucoxanthin in the water column. This may be due to smaller phytoplankton being numerically dominant over microplankton diatoms (see Riley (1957), Ryther (1958), Menzel and Ryther (1960), DuRand *et al*., (2001), Caron *et al*., (1999) and others for details of microplankton, picoplankton and nanoplankton cell abundances), with the effect of overshadowing or masking the contribution of microplankton diatoms to total Fucoxanthin concentrations.

Similar magnitude microplankton abundances were recorded for the second twelve-month period (2001 – 2002), although the spring increase was observed one month later than in 2000. This later spring increase in abundance coincides with the passage of an eddy feature close to the BATS sampling station. Although the eddy identified was older than ~4 months (and therefore unlikely to be causing biological changes), it is possible that this one month 'delay' in the timing of the spring bloom compared to spring 2000 is as a result of a different body of water (with a different origin) enclosed by the eddy feature passing the BATS area.

Although increases in total microplankton abundance are visible during the typical spring bloom period for each of the twelve month periods analysed, the variability observed just between three individual years highlights how easily any seasonal spring signal in microplankton abundance would be suppressed when abundance data is analysed on longer seasonal, or even monthly timescales. The largest variation in abundances are mostly observed in the microzooplankton groups, especially within the tintinnids. During the spring of 2000 and 2002, peak tintinnid abundance occurred at the same time as peak total microplankton abundance. The grazing nature of tintinnids suggests these abundance peaks could be indicative of a response to availability of smaller phytoplankton as prey

items due to the abundance of smaller phytoplankton reported to increase during the spring period (DuRand *et al.,* 2001; Steinberg *et al.,* 2001)

7 Results IV - Inter-annual variability

Year-over-year, changes in seasonal variability are observed as inter-annual variability. Variation in the timing of the spring bloom or the onset of stratification, over a period of several years is documented as inter-annual variability. It is appropriate to examine data on different time-scales to identify any variability within the data, and the driving factors responsible for it. Examining a decade-long data set enables inter-annual variability to be analysed, in addition to analysing long-term trends that would otherwise be missed over shorter time-series. Where correlations and regressions are calculated, a weak correlation / trend is < 0.5, a strong correlation / trend > 0.5.

7.1 Microplankton abundance

Total microplankton abundance throughout the time-series averaged 28.4 cells L^{-1}, showing a significant (but weak) long-term trend of increasing abundance ($p < 0.001$, $R^2 = 0.182$, model 1 linear regression, n = 74) (Figure 50). A weak long-term increase in abundance was recorded in autumn samples ($p = 0.022$, $R^2 = 0.228$), with strong increasing abundance trends recorded for summer ($p = 0.018$, $R^2 = 0.573$) and winter samples ($p = 0.041$, $R^2 = 0.527$). There was no significant increase observed however in spring samples throughout the time-series. Abundances were below average throughout 1999-2001, only rising above average late in 2002. From 2004 - 2008, total cell abundances ranged from ca. 7 cells L^{-1} to a maximum of ca. 125 cells L^{-1} (April 2005). Abundances declined from 2005, with below average abundances of 11-16 cells L^{-1} in autumn 2008. Prior to 2004 few positive abundance anomalies were seen, with a shift towards positive abundance anomalies seen after this time. All years were tested using a Kruskal Wallis one-way ANOVA to identify any significant differences between the years; $p < 0.001$. Low abundances from 2001 and high abundances from 2005 were primarily responsible for this significant difference (Table 8). For full results of inter-annual testing see Appendix 10.6.

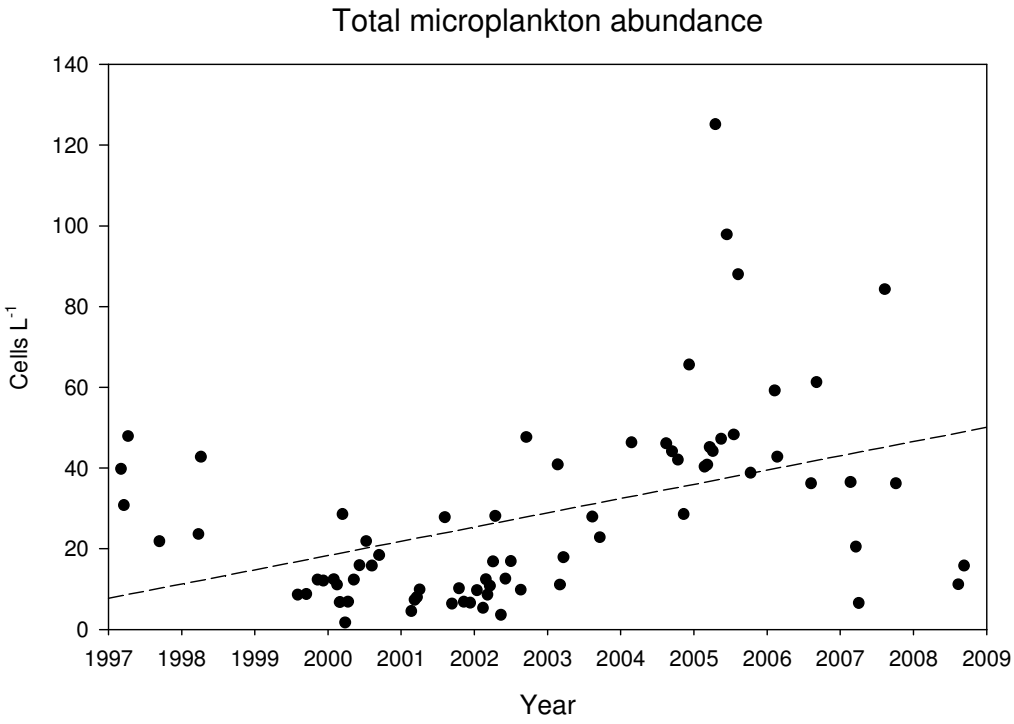


Figure 50 - Scatter plot showing total microplankton abundance (Cells L^{-1}) over the sampling period 1997 – 2009. Dashed line represents model 1 linear regression, $R^2 = 0.182$.

Significant year pairs	Q value
2001 vs. 2004	3.985, $p < 0.05$
2001 vs. 2005	4.949, $p < 0.05$
2001 vs. 2006	3.637, $p < 0.05$
2005 vs. 1999	3.411, $p < 0.05$
2005 vs. 2000	4.014, $p < 0.05$
2005 vs. 2002	4.080, $p < 0.05$

Table 8 – Pairs of years identified as being significantly different to each other (pairs responsible for Kruskal-Wallis ANOVA significant variation). Pairs identified using Dunn's Pairwise testing.

The differences in mean abundance between 2001 and 2002 can be seen in Figure 51. The largest variation observed within one year was during 2005, with a maximum recorded abundance of 125.1 cells L^{-1}, minimum of 38.8 cells L^{-1}. Average total microplankton was also highest during 2005, at 61.6 cells L^{-1}, whilst lowest average total microplankton abundance was during 2001 at 9.8 cells L^{-1}.

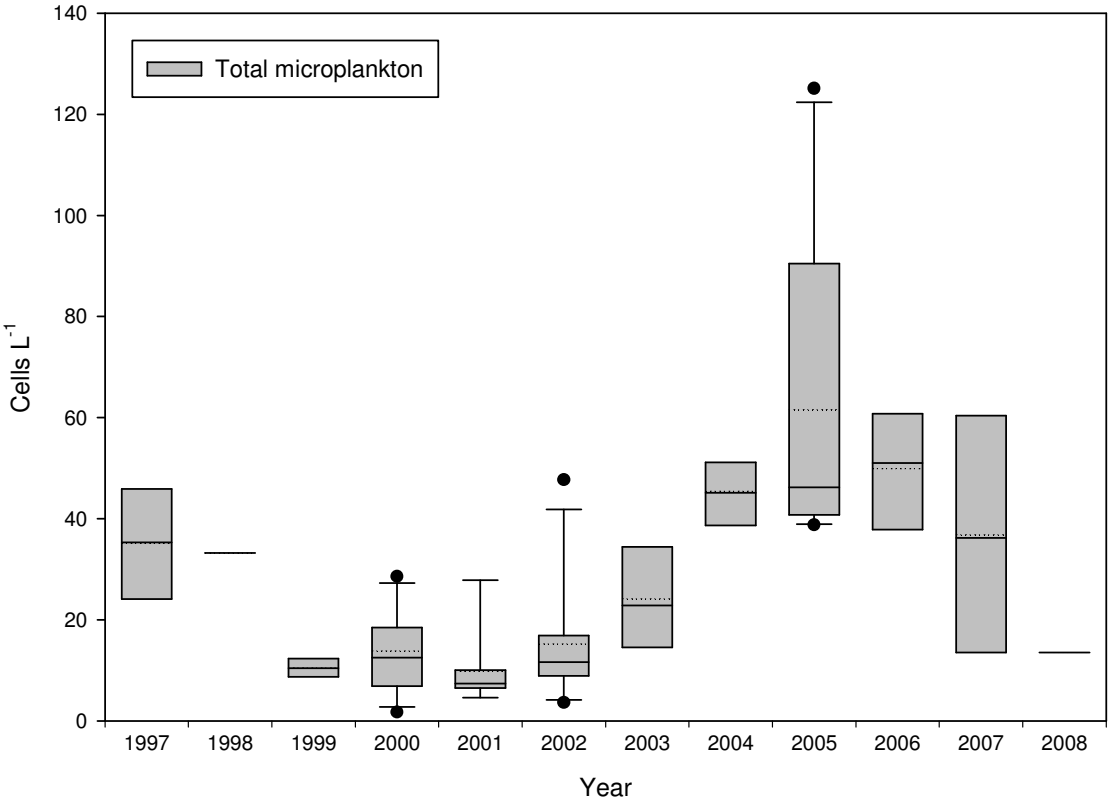


Figure 51 - Box and whisker diagram for total microplankton abundance (1997 – 2009). Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance; dotted lines represent mean abundance (circular data points show outliers). 'n' values are shown below the graph signifying the number of samples analysed in each year.

An overall significant (but weak) long-term increase in diatom abundances is recorded (model 1 linear regression, $p = 0.002$, $R^2 = 0.130$). Long-term increases are seen in autumn ($p = 0.023$, $R^2 = 0.225$) and summer diatom samples ($p = 0.018$, $R^2 = 0.575$), with no significant long-term increase in winter or spring samples. Diatom abundance ranged from 0.2-27.5 cells L^{-1} over the sampling period, averaging 7.5 cells L^{-1}. Lowest abundances were recorded during 1999-2003, highest abundances during 1998 and 2005 (Figure 52). Monthly abundance anomalies follow a similar pattern to monthly total abundance anomalies with mostly negative anomalies during the first two-thirds of the sampling period, shifting to mostly positive anomalies after 2003/4 (Figure 54). Highest average abundances were recorded in 1998 (15.4 cells L^{-1}), followed by 2005 (14.4 cells L^{-1}) (Figure 56). Lowest average abundances were recorded in 2001 (3.12 cells L^{-1}), with all years 2000 – 2002 recorded with yearly averages < 4 cells L^{-1}. Diatoms were numerically dominant in only 11% of samples analysed.

Dinoflagellates were numerically dominant in the remaining 89% of analysed samples, averaging 15.0 cells L^{-1}, with abundances ranging from 1.2 - 92.2 cells L^{-1}. Abundances were lower throughout the first two-thirds of the time-series, with an increase seen after 2004 (Figure 52). Highest abundances were recorded during 2005 (92.2 cells L^{-1}), as well as highest average abundances (36.6 cells L^{-1}) (Figure 56). Lowest average abundances were recorded during1999 and 2001 (both 4.6 cells L^{-1}). Increasing abundances until 2004 are reflected in the mostly positive monthly anomalies during 2004-2006 (Figure 54), although a mix of positive and negative anomalies are seen in the last two years of the data set, reflecting a drop in dinoflagellate abundance during this time. Throughout the course of the whole time-series however, there was a significant (but weak) increase in abundance of dinoflagellates ($p < 0.001$, $R^2 = 0.185$). A weak increase is seen in autumn samples ($p = 0.023$, $R^2 = 0.224$), with both summer and winter samples showing a strong increasing trend in abundance ($p = 0.013$, $R^2 = 0.613$ and $p = 0.018$, $R^2 = 0.636$ respectively). As with the diatoms, spring dinoflagellate abundances showed no significant increase over the duration of the sampling period.

Tintinnid abundances followed a similar pattern to that of diatom and dinoflagellate abundances, with higher abundances additionally observed in early 1997 (Figure 52). Abundance ranged from 0.3-18.5 cells L^{-1}, with a time-series average of 4.7 cells L^{-1}. Higher abundance at the start of the time-series produces a large positive monthly abundance anomaly before mostly negative anomalies seen between 1999 and 2003 (Figure 54). Average yearly abundances are highest in 2005 (8.8 cells L^{-1}) and lowest during 1999 and 2001 (Figure 56). The tintinnids also showed a significant but weak trend of increasing abundance over the time-series ($p = 0.017$, $R^2 = 0.078$), although this trend was not significant when seasons were considered individually.

Radiolaria showed lower abundances throughout the first two-thirds of the data set (including 1997 samples), before increasing to peak abundance (2.1 cells L^{-1}) in 2005 (Figure 52). High abundance (1.4 cells L^{-1}) was also recorded in late 2007. Monthly abundance anomalies show more variation than for diatoms, dinoflagellates and tintinnids (Figure 54); a higher number of positive anomalies were seen during the predominantly negative anomaly phase (1999-2003), in addition to a mix of negative and positive anomalies seen during the high-abundance years (2005 and 2007). The long-term average for radiolaria abundance was 0.28 cells L^{-1}. Yearly averaged radiolaria abundances were always < 0.60 cells L^{-1}, with the highest (lowest) averages in 2006 (1998) at 0.57 (0.10) cells L^{-1} (Figure 56). Radiolaria abundances showed a significant but weak increase over the time-series ($p < 0.001$, $R^2 = 0.168$). Contrary to the diatoms and dinoflagellates, only spring radiolaria abundances showed a significant increase over time ($p = 0.027$, $R^2 = 0.144$).

As with the radiolaria, no significant increase was observed for summer, autumn and winter foraminifera abundances. A significant but weak long-term increase in foraminifera abundance was recorded over the time-series ($p = 0.012$, $R^2 = 0.085$), driven by a time-series increase in spring foraminifera abundances ($p = 0.028$, $R^2 = 0.085$). Foraminifera were not observed to be present in all samples analysed (Figure 53) however it was noted that when they were present, it was usually at higher abundances than the radiolaria, silicoflagellates and acantharia, with a time-series average of 0.48 cells L^{-1}. The foraminifera appear to display a stronger

seasonal signal throughout the time-series than other groups, with highest abundances recorded in February samples for six out of the twelve years analysed. Low abundances throughout the first half of the time-series increased to a maximum abundance (3.4 cells L^{-1}) in spring 2004 (Figure 52). A mixture of positive and negative abundance anomalies are recorded, although 1999-2004 again show mainly negative anomalies (Figure 54). Highest yearly averaged abundances were recorded in 2004 (0.99 cells L^{-1}) and the lowest at 0.02 cells L^{-1} during 1999 (Figure 56).

Contrary to the other groups already mentioned, the silicoflagellates did not show any significant increase or decrease in abundance over the course of the time-series ($p = 0.174$). Silicoflagellates reached peak abundance (1.9 cells L^{-1}) at the same time as the foraminifera (February 2004), with the majority of abundances prior to this at an order of magnitude lower (0 - 0.2 cells L^{-1}). 2005, 2006 and 2007 all showed abundance peaks > 0.5 cells L^{-1}, although surrounding abundances remained low (Figure 52). The period between mid-2000 and late 2002 shows only negative abundance anomalies, representing very low abundances, with positive and negative anomalies fairly evenly distributed throughout the rest of the time-series representing a large variation in silicoflagellate abundance (Figure 54). For nine of the twelve years analysed, the highest abundance was recorded during the spring (seven times in February, twice in March). A time-series average of 0.14 cells L^{-1} was recorded for the silicoflagellates, which like the foraminifera were noted to be absent from a large number of samples. The highest yearly averaged abundance was recorded in 2006 with 0.48 cells L^{-1}, whilst the lowest average abundances was just 0.01 cells L^{-1} in 2008 (Figure 56).

As with the silicoflagellates, there was no significant long-term increase or decrease recorded for the acantharia ($p = 0.336$). Acantharia abundances were lowest between 1999 and 2001, reaching peak abundance of 0.6 cells L^{-1} in late 2005. High acantharia abundances were also recorded in early 2004 (0.6 cells L^{-1}). Abundances in the remaining years were generally low; not exceeding 0.4 cells L^{-1} (Figure 52). Low abundances in 1999-2001 are represented by negative abundance anomalies, also seen from autumn 2001 to autumn 2002 (Figure 54). A

mixture of positive and negative anomalies is seen for the remainder of the time-series, representing a mixture of higher and lower abundances. A time-series average of 0.10 cells L^{-1} is calculated for the acantharia, with the highest yearly average of 0.21 cells L^{-1} in 2004, and the lowest average in 2008 of just 0.008 cells L^{-1} (Figure 56).

