Developing and testing an index of change in microplankton community structure in temperate shelf seas

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$\Theta\alpha\lambda\alpha\tau\tau\alpha, \vartheta\alpha\lambda\alpha\tau\tau\alpha!!$

(greek: The sea, the sea!!)

Xenophons Anabasis, 4, 7

I dedicate this work to my friend Mareike who would have been happy to see that I did turn into a marine scientist in the end.

Abstract

Assessment of eutrophication of coastal waters has traditionally relied on bulk indicators of ecosystem state (e.g. nutrients and phytoplankton biomass as chlorophyll) and changes in phytoplankton floristic composition such as the occurrence of nuisance and harmful species. Information on these variables does not allow adequate insight into the effects of anthropogenic nutrient enrichment on ecosystem "health": i.e. the structure and functioning of the biological community. Environmental policies like the Marine Strategy Framework Directive (MSFD) require an ecosystem approach to marine management, suggesting the need for a holistic approach to assessing environmental status. Autotrophic species of microplankton are primary producers and form the base of the pelagic food web. Microheterotrophs are their immediate consumers, and this suggests that changes in microplankton community structure may be a useful indicator of pelagic ecosystem health.

The aim of this study was to develop and test an indicator to detect change in microplankton community structure in the context of eutrophication. The theoretic approach of an existing phytoplankton community index (PCI) was used to develop a microplankton community index (MCI). The theory involved the use of "lifeforms" (functional groups) and system state space theory. The approach was to select lifeforms that provided information on eutrophication, biodiversity and energy flow. These lifeforms included diatoms, dinoflagellates, micro-flagellates, and ciliates. Pairs of lifeforms were used as state variables to describe the state of the ecosystem. For each pair of lifeforms, data on abundance or carbon biomass were mapped into state space. The resulting "cloud" of points incorporated the inherent variability of microplankton populations. The index calculated as the difference between "clouds", can be used to determine whether differences occur between different sites (with different degrees of pressure) or at the same site over time (response to pressure at a single site). Three moored instrument sites were selected to develop and test the MCI. High temporal resolution sampling of physical, chemical, and microplankton components was carried out for two years (February 2008 - December 2009) in the western Irish Sea (WIS). For the mooring sites in Liverpool Bay (LBay) in the eastern Irish Sea and the West Gabbard (WGabb) in the southern North Sea data of those components were provided for the same frequency and period.

Microplankton cell abundance and carbon biomass showed that the expected seasonal cycle was coupled to hydrodynamic conditions at each site with the sub-surface light climate considered to be the main factor that controlled the start and duration of the production season at all three sites. At WIS, diatoms dominated the spring bloom and autumn period. Succession from diatoms to dinoflagellates was associated with increased stratification and micro-flagellates were abundant but without an obvious seasonal pattern. Diatoms dominated the microplankton throughout the year

at LBay and WGabb due to high nutrient concentrations and intermittently stratifying conditions. The influence of nutrient enrichment on microplankton community was investigated at the LBay ($\approx 30\mu$M winter DIN) and WGabb ($\approx 15\mu$M winter DIN) sites by using five pairs of lifeform state space plots (diatoms/dinoflagellates, autotrophs/heterotrophs, autotrophs /mixotrophs, mixotrophs/heterotrophs, and small/large sized microplankton). A clear increase in the autotroph biomass at LBay station in the autotrophs/mixotrophs comparison was observed and the MCI value of the small/large sized microplankton comparison suggested a difference between the communities at the two sites with higher biomass of the large sized lifeform at LBay. Comparisons with the heterotrophic lifeform were difficult, because few data points were available.

By including additional microplankton lifeforms the MCI extended the PCI approach and can be used to provide a more complete assessment of change in microplankton community structure. Further development and assessment is required such as what represents the optimum size of datasets for reliable application of the index and the distinction of the nutritional mode in long-term preserved microplankton samples.

A key element of the MCI application is the comparison to a reference condition. According to the MSFD such conditions should be representative of good environmental status (GES). On the basis of current understanding of microplankton ecology (biogeography, seasonal dynamics and succession) the results from this study suggest that the microplankton community at station WIS represents GES and this station is therefore proposed as a reference site for seasonally stratifying temperate shelf seas.

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Chapter 1

General Introduction

1.1 Background

The marine environment provides many resources for humans with its highly complex, diverse habitats and biodiversity. Fisheries, transport, nutrient assimilation, aquaculture, energy, and tourism are the main goods and services that the marine ecosystem provides. According to Lotze (2007) the biological resources were initially viewed limitless, but examples of decline and loss in fishery and the decrease of water quality in many coastal regions due to anthropogenic nutrient enrichment proved this view wrong. The world oceans, especially the coastal regions are facing increasing pressures from climate change and human activity such as eutrophication, pollution, commercial fishing, and alteration of coastlines. In response to all these pressures and with increasing awareness and understanding on how anthropogenic influence can degrade the marine environment, scientists and policy-makers worldwide have developed strategies to protect, conserve and re-store the world's seas and oceans. The basic legal framework is the United Nations Convention on Law of the Sea (UNCLOS, 1982) that governs the uses of the oceans. Many initiatives have been developed at international, national and regional levels since then (Parsons, 2005). Conventions such as the Oslo Paris convention for the protection of the marine environment of the North East Atlantic (OSPAR, 1992), the convention on Biological Diversity (1993) and the protection of the Underwater Cultural Heritage (UNESCO, 2001) are some of the conventions set out to help the sustainable human use of the marine environment. The Kyoto protocol adopted in 1997 and set into force in 2005 was a milestone in the evolution of directives focusing on climate change worldwide. It was the first multi-step attempt to approach the climate problem as a global issue linking across sectors and looking at six different emission gases rather than just one. Two key points were realised by scientists and policy makers in the last two decades while assessing the environmental status of ecosys-

tems. First, one pressure alone (for example anthropogenic nutrient enrichment) cannot define an undesirable disturbance to an ecosystem and second, detecting or diagnosing an undesirable disturbance requires a multi- step method providing linkages between stages (e.g. increased nutrient - increased organic matter) in the assessment of ecosystems. This increased awareness globally has led to the attention of the "ecosystem approach". It was widely recognised that the assessment of the environmental effects of human pressures required a holistic approach rather than a sector-by-sector basis. Scientists also realised that this assessment should be about structure and function of ecosystems that is, ecosystem health, which cannot be defined by simple bulk indicators alone.

The OSPAR convention launched a similar approach to the Kyoto protocol in assessing the marine ecosystem to that, implementing a suite of five thematic strategies to address the main threats that it has identified within its competence (the Biodiversity and Ecosystem Strategy, the Eutrophication Strategy, the Harzardous Substances Strategy, the Offshore Industry Strategy and the Radioactive Substances Strategy), together with a Strategy for the Joint Assessment and Monitoring Programme.

1.2 Eutrophication

One of the main human pressures that coastal marine ecosystems are facing is eutrophication which is seen as a major threat to the functioning of coastal ecosystems (Edwards, 2005) and a worldwide problem of increasing severity (McIntyre, 1995). Generally, under conditions in which microplankton growth is constrained by the availability of nutrients rather than light, nutrient input is assumed to result in the rapid growth of opportunistic, fast growing primary producers (r-selected species Margalef, 1963), elevated chlorophyll levels and therefore, the accumulation of extra biomass. Some increased production might be viewed as good because it means that more fish would be available for humans to consume. However, excessive addition of nutrients (mainly nitrogen and phosphorus) to coastal waters is expected to have an impact on the health of ecosystems. It is not so much the size of the input, but rather the uncoupling between production and use that can lead to problems associated with eutrophication (Tett *et al.* 2007). The rapid increase in biomass can result in the spread of opportunistic seaweeds, loss of seagrass meadows, increased occurrence of Toxin Producing Algae (TPA) and Harmful Algal Blooms (HABs), deep water de-oxygenation, benthic mortalities, and potential harm to fisheries and sustainable human use. In fact areas of the coastal North Sea are commonly considered to be nutrient enriched (Hickel *et al.*, 1993). Studies at several coastal areas have related increased phytoplankton biomass and production to anthropogenic enhancement of

nutrient concentrations (Allen *et al.*, 1998; Gowen *et al.*, 2000; Cadée & Hegeman, 2002). The growth of nuisance micro- and macro algae, water discolouration and generation of foaming (for example associated with the colonial stage of the micro-flagellate *Phaeocystis* sp. (Lancelot *et al.*, 1987)), increased production of organic matter that may rise to extra biochemical oxygen demand and hence increased removal of oxygen, leading to the potential risk of local anoxia, especially in enclosed waters such as sea-lochs (Tett *et al.*, 1986) and the Baltic Sea (Larsson *et al.*, 1985) and some parts of the southern North Sea like the Dogger Bank (Greenwood *et al.*, 2010) have been reported. The loss of submerged vegetation due to shading and changes in the benthic animal community structure may also be consequences of oxygen depletion or the presence of toxic microplankters (Tett, 1987b; Smayda & Reynolds, 2001; Bricker *et al.*, 2004) which can also lead to occasional or even frequent fish kills. Other consequences include shifts in species composition and community structure that are not natural, e.g. from diatoms to flagellates (Officer & Ryther, 1980; Gifford & Caron, 2000) and may in turn change ecosystem food web and nutrient cycling dynamics. All these events are seen as an undesirable disturbance (UD) to the balance of organisms (Lancelot *et al.*, 2006; Tett *et al.*, 2007; Gowen *et al.*, 2008 and references cited therein). The severe consequences are ecological and economical and affect not only coastal marine resources but other marine life and eventually human health (Nixon, 1995). Therefore, many conventions and directives were developed to identify and deal with the problem of anthropogenic eutrophication and its consequences. For EU transnational waters the EU Urban Waste Water Treatment Directive (UWWTD, 91/676/EC) the Nitrate Directive (ND, 92/43/EC), OSPAR's strategy to combat eutrophication (OSPAR, 2003), and the Marine Strategy Framework Directive (EC/56/2008) are important. To diagnose marine eutrophication as it is defined by EU Directives and OSPAR an understanding of undesirable disturbance to the balance of organisms is needed. Tett *et al.* (2007) defined undesirable disturbance as *"a perturbation of a marine ecosystem that appreciably degrades the health or threatens the sustainable human use of that ecosystem"*. When an undesirable disturbance correlates with nutrient enrichment it can result in eutrophication. The definition of eutrophication is similar for the EU Directives and OSPAR and the OSPAR definition is thus given representatively: *"Eutrophication means the enrichment of water by nutrients causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and the quality of the water concerned ..."* . But in it's origin eutrophication has a completely different meaning. The word "eutrophe" was associated with the healthy and nourished flora of German bogs invented by the German limnologist Weber in 1907 (Hutchinson, 1973) who referred to the Greek word 'eutrophos' = well-nourished. Views have changed since then and Parma (1980) formed the view that was widely held during the 1990s con-

cluding that eutrophication was an enrichment process which was a nuisance with respect to water quality management. Under the UWWTD a water body is labelled as *sensitive* when it is identified as likely to become eutrophic or when it is identified as suffering from eutrophication. The Nitrate Directive defines it as *nitrate-polluted* and the OSPAR convention calls it *a problem area*. Once consequences of eutrophication are identified, management actions are stringent treatment of urban waste water, reduction in the use of nitrate fertilizers on land, and measures to reduce or to eliminate the anthropogenic causes of eutrophication which is an explicit requirement of OSPAR's strategy (2003). To place undesirable disturbances uniquely in the context of eutrophication, two difficulties remain (Tett *et al.*, 2007). First, some disturbance of marine ecosystems occurs naturally and second, undesirable disturbance might be caused by a mixture of pressures of which nutrient enrichment is only one. It is therefore essential to understand the human influence on coastal ecosystems and to find causal links between algal changes and eutrophication. Consideration needs to be given to the effects of wider scale natural environmental changes on algal populations as change could be wrongly attributed to anthropogenic nutrient enrichment while the real modifier could be climatic in origin (Edwards, 2009).

1.3 Ecosystem Health

Undesirable disturbance can be diagnosed by accumulating evidence of ecohydrodynamic type-specific changes in the environmental status of an ecosystem. The environmental status of the marine environment can be seen as its health. While a status can be defined as a "condition" evaluated with respect to human interests, a definition for a healthy ecosystem is given by Costanza (1992) as "a system that functions well and is able to resist or recover from disturbance" similar to a healthy human body. The key elements of a healthy ecosystem are the system's structure and vigour, its resistance to disturbance and its resilience to recover from disturbance (Tett *et al.*, 2007). The vigour of an ecosystem lies in its biologically mediated fluxes of energy and materials as well as its ability to recover from disturbance by means of recolonization and population growth. Although those processes, and the food supply available to higher levels in marine food chains, depend on primary production, the relationship between production and ecosystem health is not linear. Exceeding a moderate supply of organic matter can result in a state in which eukaryotic consumers fail to deal effectively with organic input and the coupling between production and use is no longer provided (Tett *et al.* 2007). The organisation of an ecosystem is its structure comprising its biodiversity, its food web and its biophysical structure. The structural component of ecosystem health could respond non-linearly to increasing ecological pressure, such as nutrient enrichment (Tett *et al.* 2007). Pushed beyond a certain threshold, structural changes can occure rapidly,

cumulating in a radically altered state from which recovery is slow. Therefore, a key operational need is to detect a trend toward a widespread undesirable disturbance before the ecosystem has reached the limit of its resistance to nutrient and organic enrichment. This is essential, because we do not know exactly how close to the threshold our ecosystems are (Tett *et al.* 2007).

There are five main indicators which can describe the status of a marine ecosystem and thus its health (Tett *et al.*, 2007):

1. bulk indicators, e.g. chlorophyll, winter nutrients;
2. frequency statistics, e.g. HABs;
3. flux measurements, e.g. primary production;
4. indicator species, e.g. seagrasses, *Phaeocystis*;
5. structural indicators, e.g. trophic indices

The first three indicators relate to vigour and coupling and with that the functioning of an ecosystem and the last two are indicators of change in structure. Bulk measurements such as phytoplankton biomass (as measured by chlorophyll) are typically used as an indicator of nutrient enrichment (CSTT 1997; Gowen *et al.*, 1992; Malcolm *et al.*, 2002; Painting *et al.*, 2005) and are a useful expression of phytoplankton biomass (Harding, 1994). Those bulk indicators can be measured against a threshold. In the case of phytoplankton biomass measured as chlorophyll for example, a summer threshold of 10 mg Chl m^{-3} has been proposed for UK waters (CSTT, 1994). More recently, thresholds of 15 and 10 mg Chl m^{-3} for coastal and offshore waters respectively have been proposed (OSPAR, 2003). However, nutrient enrichment and accelerated algal growth are not in themselves harmful (Tett *et al.* 2007) and are therefore not seen as an undesirable disturbance. And because these bulk measurements provide little information on the extent of change in the structure of the marine ecosystem, they cannot adequately identify undesirable disturbances of nutrient enrichment. Therefore, taxon diversity (Karydis & Tsirtsis, 1996), seasonal succession (Hallegraeff & Reid, 1986; Gailhard *et al.*, 2002; Tett *et al.*, 2008), and indicator species (Edwards *et al.*, 2001; Paerl *et al.*, 2003) that collectively give insight into the ecosystem structure need to be assessed also to identify and diagnose eutrophication.

In former years eutrophication was identified by measurements of winter nutrient levels of nitrate and phosphate and summer concentrations of phytoplankton chlorophyll, which were compared with thresholds. Now, these bulk indicators are used in the screening process to identify whether a water body is at risk of eutrophication. The water body is identified as eutrophic only when evidence of all stages (undesirable disturbance to the balance of organisms caused by accelerated growth

of algae and higher plants, caused by nutrient enrichment) can be confirmed with a causal link between them. Thus, eutrophication is seen as a process of enrichment (Tett *et al.* 2007; Gowen *et al.* 2011). The implication of the OSPAR and EU definitions for eutrophication is that the disturbance is only undesirable when human-generated.

1.3.1 Marine Strategy Framework Directive

In October 2005, the European Marine Strategy Framework Directive (MSFD) was presented by the Commission of the European Union (EU) for the protection of all seas of the EU. On European levels the MSFD attempts to assess the marine environmental status with eleven descriptors that link across sectors and intends to follow the "ecological approach". These eleven quality descriptors (QD) include biological diversity (QD 1), non-indigenous species (QD 2), population of commercial fish/shell fish (QD 3), elements of marine food webs (QD 4), eutrophication (QD 5), sea floor integrity (QD 6), alteration of hydrographical conditions (QD 7), contaminants (QD 8), contaminants in fish and seafood for human consumption (QD 9), marine litter (QD 10), introduction of energy (including underwater noise) (QD 11). The MSFD has been designed to follow the ecosystem approach by taking into account habitat types, biological components, physico-chemical characteristics including those resulting from anthropogenic impact and hydromorphology. The MSFD is the first directive that uses descriptors (eleven) that link across the marine ecosystem of the whole of Europe providing this integrative approach in assessing the environmental status of the seas. It was set into force in 2006 (2008/56/EC) and is applicable to all European waters up to 200 nautical miles from the coastal baseline. There is therefore a small area of overlap with the Water Framework Directive (WFD) for one nautical mile. It includes the water column, sea bed and its sub-surface geology. The overall aim of the MSFD is to protect and where necessary re-store the European seas: ensuring sustainability for human use and providing safe, clean, and productive marine waters. For the MSFD to progress there are three important objectives, similar to those within the WFD coastal waters (see Townend, 2002; Borja, 2005). These are:

- monitoring and assess the present state of the system and rates of change (long- and short-term changes)
- system models that account for the inherent non-linearity of processes and the complexity of their interactions
- education at different levels and addressed to the citizens and to specialised high level training

By legal requirements of the Marine Strategy Framework Directives the variables that are required to be analysed include:

- physical features (bathymetry; temperature and salinity; currents and residence time)
- habitat types (identification and mapping of special habitat types with their physico-chemical characteristics)
- biological elements (including phyto and zooplankton; invertebrate fauna; fish, mammals and seabird populations; together with structural and community parameters, population dynamics and the introduction of alien species)
- pressure and impacts, such as nutrient inputs and cycling, chemical pollution, physical and non-physical disturbance as well as non-toxic contamination and biological disturbance.

With this approach the MSFD established a framework designed for all member states to achieve "good environmental status" (GES) in the marine environment by 2020 (2008/56/EC). GES is defined as *"the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive within their intrinsic conditions"*. With regard to eutrophication (QD 5) GES has been achieved *"when the biological community remains well-balanced and retains all necessary functions in the absence of undesirable disturbance associated with eutrophication and/or when there are no nutrient-related impacts on sustainable use of ecosystem goods and services"*. In the context of eutrophication, the MSFD has four key attributes that are important and that help to identify the condition of the observed water body. (i) Water clarity relates to phytoplankton biomass and is important for growth of benthic plants; (ii) primary production associates with nutrient loadings to marine waters; (iii) organic decomposition registers the fate of ungrazed production and is potential for oxygen consumption that could lead to oxygen depletion (hypoxia/anoxia); (iv) algal community structure reflects the ecological balance of primary producers, undesirable shifts in the balance can include the appearance of harmful algal blooms (HAB). Thus monitoring the structure and function of marine ecosystems to assess the status of marine European waters is of vital importance and indeed is a legal requirement under the framework of the European Marine Strategy for members of the European Union.

1.4 Microplankton as a Structural Indicator of Change

Microplankton encompasses all unicellular micro-organisms in aquatic, epiphytic and benthic life. It is responsible for the majority of primary production in the world's ocean providing an essential ecological function for life and forming the base of marine pelagic food webs. A member of the microplankton is a microplankter, and their typical size range is from 2 μm to 1 mm. The autotrophic microplankters fix solar energy by photosynthesis, using carbon dioxide, nutrients, and trace metals (Zeitschel, 1978) and although these drifting, microscopic photosynthetic organisms represent less than 1% of the photosynthetic biomass on the earth they are responsible for 46% to 50% of the global net primary production (Falkowski *et al.*, 1998; Beardall & Raven, 2004; Behrenfeld *et al.*, 2004). With more than 4,000 known marine species globally (Sournia *et al.*, 1991), microplankters occupy a key position in defining global climate, and oceans and atmospheres chemical composition. As the base of the pelagic marine food web, they influence the nature of the entire food web and the abundance of marine organisms (Tett, 1990). Because plankton are closely coupled to environmental change (Hays, 2005), it makes them sensitive indicators of environmental disturbance. Especially, the sensitivity to change of nutrient enrichment could make the pelagic microplankton community an ideal indicator of change in structure and therefore an important tool in assessing ecosystem health in the context of eutrophication.

1.4.1 Changes in Microplankton Community Structure in Response to Nutrient Enrichment

On the basis of the initial response of phytoplankton to nutrient enrichment and their key role as primary producers, their community structure evidently holds the key to diagnose eutrophication (Tett *et al.*, 2007). Increases in ratio of N:Si may cause increases in the proportion of non-silicified algae (Gillbricht *et al.*, 1988; Tett *et al.*, 2003b). But care must be taken in the use of simple ratios like this since they can underestimate the effect of nutrient pressure on well-stirred waters where diatoms are naturally dominant. Setting Ecological Quality Standard (EQS) from such ratios where % thresholds are used, tends to reflect the view that diatoms are good and dinoflagellates are bad and misunderstands the multiple roles that each group plays in marine ecosystems. Generally, it is apparent that the microplankton encompasses a wide range of biochemical, taxonomic and functional diversity (Jeffrey & Vesk, 1997; Tett *et al.*, 2003b; Delwiche *et al.*, 2004) and as pointd out by Tett *et al.* (2007) this diversity should not be ignored when assessing the health of the

plankton.

The global increase of the frequency, intensity and geographic distribution of some harmful algae and harmful algal blooms (HABs) over the past several decades could be related to the relative availability of different forms of nitrogen. According to Sellner *et al.* (2003) blooms of these organisms are attributed to two primary factors: natural processes such as circulation, upwelling, relaxation and river flow and anthropogenic: loadings leading to eutrophication. It has been recognized that many HABs appear to be related to a change not only in total nutrient availability but also in the relative abundance of the major nutrient elements (Tilman, 1977; Smayda, 1990, 1997; Anderson *et al.*, 2002; Glibert *et al.*, 2005a; Heisler *et al.*, 2008). As with all blooms, their proliferation results from a combination of physical, chemical and biological mechanisms and their interactions with other components of the food web. HAB species can be directly and indirectly stimulated by nutrient enrichment in several ways.

1.5 The PCI - an Integrative Indicator

The sensitivity of the microplankton to change of nutrient enrichment is ideal to use these organisms as indicators in the assessment of the status of marine ecosystems. Many of those indicators have been developed and found frequent application. Biodiversity indices (Shannon, 1948) or multivariate statistics (Edwards, 2005) have proven to be powerful tools in analysing microplankton data. Devlin *et al.* (2007) proposed a phytoplankton index (I_E) to classify and assess the UK marine waters under the requirements of the WFD. A phytoplankton community index (PCI) was developed by a collaboration of scientists funded by DEFRA (Department of Environmental Food and Rural Affairs) to help detect changes in phytoplankton community structure and hence undesirable disturbance in the context of eutrophication (Tett *et al.*, 2007). The index was designed to assess the state of the phytoplankton part of the pelagic ecosystem with respect to a reference condition aiming not to be a measure of structure but of change in structure (Tett, 2006) in response to environmental forcing such as anthropogenic nutrient enrichment. The use of the PCI arose from two contexts. The first one concerned the UWWTD and OSPAR's strategy to combat eutrophication. Both require management action to be taken in the event of an undesirable disturbance. The second one concerns the EU Water Framework Directive (WFD) and the new Marine Strategy Framework Directives (MSFD), which define ecological status as an expression of the quality of structure and functioning of aquatic ecosystems associated with waters to be measured by comparing the state of biological quality elements with those in a type-specific reference condition. The idea behind the PCI (Tett *et al.*, 2007) is to define an ecosystem state in terms of

values of state variables (i.e. lifeforms) taking into account the natural, especially seasonal, variability that is an essential part of phytoplankton ecology and plotting these state variables as lifeforms into a multidimensional "state variable space". The purpose of the phytoplankton part was to provide a simple parametrisation of autotrophic and heterotrophic processes with not so much emphasis on the single organism or species. At this stage, therefore, any theory of microplankton floristic composition deals not with species or genera, but with lifeforms (Margalef, 1978) in terms of functional groups, analogous to the 'guilds' of species used as a grouping device by benthic ecologists (Bolam *et al.*, 2002; Loreau *et al.*, 2002; Biles *et al.*, 2003; Hooper *et al.*, 2005) lifeforms of microplankton represent the variables that define the marine ecosystem state space.

1.6 Aim and Objectives

In this study I was concerned with the structure, that is the organisation of the microplankton part of the pelagic marine ecosystem. The overall aim was to develop and evaluate an index to identify change in microplankton community structure and relate this change to increased anthropogenic nutrient enrichment. Microplankton community structure in temperate shelf seas was chosen to be the focus of this work because temperate shelf seas are not only best studied but also most subject to anthropogenic influence (Tett *et al.*, 2003a). The index was evaluated by testing the hypothesis that different levels in anthropogenic nutrient enrichment in hydrodynamically similar coastal regions cause changes in microplankton community structure.

It has been shown that the current PCI can be used to detect a change in phytoplankton community structure in the context of eutrophication (Tett *et al.* 2007). However, it is based on diatoms and dinoflagellates and does not encompass other important lifeforms (micro-flagellates and heterotrophic protozoa) that contribute to microplankton structure neither does it discriminate the nutritional mode of dinoflagellates. As part of the development of the microplankton community index (MCI), the objectives of this study were therefore to: i) incorporate the heterotrophic and mixotrophic part of the microplankton community as well as the autotrophic organisms to make the index a more complete indicator of change in microplankton community structure; ii) evaluate the performance of the MCI as a tool for providing insight into changes in microplankton community structure in the context of anthropogenic nutrient enrichment.

Chapter 2

Microplankton

2.1 Introduction

This chapter presents an introduction to marine microplankton. The first part gives a general introduction to microplankton and leads into the origin and evolution of the microplankton, describing the four morphology and taxonomy phyletic groups focused on during this study. The second part emphasises the key role of microplankton as the base of marine food webs and describes its dynamics in temperate shelf seas. It finally introduces microplankton seasonal succession and the theory of lifeforms in preparation for the development of a microplankton community index (MCI) followed by a summary and conclusion of the chapter.