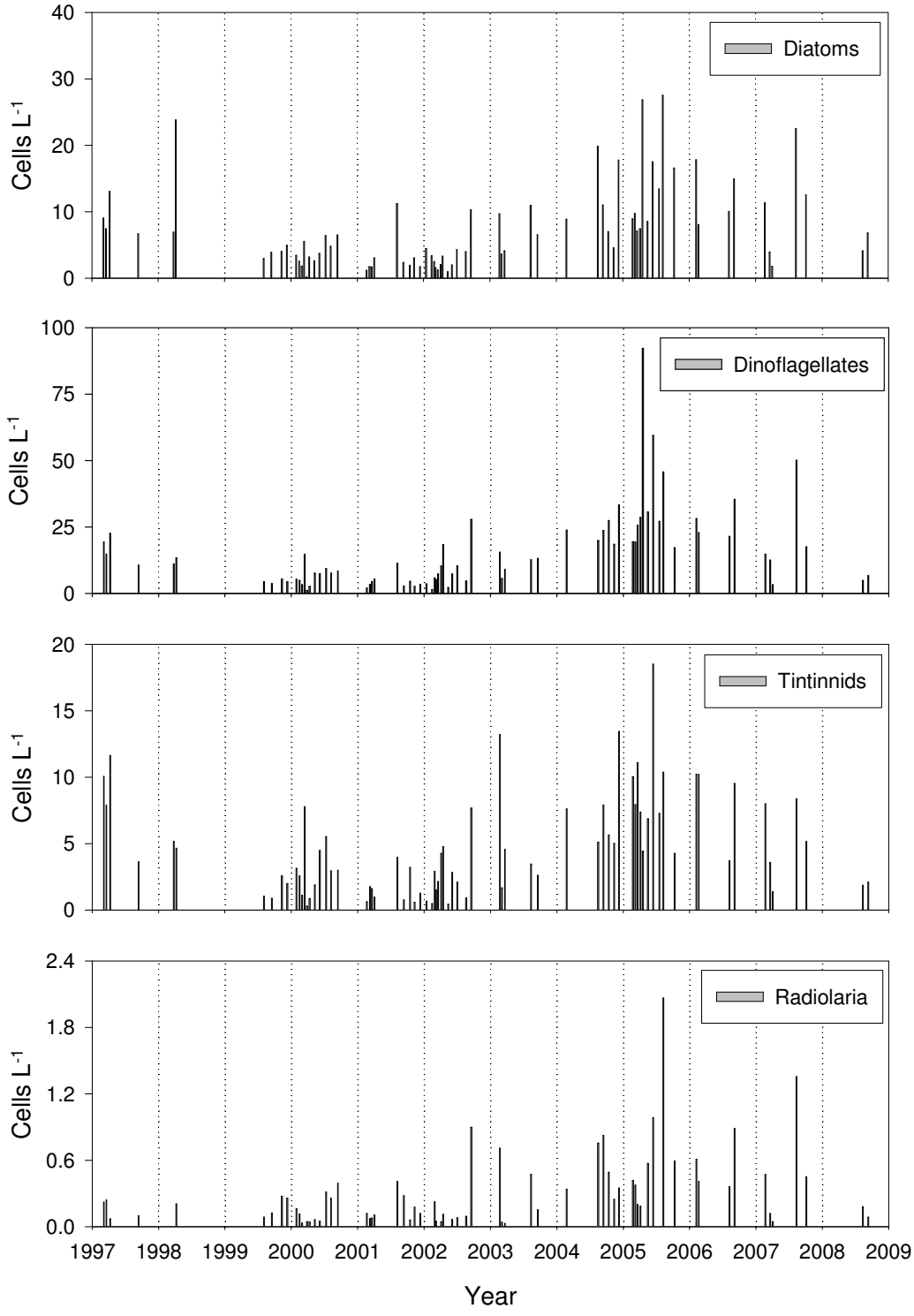


Figure 52 - Long-term changes in microplankton group abundances at BATS 1997-2008. Each bar represents microplankton group data from a separate BATS core or bloom cruise. X-axis tick marks and labelling represent January 1 of each year.

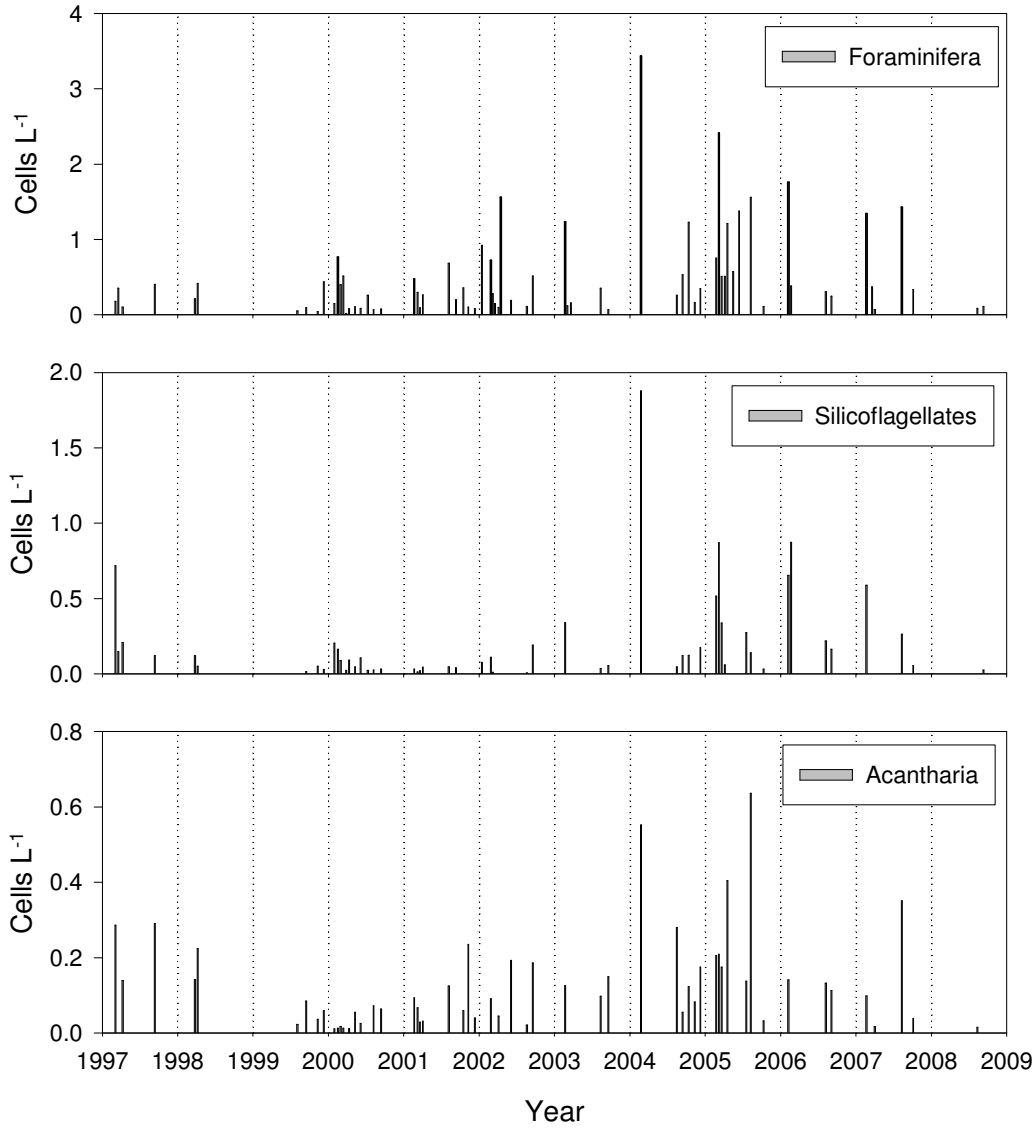


Figure 53 - Long-term changes in microplankton group abundances at BATS 1997-2008. Each bar represents data from a separate BATS core or bloom cruise. X-axis tick marks and labelling represent January 1 of each year.

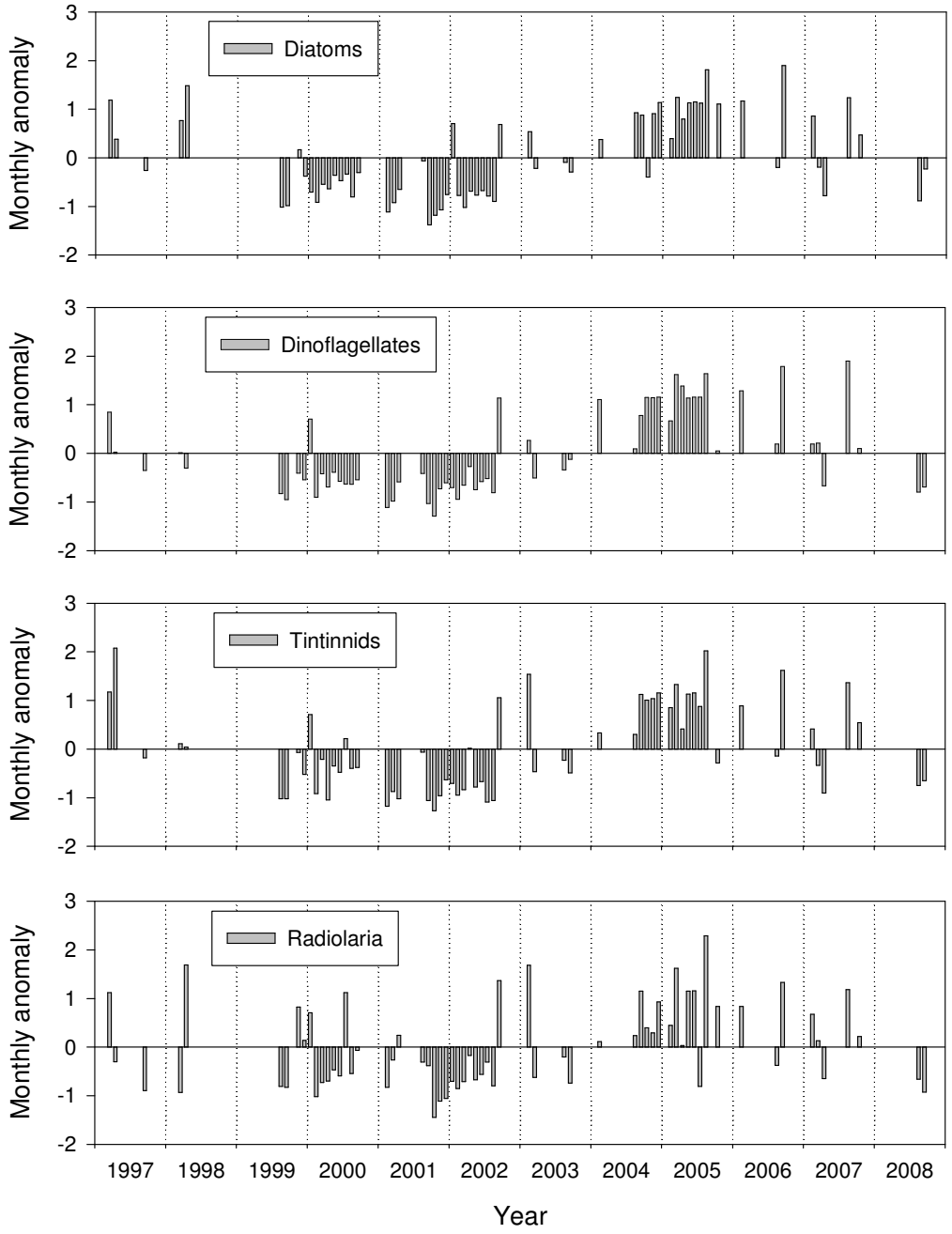


Figure 54 - Monthly anomaly of microplankton abundance for each group analysed. See Methods for anomaly calculation. X-axis tick marks represent January 1 of each year.

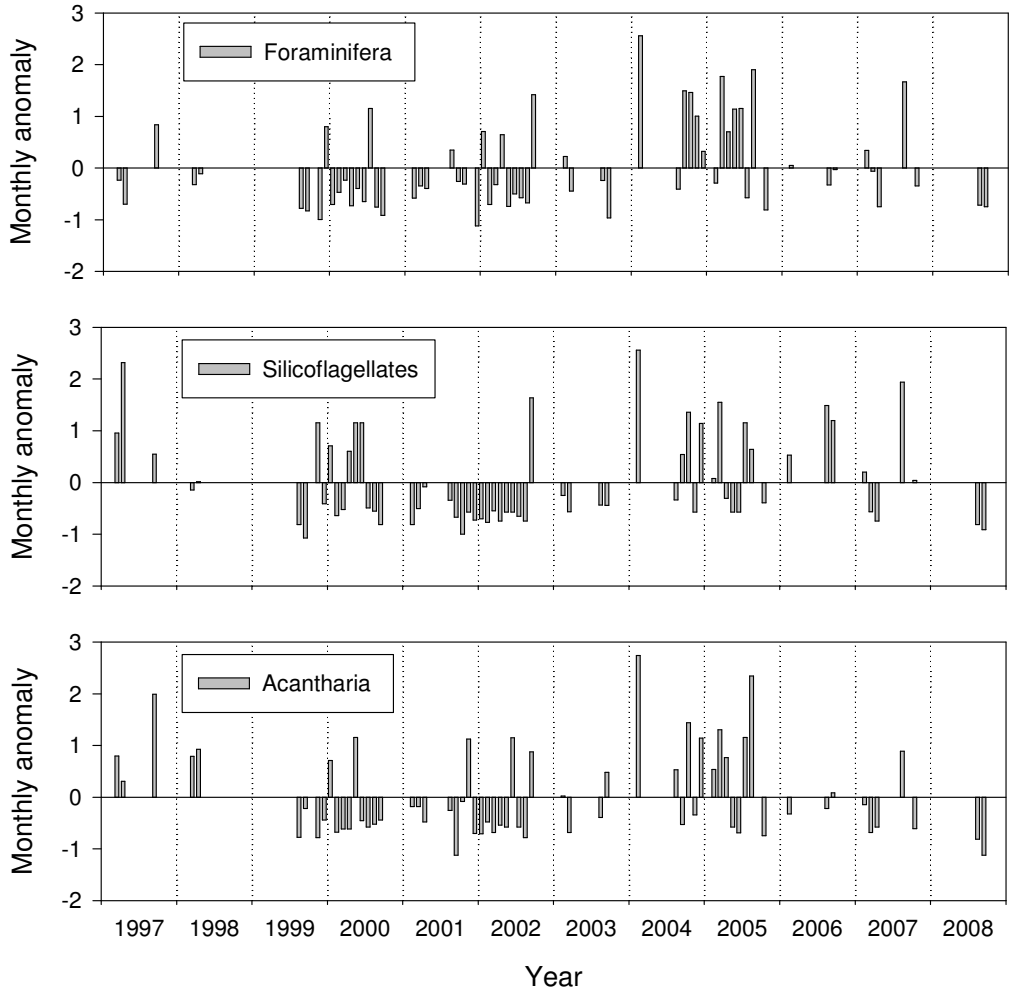


Figure 55 - Monthly anomaly of microplankton abundance for each group analysed. See Methods for anomaly calculation. X-axis tick marks represent January 1 of each year.