2.2 Microplankton

Plankton was defined by Hensen in 1887 (Ruttner, 1953; Hutchinson, 1967) and can be summarised as a comprehensive term that includes all organisms (animals and plants) that either float passively in the water, or possess such limited powers of swimming that they are carried from place to place by the water currents. The word plankton comes from the Greek word $\pi\lambda\alpha\gamma\kappa\tau\o\zeta$ (‘planktos’), meaning ‘wandering’ or ‘drifting’. Planktonic organisms are the most abundant lifeform on earth apart from bacteria and viruses and can be found in all aquatic ecosystems. In geological terms some planktonic organisms are ancient species dating back to several million years (coccolithophores, foraminiferans). The oldest planktonic organisms found are fossil diatoms being about 140 million years old (Amato, 2004). Plankton is conventionally divided into zooplankton (the animal component), phytoplankton (the plant component where the Greek term *‘phyton’* stands for ‘plant’) and bacterioplankton (the bacterial component) and can range in size from a few micrometers

(picoplankton) not visible to the naked eye, up to a few meters (jellyfish). The focus of this study lay on the phytoplankton. But phytoplankton as explained in further chapters is comprised of more than strictely the photosynthesising organisms. Different nutritional modes of phytoplankton are of vital importance as they bear directly on trophic interactions, especially the linkages between the microbial loop and the classical metazoan food web and helps to explain ecosystem functioning. In this context it seems appropriate to define the organisms that make up the microscopic part of the plankton not as phytoplankton but as microplankton incorporating autotrophs, heterotrophs, and mixotrophs. The term was originally used for this purpose by Dussart (1965) and Tett & Wilson (2000) included heterotrophic bacteria and cyanobacteria. However, this definition should not be confused with the more common usage of the term like it is often found in books referring to a specific size range of phytoplankton (e.g. Sieburth *et al.*, 1978; Lalli & Parsons, 1997). Microplankton encompasses all unicellular micro-organisms in aquatic life and is responsible for the majority of primary production in the world's ocean. It provides an essential ecological function for life and forms the base of most aquatic food webs. A member of the microplankton is a microplankter, and their typical size range is from 2 μm to 1 mm. The photoautotrophic (hereafter called autotrophic) microplankters fix solar energy by photosynthesis, using carbon dioxide, nutrients, and trace metals (Zeitschel, 1978). And although these drifting, microscopic photosynthetic organisms represent less than 1% of the photosynthetic biomass on the earth they are responsible for 46% to 50% of the global net primary production (Falkowski *et al.*, 1998; Beardall & Raven, 2004; Behrenfeld *et al.*, 2004). With about more than 4,000 known marine species globally (Sournia *et al.*, 1991), microplankters occupy a key position in defining global climate, and oceans and atmosphere chemical composition and influence the nature of marine food webs (Tett, 1990) with their dynamics, nutrient cycling, habitat condition, and fishery resources (Paerl *et al.*, 2003).

2.3 Origin and Evolution

Since the publication of the theory of evolution by Charles Darwin in 1859 the establishment of the universal tree of life has been a major quest in biology. Much of it was resolved by the entomologist Henning through the development of the cladistic method (see Hennig, 1966) but the main difficulty of the scarcity of morphological characters remained. Until comparatively recently, living organisms were divided into two kingdoms: the Animalia and the Plantae. In the 19th century, evidence began to accumulate that these were insufficient to express the diversity of life, and various schemes were proposed with three, four, or more kingdoms. One such scheme divides all living organisms into five kingdoms and is commonly used: Monera (bacteria), Protista, Fungi, Plantae, and Animalia. This coexisted with a

scheme dividing life into three main domains: the bacterial Prokaryotae (bacteria, etc.), the Archaea (archa bacteria) and the Eukaryotae (animals, plants, fungi, and protists).

However, progress toward the resolution of the tree of life remained elusive for decades because of a lack of methods (Philippe, 2004). Only in the 1980's and with the development of molecular techniques such as the study of ribosomal RNA (rRNA) was progress made (Woese, 1987) and the phylogeny (evolutionary relatedness) of organisms became much clearer.

An image of the tree of life as it is generally understood, is given in Figure 2.1. The phyla that are of interest in this study are circled.

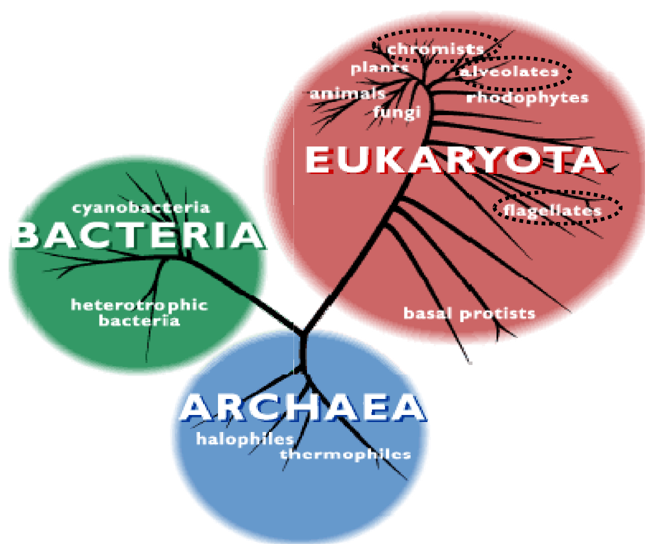


Figure 2.1: A general illustration of the tree of life with the three main domains. Circles indicate the phyla of interest for this study, the flagellates, the alveolates and the chromista. Image inspired by: http://www.ucmp.berkeley.edu/alllife/threedomains.html

2.3.1 An Introduction to the Molecular Approach to Microplankton Evolution

Recent work has shown that the Prokaryotae are far more diverse than expected and they are now divided into two domains, the Bacteria and the Archaea, as different from each other as either is from the Eukaryota. Neither group is ancestral to the others, and each shares certain features with the others as well as having unique characteristics of its own.

Within the last three decades, additional work has been undertaken to resolve relationships within the Eukaryota (e.g. Cavalier-Smith, 1995; Delwiche *et al.*, 2004; Archibald, 2008). It now appears that most of the biological diversity within the eukaryotes lies among the protists (Keeling, 2009), and many scientists (e.g. Archibald *et al.*, 2003; Adl *et al.*, 2005; Keeling *et al.*, 2005; Burki *et al.*, 2008) argue that protists should also be divided into more domains like has been done with the prokaryotes. Plankton is a good example, as it was traditionally divided into the three compartmental groups (zooplankton, phytoplankton, bacterioplankton) and then further subdivided into trophic levels on the basis of taxonomic categories well above the species level. This conventional division into animals, plants, and bacteria masks details and neglects the microscopic organisms that are now increasingly thought to be of importance in marine ecology and especially in coastal and estuarine regions. All flagellates for example were grouped phytoplankton although some of them are heterotophic organisms exploiting particulate organic matter (Tett *et al.*, 2003b). In contrast other protozoan groups, the ciliates for example, were often assigned to zooplankton. However, the ciliate *Mesodinium rubrum* with its incomplete symbionts has a high photosynthetic rate and has even be reported to form red tides (Taylor *et al.*, 1971; White *et al.*, 1977).

A diagram of the section of the eukaryotes in the tree of life most relevant to this study is given in Figure 2.2. The focus is on the Chromalveolatae as this is the lineage that most of the microplankton of interest in this study derive from.

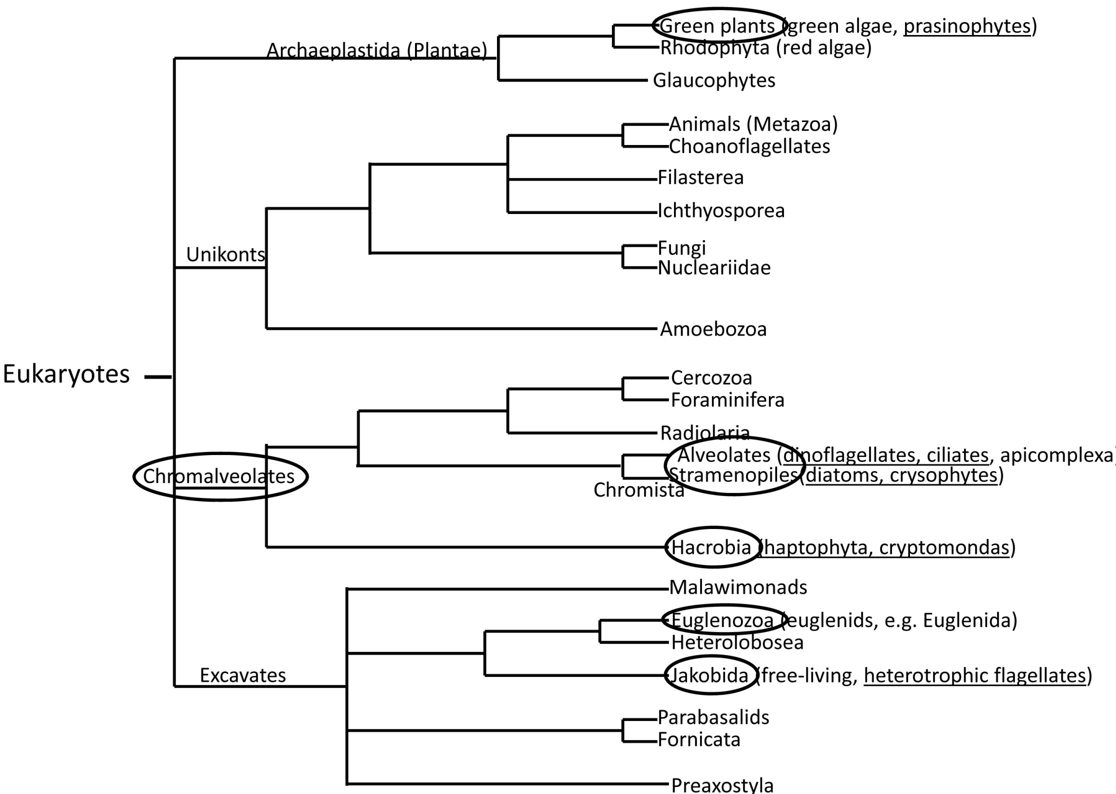


Figure 2.2: A detailed genetic tree of life for Eukaryotes. The important lineages for this study are highlighted with circles and the families are underlined. Diagram inspired by “the tree of life web project” (http://tolweb.org)

Chromalveolates

The Chromalveolates comprise six major groups of primarily single celled eukaryotes and are characterised by the presence of chlorophyll a and c (unlike the typical red algae plastids). Alveolates, chromista and a clade composed of cryptomonads and haptophytes have been proposed to constitute the chromalveolates. The alveolates are the lineage of the phylum myzozoa and within this are the dinoflagellates and ciliates (two important phyletic groups in this study). The chromista are the lineage to the stramenopiles and the phylum bacillariophyta (diatoms are also an important part in this study) are positioned within this. The alveolates are hypothesised to be related to stramenopiles and hacrobia (Cavalier-Smith, 2004; Keeling, 2009). The basis for this hypothesis is the widespread presence of plastids in these groups that are all derived from secondary endosymbiosis with a red alga. It was therefore proposed that all Chromalveolates share a common ancestor when this endosymbiosis took place (Cavalier-Smith, 1999).

The chromista lineage holds only autotrophic organisms, whereas the alveolata are a monophyletic group of primarily single-celled eukaryotes that have evolved diverse modes of nutrition, such as predation, photoautotrophy and intracellular parasitism.

Alveolates are one of the best-supported groupings that have emerged from the analysis of molecular phylogenetic data in eukaryotes (Fast *et al.*, 2001; Cavalier-Smith & Chao, 2006). The complex set of phylogenetic and cellular characteristics has made inferences about the evolutionary origins of photosynthesis in alveolates a challenging and somewhat contentious area of biodiversity research (Leander, 2008). The closest relatives of the alveolates are the heterokonts. The relationship between alveolates and heterokonts is also well supported by molecular data (Fast *et al.*, 2001; Harper & Keeling, 2003; Hackett *et al.*, 2004a).

There are other branches of the evolutionary tree that need to be mentioned in order to understand the evolution of micro-flagellates. These form the fourth group of interest in this study and have the most diverse origins. Some micro-flagellates, such as the prasinophytes are derived from the green plants belonging to the clade archaeplastida. The plastids of the archaeplastida are only surrounded by two membranes and it is therefore suggested that the plastids developed directly from endosymbiotic cyanobacteria (McCourt *et al.*, 1996). Other micro-flagellates, the haptophytes and cryptomonads have their origin in the chromalveolates and within that in the hacrobia (Okamoto & Keeling, 2009). Flagellates that were abundant in low numbers but nonetheless important in this study were euglenoids and free-living heterotrophic flagellates. Euglenida are derived from euglenozoa placed in the origin of excavates (Leander, 2008) (see Figure 2.2). The free-living flagellates belong to the division of the jakobida that also have their origin in the excavates (Simpson & Roger, 2004). Table 2.1 provides the meaning and origin of some of the terms used in this chapter.

Table 2.1: The origin and meaning of some terms used in the text

Name	**Origin**	**Meaning**
Chromalveolates	latin	Chromista + Alveolates
Alveolates	latin	"forming a channel", deeply pitted, with cavities
Chromista	latin	"coloured"
Myzozoa	latin	"sucking life"
Stramenopiles	greek	"straw-haired"
Archaeplastida	latin	plastids from the origin
Hacrobia	latin	"Ha" from haptophyta, "cr" from cryptomonads, "bia" general suffix referring to life
Euglenida	greek	"eu" = good, "glene" = eyeball
Excavata	latin	"hollow out, made hollow, removed"
Apicomlexa	latin	"apex" meaning "top" and "complexus" meaning infolds (refers to a set of organelles)
Haptophytes	greek	"hapsis" meaning touch, reflecting their use of the haptonema

Endosymbiosis

Endosymbiosis has played the key role in the nutritional evolution of microplankters and understanding of endosymbiotic processes has helped to distinguish the different clades and lineages of the tree of life. The term endosymbiosis is defined as the process by which one cell is taken up by another and retained internally, such that the two cells live together and integrate at some level, sometimes permanently. Endosymbiotic interactions have been common in eukaryotic evolution, and many such partnerships persist today (Margulis, 1981). In two cases, endosymbiotic events had far-reaching effects on the evolution of life: these are the origins of mitochondria and plastids (chloroplasts).

Mitochondria

Mitochondria are generally known as the energy-generating powerhouses of eukaryotic cells, where oxidative phosphorylation and electron transport metabolism takes place (Reichert & Neupert, 2004). They are also involved in several other processes such as oxidation of fatty acids, amino acid metabolism, and assembly of iron-sulfur clusters (Lill *et al.*, 1999; Lill & Kispal, 2000). Mitochondria are bounded by two membranes, the innermost of which is generally highly infolded to form 'cristae' that have characteristic shapes, either flat, tubes, or paddle-shaped (Taylor, 1978). The presence of mitochondria is an ancestral trait in eukaryotes (Roger, 1999; van der Giezen & Tovar, 2005), although in certain anaerobes and microaerophiles they have radically reduced or transformed functions and in some cases they are not involved in energy production at all (Müller, 1993; Tovar *et al.*, 1999; Williams & Keeling, 2003; van der Giezen *et al.*, 2005; Embley, 2006). Mitochondria can be traced back to a single endosymbiotic event of an alpha-proteobacterium (Gray *et al.*, 1982; Andersson & Kurland, 1999; Gray & Doolittle, 1999; Lang *et al.*, 1999; Gray *et al.*, 2004).

Plastids

Plastids are the photosynthetic organelles of plants and algae. The term is a general annex for organelles that include plastids such as chloroplasts (in the green lineage), rhodoplasts (in the red lineage) and leucoplasts (colourless plastids that have lost their photosynthetic abilities). In addition to photosynthesis, plastids have a diverse range of functions, including the biosynthesis of amino acids, fatty acids and isoprenoids (Harwood, 1996; Herrmann & Weaver, 1999; Rohdich *et al.*, 2001). As in the case of mitochondria, in many lineages plastids have been radically reduced or transformed, primarily through the loss of photosynthesis (e.g., the apicoplast of apicomplexa, and the relict plastids of many parasitic algae and plants (Wilson, 2002; Ralph *et al.*, 2004; Gould *et al.*, 2008). Plastids can also be traced back to a single endosymbiotic event involving a cyanobacterium and the ancestor of the

archaeplastida (Reyes-Prieto *et al.*, 2007; Rodriguez-Ezpeleta *et al.*, 2007). Unlike mitochondria, plastids spread to other eukaryotic lineages by secondary and tertiary endosymbiotic events (McFadden, 1999; Keeling, 2004; Archibald, 2005; Gould *et al.*, 2008). In these events, one eukaryotic cell took up another eukaryote that already contained a plastid (an alga). This second, endosymbiotic eukaryote was then reduced and integrated, and in most cases all that remains of this alga is the plastid surrounded by the remains of the endosymbiont's plasma membrane. However, in cryptomonads and chlorarachniophytes a tiny relict of the algal nucleus called a nucleomorph has been retained and it was the study of these that helped elucidate the complex evolutionary history of plastids (McFadden *et al.*, 1997; Douglas *et al.*, 2001; Archibald, 2005; Gilson *et al.*, 2006). Other endosymbiotic relationships based on photosynthesis are also known (Okamoto & Inouye, 2005; Johnson *et al.*, 2007; Rumpho *et al.*, 2008), but typically these are less integrated and are generally accepted as being organelles rather than endosymbionts. The endosymbiont becomes an organelle when it becomes encrypted with the DNA from the eukaryotic cell that took it up.

It is widely believed that primary plastids evolved from one single endosymbiotic event (Reyes-Prieto *et al.*, 2007 and references therein). Chloroplasts arose monophyletically from a cyanobacterium with phycobilins (pigments) and chlorophyll *a* and *b* that was phagocytosed (food particle uptake as whole cells, digested within special food vacuoles) by a biciliate protozoan host (Cavalier-Smith, 1982; 1995) that converted it to an organelle (Reumann, 1999).

There are three distinct algal lineages with primary plastids that are directly derived from the free living cyanobacteria. The glaucocystophytes, an unfamiliar and relatively rare group of mostly freshwater algae that are not discussed further in this study. The green algae, characterised by the presence of two organelles with membranes (Cavalier-Smith, 2000) (the prasinophytes evolved from the green lineage and are of interest in this study as part of the flagellate group). And the red algae, believed to have lost the chlorophyll *b* of their ancestor cyanobacterium (Cavalier-Smith, 2000) and the plastids of the algae are pigmented with chlorophyll *a* and phycobilisomes.

The red algae are a diverse and widespread group and dominate many temperate and tropical marine intertidal environments. They are important as primary producers and a food source as well as being of commercial value in industrial chemistry (Delwiche, 2004).

It is less clear how often photosynthesis has spread horizontally across the eukaryotic tree of life by secondary endosymbiosis. Finding out how organisms with secondary enbosymbiosis are related to each other by comparing the sequence of their genomes is rather complex (Archibald, 2008). Secondary plastids are distributed patchily

across organisms, often being most closely related to non-photosynthetic, plastid-lacking lineages like ciliates (Archibald, 2008). Cavalier-Smith (2000) proposed the hypothesis that a single, ancient endosymbiosis occurred in the common ancestor of all modern-day lineages harbouring a red secondary plastid. If this hypothesis is true (and if the dinoflagellate peridinin plastid is a vertical descendant of the original chromalveolate plastid), then the ancestor of all dinoflagellates was photosynthetic, and it contained the same type of plastids as the ancestor of all apicomplexans. The close relationship between dinoflagellates and apicomplexans, and the abundance of parasitic groups branching from the base of the dinoflagellate lineage (syndinians) argues for a parasitic (or perhaps mutualistic?) ancestor for the whole group. The recent discovery of a photosynthetic endosymbiont of corals, Chromera, with apicomplexan phylogenetic affinities strongly supports these two views (Moore *et al.*, 2008). It was shown that the apicomplexan apicoplast (a plastid remnant in that group) was derived from a red alga in the same endosymbiotic event that gave rise to the dinoflagellate peridinin plastid (Keeling, 2008; Moore *et al.*, 2008). This event could have been the original chromalveolate endosymbiosis. Recent data also suggest that the nuclei of organisms from at least some of the early, non-photosynthetic branches of the dinoflagellate lineage, e.g. *Perkinsus* (Stelter *et al.*, 2007; Matsuzaki *et al.*, 2008) and *Oxyrrhis* (Slamovits & Keeling, 2008) contain genes of a plastidial origin.

Whether the chromalveolate hypothesis turns out to be correct or not, at least the dinokaryotic non-photosynthetic dinoflagellates seem to have had photosynthetic ancestors. Photosynthetic and non-photosynthetic forms frequently co-occur in the same groups in phylogenetic trees, and since the typical dinoflagellate peridinin plastid is unlikely to have originated more than once, a repeated loss of photosynthetic ability in the non-photosynthetic groups is thought to be likely (Saldarriaga *et al.*, 2001; Snchez-Puerta *et al.*, 2007). The presence of cryptic plastids in ostensibly non-photosynthetic forms (Sparmann *et al.*, 2008) is significant in this regard. In some lineages, the peridinin-plastid was lost and replaced by either true plastids or plastid-like organelles with different characteristics. The molecular mechanisms that enabled this plastidial promiscuity in dinoflagellates are poorly understood and the reasons why this happens in dinoflagellates and not in other groups with secondary plastids are still unclear (Hoppenrath & Saldarriaga, 2010).

Despite the large morphological differences between ciliates, apicomplexans and dinoflagellates, alveolates do share several morphological features:

- A system of abutting membranous sacs, called ‘alveoli’, positioned beneath the plasma membrane (a characteristic that is shared by taxa and most common ancestors); the alveoli can be empty (e.g. colpodellids and apicomplexans) or filled with cellulosic material (e.g. thecate dinoflagellates and some ciliates)

- Distinct micropores through the cell surface that function in pinocytosis (ingestion of liquid into a cell by the budding of small vesicles from the cell membrane)
- Similar extrusive organelles (e.g. trichocysts)
- Closed mitosis
- Tubular mitochondrial cristae

2.3.2 Dinoflagellates

The term 'Dinoflagellate' comes from the Greek word *'dinos'* meaning 'whirling' and from the latin word 'flagellum' for 'whip' because these organisms are characterised by two flagella: one girdling the cell and the other trailing the cell. The flagella lie in surface grooves. The transverse one is called the cingulum and the longitudinal one is the sulcus, the distal portion of which projects freely behind the cell. Some dinoflagellates have a desmokont flagellation (two dissimilar flagella emerging from the anterior part of the cell) (e.g. *Prorocentrum*), whereas dinokont species (e.g. *Gymnodinium*) have two flagella that are inserted on the ventral side of the cell located in the cingulum and in the sulcus (Hoppenrath & Saldarriaga, 2010). There are distinctions between armoured dinoflagellates that have a cell wall or theca of cellulose and naked colourless dinoflagellates that lack a theca as well as plastids (Delwiche *et al.*, 2004). Naked dinoflagellates are not necessarily strictly heterotrophic. A symbiotic relationship between naked colourless dinoflagellates and photoautotrophic organisms is often apparent and defines these dinoflagellates as mixotrophic organisms.

Dinoflagellates are common in all aquatic ecosystems (Hoppenrath & Saldarriaga, 2010). Typically, dinoflagellate motile cells are biflagellated unicells, between 10 and 100 μm in length (the extreme range is 2 to 2000 μm). They are second only to diatoms as marine primary producers. Approximately 4500 dinoflagellate species have been described and these have been assigned to more than 550 genera. However, nearly three quarters of these genera and more than half of the species are fossils. Of the approximately 2000 living species, more than 1700 are marine and about 220 are freshwater (Taylor *et al.*, 2008). Between 2000 and 2007 three new dinoflagellate families, 22 new genera, and 87 new species were described (Centre of Excellence for Dinophyte Taxonomy CEDiT), although it is unclear, whether these discoveries are truly new or whether taxonomists have rearranged families, genera and species.

Recent molecular analyses have shown that there are large numbers of undescribed dinoflagellate species amongst the marine picoplankton (e.g. Moreira & Lopez-

Garica, 2002; Worden, 2006) and as symbionts (zooxanthellae) in many types of protists and invertebrates like corals (Coffroth & Santos, 2005). As phagotrophic (food particle uptake) and mixotrophic (food particle uptake and photosynthesis) organisms, dinoflagellates are important components of the microbial loop in the oceans and help channel significant energy to higher trophic levels. These organisms have novel cytoskeletal and nuclear features (e.g. permanently condensed chromosomes) that make them very distinctive among eukaryotes (Fensome *et al.*, 1993). For taxonomic purposes the two most obvious characteristics are the cingulum (transverse groove) and sulcus (longitudinal groove). The position of the flagella can also be an important feature for identification, but are often detached in preserved samples. The side on which the sulcus is located, is the ventral view, the opposite side is the dorsal view (see Figure 2.3). The part of the cell that lies anterior is termed the apex and the side opposite the apex it the antapex. For the identification of thecate dinoflagellates, the arrangement, number and shape of the thecal plates are important features. Dodge (1982) and Tomas (1995) provide detailed and in-depth information about the taxonomy of dinoflagellates.

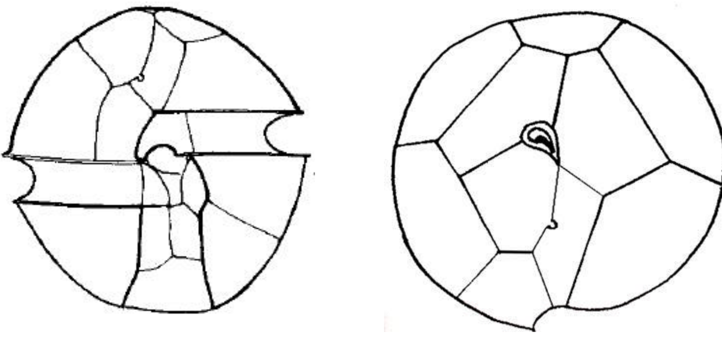


Figure 2.3: Illustrations of the ventral (left image) and apical view (right image) of a thecate dinoflagellate. Images taken from Tomas 1995.

In terms of morphology, dinoflagellates can be as varied and complex as any unicellular eukaryote. Dinoflagellates exist as plasmodia (i.e. multinucleate organisms), biflagellated cells, coccoid stages and even, in one small group, as cell arrays that approach multicellularity. Cyst formation in dinoflagellates has been observed during the sexual reproduction when the fusion of two gametes form the planozygote. This zygote starts dividing again or forms a cyst, the so-called hypnozygote, which is morphologically dissimilar from the vegetative cell (Pfiester & Anderson, 1987; Marret & Zonneveld, 2003). The cyst is very resistant to degradation and is thought to act as a resting stage that can survive adverse environmental conditions (often in seabed sediments).

Dinoflagellates are known to have endosymbiotically gained and lost photosynthesis from different prey organisms several times independently throughout their history (Saldarriaga *et al.*, 2001; Shalchian-Tabrizi *et al.*, 2006). This group is therefore nutritionally very diverse and includes autotrophic, heterotrophic, and mixotrophic species. In many studies however, dinoflagellates have been classified as phytoplankton, regardless of their actual trophic status (Lessard & Swift, 1986). This is due in part to the difficulty of reliably detecting chloroplasts in preserved species with light microscopy. Approximately half of the dinoflagellate species are photosynthetic (Delwiche *et al.*, 2004), but obligate autotrophic species are rare (Schnepf & Elbrächter, 1992). Photosynthetic dinoflagellates are generally mixotrophic and rely on a combination of photosynthesis and heterotrophic nutrition.