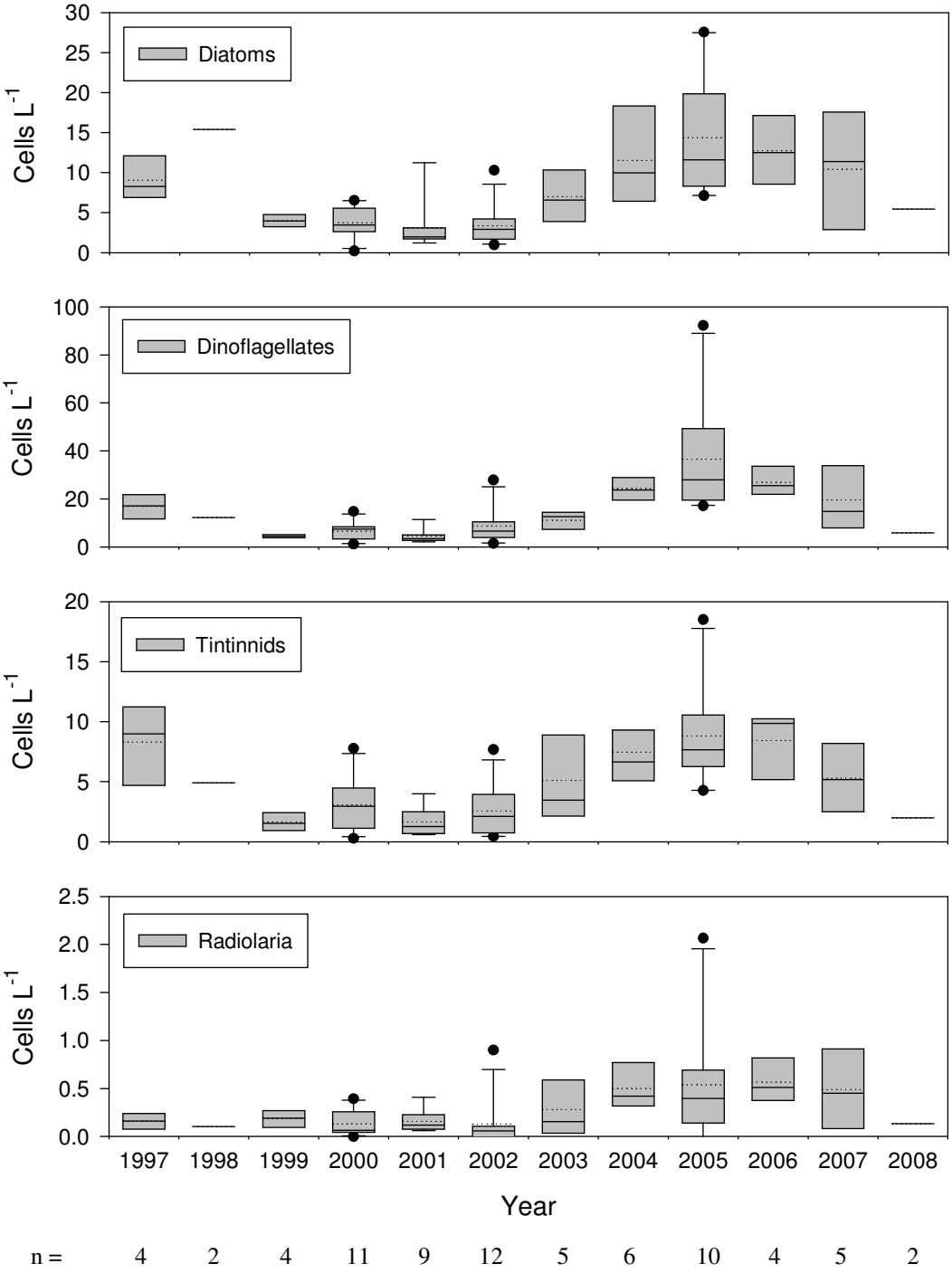


Figure 56 - Yearly box and whisker plots of microplankton abundance throughout the entire time-series (1997-2008) by group. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each year.

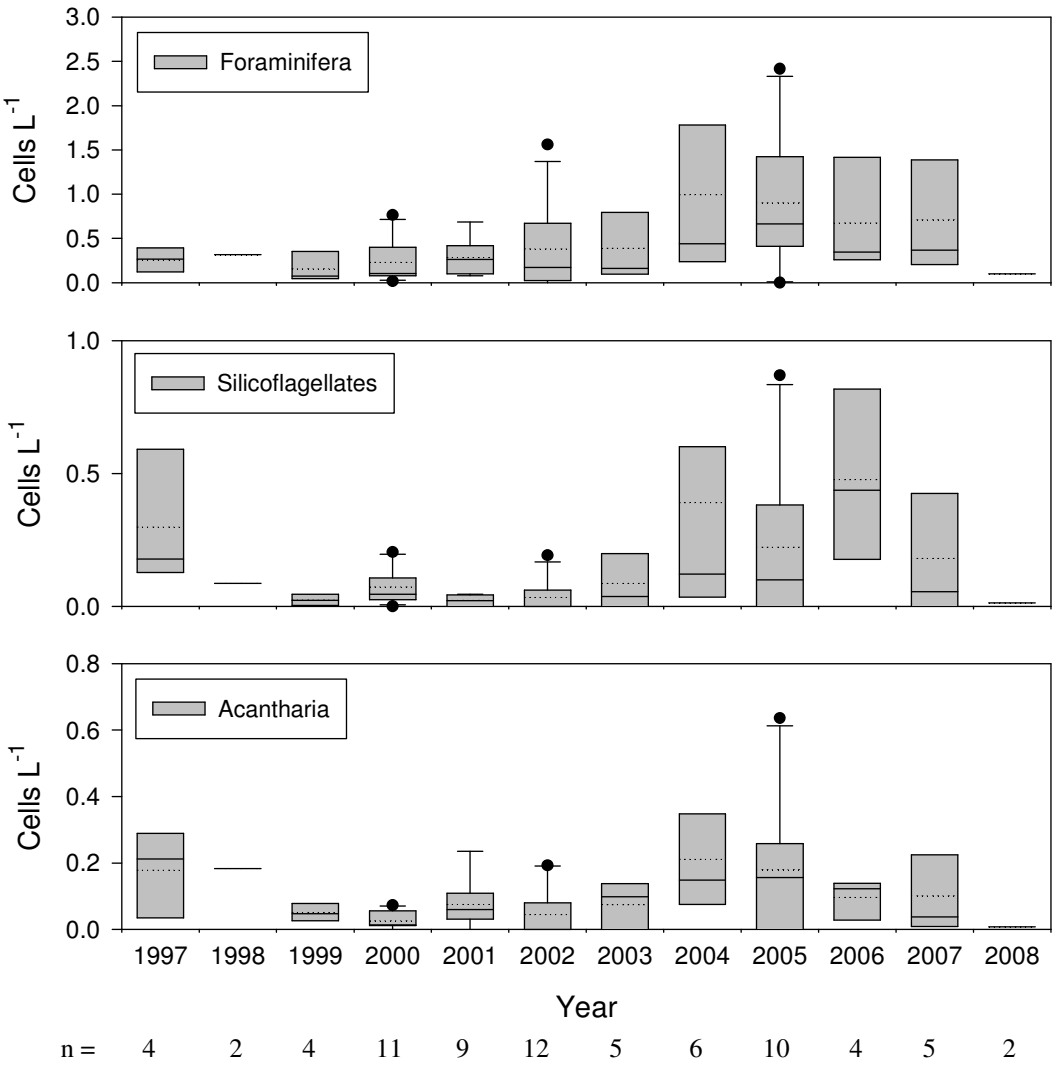


Figure 57 – Yearly box and whisker plots of microplankton abundance throughout the entire time-series (1997-2008). Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each year.

7.2 Microplankton relative abundance

On average, dinoflagellates accounted for 50.9% of the total microplankton community, followed by diatoms (27.8%), tintinnids (17.5%), foraminifera (1.9%), radiolaria (1.0%), silicoflagellates (0.5%) and acantharia (0.4%) (Figure 59). When a model 1 linear regression was applied to relative abundance data for each group, no groups except dinoflagellates and tintinnids showed any significant increase or decrease in relative abundance during the course of the sampling period. Full results can be seen in Table 9. Dinoflagellates were recorded as showing a significant but weak increase in relative abundance over the time-series ($p = 0.035$, $R^2 = 0.060$), whilst tintinnids showed a significant but weak decrease ($p = 0.036$, $R^2 = 0.055$). As these two groups are two of the top three microplankton groups in terms of numerical dominance, it is unsurprising that as one shows an increase in relative abundance, the other shows a decrease. However, as mentioned in Results IV - 7.1, the dinoflagellates showed an increase in long-term abundance for autumn, summer and winter samples, suggesting that increasing dinoflagellate abundance (rather than decreasing tintinnid abundance) is responsible for the changes observed in relative abundance data.

Microplankton group	p - value
Diatoms	0.602
Dinoflagellates	**0.035**
Tintinnids	0.036
Radiolaria	1.00
Foraminifera	0.845
Silicoflagellates	0.451
Acantharia	**0.083**

Table 9 - Results of Model 1 linear regression applied to the relative abundance of each microplankton group over the entire time-series

This lack of significant increase or decrease in many of the microplankton groups represents a microplankton community which, although changeable in group abundance, is relatively constant in terms of relative abundance. Whilst there have been long-term increases in abundance in most of the groups, only dinoflagellates appear to have increased in proportion to the other microplankton groups. This stable community composition (relative abundance) can also be seen when year-specific samples are used to create a non-metric multi-dimensional scaling ordination plot (Figure 58). The even distribution of samples from all years across the cluster shows the community composition differed little between years (data square root transformed, NMDS plot created using a Bray-Curtis similarity matrix)

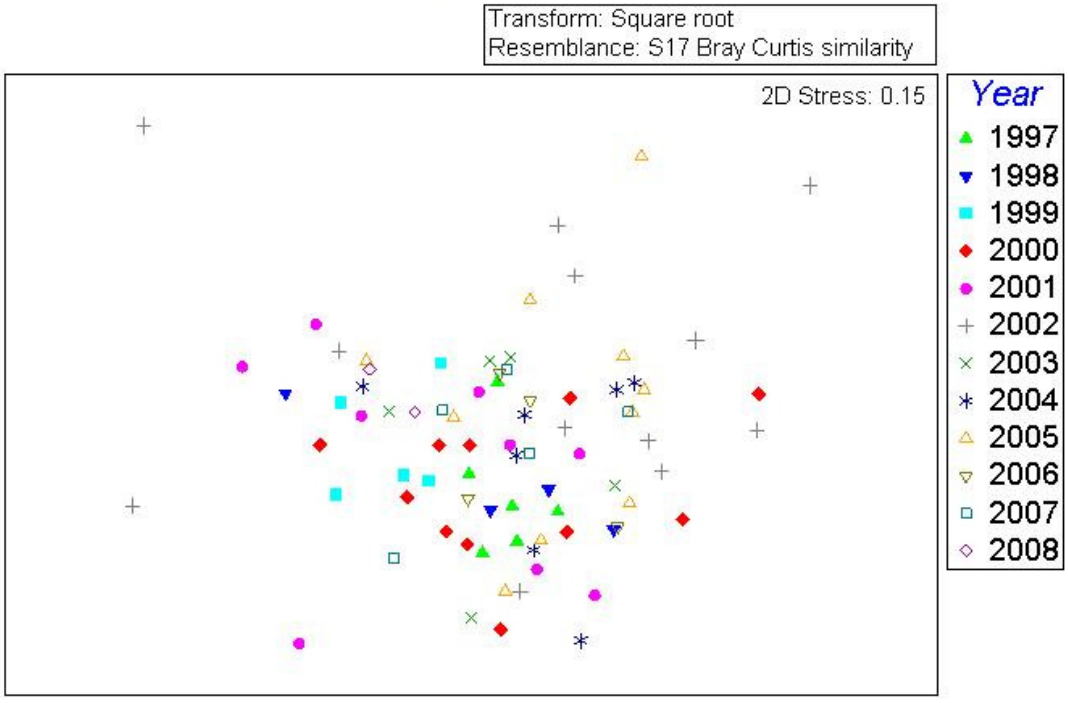


Figure 58 - Non-metric multidimensional scaling ordination based on Bray-Curtis similarities (calculated from square-root transformed, sample-averaged) of microplankton composition data for all groups. Sample ID are coded with symbols denoting the year they represent. 2D Stress = 0.15

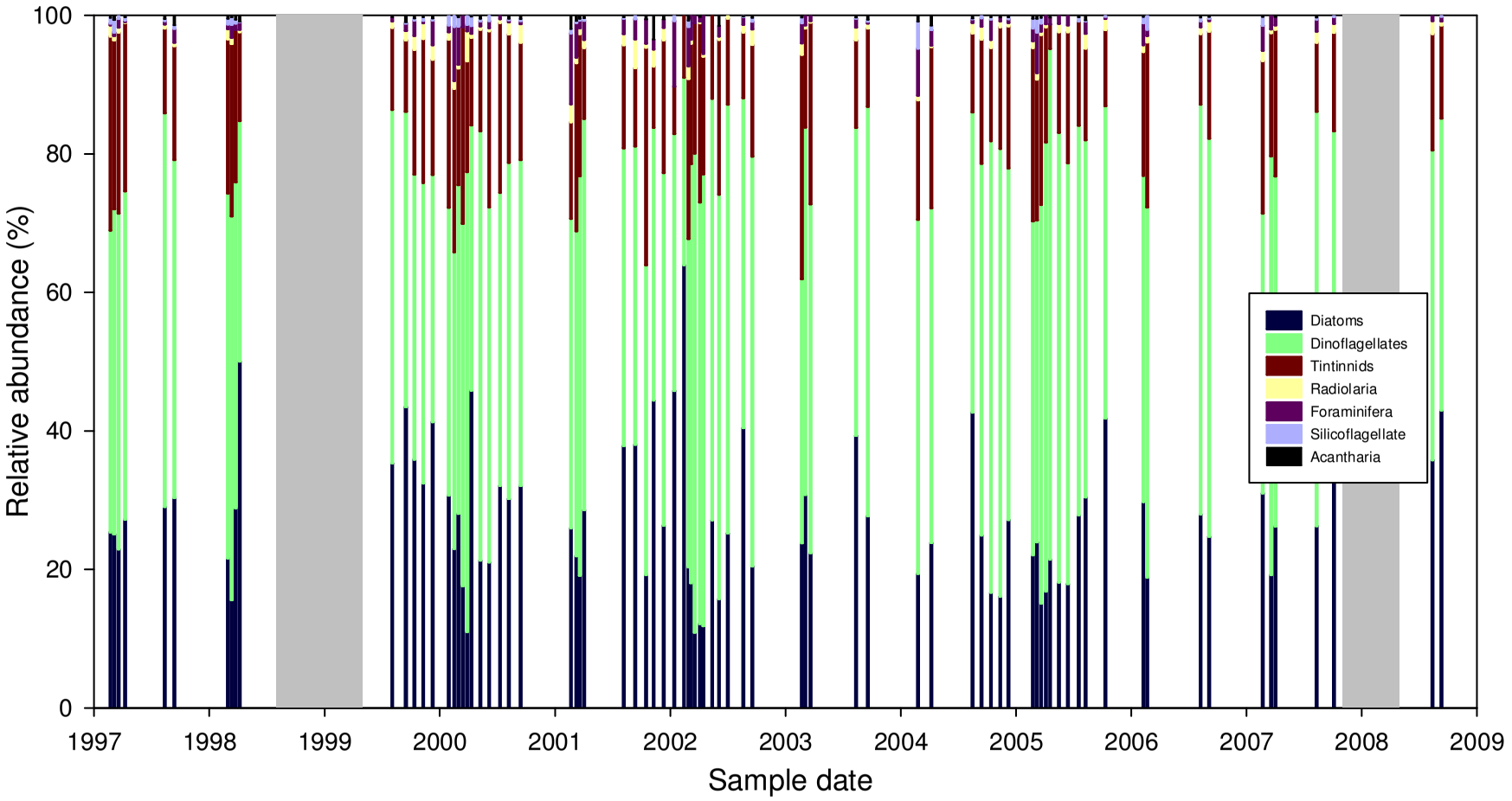


Figure 59 - Average relative abundance for microplankton groups from all samples analysed. Grey columns represent periods where no microplankton samples were collected.

7.3 Water column data

Microplankton abundance data was compared with physical and chemical data collected from the BATS site over the sampling period (1997 – 2008). Physical and chemical data was averaged over the top 150m of the water column to represent the depth of water sampled by the microplankton net. Correlations between microplankton group abundances and physical/chemical parameters are shown in Table 10. Full results of statistical testing can be found in Appendix 10.7. To enable a more detailed and reliable comparison between environmental and abundance data to be made, data should ideally be checked for independence and randomness, to remove any autocorrelation issues.

Both diatoms and radiolaria were found to have significant (but weak) positive correlations over the long-term data set with temperature, although this was not seen in any other microplankton groups (Figure 60). Only the Radiolaria had previously shown a significant correlation between monthly-averaged temperature and abundance data (Results I - 4.1, Appendix 10.4). Dinoflagellates, tintinnids and silicoflagellates all showed significant (but weak) positive correlations with salinity (with no significant correlations seen between monthly averaged salinity and abundance data for any of these three groups). Total microplankton also showed a weak but significant positive correlation, driven by the positive correlations seen for dinoflagellates and tintinnids (two of the three numerically dominant groups). Forams were the only single-celled microplankton group to show a significant correlation (weak positive) with Nitrite concentrations, whilst silicoflagellates were the only group to show a significant correlation (weak negative) with Phosphate concentrations over the sampling period (as with monthly-averaged data). No significant correlations were recorded for any of the microplankton groups for Silicate or combined Nitrate and Nitrite concentrations over the sampling period. Neither the pairings of Peridinin concentrations and dinoflagellate abundances, or Fucoxanthin concentrations and diatom abundances showed any significant correlation either, suggesting microplankton numbers of these two groups are too low for them to be contributing significantly to these pigment concentrations in the water column.

	Temperature (n=72)		Salinity (n=72)		Nitrite (n=69)		Phosphate (n=70)	
	C. coeff	p-value	C. coeff	p-value	C. coeff	p-value	C. coeff	p-value
Diatoms	0.253	0.032						
Dinoflagellates			0.277	0.019				
Tintinnids			0.351	0.003				
Radiolaria	0.405	<0.001						
Foraminifera					0.318	0.008		
Silicoflagellates			0.244	0.039			-0.345	0.004
Acantharia								
Total Microplankton			0.279	0.018				

Table 10 - Tabulated results for significant correlations between different microplankton group abundances and physical/chemical parameters measured at the BATS site, averaged over 150m. Grey shaded boxes indicate non-significant results for correlations. All correlations are Spearman Rank correlations.

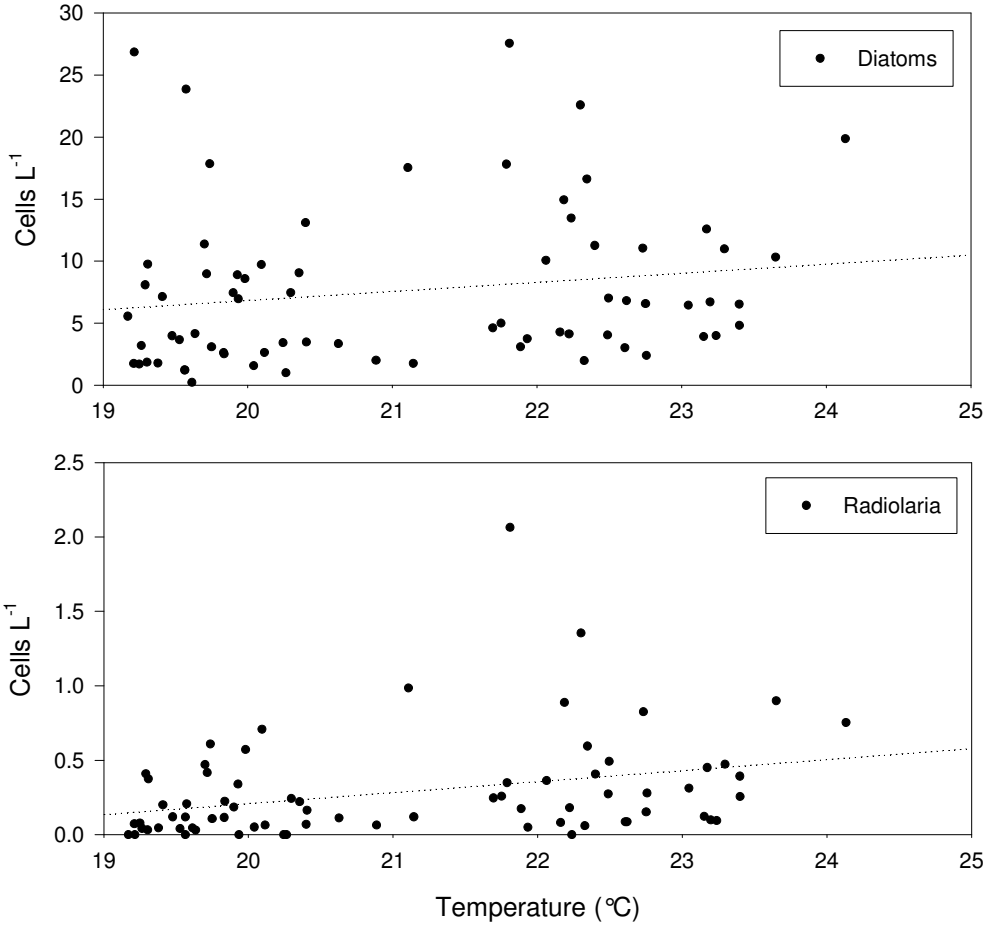


Figure 60 - Scatter plots showing correlation of diatom abundance (top) and radiolaria abundance (bottom) with water column temperature. Dotted lines show model 1 linear regression (diatoms $R^2 = 0.03$, radiolaria $R^2 = 0.10$).

7.4 Discussion

Of all the single-celled microplankton groups analysed, only the silicoflagellates and acantharia did not show a significant but weak increase in abundance throughout the course of sampling. The increases of all other groups are in accordance with recent reports of increasing chlorophyll a concentrations, primary production rates and carbon export (Lomas *et al.,* 2010), as well as increases in abundance and biomass of the larger zooplankton (Steinberg *et al.,* 2012). In most microplankton groups, this increase in abundance was driven by an increase in abundance during a particular season i.e. radiolaria showed a significant but weak increase in spring abundances, but not for any other season. Radiolaria and foraminifera are the only two groups to show significant increases in spring abundances, whilst tintinnids show an overall increase, but not significant for any particular season. Total microplankton abundance showed a significant increase in summer, winter and autumn samples, but not spring samples. This lack of significant increase for spring diatom, dinoflagellate and total microplankton abundances suggests that whilst abundances in other seasons are able to increase, there is some controlling factor acting to keep spring abundances from increasing. This may be due to various factors, including (but not limited to) the possibility of nutrient competition with the smaller, more numerically dominant prokaryotic picoplankton for example (Steinberg *et al.,* 2001). In terms of relative abundance, dinoflagellates and tintinnids were the only two groups to show any significant change over time. A decrease in tintinnid relative abundance and an increase in dinoflagellate relative abundance were seen. These changes in dinoflagellate and tintinnid relative abundance are driven by an increase in absolute abundance for dinoflagellates in all seasons excluding spring, rather than a decrease in tintinnid abundances. A general lack of significant increase or decrease in the relative abundance of microplankton groups represents a changeable (in abundance) community, but one which remains constant in terms of relative abundance of the different microplankton groups.

Diatoms and radiolaria showed a significant positive correlation to temperature, reflecting their (often) summer abundance peaks. Whilst it may be expected to

see diatoms increasing in abundance during spring samples (and therefore lower temperatures) as suggested in previous findings (Menzel and Ryther 1960; DuRand *et al.,* 2001; Steinberg *et al.,* 2001), it has been shown that fucoxanthin (as an indicator of diatom presence) is found in the water column at times other than the spring bloom, and that diatoms often also show high abundances at other times of the year (Steinberg *et al.,* 2001). Salinity showed a significant positive correlation with dinoflagellates, tintinnids, silicoflagellates and total microplankton abundances. This would indicate these groups showing a preference for existing in summer months when the water column becomes stratified and evaporation increases salinity in the surface waters. Silicate and combined nitrate and nitrite showed no significant correlations to any of the microplankton groups, whilst Nitrite showed a significant but weak positive correlation with the foraminifera, and phosphate showed a weak negative correlation with silicoflagellate abundance. The correlation of foraminifera with nitrite can be seen in the higher springtime abundances of foraminifera, when the water column is still well mixed, and nutrient rich water is mixed into the euphotic zone. The foraminifera appear to be able to utilize the input of nutrients far more quickly than other microplankton groups, for whom no significant correlations are seen between abundance and nutrient levels. The weak negative correlation between phosphate levels and silicoflagellate abundance is less easily explained; as autotrophs there should be no time-lag involved between the increase of nutrients and the increase in abundance of cells. This discrepancy may be due to the fact that they are competing for a similar 'niche' to the diatoms and autotrophic dinoflagellates, both of which occur in far greater abundances than the silicoflagellates, which are simply being outcompeted for nutrients (and therefore present in lower abundances).

Within the long term pigment data record, no significant correlations were found between diatoms and fucoxanthin, dinoflagellates and peridinin, or between chlorophyll a and either diatoms, dinoflagellates or total microplankton abundance. This is most likely to be due to the fact that the microplankton contribution to total water column chlorophyll and other pigments is too small to cause any appreciable correlation between cell abundances and nutrient concentrations; although smaller cells will have less chlorophyll in them, they are

present in far greater numbers. These smaller cells are therefore suggested to be more important in terms of contribution to pigment concentrations in the water column at the BATS site.

These long-term changes in microplankton abundance cannot be totally explained by a single driving factor, such as temperature, salinity, nutrients etc. It is therefore suggested that the microplankton community at the BATS site is linked with a whole suite of changing physical and chemical parameters, rather than a single driving factor. The lack of significant change in relative abundance of microplankton groups again suggests a stable microplankton community composition capable of withstanding high levels of variability, both in terms of short timescale and long-term nutrient availability and water column physical properties. It is still unclear however as to whether changes within the individual microplankton groups are occurring, such as the size of microplankton diatoms being replaced by smaller microplankton diatoms, or a dominant diatom species changing to a different species for example.

8 Results V - Microplankton biogeochemistry

Biogeochemical analysis was performed on the BATS microplankton samples, to determine biogenic silica and particulate calcium concentrations. These analyses are described in this chapter, with particular reference to diatom size and biogenic silica concentrations, and foraminifera size and particulate calcium concentrations. Whilst absolute abundance data is useful in determining changes in community size, using biogeochemical data enables a more in-depth observation of how individual groups may be responding in terms of size, or mineral content.

8.1 Particulate Calcium

Microplankton samples were analysed for particulate calcium as described in Methods 2.5, with cell sizes obtained using the FlowCAM software. Highest particulate calcium values were recorded in spring 2006 of 0.15 μgL^{-1}. Lowest concentrations were recorded in 2000, of < 0.002 μgL^{-1} (Figure 61). In years where both spring and autumn samples were analysed, spring samples had the highest particulate calcium concentrations in all but two years (highest spring samples shown as green dots, highest autumn samples as red dots). Over the course of the sampling period, there was a significant but weak increase in total particulate calcium concentrations ($p = 0.032$, $R^2 = 0.080$) when a model 1 linear regression was applied. Autumn samples also showed a significant but weak increase in particulate calcium over the sampling period ($p = 0.018$, $R^2 = 0.261$), although no significant increase was recorded for spring particulate calcium concentrations ($p = 0.319$).

The largest variation in monthly particulate Calcium concentrations was recorded for February samples, closely followed by August and March samples (Figure 62). Highest monthly averaged particulate calcium values were also recorded for February samples of 0.045 μgL^{-1}, with the lowest values measured in January (0.002 μgL^{-1}) and May (0.003 μgL^{-1}).

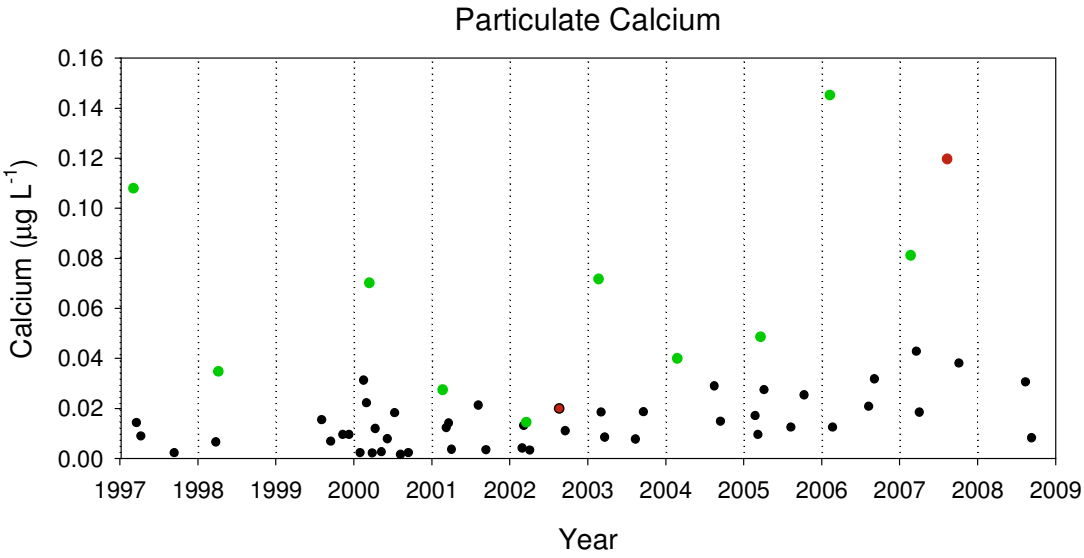


Figure 61 - Measured particulate Calcium concentrations for all spring and autumn samples throughout the sampling period. Green data points represent spring samples, red data points represent autumn samples.

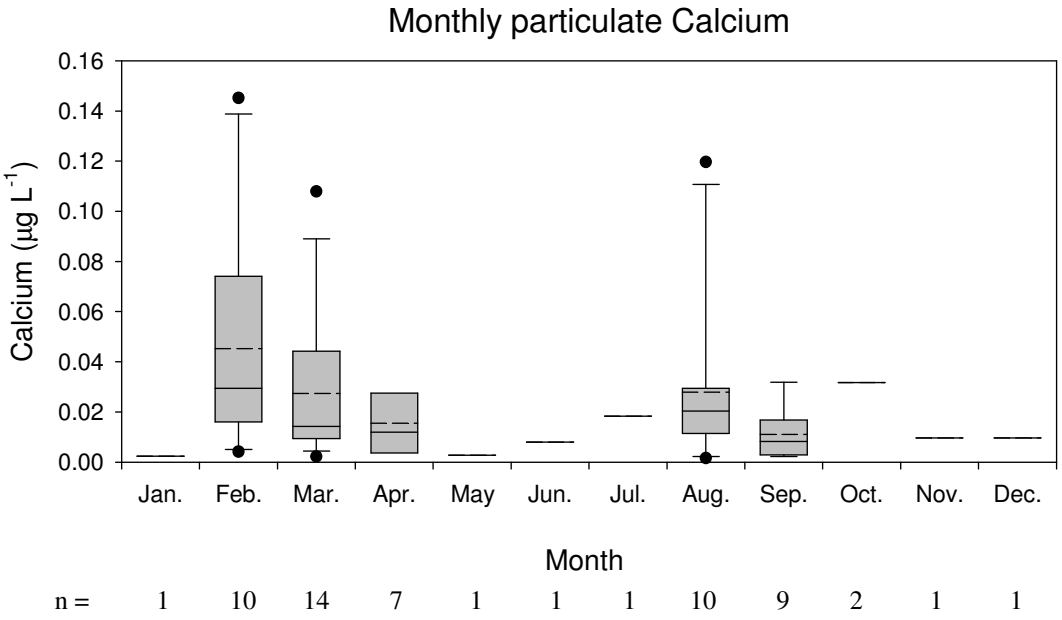


Figure 62 **-** Monthly box and whisker plot of measured particulate Calcium. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each month.

Despite the higher particulate calcium concentrations generally being recorded for spring samples, there was no significant difference found between the particulate calcium concentrations for each month (KW one-way ANOVA, p = 0.132), or between seasons (KW one-way ANOVA, p = 0.141).

Patterns in foraminifera abundance have previously been described on monthly (Results I - 4.1), seasonal (Results I - 4.2) and inter-annual (Results IV - 7.1) time scales. From these results sections, the following points can be summarised:

- Largest variation in foraminifera abundance in February samples (Figure 63 – Top plot of earlier Figure 24)
- Highest monthly averaged foraminifera abundance in February samples
- No significant differences in median abundances between months (p = 0.15) or seasons (p = 0.148)
- Significant but weak long-term increase in abundance over the time-series (p = 0.012, R^2 = 0.085) driven by a time-series increase in spring foraminifera abundance (p = 0.028, R^2 = 0.085)

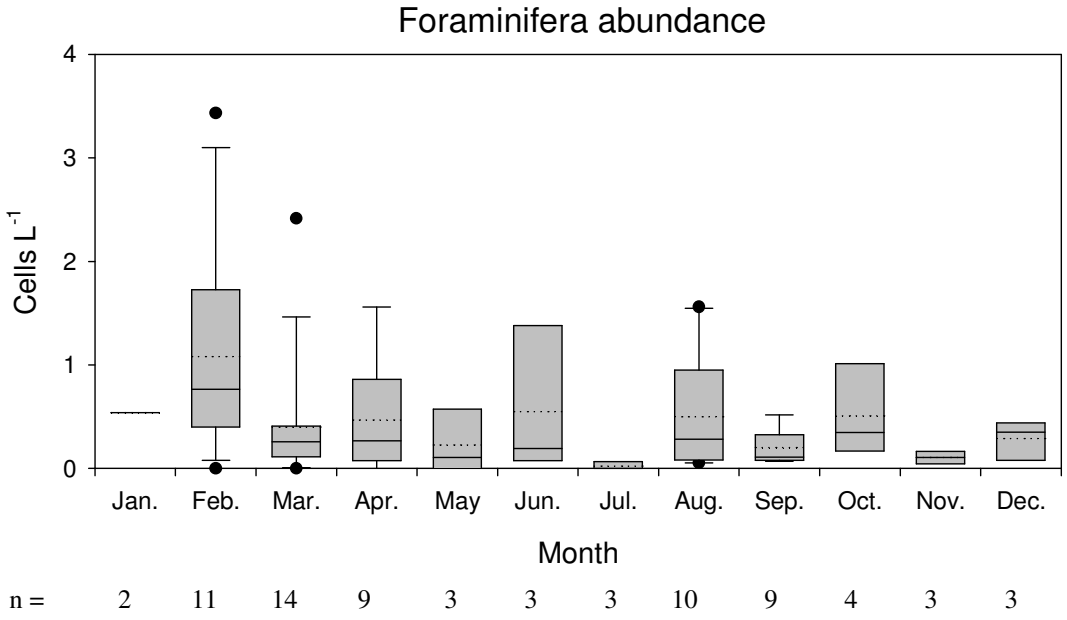


Figure 63 - Monthly box and whisker plot of foraminifera abundance. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each month.