Dinoflagellates possess chlorophyll *c* and show the greatest diversity of plastids of any eukaryotic group (Delwiche, 1999). Schnepf & Elbrächter (1999) noted that the diversity in chloroplast-types that exists within dinoflagellates is unparalleled within any other group of eukaryotes. The main type of plastid, the peridinin (the pigment peridinin is a type of carotenoid only found in dinoflagellates), is present in the large majority of photosynthetic dinoflagellates. It contains triple-membraned (sometimes double-membraned) envelopes, thylakoids usually in groups of threes and various types of pyrenoids (Schnepf & Elbrächter, 1999). No girdle lamellae are present. Photosynthetic pigments include chlorophyll *a* and *c2* as well as peridinin, carotene, small amounts of diadinoxanthin and dinoxanthin (Jeffrey *et al.*, 1975). The usual storage products in dinoflagellates are starch, produced exterior to the plastid, and oils. DNA-containing areas in peridinin plastids may be single or multiple, sometimes in prominent nucleoid-like regions (Dodge, 1973).

Autotrophic dinoflagellates exhibit autofluorescence in bright red (Lessard & Swift, 1986). Typical, autotrophic dinoflagellates belong to the genera *Gonyaulax*, *Ceratium* and *Prorocentrum*.

Dinoflagellates are referred to as heterotrophic, when they lack chlorophyll *a* autofluorescence. Heterotrophic species belong to the genera *Protoperidinium*, *Gyrodinium* and *Gymnodinium*, although some species of these genera have been found to contain chloroplasts. In fluorescent light heterotrophic dinoflagellates appear green. The cause of the green fluorescence is not precisely known but it has been suggested that it is due to carotenoid pigments which are unique to heterotrophic dinoflagellates (Balch & Haxo, 1984; Carreto, 1985).

Nearly 50% of the dinoflagellates lack chloroplasts and are obligate heterotrophs (Lessard, 1991). These dinoflagellates exhibit a range of feeding mechanisms. Some possess a peduncle (e.g. *Peridiniella danica*), others have a pallium or feeding veil (e.g. *Protoperidinium bipes*) or engulf their prey directly (e.g. *Noctiluca scintillans*), and there are even parasitic dinoflagellates (Jacobson & Anderson, 1986; Drebes &

Schnepf, 1988; Taylor *et al.*, 2008). Some heterotrophic dinoflagellates appear to have chloroplasts, but the plastids are those of ingested phototrophic organisms that remain temporarily functional (Janson, 2004).

Direct phagocytosis (food particle uptake as whole cells, digested within special food vacuoles) occurs in several species of athecate (naked) dinoflagellates. A distinct cell mouth (cytostome) is then present (e.g., *Noctiluca*, *Oxyrrhis*, *Gyrodinium*) that can be distended to capture large prey organisms. Species of *Gyrodinium* and *Noctiluca* are capable of engulfing whole diatom chains, whole copepod eggs (approx. 1-1.9 mm), and other similarly sized objects.

Myzocytosis is a different form of feeding that involves piercing the prey's cell membrane with a special organelle (the peduncle) and 'sucking' out the cell contents of the prey as if through a straw (e.g. *Paulsenella* spp., *Pfiesteria* spp., see Schnepf & Elbrächter, 1992). Thecate (armoured) dinoflagellates cannot expand in volume the same way that athecate ones can, and thus are unable to ingest large prey items directly. Instead, some species extend a delicate, pseudopodial feeding veil, the pallium, with which they surround portions of diatom chains or other large prey. Digestive enzymes are secreted by the pallium, and digestion then occurs extracellularly. The veil is retracted afterwards (Gaines & Taylor, 1984; Jacobson & Anderson, 1992).

Organisms known to be capable of both photosynthesis and active feeding are called mixotrophs (Stoecker, 1999). The number of mixotrophic species is rapidly increasing because of the increased use of epi-fluorescence microscopy. Genera that were originally thought to have been strictly heterotrophic or autotrophic species were discovered to contain mixotrophic species e.g. *Dinophysis, Prorocentrum, Ceratium, Gymnodinium* and *Scrippsiella* (Jacobson & Anderson, 1994; Skovgaard, 1996; Stoecker *et al.*, 1997; Jeong *et al.*, 2005).

Many photosynthetic dinoflagellates have unique photosynthetic organelles that differ in practically all respects from the typical peridinin plastids. However, in relation to these organelles, it is not always easy to distinguish between true plastids (i.e. organelles that include proteins encoded in their host's nucleus) and recent and/or temporary endosymbiosis of complete organisms, or fragments of organisms (plastids) that have been 'recruited' to perform photosynthesis. Temporary plastids that are taken from prey and need to be replenished regularly are called kleptochloroplasts ('stolen' chloroplasts). These are often derived from cryptomonads. Endosymbiosis between otherwise non-photosynthetic dinoflagellates and complete algal cells (not just their plastids) are also known (e.g. *Noctiluca* and its endosymbionts, members of the prasinophycean genus *Pedinomonas*), and in some cases these symbioses become so close that the host cell is only known in combination with its endosymbiont (Hoppenrath & Saldarriaga, 2010).

Yellow-orange fluorescence is normally associated with external symbionts in heterotrophic species (cyanobacteria). Observations of yellow-orange fluorescence inside the cells of some dinoflagellate species point to a cyanobacterial like endosymbiosis (Lessard & Swift, 1986). These sorts of relationships are thought to have given rise to new types of plastids. Cells of the species *Podolampas bipes* are known to have several eukaryotic endosymbionts in each dinoflagellate cell (Schweikert & Elbrächter, 2004).

Genetically, dinoflagellates are also unique. According to Hoppenrath & Saldarriaga (2010) gene products of all dinoflagellate nuclei for example are processed in a unique way: a spliced leader is trans-spliced to all mRNA molecules. The genomes of plastids and mitochondria of the group are also unique: they are atomized (i.e. the genome is split into very small fragments), and gene content is much, much lower than that of comparable organelles in other organisms.

Dinoflagellates are probably best known as causers of harmful algal blooms (HABs). About 75-80% of toxic phytoplankton species are dinoflagellates (Cembella, 2003) and they can cause red tides that often kill fish and/or shellfish either directly, because of toxin production, or because of effects caused by large numbers of cells that clog animal gills, deplete oxygen, etc. (Smayda, 1997). Dinoflagellate toxins are among the most potent biotoxins known. They often accumulate in shellfish or fish and passed up the food chain causing mortality of sea mammals and sea birds. When shellfish or fish are eaten by humans the accumulated toxins can cause diseases like paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP) and ciguatera (Lehane & Lewis, 2000). The harmful toxins have been linked to major human health concerns, especially in estuarine environments (Hoppenrath & Saldarriaga, 2010).

Some images of dinoflagellates of different nutritional modes are given in Figure 2.4.

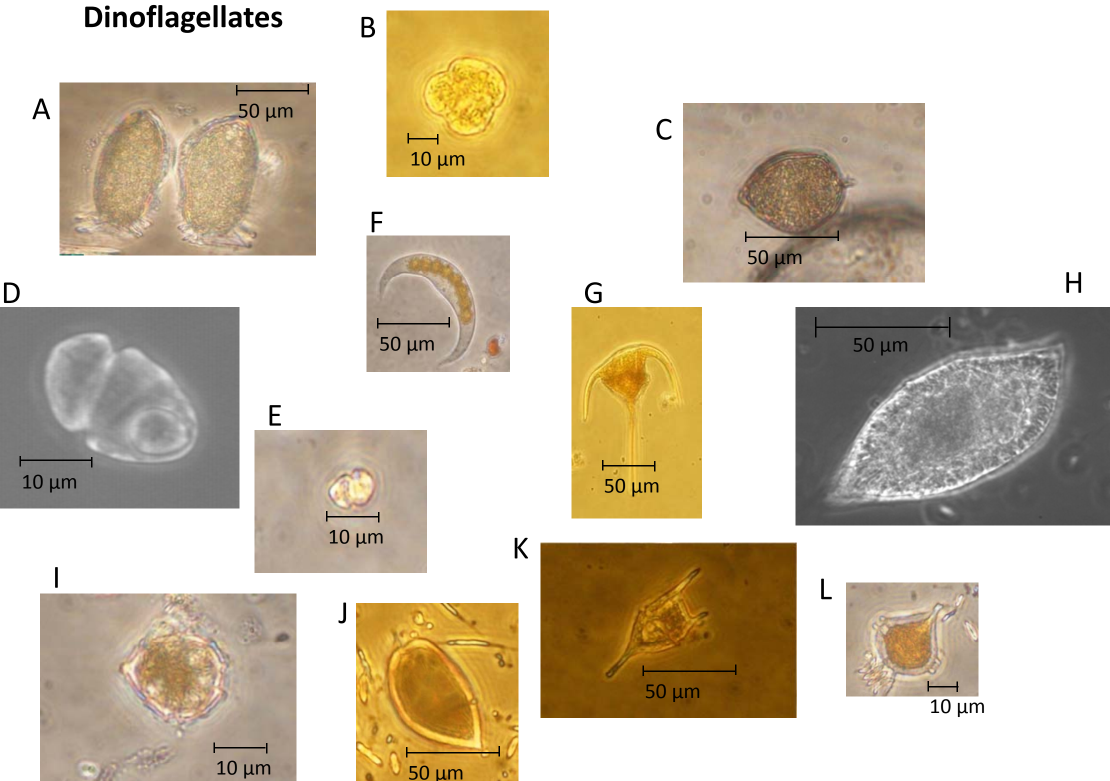


Figure 2.4: Images of dinoflagellates. A two cells of *Dinophysis acuta*, B medium sized (> 25-40μm) *Gymnodinium*, C *Prorocentrum micans*, D *Nematodinium* sp.? (photo taken and kindly provided by E. Capuzzo), E small (10-25μm) *Gymnodinium*, F *Pyrocystes lunula*, G *Ceratium tripos*, H large (> 40-75μm) *Gyrodinium* sp. (photo taken and kindly provided by E. Capuzzo), I small (10-25μm) armoured dinoflagellate, J *Prorocentrum micans*, K *Ceratium minutum*, L *Gonyaulax* spp.; if not otherwise mentioned photos taken by the author during this study.

2.3.3 Silicoflagellates

Silicoflagellates belong to the dictyochophyceae and are derived from the heterokonphyta. Although they have common ancestors with the dinoflagellates, silicoflagellates are characterised by different features and build therefore a separate group. The cells are unicellular, with several different life stages: a stage with a silica skeleton and one flagellum, an amoeboid stage and several more flagellate, naked cells (Moestrup & Thomsen, 1990). Silicoflagellates are exclusively autotrophic and appear in cold and temperate coastal and oceanic waters all year round. The species *Dictyocha speculum* and *Dictyocha fibulum* are commonly found in British and Irish coastal waters generally in low numbers throughout the year. Occasionally elevated cell abundance can occur during late winter and spring. In the mid 1980's it was believed that the species *Dictyocha speculum* had a toxic effect during their naked stage and caused fish kills in Denmark in 1983. However, Henriksen *et al.* (1993) ar-

gued that the fish-kills were associated with the *Dictyocha speculum* bloom causing anoxic conditions rather than the species producing a toxin. High density blooms of silicoflagellates (e.g. *Dictyocha speculum*), are occasionally reported to clog fish gills, causing suffocation (UNESCO, 2010).

Dictyocha speculum and *Dictyocha fibulum* were included in the identification and enumeration of microplankton during this study as they were frequently present and the only silicate users apart from diatoms.

Images of *Dictyocha speculum* and *Dictyocha fibulum* are given in Figure 2.5.

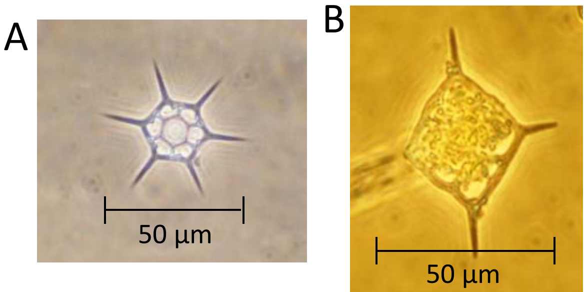


Figure 2.5: Images of silicoflagellates recorded in this study. A *Dictyocha speculum* a six spine formation, B *Dictyocha fibulum* a four spine formation. Both images taken by the author during this study.

2.3.4 Ciliates

Ciliates are protozoa approximately 200 μm in size (Dussart, 1965) that belong to the phylum ciliophora and share a common ancestor of the alveolates with the dinoflagellates. There is, however, no compelling cellular evidence that ciliates have ever had photosynthetic ancestors, despite the fact that many different lineages of ciliates are known to (temporarily) harbour photosynthetic symbionts (Johnson *et al.*, 2007). Some ciliate traits contain functional chloroplasts or whole algal cells and are therefore able to contribute to primary production (e.g. Stoecker, 1991).

Ciliates owe their name to the hair-like organelles called "cilia" that enable them to move and capture prey. Ciliates are considered to be abundant and extremely diverse taxonomic (Leakey *et al.*, 1994), phagotrophic organisms. Since the discovery of the microbial loop (Pomeroy, 1974; Azam *et al.*, 1983) ciliates have become increasingly recognised as playing an important role in the energetics and trophodynamics of plankton foodwebs (Capriulo & Carpenter, 1980; Pace & Orcutt, 1981;

Azam *et al.*, 1983; Porter & Feig, 1988; Zinabu & Bott, 2000). This is because these microzooplankters are major grazers of phytoplankton (Gifford & Caron, 2000) and are important nutrient recyclers (Berman, 1991; Caron, 1991). As prey for higher trophic consumers ciliates further constitute a trophic link between the microbial loop and the classical metazoan food web (e.g. Stoecker, 1990; Gifford, 1991). Burkill *et al.* (1987, 1993) and Leakey *et al.* (1992) have shown that ciliates are an important component of the microzooplankton community in open-ocean, coastal and estuarine waters.

The best characteristic feature for ciliates are two heteromorphic nuclei and a cell cortex containing many cilia arranged in particular configurations. Ciliates can be found in very different habitats. Some ciliates (known as tintinnids) for example, inhabit the intestinal tract of ruminants while other species are known to invade flesh of fish. Some ciliate images are given in Figure 2.6

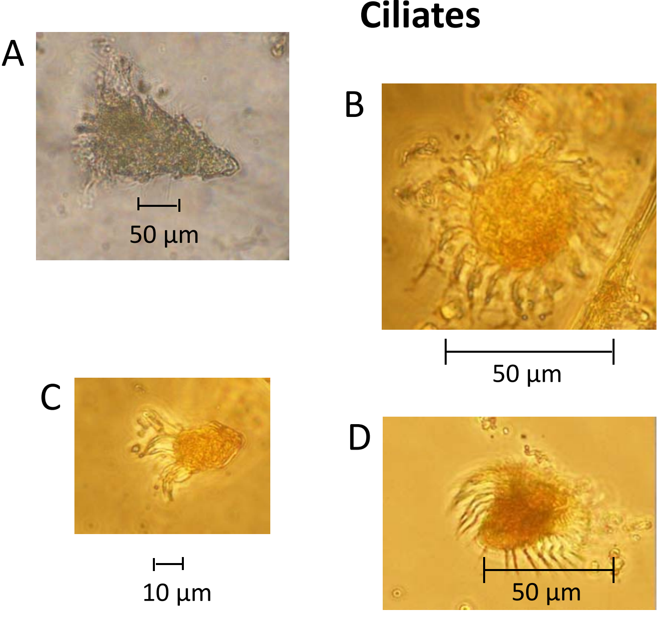


Figure 2.6: Images of ciliates recorded in this study. A *Laboea* sp., B *Strombilidium spiralis*?, C *Mesodinium*, D medium sized unidentified ciliate (possibly *Strombidium* sp.). All images taken by the author during this study.

2.3.5 Diatoms

Diatoms (bacillariophyta) are derived from the heterokont clade (stramenopiles) with tertiary endosymbiosis and have traditionally been classified as plants like most photosynthetic protists (Delwiche *et al.*, 2004). However, stramenopiles are not closely related to land plants: they are characterised by chromophyte pigmentation and contain pigments, such as chlorophyll *a*, *b*, *c*, *d* and *e*, carotinoids, xanthophyll

and phycobilines. They also lack the plasmodesmata and starch production of land plants and their relatives.

Diatoms (greek: 'cut in half') are one of the most diverse and beautiful groups of organisms in the plankton and are exclusively autotrophic. With several thousand species (Sournia *et al.*, 1991; Mann, 1999) they are among the most important aquatic micro-organisms today. Diatoms are characterized by their silica cell wall called a frustule and therefore all species have a requirement for silicate. They have an extensive fossil record going back to the Cretaceous. Some rocks are formed almost entirely of fossil diatoms, and are known as diatomite or diatomaceous earth. These deposits are mined commercially as abrasives and filtering aids. The analysis of fossil diatom assemblages can also provide important information on past environmental conditions (e.g. Harwood, 2007).

Diatoms can be divided into two types: centric (the ornamentation on the valve face is arranged in relation to a central point) and pennate (bilaterally symmetrical). Species of both types typically range in size from 5 to 500 μm but there are some exceptions of up to 1mm in length. Some diatoms build chains and many of them have cell wall processes (spines). Both features are believed to decrease sinking rates and increase protection against grazers.

All diatoms have the same basic architecture that consists of two valves. The slightly larger epivalve and the smaller hypovalve which fits into the epivalve. Each valve consists of the valve face (i.e. the top of the valve), valve mantle and cingulum which is also often called the girdle. Diatoms have three major axes, these are the apical, transapical and pervalvar axes (Figure 2.7). The transapical and apical axes describe the width and depth of the cell. In these centric diatoms with a circular cross section, width and depth are of the same size. The valve view is usually the most important view for examining the morphological feature needed for species identification (see Figure 2.7).

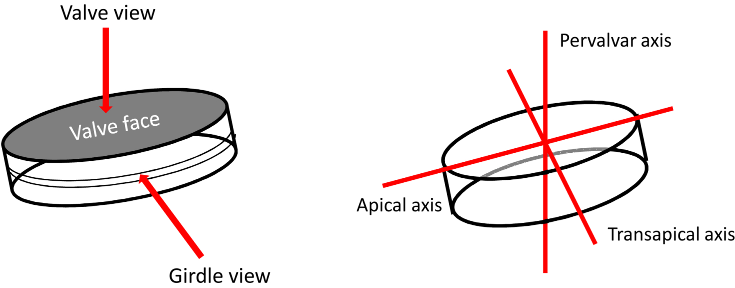


Figure 2.7: Different views of a diatom cell for examining the morphological features of diatoms.

For the identification of a diatom cell several characteristics are used including size,

morphology of the frustule, number and shape of the chloroplasts and the types of chain formation. Chain and colony formations are a common feature in diatoms. Cells may be joined directly by abutting valve faces (e.g. *Leptocylindrus danicus* see Figure 2.8) or by interlinkage of their processes (e.g. *Chaetoceros curvisetus* see Figure 2.8). In some species mucous secretions are involved (e.g. *Odontella sp.* see Figure 2.8 and *Biddulphia* sp.).

Asexual and sexual reproduction is reported in diatoms (Round *et al.*, 1990). Asexual reproduction is the most common. Each cell divides into two and the daughter cells receive half of the parent valve and a new hypovalve is produced to fit inside the parental valve. This type of reproduction can occur once a day, or faster, when conditions are favourable. However, asexual reproduction results in a decrease in average cell size while sexual reprodution is a way of restoring the original size. A common mode of the centric diatoms is oogamy, where large non-motile eggs and small motile sperm are produced. The latter are formed inside a valve and are then liberated into the surrounding medium. These gametes might sometimes be mistaken as flagellates. The sperm fertilises the egg within the parent theca and the zygote then exits the valve expanding into a large sphere bounded by the auxospore (organic membrane) in which a new large cell is formed. In pennate diatoms, isogamy (i.e indistinguishable male and female gametes) is more common. It is usually initiated by the pairing of two cells, which then undergo meiosis to produce gametes inside the original vegetative cell. In many centric and a few pennate diatom species the asexual production of resting stages are an additional feature of the life cycle (McQuoid & Hobson, 1995).

Although dinoflagellates are more commonly associated with biotoxin production, *Pseudo-nitzschia* is the one diatom known to produce toxins causing amnesic shellfish poisoning (ASP). In certain parts of the world (e.g. China) some diatom species produce blooms that are regarded as nuisance or appear in such high abundances that they discolour water (e.g. *Asterionellopsis glacialis* and *Skeletonema* spp.). Other species such as *Chaetoceros socialis* and *Chaetoceros concavicornus* are known to clog fish gill causing suffocation.

Some images of diatoms identified and counted during this study are given in 2.8.

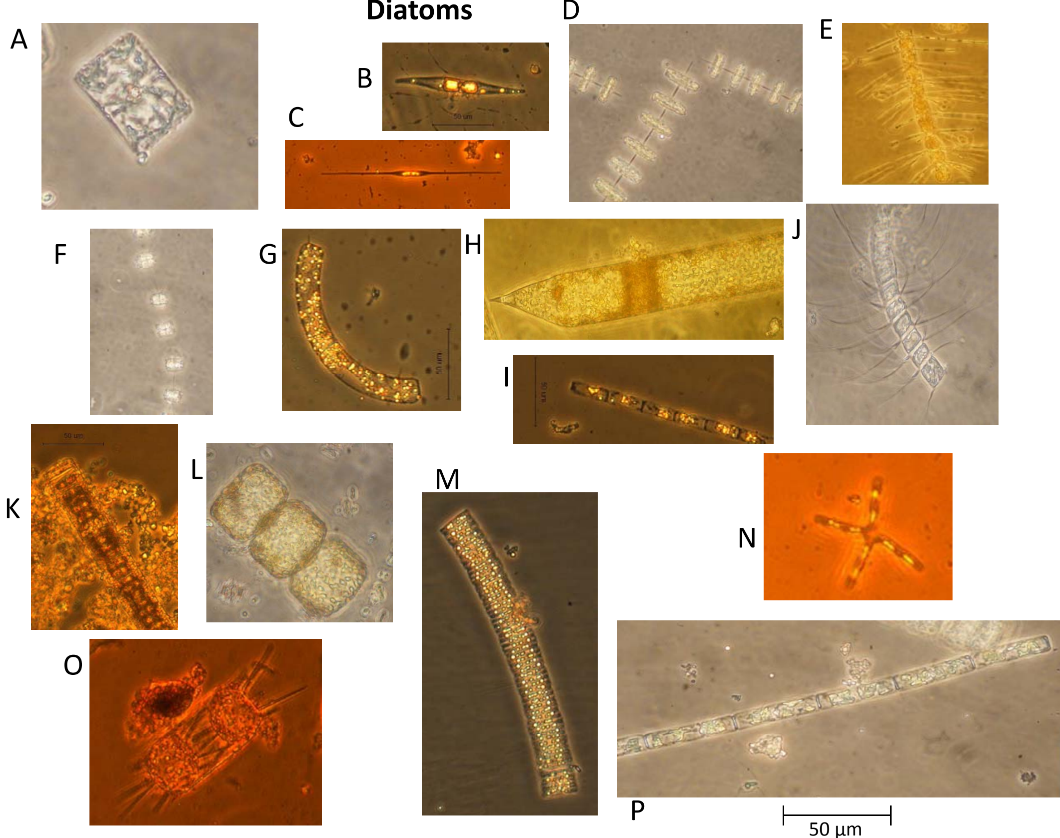


Figure 2.8: A *Meuniera membranacea*, B small pennate diatom (25μm), C *Cylindrotheca closterium*, D *Thalassiosira rotula*, E hyalochaetae (with chlorophylls in spines) *Chaetoceros*, F *Thalassiosira angulata*, G *Guinardia striata*, H *Rhizosolenia imbricata*, I *Leptocylindrus danicus*, J *Chaetoceros curvisetus*, K *Paralia sulcata*, L *Laudaria annulata*, M *Brockmanniella brockmannii*? maybe *Fragilariopsis* sp., N *Asterionella* sp., O *Odontella mobiliensis*, P *Guinardia delicatula*. All images taken by the author during this study.

2.3.6 Micro-flagellates

Micro-flagellates are a diverse group of small (approximately 10μm) organisms belonging to, for example, the raphidophtes, cryptophytes, haptophytes and euglenophytes. Although micro-flagellates have photosynthetic pigments in chloroplasts and usually use carbon dioxide as a carbon source, species are also reported to take up dissolved organic substances to cover the carbon demand for example under bad light conditions (White, 1974; Tuchmann *et al.*, 2006).

The micro-flagellates were an important part in this study and due to limited equipment, knowledge and time they were categorised into six different groups. These were the cryptomonads, *Phaeocystis sp.* (belonging to the prymnesiophyta), the prasinophytes, the euglenophytes, small heterotrophic flagellates and nanoflagellates (only distinguished by size; $< 7\mu$m).

The cryptomonads and prymnesiophytes (the latter often referred to as haptophytes) are two of the subgroups of the hacrobia. They are large and well studied lineages of important primary producers (Okamoto & Keeling, 2009). The group hacrobia was only recently proposed and is based exclusively on molecular data since no single morphology characteristic has been found that unites these groups. The cryptomonads and haptophytes were formally both proposed to be part of the chromalveolates (Cavalier-Smith, 1999).

Cryptomonads are aquatic unicellular eukaryotes that inhabit both marine and freshwater environments. Most cryptomonads are photosynthetic (and are thus referred to as cryptophytes) and possess plastids that are very diverse in pigmentation (Okamoto & Keeling, 2009). Commonly, cryptophycean species are ellipsoid to a distinct tear-drop shape with a typical furrow or depression. Two flagella arise from the end of the furrow. In genera such as *Prymnesium* (derived from the haptophytes), cells are covered by minute scales but look like naked cells when viewed under light microscopy. Typically, motile cells have two flagella and a haptonema which can sometimes appear as a third flagellum but is a special organelle, possibly involved in prey capture.

The reason why species of *Phaeocystis sp.* were distinct in this study was that *Phaeocystis* blooms were the focus of a European research project and an indicator species in the 1980s (Lancelot *et al.*, 1987). It has been given extra attention ever since in relation to nutrient loading in European coastal waters (Smayda, 1997, Cadée & Hegeman, 2002). Extensive *Phaeocystis* blooms during late spring and early summer are a feature of the southern North Sea (Lancelot *et al.* 1987) and Liverpool Bay in the Irish Sea (Jones & Haq, 1963; Gowen *et al.*, 2008) and their magnitude can cause beach fouling and foam production when cells die off and mortality in marine organisms might be caused due to oxygen depletion in the water. In waters around the UK this might not be the case (see Tett *et al.*, 2007; Gowen *et al.*, 2008). Some argue that the blooms occur naturally (Cadée & Hegeman, 2002; Jones & Haq, 1963; Gowen *et al.*, 2008) and although they might be a nuisance to tourisms (Cadée & Hegeman, 2002), *Phaeocystis* species were not included in the harmful algal list by Smayda (1997).