Foraminifera abundance and measured particulate calcium showed a significantly positive (but weak) correlation (Spearman Rank correlation coefficient = 0.461, $p < 0.001$) (Figure 64). By using foraminifera abundance data and measured particulate calcium data, an average cell calcium value for the foraminifera present in each analysed sample was calculated (Figure 65).

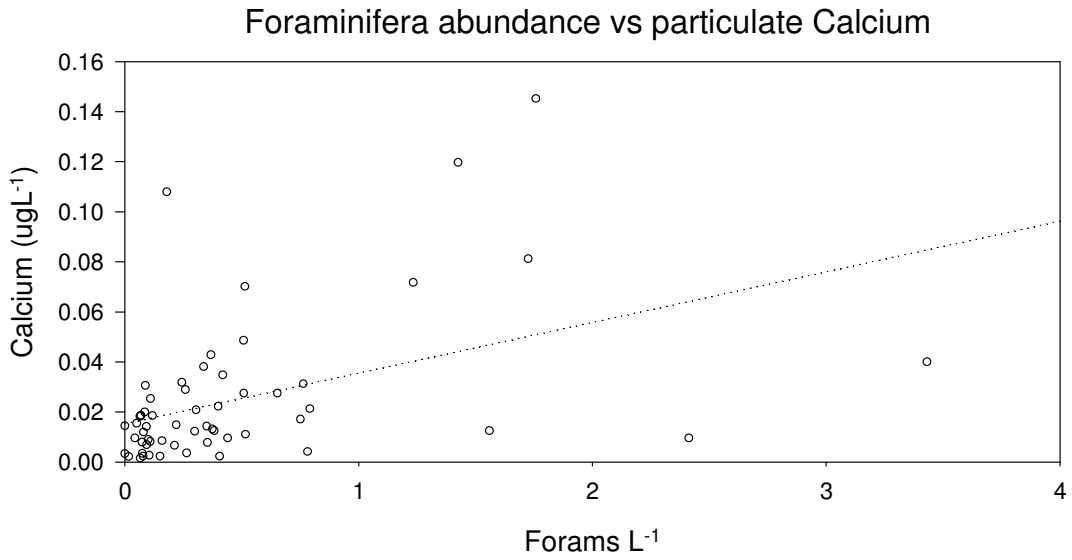


Figure 64 - Relationship between foraminifera abundance (x-axis) and measured particulate Calcium (y-axis). Dotted line represents a model 1 linear regression, $R^2 = 0.130$.

The largest variability in cell calcium values was observed in March samples, whilst the highest monthly-averaged cell calcium was recorded in July (0.28 µg cell $^{-1}$) (Figure 65). January and December had the lowest monthly-averaged cell calcium values at 0.016 and 0.022 µg cell^{-1} respectively. No significant differences in the median cell calcium values between months ($p = 0.192$) or seasons ($p = 0.744$) was found when Kruskal-Wallis one way ANOVAs were performed on the data. There was also no significant change in cell calcium over the entire time-period (Model 1 Linear regression, $p = 0.873$).

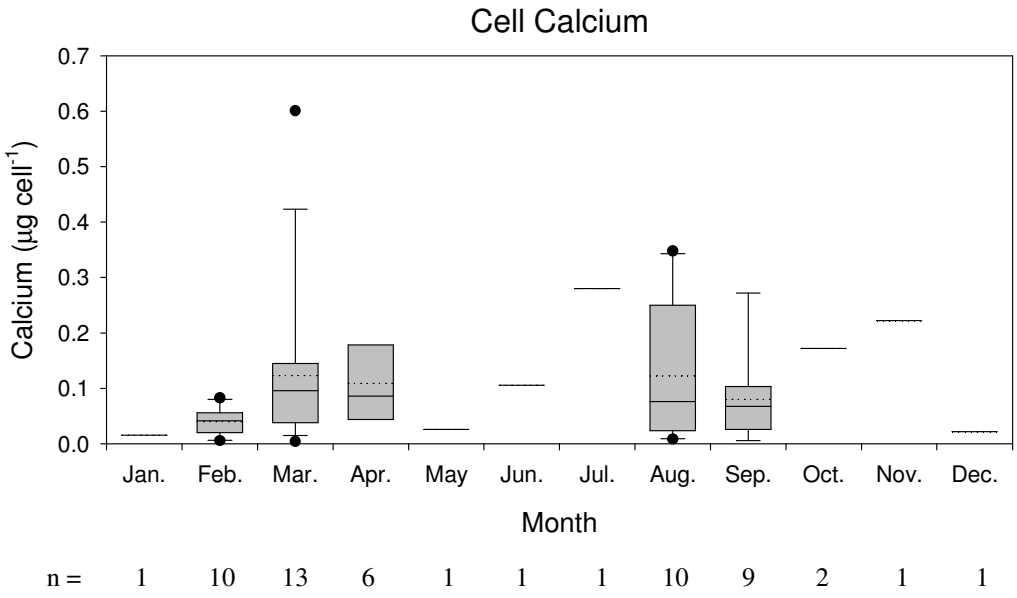


Figure 65 - Monthly box and whisker plot of calcium per cell measurements. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each month.

A similar situation can be observed in foraminifera volume. Volume data is calculated from FlowCAM measurements, based on a spherical volume calculated from cell diameter, as used in Bé *et al*., (1977) and Michaels *et al*., (1995). A high variability in foraminifera volume is observed in March samples (no volume measurements available for June or July samples, due to lack of FlowCAM size data for these months). Highest monthly average cell volumes are calculated for April and October samples, with lowest values in November and December samples (Figure 66).

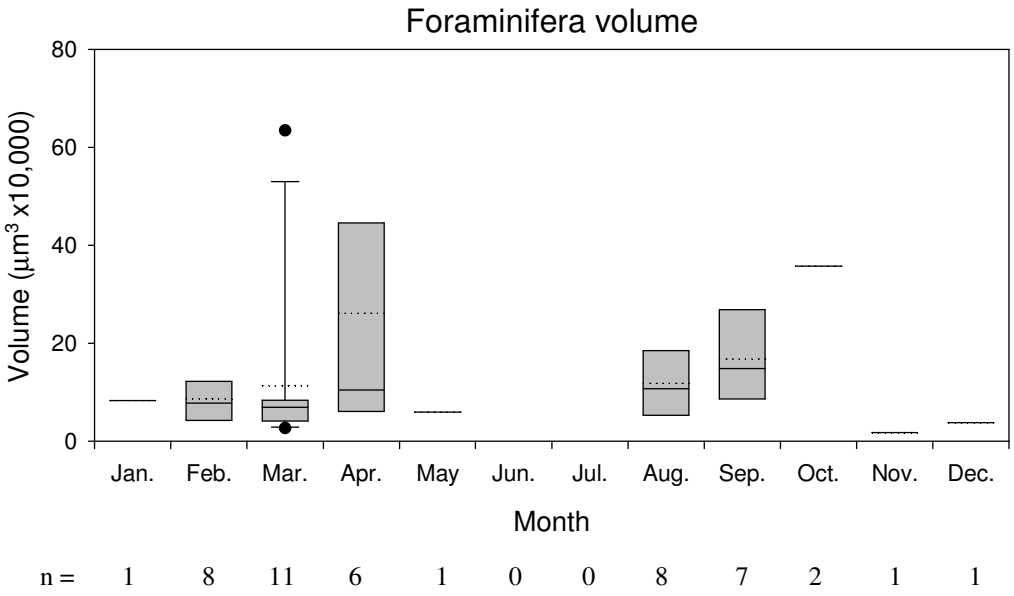


Figure 66 - Monthly box and whisker plot of average foraminifera volume per sample. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each month.

Despite the highest particulate calcium measurements and foraminifera abundances being observed in February samples, low calcium per cell values (both averages and range of values) were observed for these samples. In addition to these low cell calcium values, lower foraminifera volumes (compared to other spring and autumn samples) were observed. Autumn samples generally showed lower foraminifera abundances, mid-range particulate calcium values, and higher cell calcium and cell volume values than February samples. It is suggested that during early spring (February) samples, there are large numbers of low-calcium, small volume foraminifera present in microplankton samples, with variability in cell calcium and cell volume increasing through later spring samples. Lower abundances of high-calcium, large volume foraminifera are indicated to be present in autumn microplankton samples.

8.2 Biogenic Silica

Highest biogenic silica concentrations were generally observed in spring samples, with exceptions to this being mainly autumn samples (Figure 67). Green data points represent highest spring samples in each year, red for autumn samples, blue for winter samples. There was no significant increase or decrease in measured biogenic silica throughout the sampling period (Model 1 linear regression, p = 0.532), with highest biogenic silica measured in April 1998 (0.003 $\mu molL^{-1}$) and February 2006 (0.002 $\mu mol\ L^{-1}$). February samples showed the highest variation in measured biogenic silica, with highest monthly-averaged biogenic silica values measured in July samples (0.0010 $\mu mol\ L^{-1}$) followed by April and February samples (0.0007 and 0.0005 $\mu mol\ L^{-1}$ respectively) (Figure 68). Autumn sample averages were all < 0.0002 $\mu mol\ L^{-1}$. Despite these differences, there was no significant difference recorded in the median biogenic silica values between each month (Kruskal-Wallis one-way ANOVA, p = 0.549).

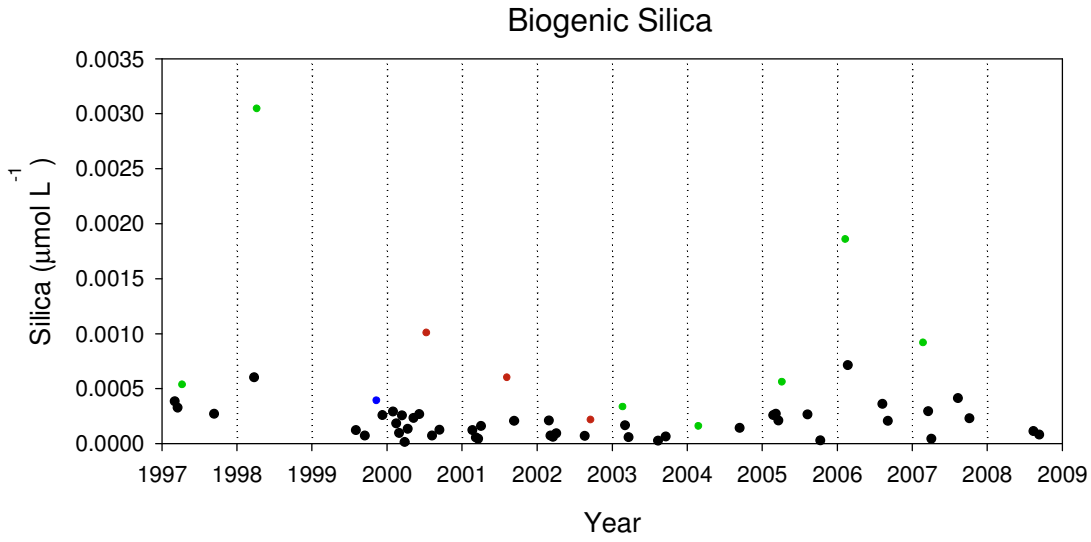


Figure 67 - Measured biogenic Silica concentrations for all spring and autumn samples throughout the sampling period. Green data points represent spring samples, red data points represent autumn samples, blue data points represent winter samples.

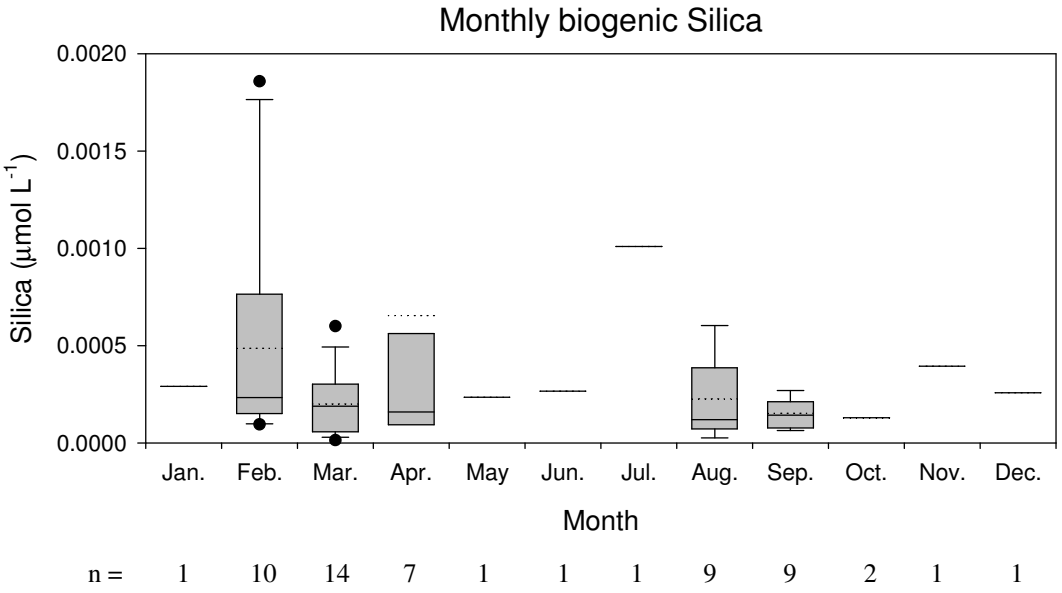


Figure 68 - Monthly box and whisker plot showing measured biogenic Silica concentrations. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each month.

When microplankton biogenic silica concentrations are compared to water column (150m depth-averaged) biogenic silica concentrations (Krause *et al.,* 2009) (Krause and Lomas, unpublished), microplankton silica concentrations are of an order of magnitude lower than water column concentrations (Figure 69). Microplankton biogenic silica concentrations were also calculated as a percentage value of the water column biogenic silica concentrations (Figure 70). The biogenic silica contribution of microplankton ranged from just 0.1 % in 2000, to maximums of 6.8 % in 1997 and 6.5 % in 2006, with an average contribution of 2.1 % throughout the time-series. Microplankton biogenic silica concentrations and water column biogenic silica concentrations did not show a correlation at a statistically significant level (correlation coefficient 0.27, $p = 0.07$).

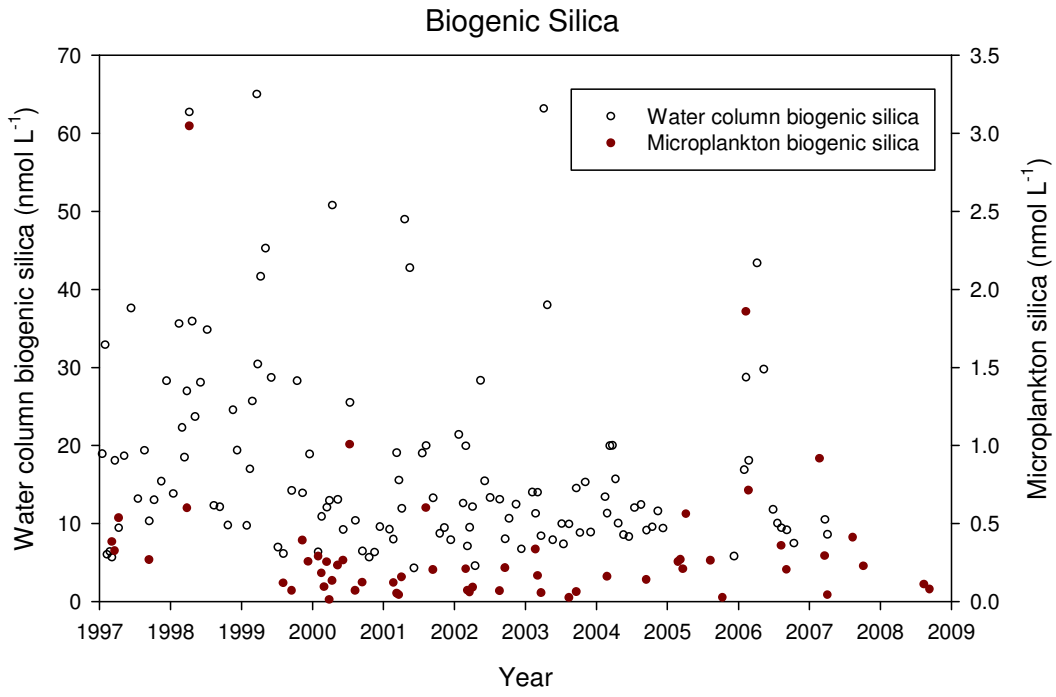


Figure 69 - Scatter plot showing water column biogenic silica concentrations (open circles), and microplankton biogenic silica concentrations (closed red circles). Water column data is shown as a single value averaged over the top 150m of the water column. Water column data provided by Jeff Krause (Krause *et al.,* 2009) (Krause and Lomas, unpublished).

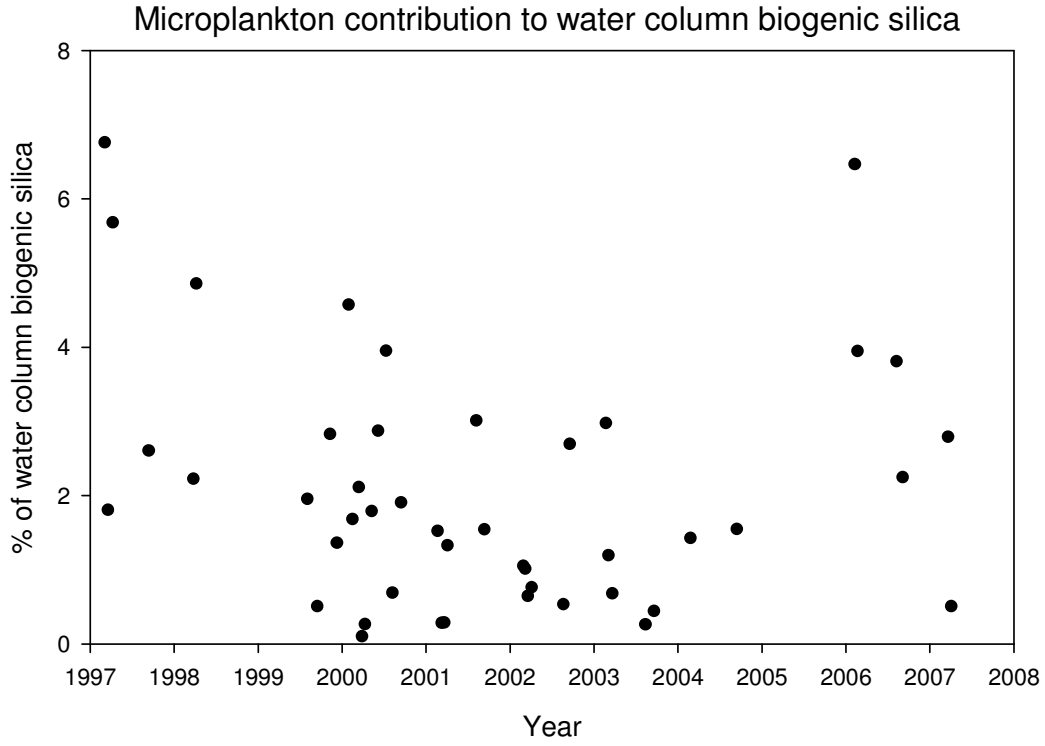


Figure 70 - Scatter plot showing microplankton biogenic silica concentrations as a percentage of water column biogenic silica (% contribution).

Patterns in diatom abundance have previously been described on monthly (Results I - 4.1), seasonal (Results I - 4.2) and inter-annual (Results IV - 7.1) time scales. From these results sections, the following points are summarized:

- Largest variation in diatom abundance in April and August samples (Figure 71 – Top plot of earlier Figure 23)
- Highest monthly averaged diatom abundance in August samples
- No significant differences in median abundances between months ($p = 0.51$)
- Significant difference between spring and autumn diatom abundances ($p = 0.028$), with higher autumn abundances
- Significant but weak long-term increase in abundance over the time-series ($p = 0.002$, $R^2 = 0.130$) driven by a time-series increase in autumn ($p = 0.023$, $R^2 = 0.225$) and summer ($p = 0.018$, $R^2 = 0.575$) diatom abundances.

A strong positive correlation between diatom abundance and measured biogenic silica was recorded (Spearman Rank correlation coefficient = 0.554, $p < 0.001$) (Figure 72).

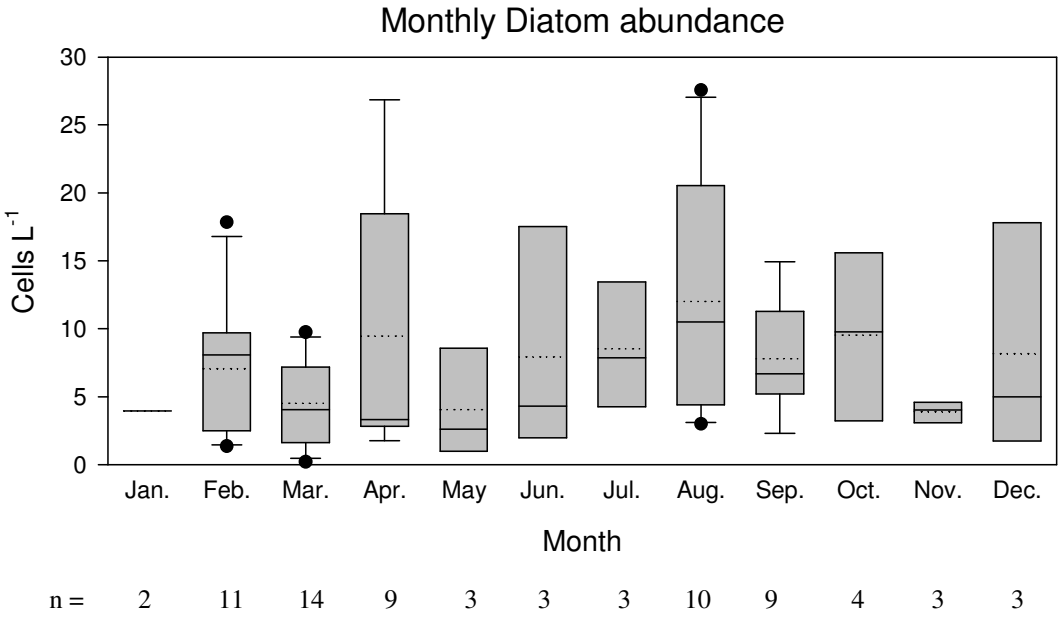


Figure 71 - Monthly box and whisker plot of diatom abundances. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each month.

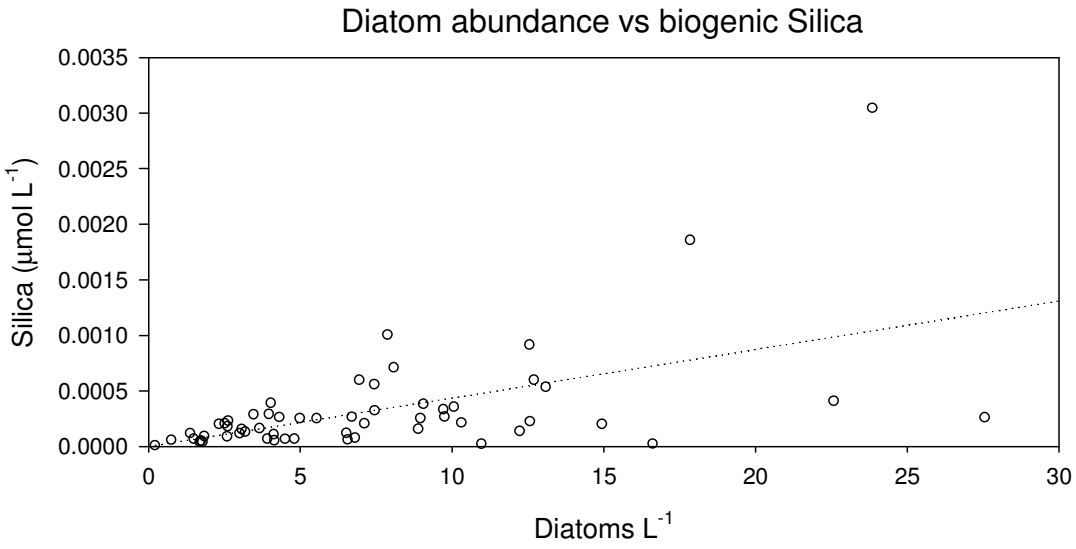


Figure 72 - Relationship between diatom abundances (x-axis) and measured biogenic Silica concentrations (y-axis). Dotted line represents a model 1 linear regression, $R^2 = 0.293$.

Diatom abundance and measured biogenic silica data were used to calculate an average silica per cell value for each sample analysed (Figure 73). In months where many samples were available for comparison, a wide variation in cell silica values is observed. Highest average cell silica values are seen in July (0.13 nmol $cell^{-1}$) and lowest cell silica values in October (0.01 nmol $cell^{-1}$). Averages for spring, summer and winter months are all greater than 0.047 nmol $cell^{-1}$, with autumn values all less than 0.026 nmol $cell^{-1}$. When tested with a Kruskal-Wallis one-way ANOVA, a significant difference in the median cell silica concentration was found between months ($p = 0.003$). This difference was driven by the cell silica values for July and October.

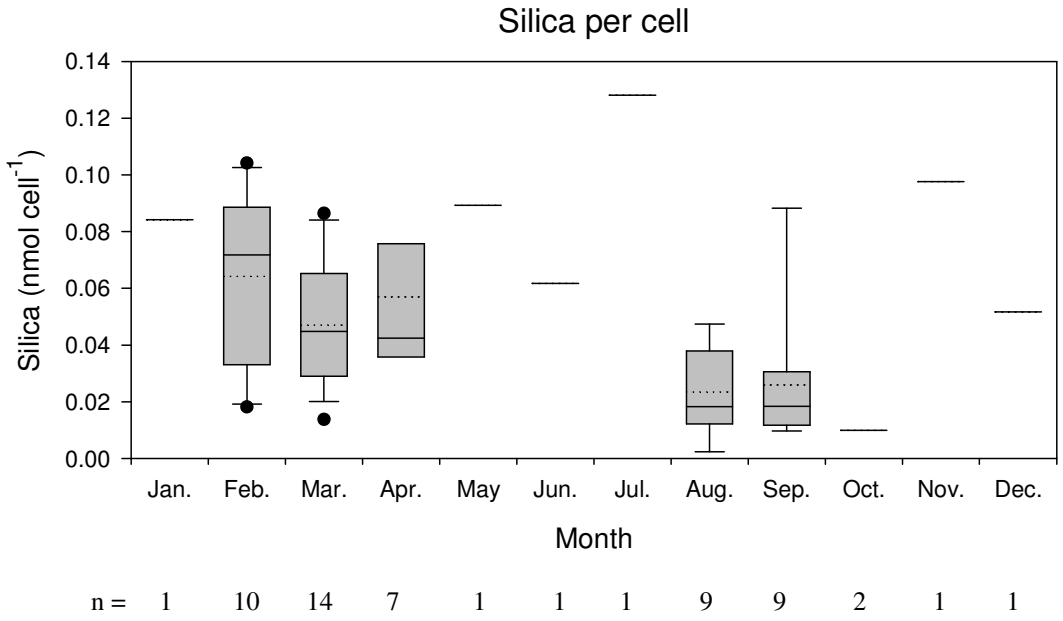


Figure 73 - Monthly box and whisker plot of average cell silica values per samples. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each month..

Cell diameter values were also measured for solitary centric diatoms in samples that were run through the FlowCAM (Figure 74). Largest centric diatoms were recorded in May samples (average diameter of 60.9 µm) with smallest average diameter cells in August (52.0 µm). The largest centric diatom average diameter was recorded in September 1999 as 74.0 µm, with the lowest sizes observed in August 2004 (43.3 µm). Centric diatom size showed a significant pattern over the time-series when a 3rd order polynomial regression was performed ($p < 0.001$, $R^2 = 0.444$) (Figure 75). Larger diameter centric diatoms are seen between 1996 and 2001, with the smallest cells observed between 2004 and 2008.

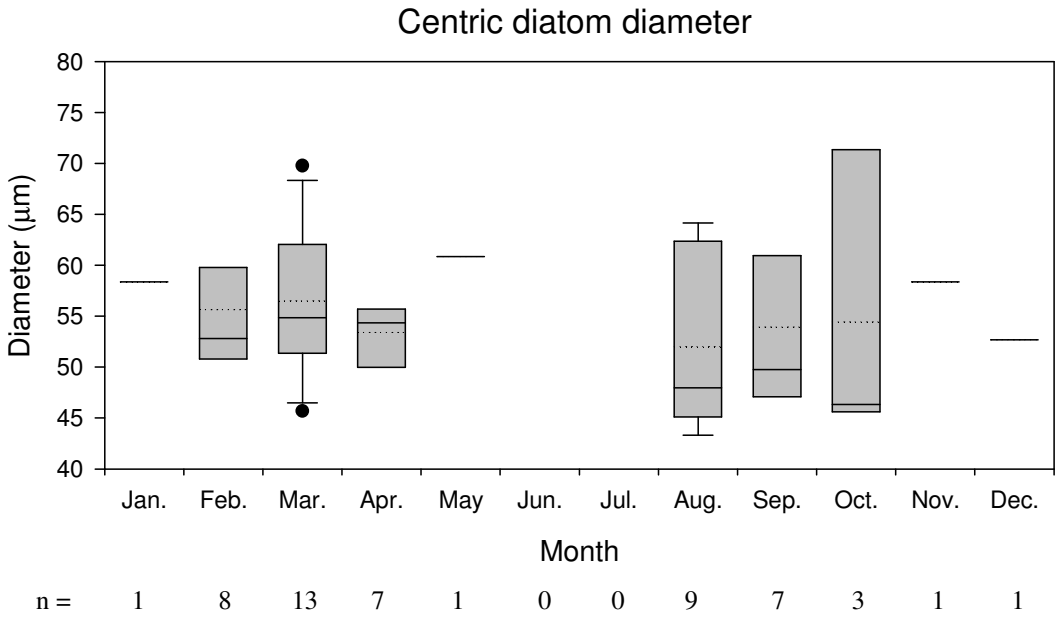


Figure 74 - Monthly box and whisker plot showing average centric diatom cell diameter measurements. Boxes represent 25th/75th percentiles; vertical bars represent 10th/90th percentiles. Solid horizontal lines represent median abundance, dotted lines represent mean abundance (circular data points show outliers). 'n' values show the number of samples analysed for each month..

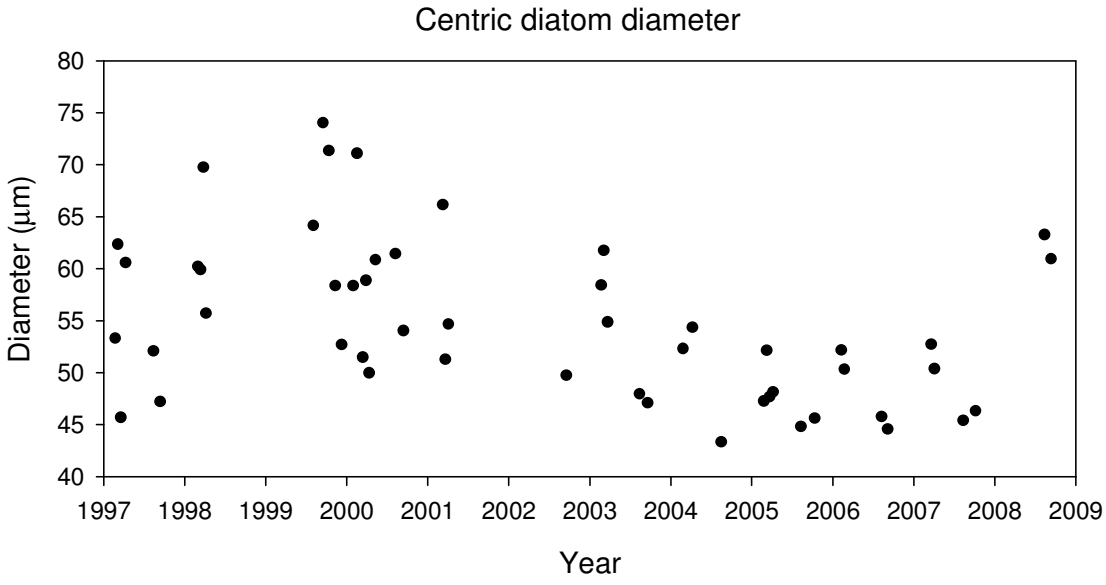


Figure 75 - Average centric diatom diameter for each sample plotted over the course of the sampling period (1997 – 2009).

When compared to the foraminifera and particulate calcium data, much less of a seasonal signal is seen in the biogenic silica and diatom data. High amounts of variability are seen in not only the total diatom abundance, but also in cell silica values and centric diatom sizes. Chain length and sizes of individual chain-forming diatoms were not recorded by the FlowCAM. The large variability described here means that although there is a significant correlation between diatom numbers and measured biogenic silica, significant patterns between abundances, sizes and cell silica (in terms of solitary centric vs. chain-forming) are difficult to identify.