The micro-flagellate *Prymnesium parvum* is know to produce a toxin that is associated with fish mortalities (Shilo & Aschner, 1953 and references therein; Holdway *et al.*, 1978 500). So also do *Heterosigma akashiwo* (Keppler *et al.*, 2005), *Chrysochromolina polylepis* (Dahl *et al.*, 1989; Lindahl & Dahl, 1990) and species of *Chattonella* (Okaichi, 1985).

In addition to being important from an ecological perspective, the hacrobia and especially the cryptophytes are of pivotal significance in terms of our understanding of endosymbiosis and the evolution of plastids. This is because cryptophytes acquired

photosynthesis by the process of secondary (i.e., eukaryote-eukaryote) endosymbiosis, having engulfed and assimilated a red algal endosymbiont at some time during their evolutionary past (Archibald & Keeling, 2002; Bhattacharya *et al.*, 2003; Gould *et al.*, 2008). As a result, cryptophytes are extremely complex from a genetic and cell biological perspective. These species have a four-membrane-bound plastid and four distinct DNA-containing cellular compartments: plastids, mitochondria, host nucleus and endosymbiont nucleus. The latter is referred to as a nucleomorph and is the focus of ongoing research aimed at understanding the pattern and process of secondary endosymbiosis (Gilson & McFadden, 2002; Archibald, 2007). A great diversity of genera is found in marine habitats. Dense populations of cryptophytes often develop in the deep-water layers of freshwater lakes (genus *Cryptomonas* Pedros-Alio *et al.*, 1995; Gervais, 1997; 1998). Due to their biliprotein light harvesting complexes, cryptophytes can photosynthesise in low light conditions (Gervais, 1997; Hammer *et al.*, 2002).

Cryptomonads were once suggested to belong to the chromista, the tripartite lineage comprised of the cryptomonads, haptophytes and photosynthetic stramenopiles (Cavalier-Smith & Swift, 1986) as these three lineages each possess a secondary plastid of red algal origin.

The prasinophytes belong to the green algae group which is characterised by the presence of only two unit membranes. Species of this lineage lost phycobilisomes and evolved thylacoid stacking instead (Cavalier-Smith, 2000). The prasinophytes are characterised by a quadrangular or ellipsoid cell shape and with one, two, four or eight flagella (Moestrup & Throndsen, 1988). The cells are either naked or with organic scales covering cell body and flagella. Some species have eyespots, located within the chloroplast (Throndsen, 1997).

The euglenozoa is a monophyletic group consisting of single-celled flagellates with very different modes of nutrition, including predation, osmotrophy, parasitism, and photoautotrophy. Characteristically, cells have an elongated sometimes ovoid outline with a canal at its anterior end. The most distinctive characteristic is the eyespot, that is present in most species belonging to this group. Predatory euglenozoans are phylogenetically widespread within the group and show a wide diversity of feeding apparatus, feeding strategies and prey preferences (Leander *et al.*, 2001; Leander & Keeling, 2004). Most predatory euglenids are adapted to move and feed on surfaces and they are important components of the microbial biota that live on the surface of many sediments. Osmotrophic euglenozoans are heterotrophs that lack a feeding apparatus and absorb nutrients directly from their environments. Photoautotrophy is restricted to a specific subclade of euglenid euglenozoans and originated via secondary endosymbiosis between a eukaryovorous euglenid and green algae (Gibbs, 1978; Leander & Keeling, 2004).

Heterotrophic flagellates belong to a small group of free-living organisms, the jakobida. There are only a dozen described species and these are generally less than 15 μm in length (mostly <10 μm). They have two flagella, which insert near one end of the cell (the anterior end in swimming cells). One of the two flagella can be difficult to see by light microscopy in some loricate species (Petersen & Hansen, 1962; Nicholls, 1984). Free-swimming jakobids have been recorded in marine and freshwater and in soil (Simpson & Patterson, 2001).

Some images of the different micro-flagellates are displayed in Figure 2.9

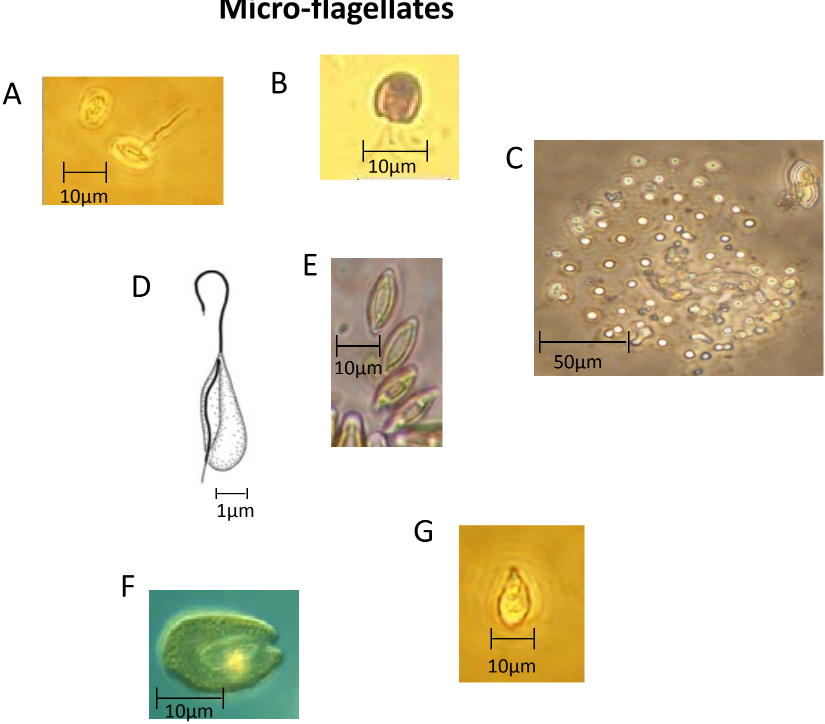


Figure 2.9: Images of micro-flagellates. A Cryptophytes, B *Chrysochromulina* (with permission of planktonnet.awi.de), C colony of *Phaeocystis* sp., D heterotrophic micro-flagellate (with permission of Patterson, 1990; http//tolweb.org), E Prymnesiophyte, F *Cryptomonas* spp. (with permission of Emden-Hoef 2009; http//tolweb.org), G *Cryptophyte*. Images that are not explicitly associated with a source were taken by the author during this study.

2.3.7 Discussion

The increasing use of various equipment such as electron microscopy has played an important role in the identification of genera and species as it has facilitated the visualization of minute structures and patterns of microplankton. The introduction of molecular techniques into the investigation of taxonomic relationships at all levels has greatly increased our ability to differentiate between species and to describe new

ones. The use of molecular techniques has allowed scientists to increase the number of informative characters to determine the evolution of organisms, but not to avoid the inherent difficulty of inferring ancient events (Philippe, 2004). The advantages of modern techniques can also bring some confusion because of the frequency with which information is updated. The classification of organisms is a very dynamic process and even at higher taxonomical levels there is often little agreement (see Williams & Kociolek, 2007). An example is the research on *Skeletonema costatum*, that was believed to be a single species (Kooistra *et al.*, 2008). However, genetic sequencing has shown that there are several distinct species (Kooistra *et al.*, 2008). The question is where do modern techniques stop being helpful and start to become confusing? It is important to know where phyla, classes, orders and even species derived from and to be aware of their evolution to understand their role in ecosystem structure and functioning. But discovering that species or genera are not exactly the same genetically does not necessarily mean that they do not belong to the same functional group or that they suddenly have different roles in the ecosystem. Do the different species of *Skeletonema*, for example, function differently and therefore play different roles in the marine ecosystem structure and functioning? The question is how much detail about microplankton species is needed to understand their ecological role and explain shifts or changes in community structure? This question is addressed by introducing the idea of lifeforms in following the sections and the literature on the principle of microplankton succession and the factors influencing microplankton and is hoped to bring more clarity.

2.3.8 Conclusion

After reviewing the literature on the origin and evolution of dinoflagellates, ciliates, diatoms and micro-flagellates one key aspect stands out. The introduction of molecular techniques in the 1980s was a milestone in the resolution of microplankton ancestry. It is apparent that the traditional division of microplankton into zooplankton and phytoplankton was too simplistic and that the microplankton is comprised of more than obligate heterotrophic and autotrophic organisms. The different nutritional modes of microplankton is of vital importance as it bears directly on trophic interactions, especially the linkages between the microbial loop and the classical metazoan food web and helps to explain ecosystem structure and functioning. This background information is useful and important when it comes to forming species and genera into lifeforms.

2.4 Microplankton in Marine Pelagic Food Webs

The marine pelagic food webs are unique as they are inherently variable, driven largely by meteorology and climate on a range of spatial and temporal scales. Microplankton as primary producers form commonly the fundamental base of marine pelagic food webs. At the simplest level a food web is a food chain. A pathway of the transfer of energy and matter between feeding or trophic levels. A food web is made up of many inter-linked food chains. The sum of trophic interactions forms an intricate web.

The classic view of a pelagic food web was that of an efficient energy transfer via a short food chain from large diatoms to copepods to fish (Hardy, 1924). But since the 1970s that classical view has been modified and changed. It had become clear that autotrophic picoplankton (0.2-2 μm sized plankton) plays an important role in primary production (Pomeroy, 1974). The discovery of the microbial loop (Williams *et al.*, 1981; Azam *et al.*, 1983) that involves the utilization of carbon by bacteria, protozoa, and small copepods (Edwards & Burkill, 1995) has added to the complexity of marine food webs and to the rates of flux of inorganic and organic material in the oceans. Picoplankters have a considerable role in the export of carbon from the surface to deeper waters (Richardson & Jackson, 2007). Joint & Pomeroy (1983) and Joint & Williams (1985) discovered that up to 75% of the primary production in the Celtic Sea in autumn was by phytoplankton smaller than 5 μm (i.e. picoplankton) and that the base of the food web in the Celtic Sea for the autumn period consisted mainly of pico-phytoplankton and autotrophic micro-flagellates. Literature labelled these micro-flagellates as nano-flagellates while it distinguished between photo-autotrophic nano-flagellates (PNAN) and heterotrophic nano-flagellates (HNAN). Studies have reported that ciliates feed on picoplankton and nanoplankton (Stoecker & Evans, 1985; Bernard & Rassoulzadegan, 1990), supplying a tight link in the transfer of energy from the microbial components in the food web to higher trophic levels (Azam *et al.* 1983; Sherr*et al.*, 1986). It is now known that heterotrophic protozoa, especially, oligotrich ciliates (Pierce & Turner, 1992), heterotrophic dinoflagellates (Hansen, 1991), and zooflagellates (Fenchel, 1984) are the main consumers of small microplankton (Tett*et al.*, 2003b). The microzooplankton might even be able to control the size of diatom blooms (Irigoien *et al.*, 2005). Thus, heterotrophy amongst the microplankton including ciliates makes the food-web interactions more complex. Legendre & Rassoulzadegan (1996) pointed out that scientists generally distinguished between two pathways in the pelagic ecosystem. A short, efficient one from large diatoms and copepods to fish (Cushing, 1989) with large energy export to higher trophic levels and another inefficient one that comprises the microbial loop, small phytoplankton, cyanobactedria, heterotrophic bacteria and protozoa. However, Legendre & Rassoulzadegan (1996) suggested that

there is in reality a continuum of trophic pathways between these extreme systems, and that various points along this continuum are characterised by specific combinations of dominant organisms and nutrient-cycling processes.

The importance of this newly discovered trophic components and the understanding of interactions has led to a re-evaluation of the pathways and rates of transfer of energy in marine pelagic food webs.

An example for a marine food web illustrating the interaction of microplankton is given in Figure 2.10.

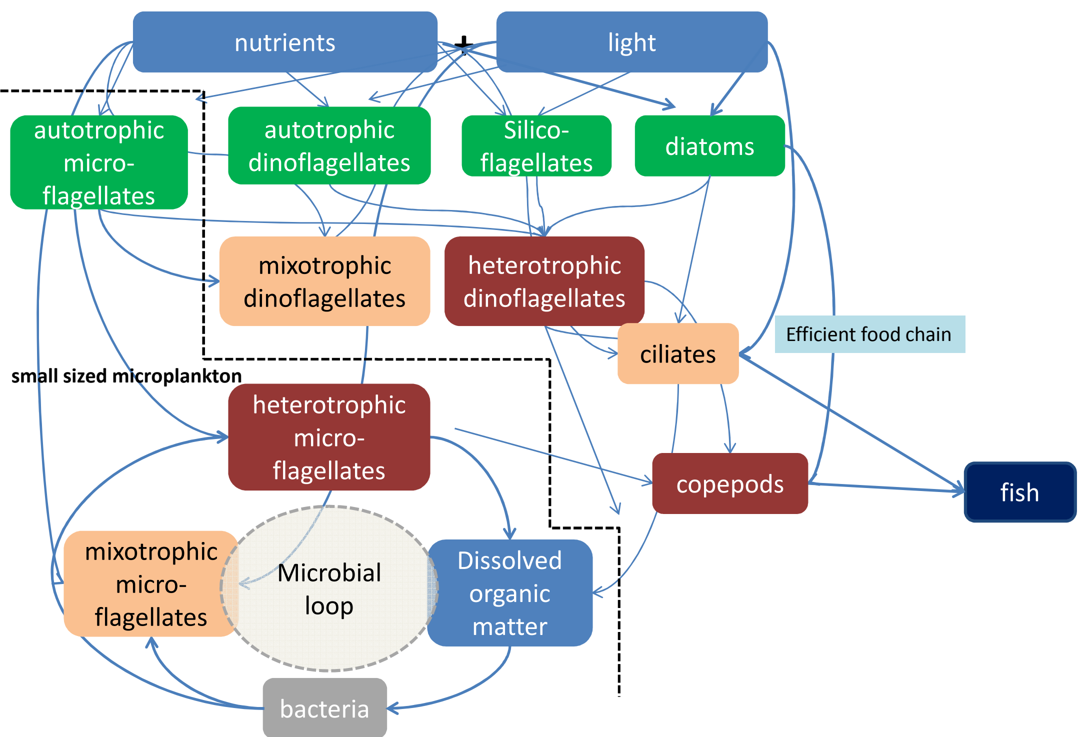


Figure 2.10: An illustration of a simplified marine food web for microplankton, including HNANs and PNANs and the microbial loop. The light blue coloured boxes represent the dissolved matter that is essential for the photosynthesising organisms (primary producers) illustrated in green. The orange coloured boxes represent the mixotrophic functional group that is capable of photosynthesis and also feeding on particulate matter while the red boxes illustrate the heterotrophic organisms that are the secondary producers. Bacteria are coloured in grey as they represent another functional group possessing the ability of N-fixation. Fish as the top predator in most marine systems are represented in dark blue. The dashed line indicates the component of small sized microplankton including the microbial loop that was added to the food web in the 1970s as an important part of a more complete marine pelagic food web.

2.4.1 Microplankton Dynamics in Temperate Shelf Seas

Microplankton growth is the result of a complex interplay of factors such as light intensity, temperature and nutrient supply affected by mixing and stratification as well as predation (Cloern, 1996).

It is generally accepted that the start and duration of the production season is controlled by the availability of light through the depth of the surface mixed layer (Gran & Braarud, 1935; Sverdrup, 1953; Pingree *et al.*, 1978; Smetacek & Passow, 1990; Tett, 1990). The euphotic zone represents the water column reaching from the surface of the sea to a depth where the light intensity falls to 1% of that at the surface (Kirk, 1983). However, Tett (1990) suggested that the base of the euphotic zone may be 0.1%. In the euphotic zone microplankton is most abundant. The bottom of the euphotic zone is called the compensation depth and its depth depends on the extent of light attenuation in the water column and the angle of the sun relative to the sea surface. Therefore, there is a critical, mean irradiance in the surface mixed layer that triggers the onset of the production season (Sverdrup, 1953; Pingree *et al.*, 1978; Smetacek & Passow, 1990; Tett, 1990). The energy at the compensation depth is such that production of organic matter by photosynthesis in respect to microplankton cells balances consumption by respiration (Zeitschel, 1978). Below this threshold, irradiance is not sufficient for net primary production. The integral above the depth where respiration (R) and production (P) are the same (R=P) is called the critical depth and was defined by Sverdrup (1953).

Behrenfeld (2010) 'abandoned' Sverdrup's hypothesis of critical depth. He demonstrated with his work with over nine years of remote sensing and satellite observation that the critical depth hypothesis of Sverdrup is an inadequate framework for understanding vernal blooms. Behrenfeld (2010) further pointed out that criticisms have been raised by others before (see e.g. Smetacek & Passow, 1990; Backhaus *et al.*, 2003). The one recuring issue was the observation of significant spring phytoplankton bloom in the apparent absence of water column stratification (see Behrenfeld, 2010 and references therein).

Wind and tidal energy are important features that influence the dynamics of water bodies in temperate shelf seas. In winter, wind generally keeps the water column vertically mixed and light is the limiting factor for microplankton growth. Nutrients accumulate from different sources such as rivers, atmosphere and local water-column and sea-bed recycling of biologically produced organic compounds while microplankton growth is minimal. Little of the solar radiation penetrates through the water column, not enough to create a euphotic zone and therefore favourable conditions for microplankton growth. Even on sunny calm winter days the onset of production is dependent on the irradiance (I_{sml}) and the depth of the surface mixed water layer

(SML). With increasing angle of the sun more light reaches down the water column creating a deeper euphotic zone. The upper part of the water column simultaneously becomes warmer due to the penetration of the sun and stratifies. The mixed layer decreases and reaches a point where it coincides with the euphotic zone. These conditions are favourable for the development of a spring bloom.

In estuaries and coastal areas where tidal energy is often too strong for the development of thermal stratification, freshwater inflow can create vertical gradients in salinity leading to haline stratification.

In low tidal energy regions when the mixed water layers slowly get replaced by stratifying waters in the beginning of spring, microplankton is exposed to higher irradiance in the upper water column and grows rapidly (Barber & Smith, 1981; Chang *et al.*, 1992) while nutrient supply rapidly decreases.

During the growing season the availability of nutrients determines the level of production. Nutrients influence phytoplankton growth and composition on two levels. The first level is qualitative as it concerns the crude distinction of what microplankters need i.e. organisms that require silicate and others that do not require silicate. The second level is quantitative and deals with the idea that optimum ratios of nutrient elements required for growth, may differ amongst species or lifeforms (Tett *et al.*, 2003a). Officer & Ryther (1980) pointed out that it is also necessary to take account of recycling rates, which are faster for P than N and faster for N than Si. With sufficient silica supply in the water typically large diatoms are the first group able to take advantage of the spring conditions as they have very short doubling time and production is higher than respiration (Smith & Kemp, 2001; Chang *et al.*, 2003). The time-scale of photo adaptation for diatoms might be short, in the order of hours such as found by Lewis & Smith, (1983) compared to the photo adaptation of dinoflagellates suggested to be in the order of days. Diatoms accumulate biomass by virtue of efficient growth within conditions of weak stratification, increasing light and high concentrations of 'new' nitrate (Cushing, 1989). Later in the year, with increasing seasonal stratification, nutrients in the euphotic zone become scarce, silica is often the limiting factor for diatom growth as it is often replaced more slowly than dissolved available inorganic nitrogen and phosphate leading to algal senescence and rapid vertical export (Tamigneaux *et al.*, 1999). Dinoflagellates and other heterotrophic/mixotrophic microplankters that do not require silicate follow the diatom bloom. Grazing zooplankton together with the limitation of nutrients contribute to the decline of the spring bloom and the summer biomass of microplankton community remains at low levels. In the post-spring bloom condition nutrients are recycled through tight heterotrophic/autotrophic linkages involving the microbial loop (Malone *et al.*, 1988; Rivkin *et al.*, 1996; Tamigneaux *et al.*, 1999). Summer stratification prevents 'new' nitrogen input from the isolated bottom water and production relies

exclusively on regenerated nitrogen. Small nano-flagellates and picoplankton often dominate by efficient utilisation of low concentrations of nitrogen (Chisholm, 1992). Under stable stratification and well illuminated water column characteristics large dinoflagellates can be successful by maintaining position near the pycnocline to alternately move to the eutrophic zone obtaining light and to the nitracline obtaining nutrients (Cullen, 1982). Because post-bloom biomass is low due to small sized organisms, relatively little algal material is exported before being consumed (Smayda, 1970; Rivkin *et al.*, 1996). This means that energy transport up the food chain is low. In autumn, wind often initiates re-mineralisation processes due to mixing and "new" nutrients are released from the bottom water into the euphotic zone exceeding the nutrient uptake of the ambient microplankton population. The availability of the released nutrients often triggers an "autumn" bloom. In coastal areas this bloom is usually dominated by diatoms as sufficient silica is normally present. However, the diatom species that dominate the autumn bloom usually differ from the species that are found in spring. In coastal freshwater influenced regions where stratification is often absent, re-mineralisation of nitrogen is less important, as "new" nitrogen is available through rivers and permanent mixing and a distinct autumn bloom is often not apparent. The decline of the autumn biomass is usually caused by light limitation (Tett & Mills, 1991; Gowen *et al.* 2000; Mills *et al.*, 2003). By winter, the microplankton cycle is complete.

2.4.2 Microplankton Succession and its Theory

Communities

Cleve (1899) recognized associations of phytoplankton in the North Atlantic Ocean which he termed "plankton types". According to Cleve (1899) discrete groups of species were characteristic of and restricted to particular marine areas. As a result the occurrence of particular species or species assemblages was taken to reflect the movement of water bodies and mixing between them. It was pointed out by Gran (1912) that this view was too rigid and Gran argued that while it was possible to distinguish species on the basis of latitude and as oceanic or coastal, it was also possible for an assemblage of species to evolve in a water body as conditions in that water body changed. Braarud *et al.* (1953) used the term "communities" for phytoplankton assemblages which characterized particular water masses in the North Sea. Williams (1981) discussed the community concept and phytoplankton and came to the conclusion that it was *"inappropriate to apply the term community to what are, in effect, more or less transient assemblages or associations having no substantial affinities between them"*. Williams (1981) proposed a more neutral term: *"species assemblage" avoiding implications other than stochastic association unless so specified.* In this study the term community is used as defined in the Oxford

dictionary: *"group of interdependent plants or animals growing or living together in natural conditions or occupying a specified habitat"*.

The Paradox of Plankton

There are about 4,000 species (Sournia *et al.*, 1991) that make up microplankton in the worlds oceans. This 'richness' has been a source of debate since the idea of competitive exclusion. Hutchinson (1961) called it the "paradox of the plankton" as it was believed that *"phytoplankton coexisted in an isotrophic or unstructured environment competing for the same sort of material"*. Hutchinson tried to explain the possibility for coexistence by the conventional niche differentiation and species exploiting the light gradient in the water column, but discounted this, viewing the water as being too well mixed (Hutchinson, 1961).

The principle in general was that no two species can occupy the same niche. The number of co-existing species cannot exceed the number of limiting resources. Richerson *et al.* (1970) tried to explain the plankton paradox with spatial and temporal variability in hydrodynamic conditions operating to prevent competitive exclusion. The presence of micro scale feature in stratified waters and associated patches of plankton referred to as 'thin layers' (Dekshenieks *et al.*, 2001) and the observation that different species may dominate different layers (McManus *et al.*, 2003) might confirm the arguments of niche differences in stratified waters. The inherent spatial and temporal variability evident in the marine environment prevents equilibrium communities to become established and enables a sufficient number of niches to exist and allow high species diversity.

Pianka (1972) advanced the niche-overlap hypothesis by stating that in rarefied environments where there is no significant competition, niches can overlap. Tilman's (1977) hypothesis of co-existence was that each species evolves to have its own optimum proportion of resource and as long as that proportion is not the same as that of another species, these species can coexist. Tilman's (1977) competitive model showed that co-existing species even increased in number when the variability of nutrient flux rates was increased (Tilman's resource-based simulation). With these results in mind, Tilman turned Hutchinson's (1961) question "why are there so many phytoplankton species in the sea?" into "why are there so few species in the sea?". The answer is supposedly found in the dynamics of competitive interactions and the resource gradient in natural systems. Margalef (1963) stated that mixed populations or biotic communities in a steady state are the exception in nature and that there is commonly a continuous shift in their structure, which is continuously readjusting to a changing environment. Margalef (1963) argued that all dynamics of communities involved in the directional change towards a stable state fall under the heading succession and in 1967 he was the first scientist to suggest that the distribution of microplankton in the oceans is "something unique". With hindsight,

it would appear that in the 1960s the views of the water column and nutrient dynamics were very simplistic. The idea of competitive exclusion was based on laboratory experiments, equilibrium conditions being reached and competition for a single resource. Scientists agree that there is no one universal hypothesis to explain "the paradox of the plankton" and Smayda (1980) stated that there is no necessity for an explanation as the paradox is merely an artefact (see also Kilham & Kilham, 1980).

Succession

Margalef (1978) believed that the variation in the supply of external energy in the form of light and turbulence was the main factor controlling the seasonal succession of microplankton in the sea. Smayda (1980) underlined Margalef's statement and emphasised that evidence has grown that species successions are, in fact, of major significance to microplankton dynamics and in coupling the microplankton community to higher trophic levels. Smayda (1980) also described the term succession as a continuous change of the taxonomic composition of microplankton communities, and the abundance and relative dominance of the different species and algal groups present. Kilham & Kilham (1980) proposed that seasonal succession sequences observed in nature are the result of inter-specific competition along resource ratio gradients which are changing over time owing to the variable supply rates for each resource. Tilman *et al.* (1982) proposed three broad factors influencing microplankton succession.

– physics: utilisation of differences in the capacity of species or lifeforms to grow in physical environments that differ especially in their vertical mixing intensity

– nutrient ratios: the relationship between the ratio of nutrient elements needed for growth and the ambient ratio of these elements

– grazing: variable loss rate due to grazing by protozoans or zooplankton that preferentially take some species or lifeforms rather than others

Officer & Ryther (1980) stated that nutrients play an important role in microplankton succession because re-mineralisation returns N, P and Si to solution at different rates and thus processes separate these nutrients. Silicate is slowest therefore it is often limiting.

In the Mediterranean Sea Margalef (1963; 1967) identified four stages of succession, with each stage dominated by the following:

1. small, colony forming flagellates and diatoms like *Skeletonema* and *Chaetoceros*;
2. medium to large sized chains of diatoms (e.g. species of *Thalassiosira* and *Guinardia*) and small to medium sized dinoflagellates like *Ceratium* and *Pro-*

rocentrum;

3. large, cylindrical celled diatoms like *Rhizosolenia* and an increasingly larger dinoflagellate population;

4. large motile dinoflagellates dominating the biomass and micro-flagellates representing the highest abundance.

According to Kilham & Kilham (1980) the fourth stage of succession that Margalef observed in the Mediterranean Sea is never reached in coastal and estuarine temperate waters. Their work also suggested that flagellates are numerically always important, but are only recognised as such when the total microplankton population decreases.

In 1978 Margalef developed the first conceptual model of succession, Margalef's 'Mandala' (Mandala defined by the Oxford Dictionary: *"graphical representation of the diagram of life"*). With this model, Margalef introduced 2 key elements: r and K strategists and lifeforms. The basic theory of succession is that there are r-selected fast growing species that require high nutrients (opportunistic species, where evolution favours productivity) and K-selected species with a slow but persistent accumulation of biomass at low nutrient levels (where evolution favours efficiency). The r-selected species are found in rarefied environments, whereas the K-selected species represent the equilibrium. Succession occurs between r-selected species when D/S ratio (demand to supply ratio) for various nutrients is increased by nutrient utilization by the species themselves. r-selected species compete for increasingly scarce limiting nutrients while K-selected species are unaffected and therefore fitter and more competitive. When the D/S ratio is close to 1, K-selected species are dominant and when conditions improve and ratios change, a shift takes place within the equilibrium community.

It is generally accepted that dinoflagellates appear to be K-strategists (Wyatt, 1974) as their maximum growth rate is low (Kain & Fogg, 1958; Eppley *et al.*, 1970; Seliger *et al.*, 1971). Further, dinoflagellates are less abundant during early stages of succession than for example diatoms that are mostly seen to be r-selected and increase in importance as nutrients decline and stratification grows stronger (Kilham & Kilham, 1980). Only very specific environmental conditions permit dinoflagellates to out-compete r-selected species (i.e. most diatoms). Eppley & Harrison (1974) hypothesized red tides to occur in nutrient-depleted surface water when a steep, shallow thermocline is underlain by nutrient-rich water.

Lifeforms

Margalef's theory of marine microplanktonic lifeforms dates back to ideas about biomes in terrestrial plant communities and the origin of the lifeform concept in Raunkiaer's classification of plant life in 1903 (see Tett, 1987). The terrestrial biome

is *"characterized by a uniform life form of vegetation, such as grass or coniferous trees"* (Smith, 1992). Biological communities like woodlands or coral reefs have a recognisable physical structure and a diverse collection of primary producers. Both can be seen as contributing to the community structure. In European woodlands for example, the member species belong to tree (top level), shrub (understorey) and herb (ground) layers of vegetation. Different lifeforms characterise these layers. In the case of microplankton the meaning of community structure includes microplankters but not the physical structure element as this is absent in pelagic systems. The seasonal changes and therefore the succession in dominant species can be considered to represent the same ecological function as layers in woodland and therefore its structure.

Margalef's 'Mandala' (Margalef, 1978) described the interactive effects of turbulent mixing and nutrient conditions on the selection of lifeforms and their seasonal succession including a red tide trajectory (see Figure 2.11). Microplanktonic lifeforms lay along a continuum from r-selected species to K-selected species.

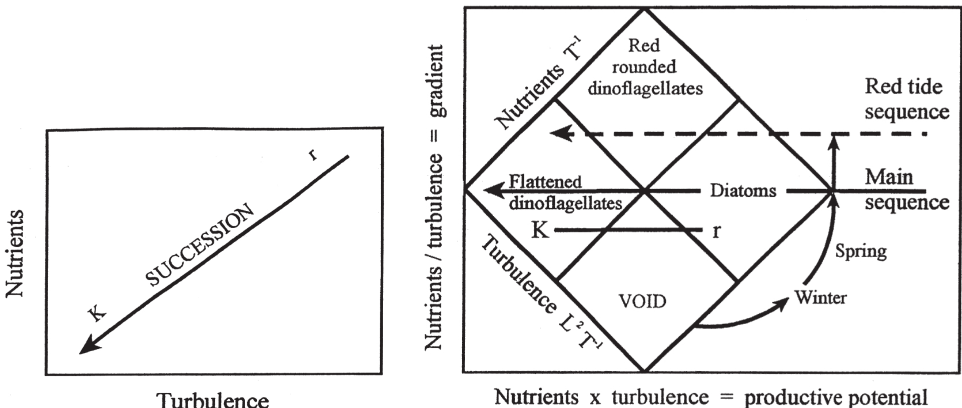


Figure 2.11: From a matrix summarizing the sequence of phytoplankton as a function of diminishing turbulence and nutrient availability (Margalef 1978) Margalef *et al.* (1979) developed a Mandala including a red tide trajectory.

Various authors such as Pingree *et al.* (1978), Bowman *et al.* (1981), and Jones & Gowen (1990) tested Margalef's 'Mandala' and demonstrated how the succession and distribution of different lifeforms of microplankton can be related to their physical and chemical environment.

Pingree *et al.* (1978) while not specially referring to lifeforms, described the effects of vertical stability on different population types representing successional series in an $s - kh$ diagram (where s represents the Simpson-Hunter stratification index, h water column depth and k the diffuse attenuation coefficient of photosynthetically active radiation) and explained how nutrient conditions suitable for a rapid growth of microplankton can be created by physical processes such as wind and tides.

Bowman *et al.* (1981) used a stratification and depth-scaled-by-light diagram to characterise the preferred physical environments of diatoms and flagellates.

Jones & Gowen (1990) used a similar approach to investigate the effect of interaction between stratification and irradiance regimes on phytoplankton community structure. Unlike Bowman *et al.* (1981) who used cell abundance, Jones and Gowen showed by using carbon biomass that diatoms prefer well-mixed water columns and dinoflagellates dominate stratified waters whereas microflagellates do not show any preference.

In some cases a lifeform based on biogeochemical or ecological function can include organisms from different taxa. Sieburth *et al.* (1978) proposed that the division into useful groups is to forget the taxonomic hierarchies and to split them when necessary into vernacular groupings on the basis of the level of organisation and the mode of nutrition. Ryther & Officer (1981) for example listed seven phytoplankter types which they ranked from the most beneficial, centric diatoms, by way of naked and scaled flagellates, green flagellates, pennate diatoms, dinoflagellates and non-motile greens, to the most undesirable bluegreens (i.e. cyanobacteria). Smetacek (1986) noted the category of tychopelagic diatoms for the large heavily silicified centric diatoms of shallow turbulent waters which are equally capable of living on the sea bed. Riegman (1998) distinguished large diatoms, small diatoms, haptophyta (Prymnesiophyceae, including coccolothophorids and the colonial *Phaeocystis*), dinoflagellates, mixotrophic algae and cyanobacteria by ecophysiological properties shared with other members of the same taxonomic group. Within each group may evolve specialists in nitrate- ammonium-, phosphate-, light-limited growth or with different temperature optima, and this may have lead to ecological clusters, meaning lifeforms that are cutting across taxonomical groupings. Reynolds (1987, 1996) and Reynolds *et al.* (2001) used the CSR conceptual model by Grimes (1979), and morphological characteristics, to distinguish a variety of lifeforms of freshwater phytoplankton. Where the CSR species are defined as:

C = invasive, r-selected, small, fast-growing, high surface-to-volume colonist species

S = acquisitive, large, slow-growing but biomass-conserving, K-selected, nutrient stress tolerant species

R = attuning, light-harvesting, attenuated, disturbance-tolerant ruderal species

Reynold's 'Intaglio' model (Reynolds, 1996) (Intaglio defined by the Oxford dictionary: *"engraved material or pattern"*) does not link the nutrient and turbulence axes as does Margalef's 'Mandala' but treats them as independent variables. Reynolds expanded Margalef's two dimensional concept to three dimensions (C-R-S concept). In his model Reynolds accounted for irradiance and its attenuation with depth as well as for nutrients and turbulence. This approach was applied to marine phyto-

plankton (Smayda & Reynolds, 2001).

Smayda & Reynolds (2001) used Margalef's 'Mandala' and Reynold's 'Intaglio' to develop a model explaining the apparent changing character of bloom species being selected and their blooms as indicators of specific habitat conditions along an onshore-offshore, mixing-nutrient gradient and separated the dinoflagellates into nine different lifeforms.

Weithoff (2003) proposed six traits as valuable for characterising lifeform aspects of freshwater phytoplankton including size, nitrogen fixation, demand for silica, phagotrophy, motility and shape. With this approach he referred to processes that were also reflected in the dynamic PROTECH model (see Elliott *et al.*, 1999; Reynolds *et al.*, 2001; Elliott *et al.*, 2001).

The discussion of lifeforms amongst heterotrophic as well as autotrophic proticts, leads on to the concept of plural micro planktons, the idea that there are persistent associations or reliable correlations between particular autotrophs and heterotrophs. Legendre & Rassoulzadegan (1996) pointed out that biological oceanographers have generally distinguished between two trophic pathways in the pelagic environment. One going from large phytoplankton to zooplankton to fish and one comprises small eukaryotic algae and cyanobacteria as well as heterotrophic bacteria and protozoa. Legendre & Rassoulzadegan (1996) suggested that there is a continuum of trophic pathways between these extreme systems in reality, and that various point along this continuum are characterised by particular combinations of dominant organisms and nutrient-cycling processes. Lee *et al.* (2003) have distinguished microplankton on their silicate requirement i.e. silicate users and non-silicate users.

Other developments in lifeform theory were reviewed by Tett & Wilson (2003) based on function and taxonomy. Tett & Wilson (2003) distinguished groups of factors that could identify and distinguish lifeforms in relation to ecosystem sustainability. There are four examples:

1. Their functionality in relation to biogeochemical cycling of bio-limiting elements like C, N, P, Si, S, O and perhaps Fe and Co. There are two levels that could explain variations in microplankton composition here. The first one is qualitative, and concerns the distinctions between algae that require silica and those that do not. The second level is quantitative, and concerns the idea of optimum ratios of nutrient elements required for growth which may differ amongst lifeforms.

2. The functionality of organisms in relation to the marine foodweb. Distinction here was made between prey as primary producers (e.g. diatoms) and predators (e.g. ciliates, some dinoflagellates and flagellates).

3. The relationship to the physical environment (e.g. turbulence, velocity, light)

as considered by Margalef (1978).

4. Taxonomy with differences between for example, organisms possessing thick silical cell walls (e.g. diatoms) or cellulose theca (e.g. armoured dinoflagellates) and whose that lack these (e.g. naked dinoflagellates, microflagellates).

Aspects that could also influence the separation of groups within different lifeforms are size and pigmentation. The idea of lifeforms that was focused on in this project was to categorise species with common ecological functions and common requirements and relate changes in lifeform communities to changes of their physical and chemical environment. As there are no guidelines or specifications for lifeforms different characteristic features can be taken to distinguish groups into lifeforms.

2.4.3 Considered Lifeforms

In the approach of categorising species into lifeforms, pigmentation was accounted for with the background of different nutritional modes of the microplankters with respect to primary production and carbon cycling. Size distinction was made with the focus on the discrimination of prey size and predators with respect of different trophic levels within the food web and the microbial loop. Taxonomical distinction was accounted for with respect to the origin of the organisms and the "deep taxonomy" of the phylian groups diatoms, dinoflagellates, micro-flagellates and ciliates with the addition of silicate and non-silicate users (as proposed by Lee *et al.*, 2003). On the basis of the literature review on the origin of microplankton and their functional role in the marine food web, the following lifeforms were considered in this study: functionality silicate and non-silicate users while the silicate users were distinguished between pelagic and tychopelagic (heavy diatoms such as *Pleurosigma*) diatoms, chain forming and solitary diatoms, and silico-flagellates. In the non-silicate user lifeform it was distinguished between dinoflagellates and within that naked and armoured dinoflagellates, micro-flagellates that categorised into *Cryptophytes*, *Prasinophytes*, *Premnesiophytes*, *Euglenophytes*, heterotrophic micro-flagellates and nano-flagellates, and ciliates and tintinnids. Further I made the distinction between autotrophic, heterotrophic, and mixotrophic, and small ($< 40\mu$m) and large ($\geq 40\mu$m) sized microplankton.

2.5 Summary and Conclusion

All microplankton undergo seasonal succession which is influenced by physical, chemical and biological factors. The great diversity of microplankton is not a paradox but a consequence of species evolutional adaptation to the fine differences of

the ecohydrodynamic conditions that are found in marine pelagic ecosystems.

The theory of lifeforms follows the idea of certain species or taxa sharing criteria that characterise them as one lifeform and describes their seasonal succession even beyond their taxonomic hierachy. Proposed models and indices by various authors have shown that the appearance and non-appearance of lifeforms (or species) are related to the hydrodynamics in aquatic ecosystems and that lifeforms can display aquatic ecosystem structure like plants communities display terrestrial ecosystem structure with the physical ability on land.

The question raised in the first part of this chapter as to 'how much detail is needed to understand the ecological functioning and importance of marine microplankton' might be answered by the following: A lot of detailed knowledge is needed to understand succession of microplankton and why and when species become dominant or disappear. It is also necessary to understand that the food web is a very complex system with a lot of interaction between organisms, tight linkages, energy transfer through the system and physical and chemical factors that have an impact on the organisms growth and cycling. Engaging in this knowledge and for the purpose of detecting a change in ecosystem structure it is essential to choose the categorisation into lifeforms very carefully. However, I believe it is not essential to be able to identify all different species of e.g. *Skeletonema* as long as it is certain that these species do not differ in their functional role as small (less than 40μm), autotrophic, silicate-user, chain-forming, opportunistic members of the community in pelagic marine ecosystems.

After reviewing the literature on the evolution of microplankton, diversity and seasonal succession, pelagic marine food webs and microplankton dynamics in temperate shelf seas with respect to all influencing factors, one conclusive key element is that microplankton ecology needs to be approached in the mechanistic (proximate) and evolutionary (ultimate) way in an attempt to understand its functioning. On a short-term basis for example, species' interactions based on nutrient competition may determine species shifts in relative abundances, but factors influencing the long-term survival of a species may be most important in determining the size, shape, or metabolic efficiency of a particular organism.

The proposal of this project is to use lifeforms (rather than species) to detect change in microplankton community structure caused by different nutrient loadings. Results of a microplankton community structure model as well as the results of the following chapters will show whether this is a satisfactory approach.

Chapter 3

Materials and Methods

3.1 Introduction

This chapter sets out the rationale for selecting sampling sites, describes the fieldwork that was carried out with its sampling routine, method development and the evolution of sampling procedures based on a series of experiments. The use of a high temporal resolution remote sampling system (hereafter high frequency sampling) was a particular challenge in terms of the need to develop a procedure to distinguish autotrophic and heterotrophic microplankton in these preserved samples. A discussion and conclusion of the method developments sums up the findings at the end of the chapter.

3.2 Selection of Sampling Sites

The sites chosen for this project had to meet certain criteria. The first sampling site needed to be 'undisturbed' from anthropogenic nutrient enrichment and one at which high resolution sampling could be conducted to develop a detailed microplankton data base for the development of a Microplankton Community Index (MCI). The second and third sampling sites had to be suitable to apply the MCI to test the hypothesis that anthropogenic nutrient enrichment influences the structure of microplankton communities.

The selection criteria for the sites were based on the need to:

- undertake high frequency sampling of microplankton;
- carry out the sampling within the project budget;
- collect supporting environmental (physical and chemical) data with the same or a similar sampling frequency;

– find an area with low anthropogenic influence that could be representing good environmental status as required by the MSFD;

These criteria were important for the site that delivered the data for the MCI development. For the two test sites the MCI was going to be applied to, it was important that the above criteria were fulfilled (except the last one) and also that the prevailing hydrodynamical conditions were similar and the nutrient conditions were different, preferrably with low anthropogenic nutrient influence at the reference site.

The project could have been performed with a sampling bottle and a hand-held conductivity, temperature, depth (CTD) instrument from a pier in Belfast Lough. This would have delivered high frequency sampling and supporting environmental data. It would have been easily accessible, and the costs would have been low. However, it was impossible to find two sites in Belfast Lough that would have had similar hydrodynamic conditions but different nutrient levels. Sampling in other sea loughs in Northern Ireland and the Republic of Ireland with small boats was considered but excessive travelling time and cost to achieve high frequency sampling ruled this option out. Another option was to embed the project in a large ongoing research programme with logistical and data support, cost efficiency and a certain assurance that the study could be achieved. This was considered the best option because it provided the convenient option for this project as all sites were embedded in fully funded programmes. The AFBI mooring site (station 38A) in the western Irish Sea (see Figure 3.1) was chosen to be the sampling site for developing the tool. This site has been used as part of ongoing biological oceanographic and fisheries investigations conducted by AFBI and so an extensive knowledge about its chemical and physical characteristics was already present. The second and third sampling site (Liverpool Bay in the eastern Irish Sea and West Gabbard in the Outer Thames estuary, southern North Sea (see Figure 3.1)) were both mooring sites chosen for applying the tool and testing the hypothesis. The sites were part of a fully funded Cefas Monitoring Programme.

The first site (station 38A) was chosen at the beginning of the project (in February 2008) to ensure a high sampling frequency for a period of two years. The other two sites were chosen later when project objectives were finalized. This raised an issue in relation to the inter-calibration of microplankton analysis between me and the analysts from Cefas. The environmental data from station 38A were kindly provided by AFBI. Cefas kindly provided the environmental and microplankton data for Liverpool Bay and partly for the West Gabbard.

3.2.1 The Irish Sea and the southern North Sea

General Oceanography of the Irish Sea

The Irish Sea is located on the north western European continental shelf and bounded by the land masses of Ireland and the UK. The geographical borders of the sea are taken between 52°N (St David's Head to Carnsore Point) and 54°40' (Mull of Galway). The Irish Sea is a small coastal sea with a volume of 2430km^3, which is less than 10% of the volume of the North Sea. The water in the region to the east is generally less than 50m deep with some extensive shallow (approximately 20m) coastal areas. Waters towards the west are generally deeper and a trough 80 to 100m deep extends north south through the western Irish Sea. Transport through the region is generally considered to be northwards with the main mass of water passing to the west of the Isle of Man, leaving via the North Channel. Water from the Atlantic and Celtic Sea provide the source water for the Irish Sea. The overall residence time of water in the Irish Sea is about 12 months although in some regions water may remain longer (Dickson & Boelens, 1988). It is evident, that local meteorological conditions, particularly wind forcing, have a major impact upon flow through the two channels and hence volume transport and residence time (Knight & Howarth, 1999). The regional differences in tidal amplitude and freshwater input as well as the bathymetry of the Irish Sea result in distinct hydrographic areas. Lowest salinities are measured in the eastern Irish Sea reflecting the pattern of freshwater inflow (Gowen & Stewart, 2005). The spatial distribution of salinity to the west of the region indicates a tongue of more saline water extending northwards through the western Irish Sea. This appears to be a consistent winter feature (Gowen & Stewart, 2005 and references cited therein). The distribution of salinity also suggests limited exchange between the eastern and western Irish Sea although radionuclide distributions indicate some east west transport (Leonard *et al.*, 1997) probably north of the Isle of Man. Due to deep water and weak tidal flow (25 cm s^{-1}) south west of the Isle of Man, temperature stratification develops during the summer (Gowen *et al.*, 1995; Horsburgh *et al.*, 2000). In the seasonal stratification area, bottom density gradients are associated with a dome of cold bottom water and there is a near surface gyre (Hill *et al.*, 1994) that may be important in retaining planktonic organisms within the stratified region (White, 1988). The transition between the stratified and mixed waters is marked by a tidal mixing front (Simpson & Hunter, 1974). In the two channels, turbulence generated by strong tidal flows is sufficient to keep the water column vertically mixed most of the year. According to Gowen & Bloomfield (1996) the microplankton production is well defined by the different hydrodynamics of the Irish Sea. For example, there appears to be a wave of production beginning in shallow Irish coastal waters (<50m) in March/ April. This wave extends to offshore waters (>50m) of the western Irish Sea in response to stratifi-

cation of the water column in April/ May. Microplankton production occurs even later and for a shorter time in coastal waters off Northern Ireland and in the North Channel.

Although the Irish Sea is fairly small its natural resources are of considerable economic importance. According to Connolly & Molloy (2000) the value of fish and *Nephrops norvegicus* (Norwegian lobster) landings in 1998 for example has been estimated as £43 million. Estimates for 2008 have been £24 million for 19,529 tonnes of fish, crustacea and shellfish (Northern Ireland State of The Seas Report 2011).

General Oceanography of the North Sea

The North Sea is part of the wider north west European shelf with a total area of 575.000 km^2. It extends from the English Channel in the South, and the Orkney and Shetland Islands in the Northwest, with a broad open boundary to the Atlantic, connecting to subarctic waters in Norwegian regions where it meets the Norwegian Sea in the North (56°N, 3°E). It is bounded by the Orkney Islands and East coasts of England and Scotland to the West and the coasts of Belgium, Netherlands, Germany, and Denmark to the East and South. It is a semi-enclosed basin with a general counter clockwise circulation, primarily open to the Atlantic in the North (Hill, 1973). Water from the Atlantic provides the main input, although there is an inflow through the English Channel and the Baltic Sea via Skaggerak with significantly low salinity influence (Hill, 1973). The northern and central regions of the North Sea typically stratify throughout summer (Pingree *et al.* 1978) with only the deepest parts of the northern North Sea ($>$ 80m depth) remaining stratified at the end of autumn (Hill, 1973). Due to its shallow depth and strong tidal currents the southern part of the North Sea is well-mixed year round normally opposing summer stratification (Howarth *et al.* 1993).

The mainly shallow features of the southern North Sea ($\approx$ 40m) provide excellent habitats for fish production and this region is still an important fishing ground in Europe (Mills *et al.*, 2005).

3.2.2 Location and Description of the Sampling Sites

The three sampling sites (see Figures 3.1, 3.2, 3.3) chosen were:

- AFBI mooring station 38A (38A) in the western Irish Sea (53°47'0 N 005°38'0 W)
- Cefas mooring station (LBay) in Liverpool Bay in the eastern Irish Sea (53°32'0 N 003°21'8 W)
- Cefas mooring station West Gabbard (WGabb) in the Outer Thames estuary, southern North Sea (51°59'0 N 002°05'0 E)

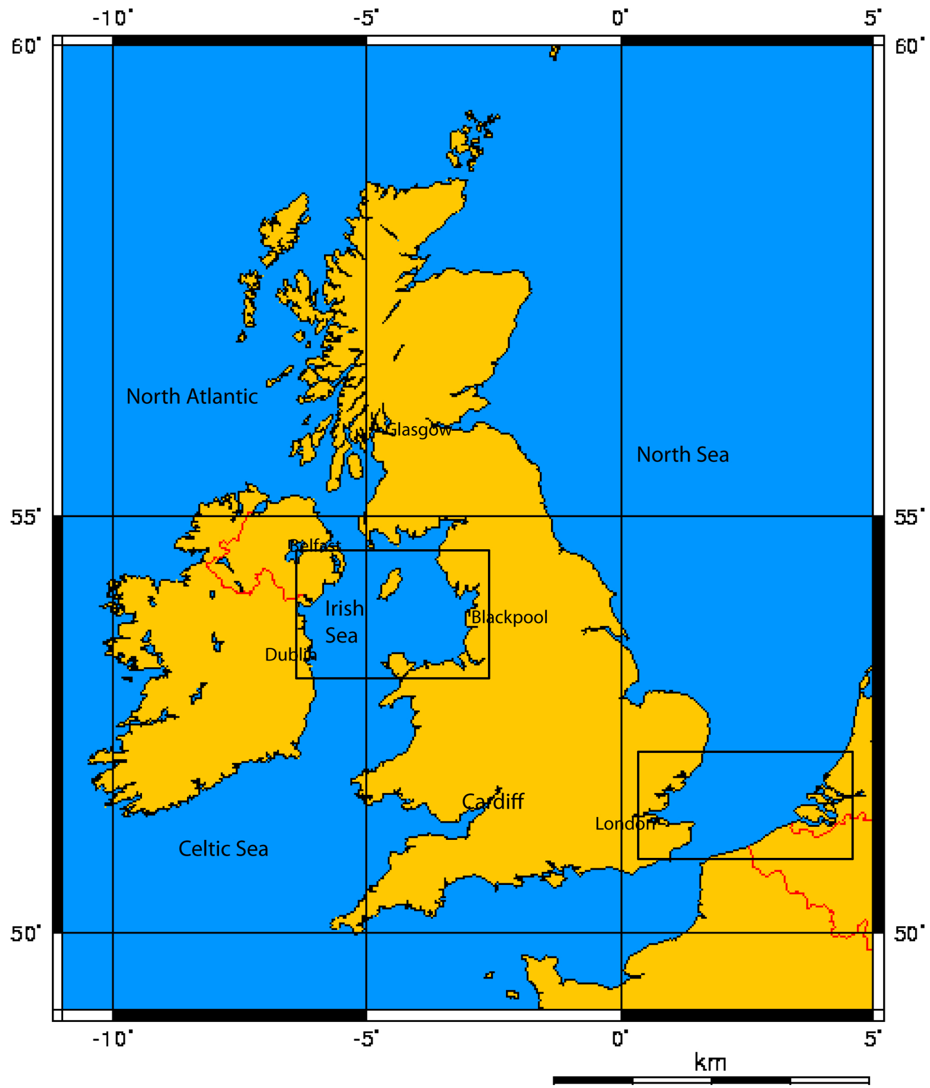


Figure 3.1: A map showing the United Kingdom and Ireland with their associated coastal seas and adjacent oceanic waters. The black boxes indicate the enlarged areas where the mooring sites are located in Figure 3.2 and 3.3. This map and the two following maps were created using the website www.aquarius.ifm-geomar.de, visited 24/02/2010.