8.3 Discussion

Within the biogeochemical data, a significant long term increase in total particulate calcium was recorded, with highest values mostly occurring in spring samples. This coincides with highest foraminifera abundances, with the relationship between foraminifera abundance and particulate calcium as a significant positive correlation. However, once cell calcium was calculated, there was found to be no significant difference between different months or different seasons, and no significant long-term increase or decrease. Autumn samples generally have lower abundances of forams with higher (and more variable) cell calcium and cell volume values. This suggestion of low volume, low calcium foraminifera being recorded in spring samples would suggest the presence of juvenile foraminifera, corresponding with the input of nutrients to the euphotic zone with continued winter/spring mixing of the water column. Previous studies have reported that individual foraminifera sampled in the upper 300m of the water column are generally found to be smaller, with thin walled, smooth tests (calcium carbonate skeletons), indicative of juvenile foraminifera (Bé and Ericson 1963; Michaels *et al.,* 1995). These thin, smooth cell walls are also ideal for harbouring photosynthetic symbionts, which are not always seen in the deeper dwelling more calcified individuals (Bé and Ericson 1963; Michaels *et al.,* 1995). Large, thick-shelled (and therefore heavily calcified) individuals are reported mainly from depths greater than 500m (Bé and Ericson 1963). Results from the analysis and life cycle observation of a North Atlantic species (*Globorotalia truncatulinoides*) suggest reproduction to occur in deeper water levels around late November, with an increase in juveniles ascending to the euphotic zone accounting for increased populations in January and February (Bé and Ericson 1963), a theory that would explain the higher abundances of foraminifera observed in February microplankton samples at BATS.

Variations in biogenic silica and diatom abundances are not so easily explained as foraminifera variability; although a significant correlation was reported between diatom abundance and measured biogenic silica, there was no significant increase or decrease in biogenic silica over the course of the time-series, and no

significant difference measured in biogenic silica between months. Cell silica values did show a significant difference between months, with high July values and low October values. Largest solitary centric diatoms were recorded in May, with the smallest average size in August samples. Over the course of the sampling period, there appears to be a large variation between larger diatom sizes and smaller sizes, with larger diatoms observed in the middle of the time-series, smaller diameter centric diatoms towards the end of the time-series. Biogenic silica measurements previously analysed in the Sargasso Sea show an increase in biogenic silica concentrations in the upper water column (top 160m) during the annual diatom bloom occurring each year between January and April (Brzezinski and Nelson 1995). As a lack of a clear spring bloom signal in the abundance of microplankton diatoms has been discussed above, it is therefore unsurprising that a lack of seasonal cycle is also observed in the biogenic silica of this group. It is again suggested that numbers of these microplankton diatoms are too low to show a significant seasonality when averaged out throughout the water column. Diatoms of this size are known to be present at the base of the euphotic zone (Goldman 1988; Goldman 1993); with a standard 150m net haul it is conceivable that localised high abundance patches of these large diatoms are being missed by the sampling procedure currently in use. As microplankton diatom biogenic silica makes up only a small proportion of the total water column biogenic silica, it is not surprising that no significant correlation is recorded between the two sets of biogenic silica data. This is the first study to report contribution of the microplankton size-fraction diatoms to water column biogenic silica, however the low percentage contribution indicates that smaller size fractions are responsible for a larger proportion of biogenic silica production in the upper water column at the BATS sampling site. Whilst it is observed that the large microplankton diatoms contribute little to the total pool of biogenic silica in the water column, it is still unclear as to how much of the remaining biogenic silica is attributed to differing sizes of smaller diatoms. It is suggested that the smaller size fraction diatoms at the BATS site are responsible for the majority of the measurable pool of biogenic silica, and not the larger microplankton diatoms.

9 Summary

This study has examined the variability of single-celled microplankton at the BATS site in the Sargasso Sea, both in terms of total abundance, and relative abundance (composition), on a variety of timescales. The aim was to identify patterns of temporal variability present in the abundance of seven different microplankton groups, and where possible, to elucidate the driving factors behind these patterns. This is the first study to specifically focus on the microplankton community at the Bermuda Atlantic Time-series Study site, and for many of the groups examined this is the first detailed study of their size fraction in the Sargasso Sea.

Of the seven named microplankton groups analysed, the radiolaria showed the most significant trends, being the only microplankton group to show a significant difference in abundance between months (low January samples and high August samples), with a strong positive correlation to monthly averaged water-column temperature. These high abundances noted with higher water temperatures are in agreement with earlier studies of sarcodine abundance in the Sargasso Sea (Swanberg and Caron 1991). Despite a lack of statistically significant differences between months, the silicoflagellates were observed to have their highest abundances in spring samples for nine out of the twelve years analysed. A spring increase in microplankton abundance was observed in all three twelve-month sampling periods analysed, although the magnitude of this increase does not indicate the presences of a typical "spring bloom" scenario as seen in other phytoplankton size groups (Steinberg *et al*., 2001; DuRand *et al*., 2001) and environments (Sverdrup 1953; Riley 1957). During one of these twelve-month sampling periods, a distinct shift is seen from a dinoflagellate-dominated microplankton sample in March, to a diatom-dominated sample in April, driven by a decrease in abundance of dinoflagellates and tintinnids rather than a dramatic increase in diatom abundances. A lack of significant increase or decrease in many of the microplankton groups on these shorter time-scales represents a microplankton community which, although changeable in group abundance, remains relatively stable in terms of relative abundance of the composition

(relative abundance of the different microplankton groups). Whilst changes on the monthly and seasonal timescales were identified, more of the microplankton groups showed significant increases in abundance over the longer inter-annual variability timescale. All groups except the acantharia and the silicoflagellates showed these significant increases in abundance over the entire sampling period (1997 – 2009). These increases are in accordance with more recent published reports of increasing chlorophyll a concentrations, primary production rates and carbon export (Lomas *et al.,* 2010), as well as increases in abundance and biomass of the larger zooplankton (Steinberg *et al.,* 2012). This highlights the importance of maintaining open-ocean time-series over a sustained period of time, as it allows these long-term, inter-annual observations to be made.

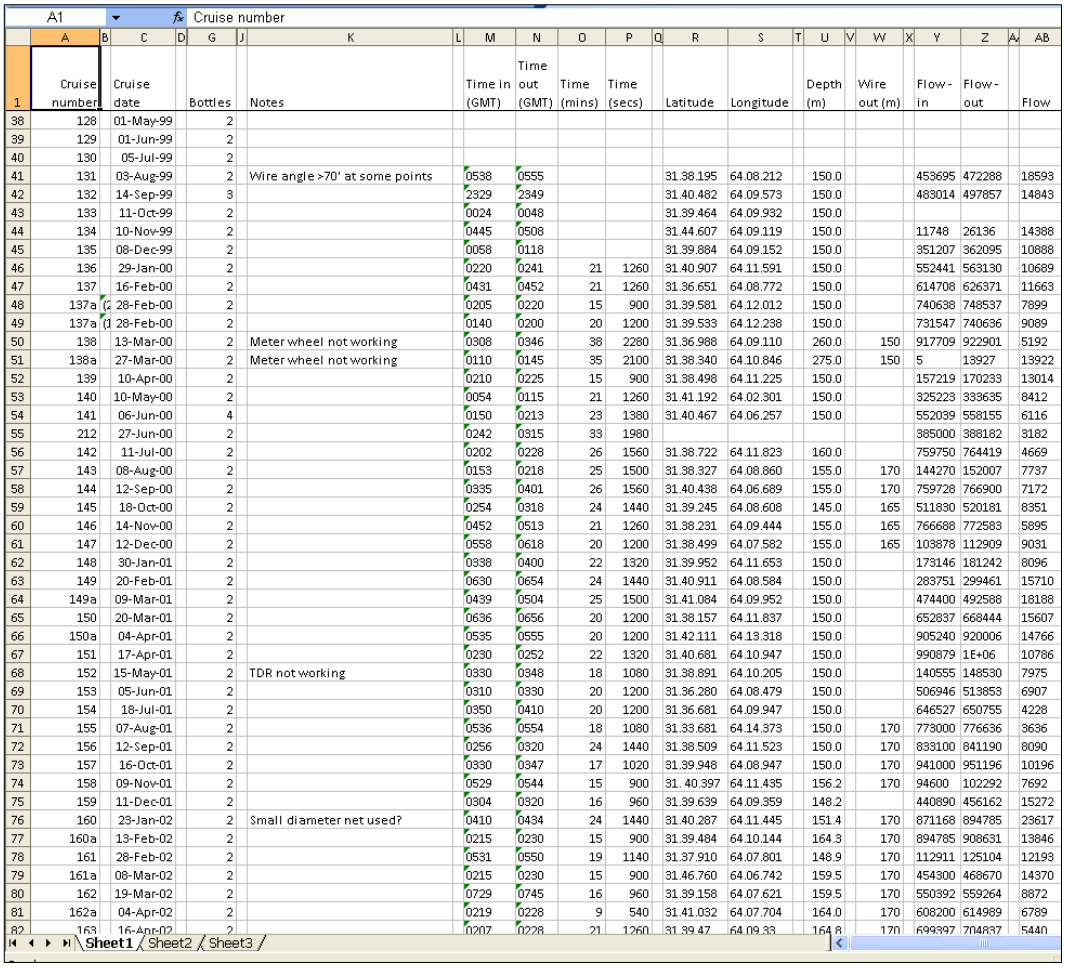
It is already suggested that eddies play an important role in the oligotrophic ocean gyres, as a mechanism of nutrient transport into the euphotic zone through convective mixing (McGillicuddy Jr and Robinson 1997), with a number of eddy features in the Sargasso Sea having been previously studied (see McGillicuddy Jr *et al*., 2007; Bibby *et al*., 2008; Ewart *et al*., 2008; Li and Hansell 2008), with various patterns (and high levels of variability) of production, chlorophyll and biomass recorded between (and within) different eddies. The presence of transient mesoscale features such as eddies was also discerned to have an effect on the microplankton communities, although the specifics of the interactions appear to vary greatly between individual eddy features, dependent on eddy age, eddy type and path of the eddy through the sampling area. As highlighted by McGillicuddy Jr *et al*., (1999), it is difficult to make directly observe the impact that an eddy feature has on a biological community due to the mismatch in physical and biological timescales. Both radiolaria and diatom abundances were positively correlated with sea level anomaly data, however further comparison between microplankton groups and eddy types and ages proved inconclusive.

Combining abundance and size data of the diatoms and foraminifera with biogeochemical data enabled a more detailed analysis of these two microplankton groups. Particulate calcium concentrations were highest in spring samples for 8 of the 12 years when samples were analysed, exceptions being 2002 and 2007. A significant correlation was recorded between particulate calcium concentrations

and foraminifera abundance over the course of the time series. A combination of high foraminifera abundances and high particulate calcium concentrations in February samples, combined with low foraminifera cell volume measurements indicate a high abundance of small volume, lightly calcified foraminifera cells. Patterns in the diatom abundance and biogenic silica concentrations were not so clearly defined as with the foraminifera and particulate calcium, although a strong positive correlation was recorded between diatom abundance and measured biogenic silica. Largest solitary centric diatoms were measured in May samples, averaging nearly 10µm more in diameter than diatoms from August samples. The large variability observed in cell silica values, centric diatom sizes and microplankton diatom abundance means that strong trends in the data are extremely difficult to identify. When compared to total water column biogenic silica, it was found that the microplankton diatoms contributed less than 8% of total biogenic silica concentrations measured, suggesting smaller size fraction diatoms are responsible for the majority of the water column biogenic silica measured.

10 Appendices

Appendix 10.1 – Screenshot of BATS microplankton tow spreadsheet



A1 Cruise number

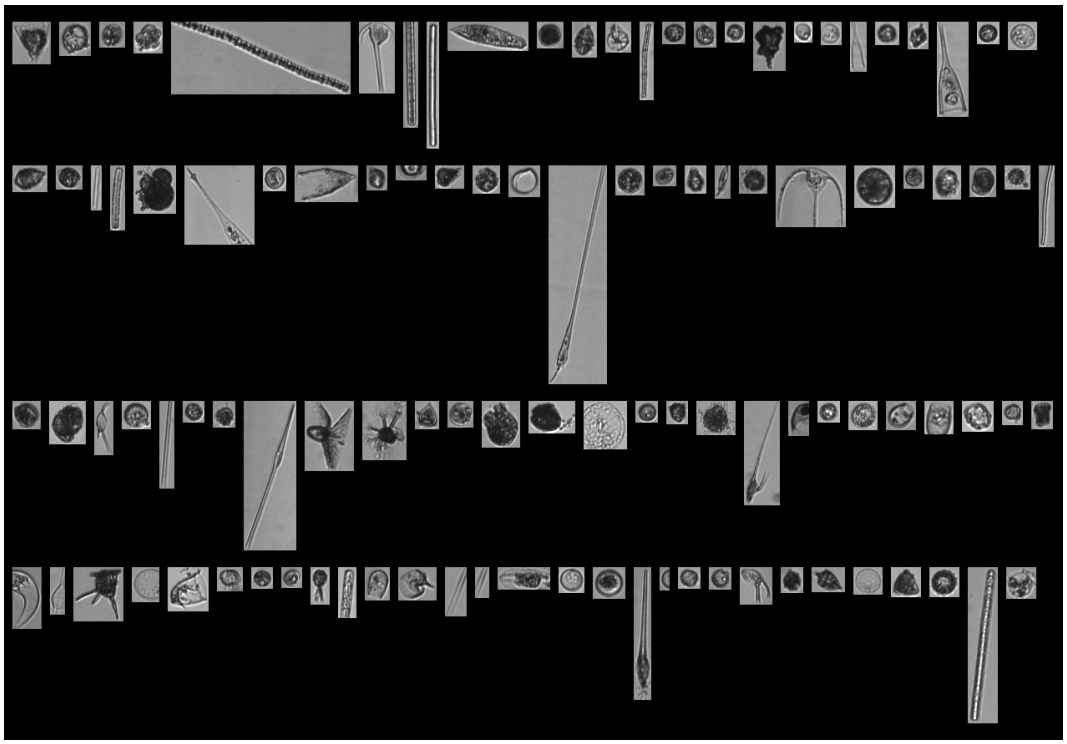
	A	C	G	K	M	N	O	P	R	S	U	W	Y	Z	AB
1	Cruise number	Cruise date	Bottles	Notes	Time in (GMT)	Time out (GMT)	Time (mins)	Time (secs)	Latitude	Longitude	Depth (m)	Wire out (m)	Flow - in	Flow - out	Flow
38	128	01-May-99	2												
39	129	01-Jun-99	2												
40	130	05-Jul-99	2												
41	131	03-Aug-99	2	Wire angle >70° at some points	0538	0555			31.38.195	64.08.212	150.0		453695	472288	18593
42	132	14-Sep-99	3		2329	2349			31.40.482	64.09.573	150.0		483014	497857	14843
43	133	11-Oct-99	2		0024	0048			31.39.464	64.09.932	150.0				
44	134	10-Nov-99	2		0445	0508			31.44.607	64.09.119	150.0		11748	26136	14388
45	135	08-Dec-99	2		0058	0118			31.39.884	64.09.152	150.0		351207	362095	10888
46	136	29-Jan-00	2		0220	0241	21	1260	31.40.907	64.11.591	150.0		552441	563130	10689
47	137	16-Feb-00	2		0431	0452	21	1260	31.36.651	64.08.772	150.0		614708	626371	11663
48	137a (2	28-Feb-00	2		0205	0220	15	900	31.39.581	64.12.012	150.0		740638	748537	7899
49	137a (1	28-Feb-00	2		0140	0200	20	1200	31.39.533	64.12.238	150.0		731547	740636	9089
50	138	13-Mar-00	2	Meter wheel not working	0308	0346	38	2280	31.36.988	64.09.110	260.0	150	917709	922901	5192
51	138a	27-Mar-00	2	Meter wheel not working	0110	0145	35	2100	31.38.340	64.10.846	275.0	150	5	13927	13922
52	139	10-Apr-00	2		0210	0225	15	900	31.38.498	64.11.225	150.0		157219	170233	13014
53	140	10-May-00	2		0054	0115	21	1260	31.41.192	64.02.301	150.0		325223	333635	8412
54	141	06-Jun-00	4		0150	0213	23	1380	31.40.467	64.06.257	150.0		552039	558155	6116
55	212	27-Jun-00	2		0242	0315	33	1980					385000	388182	3182
56	142	11-Jul-00	2		0202	0228	26	1560	31.38.722	64.11.823	160.0		759750	764419	4669
57	143	08-Aug-00	2		0153	0218	25	1500	31.38.327	64.08.860	155.0	170	144270	152007	7737
58	144	12-Sep-00	2		0335	0401	26	1560	31.40.438	64.06.689	155.0	170	759728	766900	7172
59	145	18-Oct-00	2		0254	0318	24	1440	31.39.245	64.08.608	145.0	165	511830	520181	8351
60	146	14-Nov-00	2		0452	0513	21	1260	31.38.231	64.09.444	155.0	165	766688	772583	5895
61	147	12-Dec-00	2		0558	0618	20	1200	31.38.499	64.07.582	155.0	165	103878	112909	9031
62	148	30-Jan-01	2		0338	0400	22	1320	31.39.952	64.11.653	150.0		173146	181242	8096
63	149	20-Feb-01	2		0630	0654	24	1440	31.40.911	64.08.584	150.0		283751	299461	15710
64	149a	09-Mar-01	2		0439	0504	25	1500	31.41.084	64.09.952	150.0		474400	492588	18188
65	150	20-Mar-01	2		0636	0656	20	1200	31.38.157	64.11.837	150.0		652837	668444	15607
66	150a	04-Apr-01	2		0535	0555	20	1200	31.42.111	64.13.318	150.0		905240	920006	14766
67	151	17-Apr-01	2		0230	0252	22	1320	31.40.681	64.10.947	150.0		990879	1E+06	10786
68	152	15-May-01	2	TDR not working	0330	0348	18	1080	31.38.891	64.10.205	150.0		140555	148530	7975
69	153	05-Jun-01	2		0310	0330	20	1200	31.36.280	64.08.479	150.0		506946	513853	6907
70	154	18-Jul-01	2		0350	0410	20	1200	31.36.681	64.09.947	150.0		646527	650755	4228
71	155	07-Aug-01	2		0536	0554	18	1080	31.33.681	64.14.373	150.0	170	773000	776636	3636
72	156	12-Sep-01	2		0256	0320	24	1440	31.38.509	64.11.523	150.0	170	833100	841190	8090
73	157	16-Oct-01	2		0330	0347	17	1020	31.39.948	64.08.947	150.0	170	941000	951196	10196
74	158	09-Nov-01	2		0529	0544	15	900	31. 40.397	64.11.435	156.2	170	94600	102292	7692
75	159	11-Dec-01	2		0304	0320	16	960	31.39.639	64.09.359	148.2		440890	456162	15272
76	160	23-Jan-02	2	Small diameter net used?	0410	0434	24	1440	31.40.287	64.11.445	151.4	170	871168	894785	23617
77	160a	13-Feb-02	2		0215	0230	15	900	31.39.484	64.10.144	164.3	170	894785	908631	13846
78	161	28-Feb-02	2		0531	0550	19	1140	31.37.910	64.07.801	148.9	170	112911	125104	12193
79	161a	08-Mar-02	2		0215	0230	15	900	31.46.760	64.06.742	159.5	170	454300	468670	14370
80	162	19-Mar-02	2		0729	0745	16	960	31.39.158	64.07.621	159.5	170	550392	559264	8872
81	162a	04-Apr-02	2		0219	0228	9	540	31.41.032	64.07.704	164.0	170	608200	614989	6789
82	163	16-Apr-02	2		0207	0228	21	1260	31.39.47	64.09.33	164.8	170	699397	704837	5440