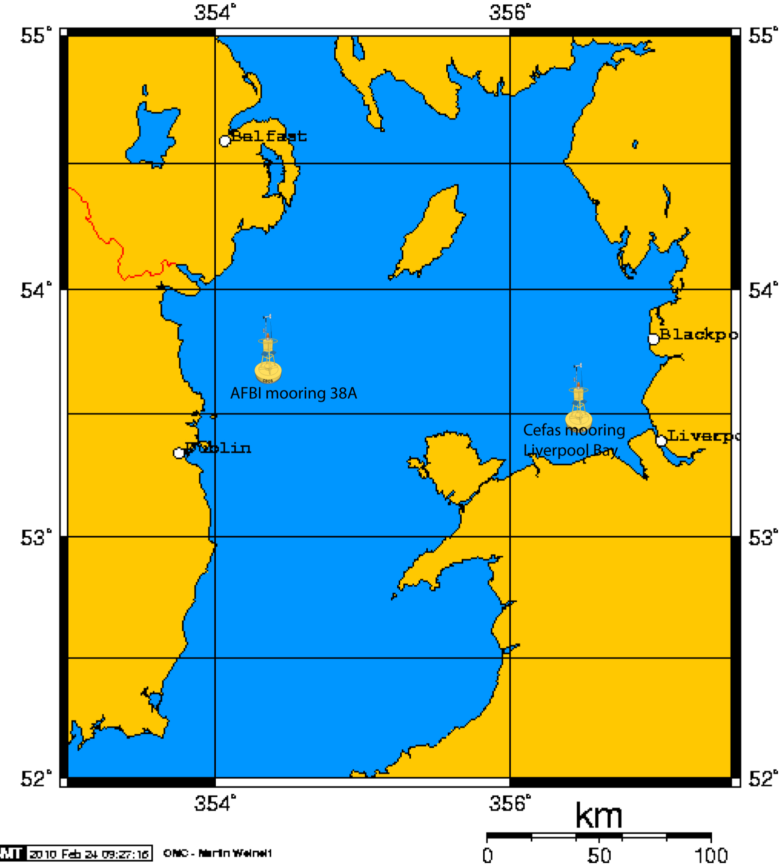


Figure 3.2: Map showing an enlargement of the Irish Sea with the location of the sampling sites AFBI station 38A and the Cefas mooring station Liverpool Bay.

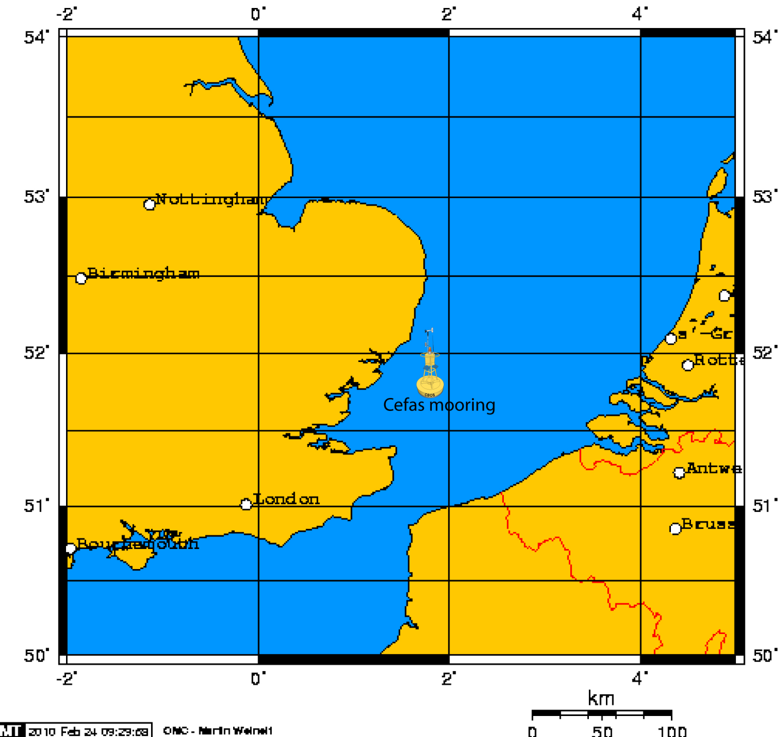


Figure 3.3: Map showing an enlargement of the location of the Cefas sampling West Gabbard site in the Outer Thames Estuary in the southern North Sea.

AFBI mooring station 38A

The AFBI mooring station 38A in the western Irish Sea, that was accessible during regular oceanographic surveys to the site, accommodated an automated access sampler attached to the 'U-shaped' leg of an instrumented mooring for high frequency sampling at a depth of approximately 14m (see Figure 3.4).

The sampling site was located ≈30 kilometres offshore in a water depth of 94 m. The mooring was in that part of the western Irish Sea that seasonally stratifies and thermal stratification of the water column in this region typically began in late April/early May. The bottom water remained isolated from surface water for four to five months (Horsburgh *et al.*, 2000; Gowen *et al.*, 2008). Winter nutrient levels of 0.72 μM dissolved available inorganic phosphate (DAIP), 7.7 μM, dissolved available inorganic nitrogen (DAIN as nitrate, nitrite, and ammonium), and 6.7 μM Silicate (SiO_2) are typical for this region (Gowen *et al.*, 2008). A typical observed seasonal production cycle lasts four to five months and coincides with the onset of thermal stratification (Gowen *et al.*, 1995).

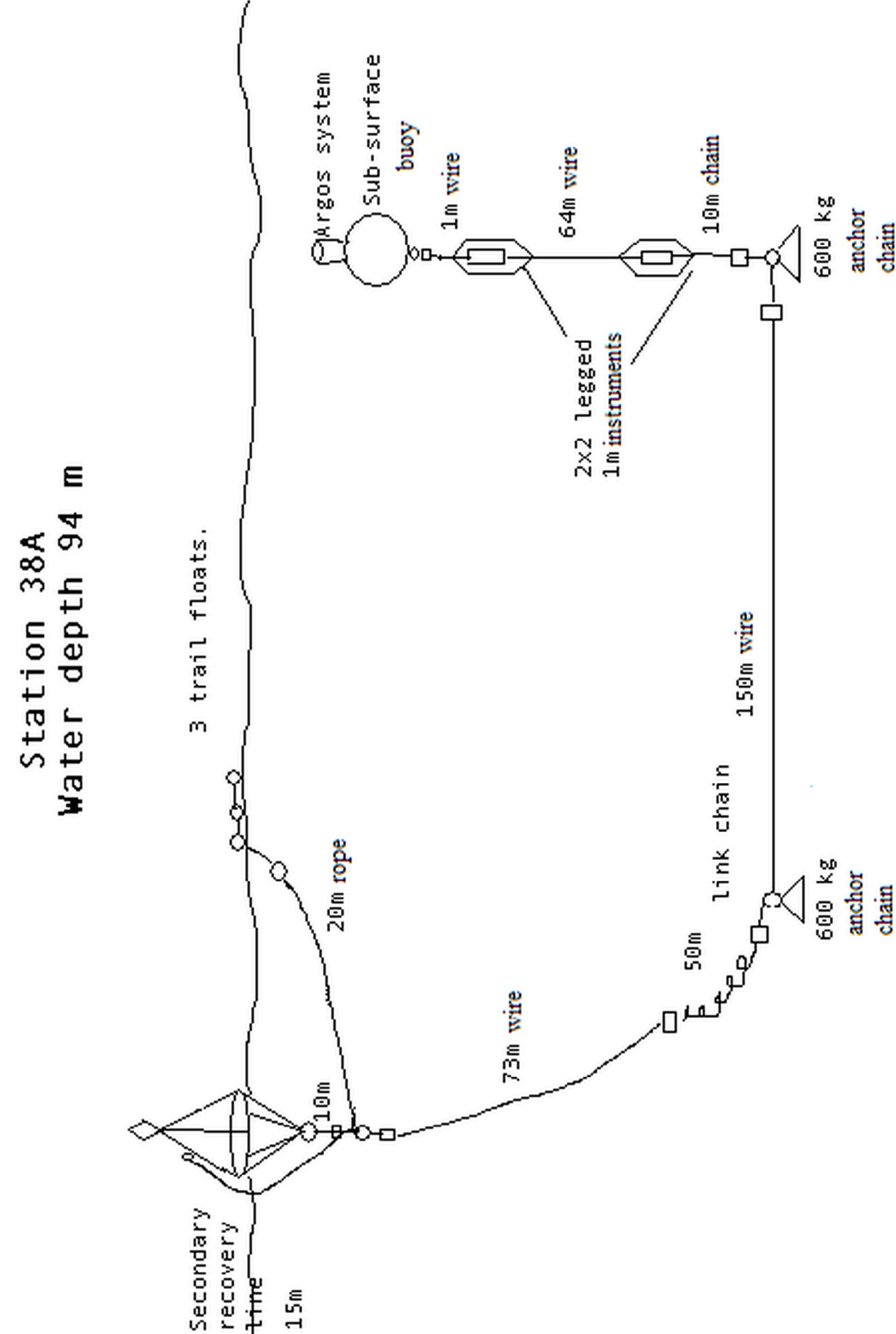


Figure 3.4: A schematic of a 'U'-shaped mooring like that operated by AFBI at station 38A in the western Irish Sea.

Cefas mooring station Liverpool Bay

The Cefas mooring station in Liverpool Bay (LBay) was located in a shallow (approximately 30m) coastal, generally mixed, freshwater influenced region (Region of freshwater influence, ROFI) (Tett *et al.*, 1993). Coastal waters of the eastern Irish Sea typically showed intermittent salinity stratification during the summer (Gowen *et al.*, 2000). Winter nutrient levels are high (2.0 μM DAIP, 30 μM DAIN and 17 μM SiO_2 (Gowen *et al.*, 2008)). Liverpool Bay also had a typical seasonal production cycle for a period of approximately five months starting in early May, but Gowen *et al.* (2000) concluded that anthropogenic nutrients had fuelled elevated spring and summer production. The Liverpool Bay mooring site was chosen to be the comparison site of the two test sites representing a site with high nutrient levels caused by anthropogenic influence. Figure 3.5 shows a schematic of a single point mooring equipped with a Smartbuoy used at both Cefas operated mooring sites. The West Gabbard mooring site was chosen to be the reference site of the two test sites representing a site with medium high nutrient levels.

Cefas mooring station West Gabbard

The Cefas mooring station West Gabbard (WGabb) was located in the Outer Thames estuary. The Thames estuary connects the Thames plume with the southern North Sea east of the English coast and is characterized by a moderately turbid, medium nutrient enriched and well-mixed aquatic ecosystem (Sanders *et al.*, 2001). The mooring site was located in approximately 35 m of water in a freshwater influenced region with intermittent salinity stratification. There were no published nutrient concentrations or seasonal production values available for this site as it was a newly established monitoring site. However, winter nutrient levels for a mooring site in the plume zone of the Thames (51°31'5 N 001°01'9 E) were 20μM SiO_2 and between 7.8 and 36.5 μM TOxN (total oxidisable nitrogen as nitrate and nitrite) (Suratman *et al.*, 2010). The West Gabbard mooring site was chosen to be the reference site of the two test sites representing a site with medium high nutrient levels.

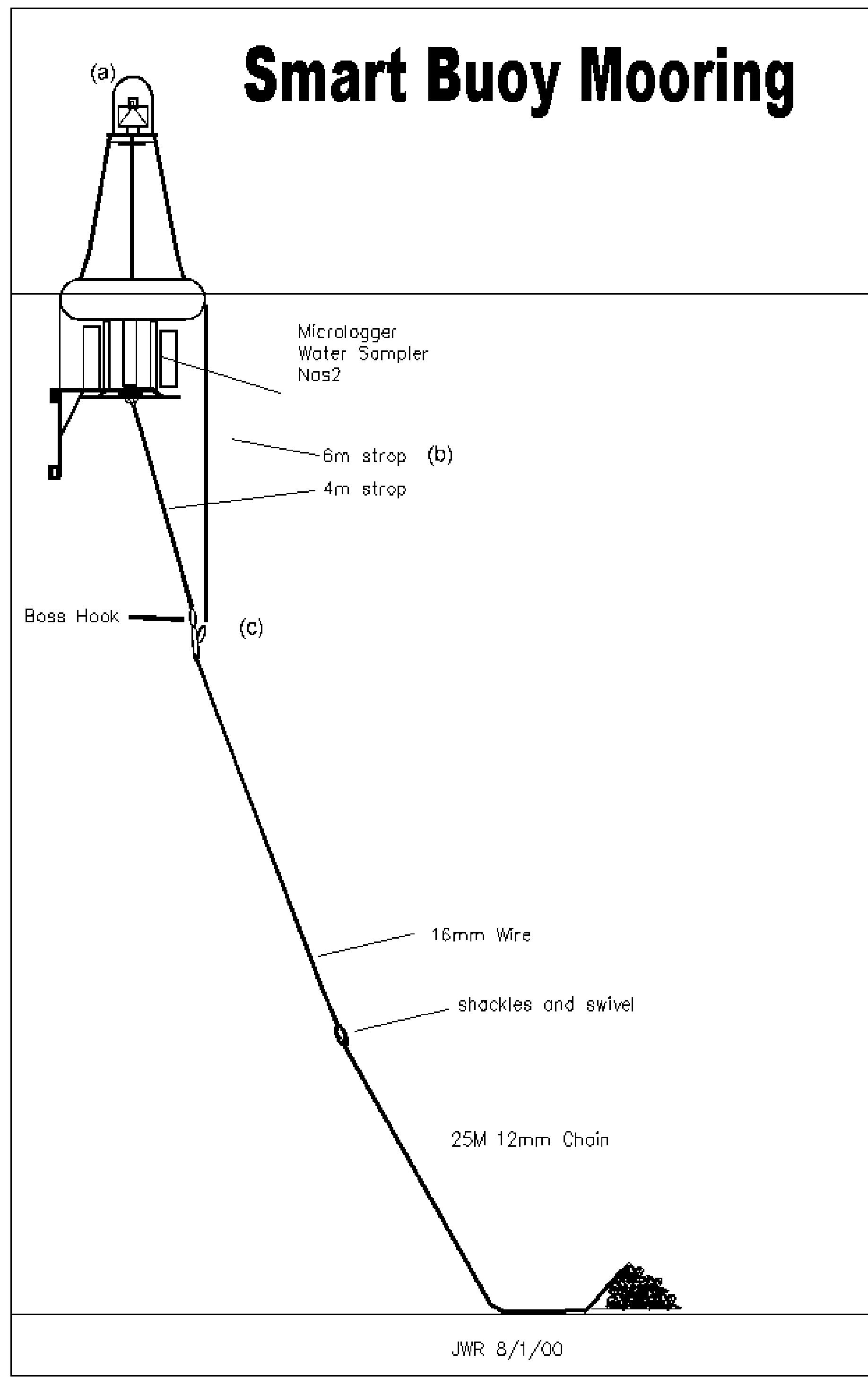


Figure 3.5: A schematic of the smart buoy 'single point mooring' used at the Cefas sampling stations in Liverpool Bay in the eastern Irish Sea and the West Gabbard site in the Outer Thames of the southern North Sea.

3.3 Sampling

The environmental data that were collected and used to support the microplankton data were: temperature, salinity, nutrients, in vivo fluorescence, and chlorophyll. For the Cefas stations photosynthetically available radiation (PAR) data were also available and so the euphotic zone could be determined after calculating K_d. Data collection was performed by two different methods. Details for all three sites are summarised in Table (3.1).

Table 3.1: Overview of the data collection; the instruments, the variables, and the frequency of collection for all three sites. Microplankton was given in cells L^{-1} and mg C m^{-3}, temperature in °C, nutrients in μM, fluorescence in relative fluorescence units (RFU), chlorophyll concentration in mg m^{-3} and column chlorophyll in mg m^{-2}, and irradiance in photosynthetically available radiation (PAR) to calculate K_d and with that the euphotic zone (z_{eu})- only available for the two Cefas mooring sites.

Instrument	Variable	38A	LBay	WGabb
RAS500(MI[1])	microplankton	daily-weekly	—	—
WMS-AquaMonitor	microplankton	—	monthly	every fortnight
Thermistors[2](MI)	Temperature	every 3 hrs	—	—
CTD (MI)	Temperature	every 3 hrs	every 2 hrs	every 2 hrs
CTD (RS[3])	Temperature	6-8 weeks	6-8 weeks	8-10 weeks
CTD (MI)	Salinity	every 3 hrs	every 2 hrs	every 2 hrs
CTD (RS)	Salinity	6-8 weeks	6-8 weeks	8-10 weeks
RAS100 (MI)	Nutrients	every 2 days	—	—
WMS-2 AquaMonitor	Nutrients	—	every 2 days	every 2-4 days
RS water sampler	Nutrients	6-8 weeks	6-8 weeks	8-10 weeks
CTD (RS)	Fluorescence	6-8 weeks	6-8 weeks	—
Fluorometer (MI)	Fluorescence	—	—	every 2 hrs
RS water sampler	Chlorophyll	6-8 weeks	6-8 weeks	—
Fluorometer (MI)	Chlorophyll	—	—	daily
Light sensors (MI)	PAR Irradiance	—	every 2 hrs	every 2 hrs

[3]MI = moored instrument with a fixed depth
[3]five thermistors attached to mooring wire from surface to bottom every 20 m
[3]RS = rosette sampler (with CTD attached) with vertical profiles during mooring surveys

Data collection at station 38A began on 17th February 2008 and ended on 31st December 2009. High frequency temperature data were recorded every three hours by thermistors attached to the mooring every 20m throughout the water column. Near surface conductivity and temperature were recorded with a CTD attached to the near surface buoy providing daily temperature and salinity data. Nutrient data were provided every two days by a remote access sampler (RAS-100) collecting water that was used to measure nutrients (dissolved inorganic phosphorous, dissolved inorganic nitrogen, and SiO_2 were analysed). Water samples for microplankton counts and species composition were taken on a daily to weekly basis (depending on the season) with the aid of a remote access sampler (RAS-500) the containers were pre-filled with 4 ml acidified Lugols iodine and every forth sampling bag contained 10 ml formalin. All moored instruments (except the thermistors) were sampling in a known but notional water depth of 12- 14 m.

Periodic sampling every six to eight weeks during surveys provided vertical conductivity, temperature, and depth (CTD) profiles as well as chlorophyll, in vivo fluorescence, and nutrients data for station 38A. These environmental data were kindly provided by AFBI.

The dataset from the LBay station accounts for a period between 1st January 2008 and 31st December 2009. TOxN and silicate measurements were made every two days with a WMS-2 AquaMonitor. Water samples for microplankton counts and species composition were also collected with an AquaMonitor instrument containing acidified Lugols iodine as preservative. Environmental measurements included conductivity and temperature, recorded hourly with CTD instruments to aid interpretation of the microplankton data during this study. Photosynthetically Active Radiation (PAR) was measured with a LiCor PAR irradiance sensor at 1 and 2 m depth every 2 hrs. All instruments were part of a Smartbuoy mooring and sampled at a water depth between 1 and 2 m and the sensor data were stored on a solid state data logger (ESM2). The nutrient analyser and water sampler were self-contained with their own power and logging capability. All data from the Liverpool Bay mooring used in this study (environmental and microplankton) were kindly provided by Cefas.

Periodic sampling every six to eight weeks at this station was made possible during AFBI oceanographic surveys in the eastern Irish Sea and provided vertical profiles of conductivity, temperature, and depth (CTD) as well as chlorophyll and nutrients.

At the WGabb station data collection began on 17th January 2008 and ended on 31st December 2009. Environmental data included conductivity and temperature measurements by CTD instruments to aid interpretation of the microplankton counts during this study. PAR was measured with a LiCor PAR irradiance sensor at 1 and

2 m depth every 2 hrs. Chlorophyll fluorescence was measured with a fluorometer attached to the Smartbuoy near the surface. TOxN and silicate measurements were made every two days with a WMS-2 AquaMonitor. Water samples for microplankton counts and species composition were also collected with an AquaMonitor instrument containing acidified Lugols iodine as preservative. Twenty- three microplankton samples collected between December 2008 and November 2009 were analysed by me during a visit to Cefas in Lowestoft.

Periodic sampling every eight to ten weeks at this station was operated by the Cefas Smartbuoy team during surveys and provided ten vertical profiles for salinity, temperature and fluorescence. All environmental data and the additional microplankton data were kindly provided by Cefas.

3.3.1 High Frequency Sampling using Remote Access Samplers and Moored Instruments

For the high frequency sampling (daily to weekly) of water samples for nutrients and microplankton, remote access samplers were deployed at the mooring sites. Data were down loaded during servicing of the mooring every six to eight weeks at station 38A. At the Liverpool Bay and the West Gabbard station the water samples for nutrients and microplankton were collected with a WMS-2 AquaMonitor instrument that was serviced every eight to ten weeks during surveys conducted by Cefas.

Remote Access Sampler (RAS-500) for Microplankton

To collect water samples for microplankton counts and species composition at high frequency at station 38A an automated remote access sampler (RAS)-500 (McLane Research Laboratories Inc., East Falmouth, Massachusetts) was attached to the submerged leg of the ‘U’-shaped mooring at the station in the western Irish Sea. It was attached at a notional but known depth of 14 m for practical reasons (space availability) and two meters beneath a similar water sampler (RAS-100) that was positioned to collect water samples for nutrient analysis. The RAS-500 was a time-series sampler designed to collect up to 48 individual 500 mL water samples and operate over a wide temperature (0 - 50°C) and depth range (up to 5500 meters). Pre and post sample acid cleaning cycles remove bio-fouling and other contaminants of the inlet tubing to keep the samples pure. The RAS-500 user interface controls a multi-port valve and displacement pump, directing the acid wash, cleaning cycles and fluid volume. During deployment, the system logged data such as battery voltage, sampling timing and performance (e.g. volume rate of sampling and volume flow of sampling). These data were downloaded after the RAS-500 was recovered to check the performance of the sampler for errors or defects. All components like the controller housing, pump assembly, multi-port valve, and sample containers were

mounted inside a protective stainless steel frame. The sampler was 128 cm high, 73 cm wide and 73 cm long. Images of the sampler set-up and the sampler attached to the u-shaped mooring are given in Figure 3.6.

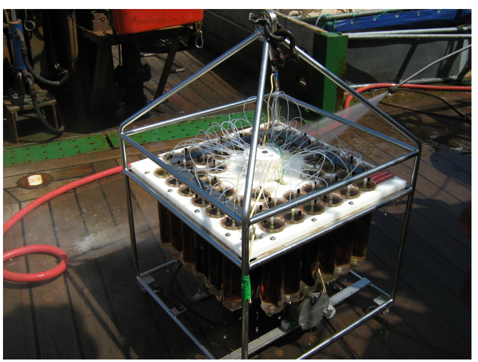


Figure 3.6: The 'RAS-500' on the deck of the *RV Corystes* (left image). And the re-deployment of the'RAS-500' instrument (right image).

The RAS pump was used to draw seawater into the sample containers and a multi-port valve directed the seawater to a particular sample bag. Each bag was connected in series between the intake head and the exhaust head. After each of the 48 samples was filled, the multi-port valve returned to the Home Port (0), sealing the sample in the bag (Figure 3.7). The whole system was operated by an alkaline battery pack, that had to be replaced when the voltage dropped to less than 28.0 volt. The programme for the sampler set-up (`ras.exe`) was activated and re-set once the sampler was recovered and connected to a computer. An example sheet for a sampling schedule for a particular programme and a particular time is given in the appendix listing 1 A.5.1.

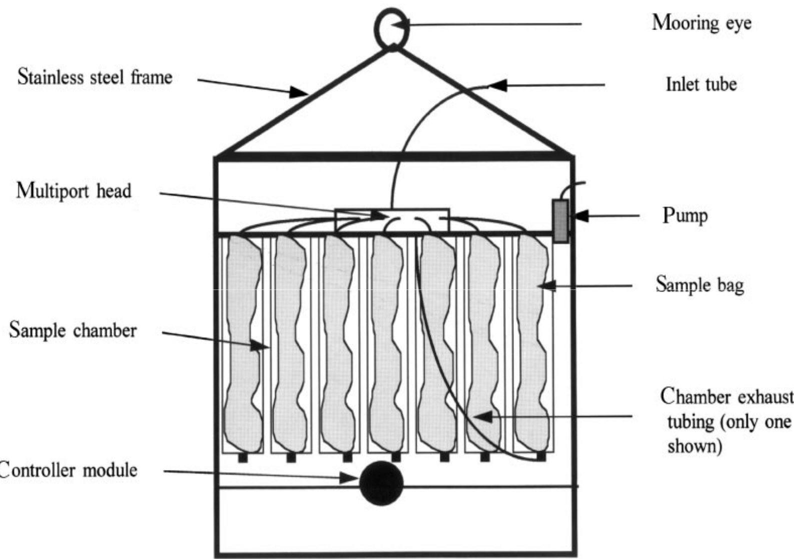


Figure 3.7: A schematic of the remote access sampler (RAS) that was used to collected water samples for microplankton and nutrient analysis.

AquaMonitor for Nutrients and Microplankton

The Cefas operated Smartbuoys were equipped with Envirotec water and microplankton sampler (WMS) AquaMonitors (Virginia, USA) for the water collection for nutrients and microplankton analysis. This instrument is for use within towed bodies and aboard taut-line moorings or monitoring buoys. The WMS takes up to 50 discrete water samples of up to 1000 ml each (Cefas used 200 ml for microplankton and nutrient analysis). The samples are acquired via a syringe-based mechanism operating with a 50 port valve assembly. A single stroke of the syringe is sufficient to fill each container. The sample storage containers are normally flexible transfusion bags which can be easily stowed in the towed body or, in the case of moored deployments, in a detachable housing. These containers are pre-loaded with preservatives as required (for microplankton and nutrients analysis Cefas used acidified Lugols iodine and mercuric chloride, respectively). The device can be programmed for autonomous sampling or operated as a "slave" within an integrated system such as a towed-vehicle. The sample volume and the time period of sampling can be determined manually and for in-situ sample processing. The instrument weighs about 8 kg with a dimension of 146 mm diameter x 623 mm and is neutrally buoyant in the water. It can be applied to water depth of up to 2500 m. An illustration is given in Figure 3.8.

Figure 3.8: An image of a water and microplankton sampler (WMS) Enicrotec AquaMonitor like that used by Cefas attached to the Smartbuoy moorings station Liverpool Bay and West Gabbard.

Microplankton Sample Recovery

The RAS 500- sampler for the microplankton sampling was attached to the AFBI mooring and deployed on 17th February 2008. From that time on, water samples were taken daily (during spring), every two (during summer), three or four days (during autumn). Weekly samples were taken during the winter period. Table 3.2

gives an overview of the frequency of sampling and period of the year, the time the samples were taken and the volume of each sample pumped into the sample bags. In the winter months, the volume of each sample was increased from 400 mL to 1200 mL (in this case 3 containers were used to achieve this volume) to ensure sufficient microplankton was collected. The sample bags were filled with 4 mL acidified Lugol's iodine before every deployment to give a final concentration of approximately 1%. From the third deployment (May 2008) onwards, every fourth bag was filled with 10 mL of formalin to give a final concentration of approximately 1% formaldehyde. This decision was made after it was found that Lugol's masks the chlorophyll flourescence of cells (Gifford & Caron, 2000). The autofluorescence of cells was important for the distinction of heterotrophic and autotrophic organisms. To account for the formaldehyde preserved samples, the sampler programme was altered to take two samples every third sampling date leaving one hour between each sampling (see sampler programme in appendix listing 1 A.5.1).

Table 3.2: Overview of the sampling time, frequency and sample volume for spring, summer, autumn and winter in 2008 and 2009 at station 38A.