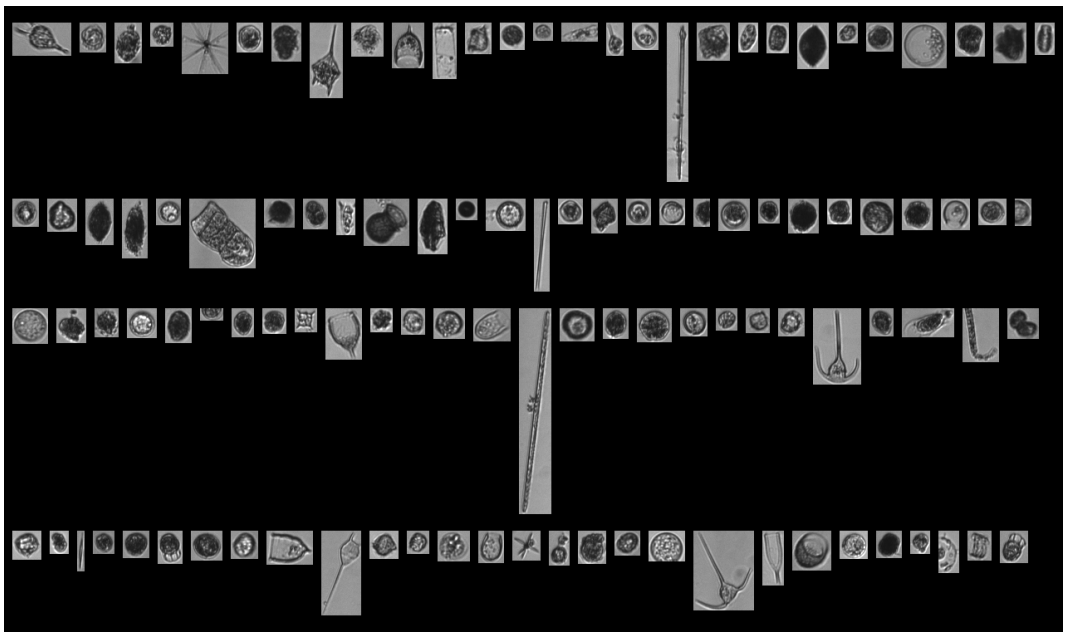
Sheet1 / Sheet2 / Sheet3

Appendix 10.2 - Table showing list of genera and species identified to date in BATS microplankton samples

Diatoms	**Dinoflagellates**	**Tintinnids**	**Foraminifera**	**Silicoflagellates**
Asterolampra marylandica *Asterolampra* spp. *Asteromphalus flabellatus* *Chaetoceros* spp. *Ditylum brightwellii* *Eucampia zodiacus* *Guinardia striata* *Leptocylindricus* spp. Melosiraceae family *Paralia* spp. *Planktionella sol* *Pseudoguinardia recta* *Thalassiosira* spp.	*Acanthogonyaulax spinifera* *Amphisolenia bifurcate* *Ceratium gravidum* *Ceratium paradoxides* *Ceratium playcorne* *Ceratium* spp. *Ceratocorys horrida* *Dinophysis rapa* *Ornithocercus quadratus* *Ornithocercus splendidus* *Ornithocercus steinii* *Oxyphysis oxytoxoides* *Palacroma cuneus* *Phalacroma mitra* *Phalacroma rotundatum* *Podolampas elegans* *Podolampas palmipes* *Protoperidinium oblongum* *Protoperidinium pellucidum* *Protoperidinium steinii* *Pyrocystis lunula* *Pyrocystis noctiluca* *Pyrophacus steinii*	*Amphorides quadrilineata* var. *brasiliensis* *Amplectella collaria* *Amplectella monocollaria* *Codonella amphorella* *Codonella apicata* *Codonella elongata* *Codonella galea* *Codonellopsis orthoceras* *Cytarocylis brandtii* *Dictyocysta elegans* var. *lepida* *Epicancella nervosa* *Epiplocylis lata* *Epiplocylis undella* *Eutintinnus birictus* *Rhabdonella elegans* *Rhabdonellopsis apophysata* *Salpingella attenuata* *Salpingella glockentoegerii* *Undella claparedei* *Undella columbiana* *Undella globosa* *Undella hyalina* *Xysteonellopsis* spp. *Xystonellopsis gaussi* *Xystonellopsis paradoxa*	*Globigerinella* spp. *Globigerinella siphonifera* *Hastigerina pelagic*	*Dictyocha fibula*

Appendix 10.3 – Sample of FlowCAM-generated images for two microplankton samples.





Appendix 10.4 – Spearman Rank correlation coefficients between monthly averaged microplankton abundance data and monthly averaged (top 150m) physical and chemical data

	Correlation coefficient	p – value	Significant?
Temperature			
Diatoms	0.322	0.295	
Dinoflagellates	0.238	0.442	
Tintinnids	-0.196	0.527	
Radiolaria	0.601	0.036	Yes
Foraminifera	-0.301	0.329	
Silicoflagellates	-0.245	0.429	
Acantharia	0.217	0.484	
Total microplankton	0.133	0.667	
Salinity			
Diatoms	-0.210	0.498	
Dinoflagellates	-0.098	0.749	
Tintinnids	0.042	0.886	
Radiolaria	-0.517	0.080	
Foraminifera	0.399	0.189	
Silicoflagellates	0.077	0.800	
Acantharia	-0.189	0.542	
Total microplankton	0.007	0.940	
Nitrate + Nitrite			
Diatoms	-0.014	0.956	
Dinoflagellates	0.077	0.800	
Tintinnids	-0.224	0.470	
Radiolaria	0.014	0.956	
Foraminifera	-0.720	0.007	Yes
Silicoflagellates	-0.566	0.051	
Acantharia	-0.273	0.377	
Total microplankton	-0.119	0.699	
Nitrite			
Diatoms	-0.329	0.284	
Dinoflagellates	-0.483	0.105	
Tintinnids	-0.098	0.749	
Radiolaria	-0.601	0.036	Yes
Foraminifera	0.154	0.619	
Silicoflagellates	0.301	0.329	
Acantharia	-0.266	0.389	
Total microplankton	-0.420	0.160	

	Correlation coefficient	p - value	Significant?
Phosphate			
Diatoms	0.035	0.904	
Dinoflagellates	0.084	0.783	
Tintinnids	-0.049	0.869	
Radiolaria	0.308	0.317	
Foraminifera	-0.441	0.143	
Silicoflagellates	-0.580	0.045	Yes
Acantharia	-0.084	0.783	
Total microplankton	-0.042	0.886	
Silicate			
Diatoms	-0.203	0.513	
Dinoflagellates	-0.420	0.165	
Tintinnids	0.126	0.683	
Radiolaria	-0.587	0.042	Yes
Foraminifera	-0.238	0.442	
Silicoflagellates	0.552	0.058	
Acantharia	-0.105	0.733	
Total microplankton	-0.301	0.329	
POC			
Diatoms	0.070	0.817	
Dinoflagellates	0.406	0.181	
Tintinnids	0.280	0.364	
Radiolaria	-0.049	0.869	
Foraminifera	0.343	0.263	
Silicoflagellates	0.007	0.974	
Acantharia	0.035	0.904	
Total microplankton	0.378	0.215	

Appendix 10.5 – Results of Mann-Whitney U tests between pairs of seasons for each microplankton group

Season pair	p – value	Significant?
Diatoms		
Spring vs. Summer	0.870	
Spring vs. Autumn	0.028	Yes
Spring vs. Winter	0.987	
Summer vs. Autumn	0.154	
Summer vs. Winter	0.962	
Autumn vs. Winter	0.061	
Dinoflagellates		
Spring vs. Summer	0.560	
Spring vs. Autumn	0.402	
Spring vs. Winter	0.269	
Summer vs. Autumn	0.900	
Summer vs. Winter	0.136	
Autumn vs. Winter	0.067	
Tintinnids		
Spring vs. Summer	0.941	
Spring vs. Autumn	0.631	
Spring vs. Winter	0.298	
Summer vs. Autumn	0.867	
Summer vs. Winter	0.312	
Autumn vs. Winter	0.183	
Radiolaria		
Spring vs. Summer	0.988	
Spring vs. Autumn	<0.001	Yes
Spring vs. Winter	0.235	
Summer vs. Autumn	0.026	Yes
Summer vs. Winter	0.469	
Autumn vs. Winter	0.099	
Foraminifera		
Spring vs. Summer	0.060	
Spring vs. Autumn	0.226	
Spring vs. Winter	0.173	
Summer vs. Autumn	0.180	
Summer vs. Winter	0.596	
Autumn vs. Winter	0.573	

Season pair	p - value	Significant?
Silicoflagellates		
Spring vs. Summer	0.109	
Spring vs. Autumn	0.800	
Spring vs. Winter	0.426	
Summer vs. Autumn	0.087	
Summer vs. Winter	0.477	
Autumn vs. Winter	0.618	
Acantharia		
Spring vs. Summer	0.244	
Spring vs. Autumn	0.111	
Spring vs. Winter	0.674	
Summer vs. Autumn	0.039	Yes
Summer vs. Winter	0.169	
Autumn vs. Winter	0.378	
Total Microplankton		
Spring vs. Summer	0.622	
Spring vs. Autumn	0.384	
Spring vs. Winter	0.414	
Summer vs. Autumn	0.900	
Summer vs. Winter	0.163	
Autumn vs. Winter	0.119	

Appendix 10.6 – Results of Kruskal-Wallis One-way ANOVA between pairs of years for total microplankton abundance. Q values are shown (top value) in addition to significance; if p value < 0.05 (bottom). Red text highlights statistically significant results (as displayed in Table 8).

	1998	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**
1997	0.094 p>0.05	2.055 p>0.05	2.036 p>0.05	2.844 p>0.05	2.047 p>0.05	0.814 p>0.05	0.606 p>0.05	0.955 p>0.05	0.674 p>0.05	0.579 p>0.05	1.275 p>0.05
1998		1.584 p>0.05	1.441 p>0.05	2.082 p>0.05	1.441 p>0.05	0.556 p>0.05	0.579 p>0.05	0.834 p>0.05	0.644 p>0.05	0.367 p>0.05	1.023 p>0.05
1999			0.452 p>0.05	0.426 p>0.05	0.470 p>0.05	1.352 p>0.05	2.857 p>0.05	3.411 p<0.05	2.729 p>0.05	1.587 p>0.05	0.403 p>0.05
2000				1.157 p>0.05	0.017 p>0.05	1.191 p>0.05	3.114 p>0.05	4.014 p<0.05	2.853 p>0.05	1.484 p>0.05	0.110 p>0.05
2001					1.195 p>0.05	2.084 p>0.05	3.985 p<0.05	4.949 p<0.05	3.637 p<0.05	2.368 p>0.05	1.195 p>0.05
2002						1.194 p>0.05	3.146 p>0.05	4.080 p<0.05	2.873 p>0.05	1.491 p>0.05	0.101 p>0.05
2003							1.549 p>0.05	2.029 p>0.05	1.525 p>0.05	0.250 p>0.05	0.667 p>0.05
2004								0.336 p>0.05	0.132 p>0.05	1.288 p>0.05	1.832 p>0.05
2005									0.149 p>0.05	1.740 p>0.05	2.155 p>0.05
2006										1.289 p>0.05	1.826 p>0.05
2007											0.856 p>0.05

Appendix 10.7 – Spearman Rank correlation coefficients between long-term microplankton abundance and 150m averaged physical and chemical data

	Correlation coefficient	p – value	Significant?
Temperature (n = 72)			
Diatoms	0.253	0.032	Yes
Dinoflagellates	0.094	0.430	
Tintinnids	0.006	0.962	
Radiolaria	0.405	<0.001	Yes
Foraminifera	-0.223	0.060	
Silicoflagellates	0.002	0.985	
Acantharia	0.158	0.183	
Total microplankton	0.111	0.354	
Salinity (n = 72)			
Diatoms	0.143	0.228	
Dinoflagellates	0.277	0.019	Yes
Tintinnids	0.351	0.003	Yes
Radiolaria	0.010	0.936	
Foraminifera	0.227	0.055	
Silicoflagellates	0.244	0.039	Yes
Acantharia	0.038	0.751	
Total microplankton	0.279	0.018	Yes
Nitrate + Nitrite (n = 70)			
Diatoms	0.038	0.756	
Dinoflagellates	0.070	0.565	
Tintinnids	-0.058	0.633	
Radiolaria	-0.077	0.524	
Foraminifera	0.087	0.472	
Silicoflagellates	-0.083	0.496	
Acantharia	-0.001	0.991	
Total microplankton	0.026	0.828	
Nitrite (n = 69)			
Diatoms	0.027	0.823	
Dinoflagellates	0.138	0.257	
Tintinnids	0.149	0.221	
Radiolaria	-0.088	0.471	
Foraminifera	0.318	0.008	Yes
Silicoflagellates	0.076	0.533	
Acantharia	0.004	0.974	
Total microplankton	0.108	0.375	

Phosphate (n = 70)			
Diatoms	-0.197	0.102	
Dinoflagellates	-0.111	0.361	
Tintinnids	-0.206	0.087	
Radiolaria	-0.158	0.190	
Foraminifera	-0.065	0.594	
Silicoflagellates	-0.345	0.004	Yes
Acantharia	-0.135	0.264	
Total microplankton	-0.159	0.187	
Silicate (n = 69)			
Diatoms	0.209	0.085	
Dinoflagellates	0.165	0.174	
Tintinnids	0.162	0.183	
Radiolaria	0.036	0.770	
Foraminifera	0.109	0.370	
Silicoflagellates	0.153	0.207	
Acantharia	-0.032	0.796	
Total microplankton	0.154	0.207	
Peridinin (n = 69)			
Dinoflagellates	0.124	0.311	
Fucoxanthin (n = 69)			
Diatoms	0.081	0.506	
Chlorophyll a (n = 69)			
Diatoms	-0.117	0.338	
Dinoflagellates	0.021	0.865	
Silicoflagellates	0.105	0.390	

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