Period	**Time (GMT)**	**Frequency**	**Sample volume**
spring	12:00:00	daily	400 mL
summer	12:00:00	2 days	400 mL
autumn	12:00:00	3 to 4 days	400 mL
winter	12:00:00	weekly	1200 mL

The RAS-500 was attached to the mooring for a period of 22 months (February 2008 - December 2009). Every six to eight weeks the sampler was serviced during visits to the sampling site. During these mooring services, each bag containing a preserved water sample was removed from its supporting container, transferred into a 250 mL amber glass bottle, and stored in the dark until further processing. The empty supporting containers were cleaned and topped up with freshwater, before new bags containing preservatives (either Lugol's or formaldehyde) were attached. A new control programme was initiated and the system was deployed after the instrument was cleaned and checked for defects. At the end of each survey the microplankton samples were transported to AFBI's laboratory at Newforge Lane in Belfast where they were stored in plastic boxes in a dark unheated storeroom until processed. In December 2009, sampling finished and the sampler was brought back on land, cleaned and stored away.

The following flow diagram (Figure 3.9) shows the procedure of sample processing

from the recovery of the RAS sampler on board the *RV Corystes* to the analysis of microplankton samples with an inverted microscope in the laboratory.

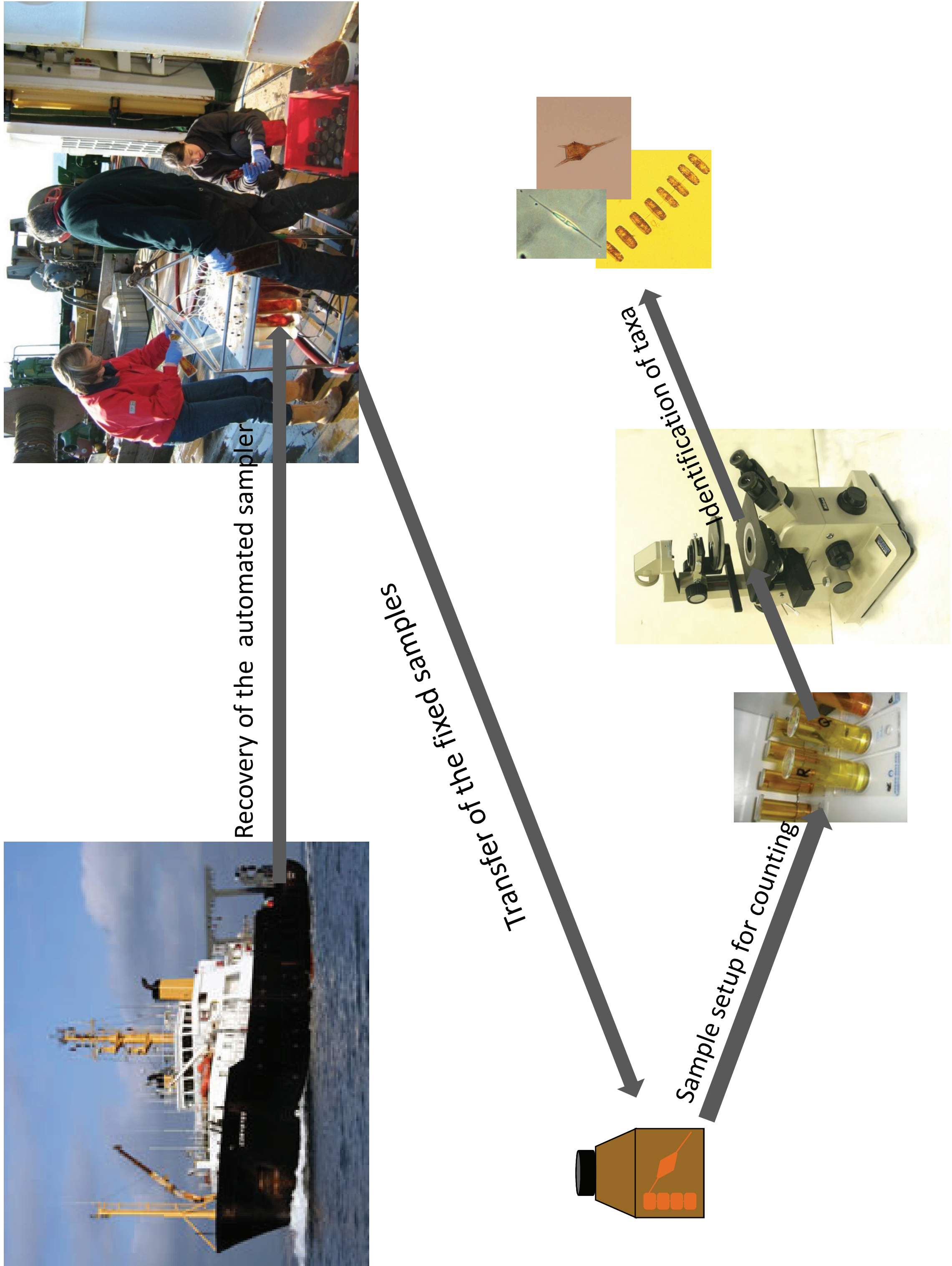


Figure 3.9: A flow diagram showing the procedure from the sample recovery at the mooring site to settling and analysing the samples in the laboratory.

Remote Access Sampler (RAS-100) for Nutrients

A remote access sampler RAS (McLane Research Laboratories Inc., East Falmouth, Massachusetts) was used to collect water samples that were used for the determination of dissolved inorganic nutrient concentrations at station 38A. The operating system of the RAS-100 nutrient sampler was similar to that of the RAS-500 and is therefore not described here. For the schematic of a RAS-100, see Figure 3.7. Sample bags for nutrients were pre-filled with 0.6 mL of 2 g L^{-1} mercuric chloride. Two 60 mL water samples were taken every two days at 12:00:00 GMT. One of the samples was taken through a polycarbonate filter (0.2mm) and one was taken without a filter. The reason for that was that, in the past, the filters were often blocked by particles and prevented sea water entering the sample bag. The sample container without the filter was therefore a “backup”. The preserved samples were transferred from the bags into Polycon vials and kept frozen at -20 °C until analysed.

Water samples for nutrient analysis at the Cefas operated moorings were collected with a WMS AquaticMonitor like that described above for water samples for micorplankton analysis.

Nutrients Analysis

Analyses were carried out following the AFBI SOPs (Standard Operation Procedures) MARCHEM002v3, MARCHEM003v3, MARCHEM004v3, for phosphate, oxidised nitrogen, and soluble silicate. A continuous flow analyser (α alpha-laval Bran & Luebbe TRAACS 800) was used for the analysis. Before analysis, samples were thawed, mixed and transferred to 5 mL sample cups. The three nutrients were analysed simultaneously and determined colorimetrically.

Orthophosphate and other labile phosphates reacted with acidic molybdate in the presence of catalytic amounts of antinomy to form a yellow heteropolyphosphomolybdate complex. The complex was reduced with ascorbic acid to molybdenum blue. The blue dye was determined at a wavelength of 880nm.

Nitrate was oxidised and reduced to nitrite with the use of a copper/cadmium reductor coil and then determined under acidic conditions with sulphanilamide hydrochloride and N-1-naphthylethyl-enediamine dihydrochloride to form an azo dye. The pink dye was determined at a wavelength of 550nm.

Silica reacted with acidic molybdate to form a yellow silicomolybdic complex. The complex was reduced by ascorbic acid to an intensely coloured blue complex. The blue dye was determined at the same wavelength as phosphate (880nm) but the interference was avoided by the addition of oxalic acid which suppresses the formation of the blue coloured phosphomolybdate heteropoly acid.

Moored Instruments

Thermistors

Five thermistors were attached to the mooring at station 38A at intervals of approximately 20 m from near the surface (surface buoy) to the bottom (wire of the anchor chain). The thermistors were set to record temperature every three hours throughout the year. The data were downloaded during service cruises using `minilog` operating software. The temperature data were stored in `ascii` files for later use in excel. The data delivered by the thermistors were also used to calculate Δt which is the value of the difference between the near surface temperature (at 2m) and near bottom temperature. Δt is a stratification index that indicates whether a water column is temperature stratified (Talling 1971). For the Cefas sites thermistor data were not available so Δt was determined using vertical temperature profiles taken during surveys to the mooring stations.

CTD Instrument

A Sea-Bird 19plus V2 CTD instrument (Sea-Bird Electronics, Inc., Washington USA) was attached to the instrument leg of the mooring at station 38A underneath the RAS-100 nutrient sampler. At both Cefas sites SmartBuoy moorings were used. Both were equipped with a CTD (Aanderaa 3919B), two light meter LiCor (flat sensor) Underwater Quantum Sensors, Envirotec WMS-2 AquaMonitor (Virginia, USA) samplers for nutrients and phytoplankton, and a Seapoint (Origin) chlorophyll fluorometer. The metal frame was build in house by Cefas.

Light Meters

The light meters were attached to the top of the toroid to an extended metal arm of the Cefas SmartBuoys (LBay and WGabb) underneath the surface buoy at 1 m and 2 m water depth. The sensors delivered values for the photosynthetically available radiation (PAR) every 3 hours during day light. These data were kindly provided by the Cefas Smartbuoy team. With the PAR values, K_d was calculated by the following formula:

$$[K_d] = \ln PAR1 / \ln PAR2 \qquad (3.1)$$

where PAR1 is PAR at 1 m depth and PAR2 is PAR at 2 m depth.

According to Kirk (1983) significant microplankton photosynthesis takes place only down to a depth at which PAR falls to 1% of that just below the surface. This depth is called euphotic zone (z_{eu}). With the assumption that K_d is approximately

constant with depth, the value of z_{eu} is given by:

$$[z_{eu}] = 4.6/K_d \tag{3.2}$$

The mooring site in the western Irish Sea was not equipped with light sensors during the sampling years. To have some comparable data for K_d at station 38A values were taken from Gowen *et al.* (2000) and more recent measurements in July 2010 (AFBI, unpubl. data).

3.3.2 Survey based Sampling

On visits to station 38A every six to eight weeks, water samples for nutrient and chlorophyll analysis were collected using a rosette water sampler with 12 5L water bottles assembled by Sea-Bird Electronics, Inc., Washington USA with a CTD instrument underneath. Up to May 2008, vertical profiles of depth, temperature and conductivity were recorded using a Falmouth Scientific International (FSI) 8001. Thereafter, a the Seabird SBE 32 (Sea-Bird Electronics, Inc., Washington USA) with a SBE 9plus CTD was used taking 25 measurements per meter. The salinity values that derived from temperature and conductivity measurements by the vertical CTD profiles were also used to calculate Δs, the surface to bottom difference in salinity. In recognition of the importance of vertical gradients in salinity (Gowen *et al.* 1995), a Δt+s was calculated to indicate stratification characteristics assuming that 0.1 salinity unit has the same density effect as 0.5° C.

Rosette Sampler and CTD Instruments

Figure 3.10 shows a schematic of a fully equipped SBE 32 similar to the model that was operated during oceanographic service cruises to station 38A from May 2008 onwards.

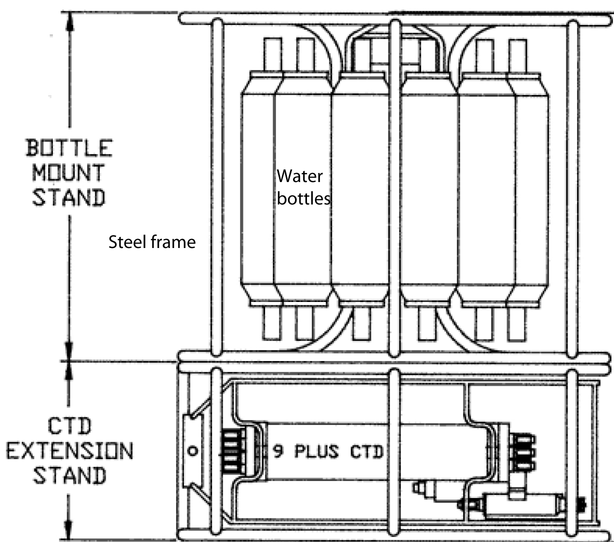


Figure 3.10: A schematic of the SBE 32 with which conductivity, temperature, and *in situ* fluorescence were measured and water samples were taken for nutrients and chlorophyll analysis.

Figure 3.11 shows the SBE 9plus CTD instrument. The CTD also carried a fluorometer for measuring in vivo fluorescence.

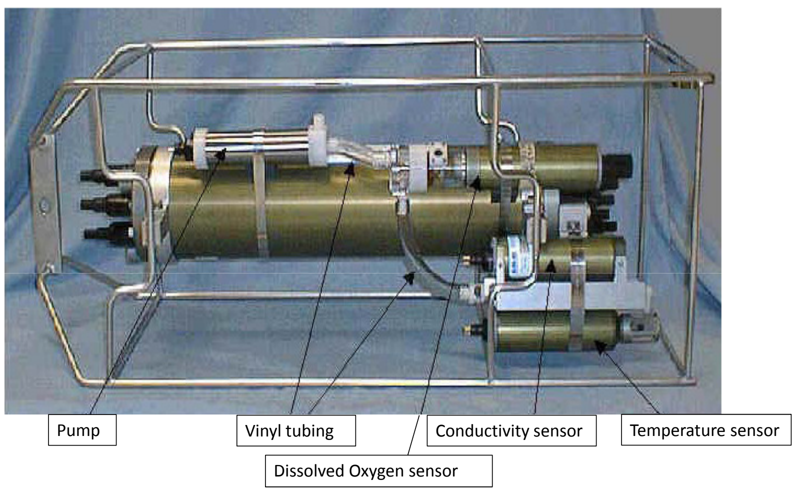


Figure 3.11: An image of the SBE 9plus CTD like that used at station 38A measuring conductivity, temperature, and *in situ* fluorescence.

The programme used to operate the instrument was SeaBirdElectronics (SBE) 11 plus V2 data processing software that converted `.hex` files into `.ascii` files. These were then converted in `.cnv` files which were readable by excel. Because the CTD

took approximately 25 measurements per meter during the complete "CTD cast" from the bottom to the surface of the water column, the data were averaged (running average) for every 0.5 m and used in later analysis. This made it easier to work with. The original data were retained.

Chlorophyll Analysis

Chlorophyll was determined according to Tett (1987). Water samples were collected from different depths using the rosette sampler. Most of the samples were immediately processed and analysed on board following AFBI's SOP MARCHEM008v3. Sub-samples of 250 mL were filtered through 2.5 cm GF/F Whatman filters and the pigments were extracted in 8 mL of 90% acetone under low light conditions at 4 °C for 24 hours. Measurements of the extracted pigments were carried out using a Turner Designs model 10A-U-005-CE filter fluorometer. Two readings were recorded. The first, Fo before and the second Fa after the addition of two drops of 8% HCL this was done to distinguish chlorophyll from phaeopigments. Chlorophyll and phaeopigment concentration (mg m^{-3}) was calculated using the following equations:

$$[C] = \text{K-(Fo-Fa) E/V} \tag{3.3}$$

$$[P] = \text{K ((H} \cdot \text{Fa)-Fo) E/V} \tag{3.4}$$

Where E is the extract volume in mL and V is the sample volume in L; K is the calibration coefficient for each sensitivity range of the instrument and H is the ratio of the specific fluorescence coefficient of chlorophyll and phaeopigment. The fluorometer was calibrated using an optical filter every time the instrument was used. When samples could not be processed immediately, they were filtered and the filters were frozen at -20°C until later analysis.

To estimate chlorophyll standing stock, discrete estimates of chlorophyll were used to derive values for every 1 m from the surface to the base of the euphotic zone. A MatLab script (`chlostock.m`) was used to calculate the chlorophyll concentration (mg Chl m^{-3}) every 0.5 m by linearly interpolating the chlorophyll data available. The chlorophyll values were then multiplied by 0.5 m to obtain chlorophyll standing stock (mg Chl m^{-2}). The sum of the chlorophyll concentrations gives the chlorophyll standing stock in mg Chl m^{-2}. The chlorophyll standing stock was used to illustrate the seasonal cycles of total column chlorophyll. The values that were available from the vertical profiles of the periodical sampling were used for this (for the WGabb station no calibrated chlorophyll values were available, thus uncalibrated fluorescence data were used instead at this site).

Nutrient Procedure

For vertical profiles of nutrients, water was collected from the different depths by the rosette sampler and was filtered through a polycarbonate filter (0.2mm) into 25 mL Polycon vials using a syringe. All samples were fixed with a final concentration of 20mg L^{-1} of mercuric chloride mixed and frozen at -20 °C until analysed. The analytical procedure were as described in nutrient analysis above (page 65).

Temperature and Salinity

Temperature was recorded by a temperature sensor taking 25 measurements every meter with an accuracy of 0.001° C (reference taken from the Seabird manual 2008). Salinity was calculated from conductivity, temperature, and pressure data.

3.3.3 Instrument Calibration

Regular calibrations of the instruments were performed. The CTD instruments were sent to the manufacturer every two years for re-calibration. Calibration of the temperature sensors was an ongoing process. An additional temperature sensor (Oceanographic Temperature Module or OTM) was attached to the moored CTD. The OTM and the Seabird temperature sensor delivered values with an accuracy of 0.0001 °C in 30 °C (Seabird Manual 2008). Salinity values derived from the CTD were calibrated using salinity measurements made on discrete samples using a bench salinometer. Water samples for this were collected during each survey.

The thermistor temperature calibration was a secondary calibration. Temperature values from the thermistors were calibrated against the corrected Sea-Bird CTD temperature values taken from depths corresponding to that of the thermistors.

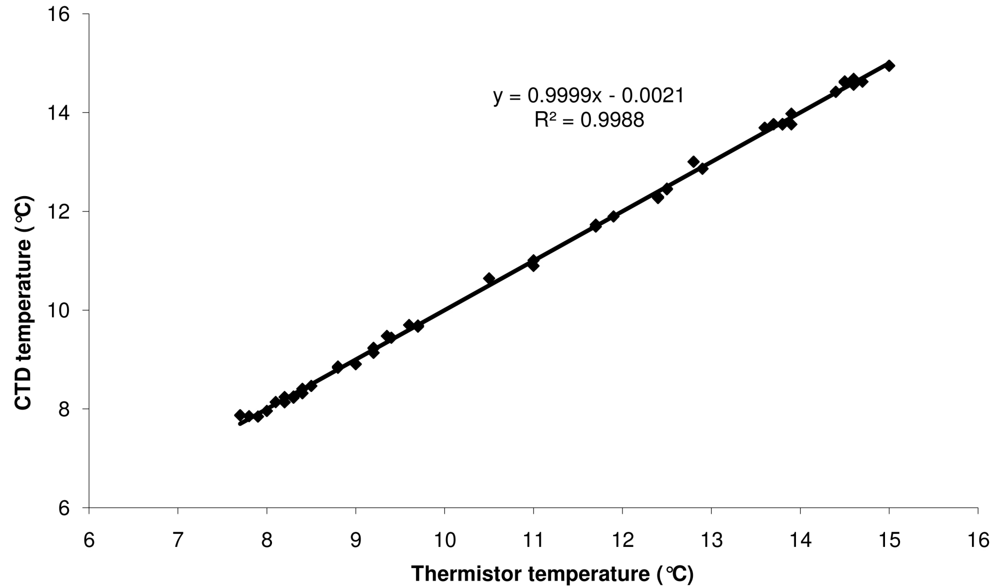


Figure 3.12: Regression of the calibration of thermistors against corrected CTD temperature values.

The Turner fluorometer was calibrated against a pure chlorophyll standard (Sigma chemical, London) and with a dilution series a regression plot was created. The instrument was checked prior to use. Fluorometer readings (before and after acidification) were regressed against concentration to derive calibration coefficients.

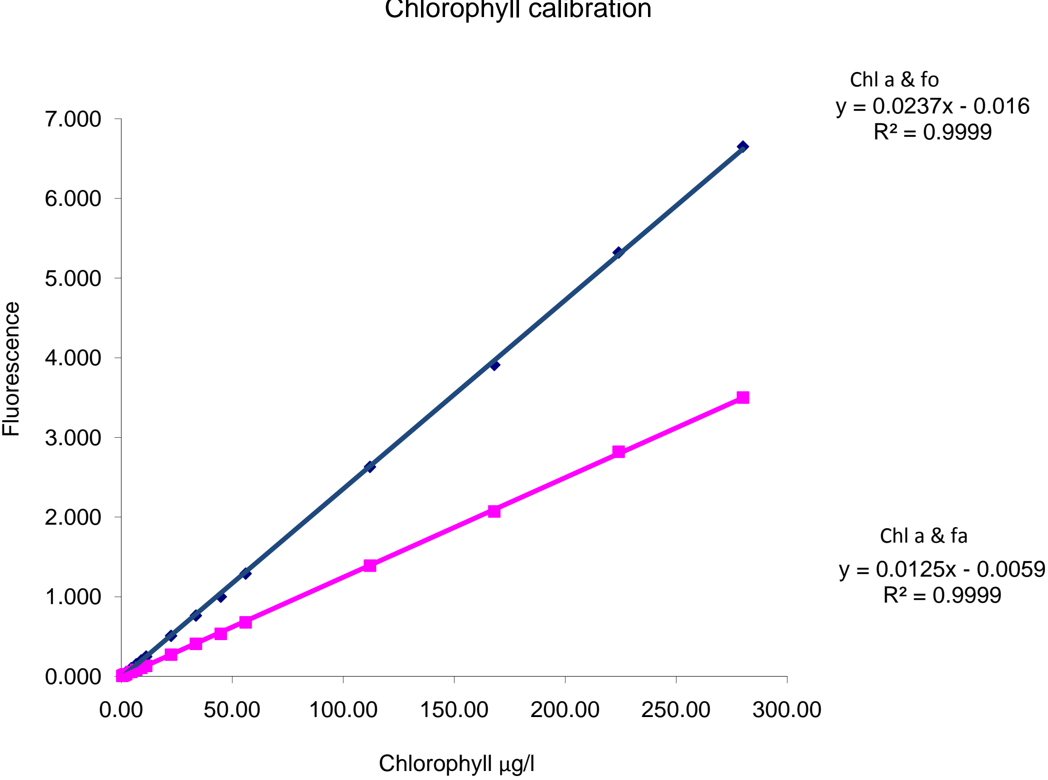


Figure 3.13: Calibration of the fluorometer performed with a chlorophyll dilution series.

Quality Assurance of Nutrient and Chlorophyll Data

Nutrients were standardised and quality assured with internal and external standards. The program 'quality analyst' was used and standard reference material was measured to assess the quality of the standards. Where the standard values did not fall within the 'two standard deviations' ('golden standard') the assessment was repeated. If repeatedly outside the 'golden standard' the standard was replaced with a new internal standard and the process was repeated. Analysis of the samples followed.

Season Categorisation

To identify seasonal patterns of lifeforms (functional groups such as diatoms, dinoflagellates, micro-flagellates and ciliates), the microplankton samples analysed were categorised into winter, spring, summer and autumn (Figure 3.14). The separation into these seasons was made using the total oxidised nitrogen (TOxN) concentration during these periods. Winter was defined for the period when TOxN exceeded 5 μM (December - March). The spring period was deemed to have started when the TOxN concentration dropped below 4 μM (April - May). Summer was defined as the period when TOxN remained below 1 μM (June-August). The autumn period was defined as the period when the TOxN concentration was between 1 and 5 μM (September - November). For comparison reasons the definition for the seasons

that was made using the TOxN concentrations at station 38A were kept the same for the other two stations although the concentrations of TOxN were occasionally higher at these two sites.

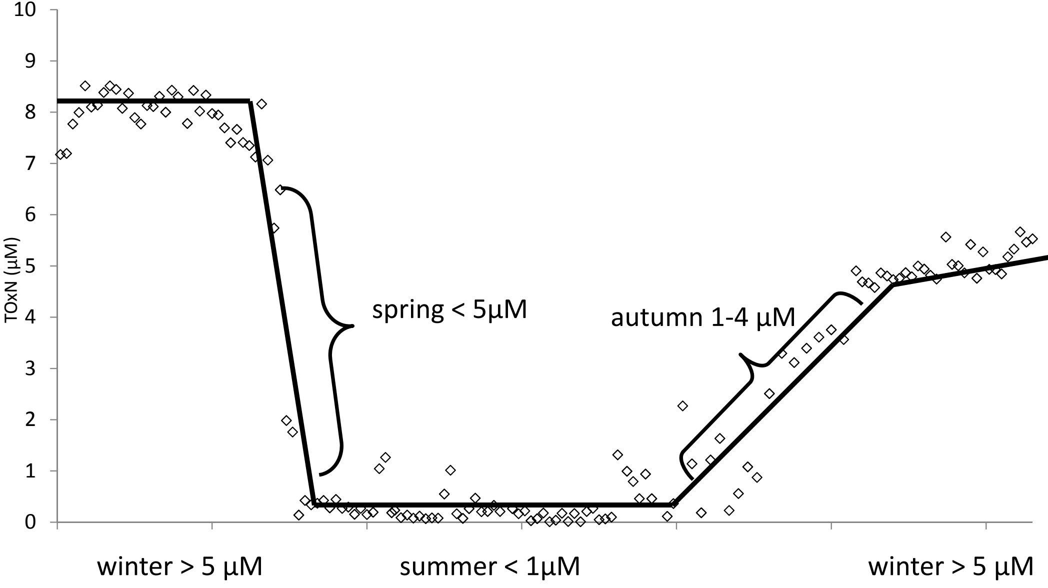


Figure 3.14: An illustration of the categorisation into winter, spring, summer, and autumn on the basis of the TOxN concentration levels from 2008 and 2009 at station 38A. The categorisation into seasons was made to help identify the seasonal patterns of lifeforms.

3.4 Microscopy

3.4.1 General Introduction

Microscopy was used to identify and enumerate the microplankters in the samples collected. An inverted microscope (Nikon Diaphot) with epi-fluorescence attachments was used for the analysis of the samples from station 38A. For the samples from the West Gabbard station provided by Cefas an inverted light microscope (OLYMPUS PE100) was used. The general identification and enumeration of the microplankton assemblage preserved in acidified Lugol's iodine was performed using classical light microscopy (100 watt halogen light bulb). For the distinction of the nutritional mode of the microplankters the samples were preserved in formaldehyde and examined with epi-fluorescence. This was only done with the samples from station 38A.

3.4.2 Preservatives

Lugol's Iodine

Acidified Lugol's iodine solution was chosen as a preservative for species identification and enumeration with the light microscope technique because it is the most widely used. It is a gentle preservative for microplankton and it makes the organisms settle faster than other fixatives (Edler, 1979). Another reason for using this fixative was to enable a direct comparison with data from earlier studies at these sites that used this preservative.

Lugol's iodine, also known as Lugol's solution, was first made in 1829 and is named after the French physician J.G. Lugol. It is a solution of elemental iodine (I_2) and potassium iodide (KI) in water and makes up a brown solution. It is often used as an antiseptic or disinfectant. In plankton biology it is a popular preservative to fix samples as it is harmless compared to aldehyde-based fixatives (Strueder-Kypke, 2003). The addition of acid used in the samples here makes the preservative last longer in the sample.

Lugol's iodine stains cells a dark brown colour but it does not necessarily preserve the shape and size of microplankters. It should also be mentioned that the solution not only fixes the cells but will dissolve hard structure such as coccoliths and diatom frustles. It is therefore not ideal for long-term storage (Gifford & Caron, 2000). However, Lugol's is an ideal stain for accurately quantifying ciliates (Stoecker & Evans, 1984) and increases the sinking rate of the organisms (Edler, 1979) which is an advantage when using the Utermöhl settling method (Utermöhl, 1958).

In this study, samples fixed and preserved with acidified Lugol's were not stored longer than one year because the effectiveness of the preservative decreases considerably over time. The samples fixed with this preservative were checked during storage as iodine is oxidised with time (Edler, 1979). Where necessary, samples were topped up with a few drops of the solution.

Formaldehyde

Formaldehyde was introduced to the sampling programme as a preservative on the 3rd recovery of the automated sampler (May 2008) at station 38A. By that stage it had become clear that the distinction between heterotrophic and autotrophic microplankters was not possible with Lugol's preserved samples as it masks the chlorophyll fluorescence of cells (Gifford & Caron, 2000). The attempt to bleach Lugol's iodine with saturated sodium thiosulphate, which reduces the darkness of the Lugol's fixed cells and enhances the brightness of the fluorescence (Strueder-Kypke *et al.*, 2003), failed.

A 100% solution of formalin consists of a saturated solution of approximately 40%

formaldehyde in water, stabilised with a small amount of methanol to limit oxidation and polymerisation. Formaldehyde fixes or preserves tissue or cells by irreversibly cross-linking primary amino groups in proteins with other nearby nitrogen atoms in protein or DNA through a CH_2-linkage. The chemical structure for formaldehyde is given in Figure 3.15.

Figure 3.15: Chemical structure of formaldehyde, preservative used to fix microplankton samples that were analysed with epi-fluorescence microscopy during this study.

4', 6-diamidino-2-2phenylindole (DAPI)

4', 6-diamidino-2-2phenylindole (DAPI) is a stain that binds strongly to DNA. It is extensively used in fluorescence microscopy (Porter & Feig, 1980). Since DAPI passes through an intact cell membrane, it may be used to stain both live and preserved cells. The chemical structure of DAPI is given in Figure 3.16.

Figure 3.16: The chemical structure of 4', 6-diamidino-2-2phenylindole (DAPI). DAPI was introduced during this study to prolong the fading of autofluorescence in preserved microplankton.

DAPI was introduced to the sampling procedure because during a visit to the Scottish Association of Marine Science (SAMS) in Oban in April in 2008, it was found that when DAPI stain was added to a freshly formalin preserved sample, the auto-fluorescence of microplankton was more intense and persisted for longer (up to 9 months) than without the DAPI stain. Therefore, it promised to be a useful stain to enhance and prolong the auto-fluorescence of organisms in long-term preserved samples.

3.4.3 Light Microscopy

Introduction

The counting technique for microplankton sample analysis was based on the description by Utermöhl in 1931 (Utermöhl, 1958) and the internal AFBI laboratory SOP Code MAERECOL010v1 (McKinney, 2007). Based on these guidelines and also by following the Standard Operating Procedures of the OAERRE (Oceanographic Applications to Eutrophication in Regions of Restricted Exchange) project Microbial Analysis Methods (Leakey, 2000), I developed my own SOP (see appendix A.3.1).

Identification and Enumeration

Sub-samples of 50 mL were settled following the method described by Utermöhl in 1931 (Utermöhl 1958). Identification to species level was conducted where possible. Where organisms could not be identified to species level, they were distinguished by size and genus or broad taxonomic categories (e.g. large pennate diatom 70 μm). Authorities for species names are given in Tomas (1995) and Dodge (1982). For the identification of flagellates and other microplankters, Tomas (1993) and the internal AFBI laboratory handbook collection was used. The abundance of species was calculated in cells L^{-1}. Species lists from all three sampling sites are given in the appendix listing 1(Appendix A.1, A.2, A.3).

To save time and to achieve reasonable accuracy in counting, samples were first examined superficially for abundance and size of organisms using a magnification of x10. This initial check determined the method that was used. Cells could either be counted on the whole base plate of the chamber, or in a central strip across the base plate of the chamber, or in fields of view (FOV). A flow diagram showing the decision pathways for adopting a particular counting strategy is given in Figure 3.17.

On completion of counting, cell numbers were entered into an excel table with the date of collection and analysis and the method used (e.g. central strip, 10 fields of view). For the calculation of cells L^{-1} different equations were used:

For cells counted in fields of view (FOV):

$$\left(\frac{\text{total number of cells}}{\text{Number of FOV}}\right) \cdot \text{Microscope field factor} \cdot \left(\frac{1000}{\text{volume settled (mL)}}\right) \qquad (3.5)$$

For cell counted in a central strip:

$$(\frac{\text{total number of cells}}{\text{area of central strip}}) \cdot \text{Microscope field factor} \cdot (\frac{1000}{\text{volume settled (mL)}}) \qquad (3.6)$$

For cell counted on the chamber base plate:

$$\text{total number of cells} \cdot (\frac{1000}{\text{volume settled (mL)}}) \qquad (3.7)$$

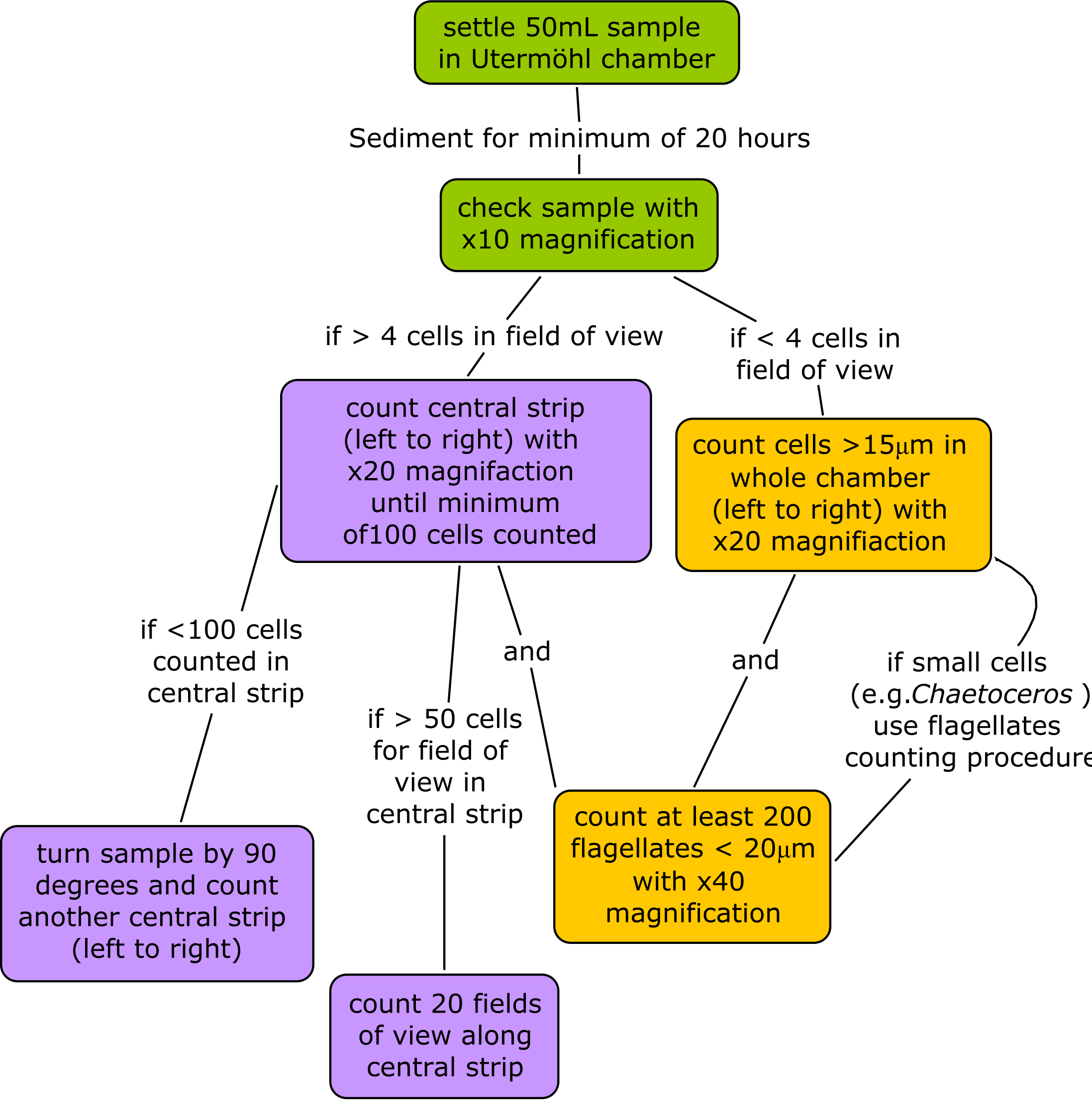


Figure 3.17: A flow diagram showing the inverted light microscope procedure for settling and counting microplankton samples fixed with acidified Lugol's iodine.

FOVs and the whole chamber were counted as shown in Figure 3.18.

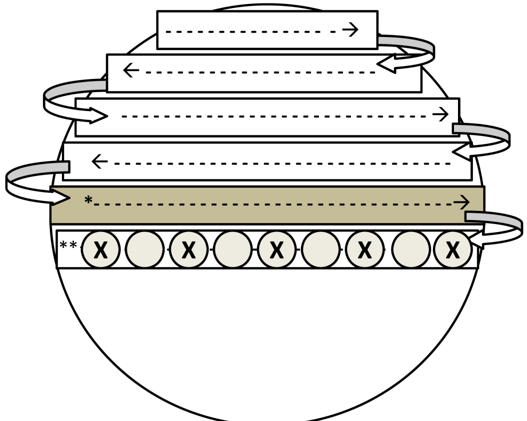


Figure 3.18: An image showing the different procedures used to count cells on the base plate of a settling chamber. One star illustrates the counting method for the central strip; two stars illustrate the counting method for fields of view (FOVs).

Biomass Calculation

Microplankton biomass (mg Carbon m^{-3}) was calculated in three steps:

- microplankton cell measurements
- calculation of cell volume
- estimation of carbon content

During microscopic analysis, size measurements of cells were taken from every 3rd sample using an eyepiece micrometer. A single cell of each species was measured at least once. Using the measurements collected from all of the samples, average measurements of each taxon were calculated. The average cell volume (mm^3) of each taxon was determined using the geometric formulae given by Edler (1979). Hillebrand *et al.* (1999) observed that different geometric models gave the same results for simple centric diatoms, while the calculated cell volumes were different for taxa with more complex shapes such as some pennate diatoms (e.g. *Licmophora*), centric diatoms (e.g. *Ditylum*) and dinoflagellates (e.g. *Ceratium*). In consideration of the findings of Hillebrand *et al.* (1999), microplankton cell volumes in this study were calculated using Edler's (1979) equations except for the genus *Ditylum* and *Ceratium* where Hillebrand's formulae seemed more accurate. *Ditylum* cell volume was calculated according to Hillebrand *et al.* (1999) and *Ceratium* volume according to Thompson *et al.* (1991). Pennate diatoms belonging to the genus *Licmophora* represented less than 0.4% of the microplankton abundance in this study so the error derived from using Edler's simpler equations was considered small.

For the different shapes of microplankton, the following equations were used.

Cylinder volume (cy) (e.g. centric diatoms)

$$\mathrm{cy} = \pi/4 \cdot (\mathrm{d}^2 \cdot \mathrm{h}) \tag{3.8}$$

Parallelepiped volume (p) (e.g. pennate diatoms)

$$\mathrm{p} = \mathrm{l} \cdot \mathrm{w} \cdot \mathrm{h} \tag{3.9}$$

Cone (c) + sphere (s) volume (e.g. armoured dinoflagelattes)

$$\begin{aligned} \mathrm{c} &= (\pi/12 \cdot \mathrm{d}^2\ \mathrm{l}) + \\ \mathrm{s} &= (\pi \cdot \mathrm{d}^3/6) \end{aligned} \tag{3.10}$$

where d = diameter of the cell; h = height of the cell; l = length of the cell; w = width

To convert cell volume to carbon biomass (pg C cell $^{-1}$) the equations of Menden-Deuer & Lessard (2000) were used:

diatoms

$$\text{biomass pg C cell/L} = 0.288 \cdot \text{cell volume}^{0.811} \tag{3.11}$$

for other protist plankton

$$\text{biomass pg C cell/L} = 0.216 \cdot \text{cell volume}^{0.939} \tag{3.12}$$

The reason why two different equations were used for diatoms and non-diatom protists was that diatoms are significantly less carbon dense than dinoflagellates, meaning their C : vol ration is lower. The other protists show no significant differences to dinoflagellates in their carbon density consequently one C : vol ratio was determined by Menden-Deuer & Lessard (2000) as given in 3.12. To convert the results from pg to mg Carbon m^{-3} value were multiplied by 10^{-3}.

Quality Assurance for Microplankton Counts

To assure the quality of enumeration and identification of samples I took part in three quality assurance (QAs) schemes. The first scheme was the BEQUALM ring test in 2009 and included identification of several marine phytoplankton species by photo-graphical and video image, enumeration and identification of mixed cultures, and morphological features. The ring test included a workshop held in Galway (Ireland) where results and counting techniques were discussed. I passed the ring test successfully.

Quality assurance for Liverpool Bay and the West Gabbard was needed because the aim was to combine existing microplankton data from these sites with data from samples that I analysed. Therefore, internal-calibration workshops were organised with Dr. Lars Edler, a phytoplankton taxonomist (who processed most of the Liverpool Bay microplankton data), and with Mr. Thomas McGowan a trained analyst in the UKAS (United Kingdom Accredation Scheme) accredited plankton laboratory at Cefas, Lowestoft.

The scheme with Dr Lars Edler involved a 4- day informal AFBI inter-comparison exercise in March 2008 and a follow up workshop of 5 days in May 2009. Samples were counted, results and counting procedures were discussed and a phytoplankton picture quiz was performed. The intense training with Dr Lars Edler was aimed at adopting his counting procedures and to minimise differences in identification and enumeration. By the end of the two workshops I adopted the Edler counting method and there were only small differences of opinion on species identification. The adopted method was applied to all samples subsequently counted. The results from samples counted prior the workshops were evaluated and where necessary recounted to ensure a certain level of accuracy.

The scheme with Mr. Thomas McGowan was an inter-calibration exercise when it became clear that the Cefas mooring station at the West Gabbard in the Outer Thames estuary was going to be used as the reference site for the Liverpool Bay station. It was decided that I would contribute to the Cefas data base by analysing samples collected from the West Gabbard mooring site in 2009. To assure the quality of identification and enumeration of counts for the samples from the site the inter-calibration exercise was carried out in November 2009 as follows. Six samples collected from the West Gabbard between December 2008 and November 2009 were randomly chosen. Sub-samples (25 mL) of each sample were settled following the Utermöhl method and the Cefas SOPs. The samples were counted independently and the results and counting methods were compared and discussed.

The results are presented in full in appendix A.6 - A.12. Here, two examples are shown. A comparison of the two counts of *Paralia sulcata* and *Prorocentrum micans*

is shown in Figure 3.19 and Table 3.3.

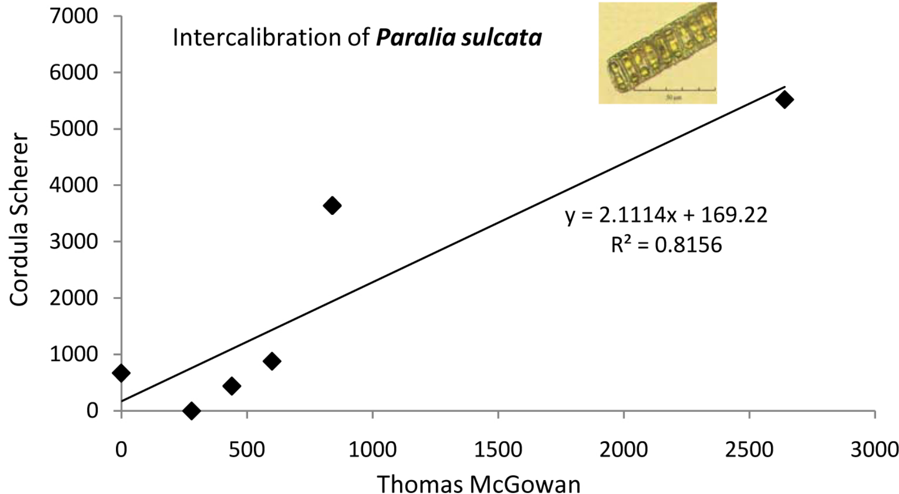


Figure 3.19: A graph showing *Paralia sulcata* abundance (cells L^{-1}) counted in six samples used for inter-calibration with Mr. McGowan.

Table 3.3: *Prorocentrum micans* abundance (cells L^{-1}) counted in the six samples during the inter-calibration with Mr. McGowan.

Sample date	McGowan	Scherer
10 Jan 2009	0	0
18 Jan 2009	40	40
22 Apr 2009	0	0
24 May 2009	40	80
19 Jul 2009	600	600
12 Aug 2009	640	600

My counts were in general more detailed at the level of speciation and gave slightly higher cell abundances compared to Mr. McGowan's counts. The reasons for this was that Mr. McGowan followed the Cefas SOP for counting phytoplankton that did not require as much detail at species level. Instead of identifying organisms to species level (e.g. different *Chaeotoceros species*) the Cefas counting SOP only require the distinction between sizes and whether they had chloroplasts in their spines (Phaeroceros) or not (Hyalochaetae). However, this was not a major problem because my study was based on lifeforms and size, so identification to the species level was not necessarily required although favoured. Mr. McGowan also only counted fully intact cells whereas I counted empty cells when they appeared in chains as in *Paralia sulcata*. This chain building pennate diatom (up to 42 cells in a chain)

is easy to identify but difficult to count as the cells often have no distinct border or space in between the cells. Furthermore, the Cefas SOP excludes the counting of single celled *Pseudo-nitzschia* spp. as they could be mistaken for *Nitzschia* or *Pleurosigma*.

Confronted by the two different SOPs (Cefas and mine) the results of the inter-calibration with Mr McGowan showed some real discrepancies and the data sets had to be standardised. The following decisions were made to achieve this:

- Species with the most disagreement in cell counts and identification were left out when present in low numbers throughout the year.
- Where organisms were not identified down to species level, they were generally categorised into lifeforms.
- Species of the *Chaetoceros* group were categorised into sizes (10-20 μm; 21-40 μm; <40μm) and into Phaeroceros or Hyalochaetae.
- Similar procedures to that of group and size categorisation were applied to other species groups that were identified to species level by one analyst but not the other.

The overall effect was to minimise discrepancies and allow a bigger data set to be used in this study.

3.4.4 Epi-fluorescence

Introduction

Fluorescence microscopy was first suggested by Wood (1956) as a tool to discriminate chlorophyll-bearing and heterotrophic micro-organisms (Lessard & Swift, 1986). Epi-fluorescence microscope methods have been developed since for the differentiation of heterotrophic, mixotrophic and autotrophic microplankton (e.g. Sherr & Sherr, 1983). Epi-fluorescence microscopy is a method where excitation and observation of the emitted fluorescence are from above the organism (from the Greek word "epi" meaning "above"). In this process the excitatory light is passed through the objective onto the organisms instead of being transmitted through the organism. Since only reflected excitatory light filters through, the transmitted light is filtered out, giving a much higher intensity. The organism is illuminated with light of a specific wavelength (or wavelengths) that is absorbed by the fluorophores (component of a molecule which causes a molecule to be fluorescent) of the organism, causing them to emit longer wavelengths of light. The illumination light is separated from the much weaker emitted fluorescence through the use of an emission filter. To take advantage of the fluorescent features of cells a microscope requires several

components. These are:

- light source (Xenon or Mercury arc-discharge lamp (in this study a super high pressure mercury lamp, model HB-10101AF was used)
- excitation filter
- dichroic mirror (or dichromatic beamsplitter)
- emission filter

The light that passes through the excitation filter excites any pigment within the cells in a wavelength range between 450 and 490 nm. The emission wavelength range is between 620 and 750 nm, making autotrophic organisms appear red and heterotrophic organisms greenish-blue. Mixotrophic organisms appear orange or greenish-blue with clearly visible red pigments. Figure 3.20 shows a general schematic of the epi-fluorescence principal.

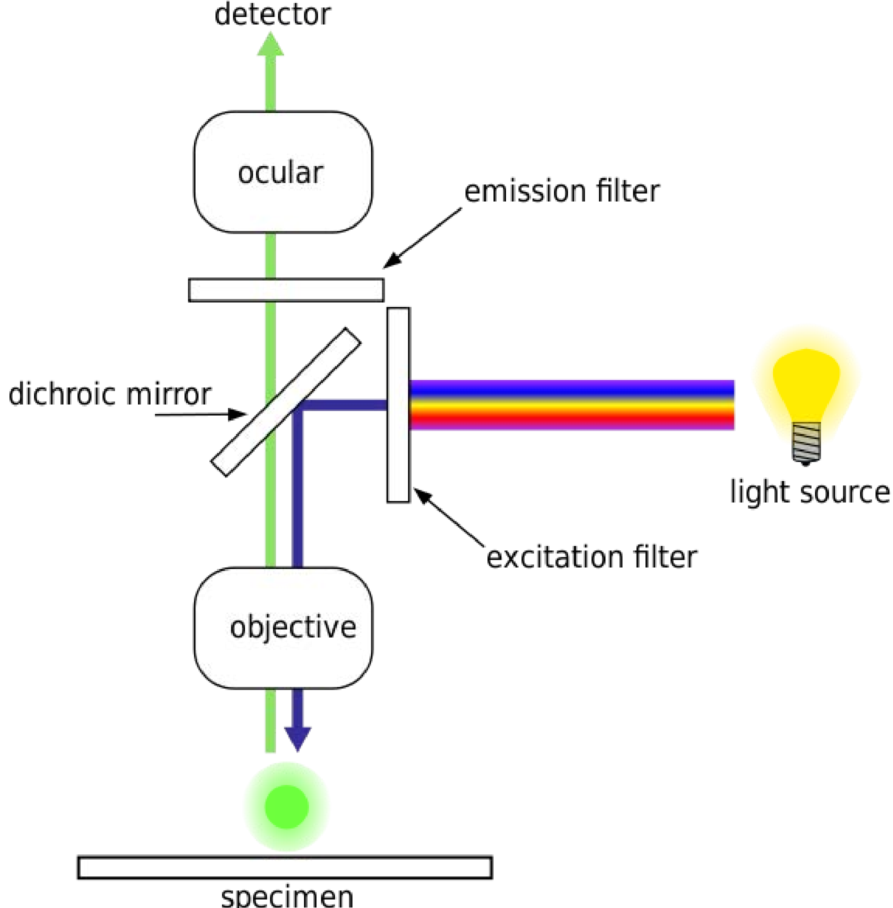


Figure 3.20: A schematic of an epi-fluorescence microscopy. (Image published by 'Free software Foundation, Inc 1.3.')

Although the nutrition of microplankters can be identified by fluorescence, the ability of cells to emit this fluorescence fades exponentially within a few days/weeks of preservation (Dr R. Leakey, SAMS pers. comm.). Reliable results from preserved

samples stored for up to 1 month were reported by Lessard & Swift (1986), when the samples were preserved with 1% unbuffered glutaraldehyde and stored in a dark cool (4°C) place. After that the chlorophyll fluorescence was too diminished to reliably identify the trophic status of the microorganisms.

This was one of the problems that had to be dealt with during this project because samples were collected remotely and were therefore between two and 12 weeks old. The preservatives available for use were acidified Lugol's iodine and formaldehyde. Lugol's iodine masks the auto-fluorescence of organisms and therefore makes it very difficult to identify the nutritional mode of the different species.

There are many publications on methods to investigate and distinguish the auto-fluorescence in live or fresh material, but not for preserved samples. Hall (1991) for example, presents a method that fixed fresh samples with 0.2% paraformaldehyde and preserved the auto-fluorescence of picoplankton for up to 15 weeks. This procedure could not be tested as paraformaldehyde was not allowed on AFBI facilities for health reasons. Vaulot *et al.* (1989) published a method to preserve phytoplankton for flow cytometric analyses using glutaraldehyde to fix fresh samples followed by immediate storage in liquid nitrogen. It was not possible to take liquid nitrogen on board of the research vessel for health and safety reasons. Sherrard *et al.* (2006) proposed a method to assess phytoplankton community structure by pigment concentration using HPLC. To prepare the samples for HPLC analysis fresh seawater had to be filtered and the filters were frozen in liquid nitrogen. Both requirements were not possible in this study.

The aim of using epi-fluorescence was to distinguish the nutritional mode of dinoflagellates and micro-flagellates and to find an appropriate method that could be applied to samples taken remotely. The following experiments were designed to find a suitable fixative and or stain for the epi-fluorescence method.

3.4.5 Nutritional Library for Dinoflagellates

For the discrimination of the nutritional mode of dinoflagellates, a library was established. A thorough internet search was done for the characteristic nutrition of dinoflagellates and catalogued into a library. Additional to that, in February, May, July, and September 2009 live samples were taken and the nutrition of all identifiable dinoflagellates was distinguished by epi-fluorescence and added to the library (see appendix A.4 and A.5).

3.4.6 Experiments to identify the Nutrition of Micro-flagellates

In the following, the method development to identify the nutrition of micro-flagellates is presented. Experiments were carried out to establish a routine method for the distinction of the nutritional mode of micro-flagellates that could be applied to microplankton samples collected remotely, preserved and stored for up to twelve weeks.

Experiment 1: Choosing the preservative

One task prior to the nutrition experiments was to choose a suitable preservative for the experiments. When fieldwork was started it was uncertain whether formaldehyde could preserve samples with the same efficiency as glutaraldehyde and therefore replace the more harmful preservative that was not allowed in AFBI facilities.

The fixatives glutaraldehyde and formaldehyde were tested using 20 samples. The work was conducted at the Scottish Association for Marine Science (SAMS) in Oban where use of glutaraldehyde was still allowed. Ten samples were fixed with glutaraldehyde and the other ten with formaldehyde. After comparing the intensity of the autofluorescence of organisms in epi-fluorescent light with the two chemicals after one, two, four, six and ten days, I concluded that for the purposes of this study there was no clear difference between formaldehyde and glutaraldehyde. Therefore, formaldehyde was used was a fixative during fieldwork.

Experiment 2: Testing the suitability of formaldehyde

After finding no clear difference in the quality of formaldehyde and the more harmful glutaraldehyde as preservatives, formaldehyde was introduced to the microplankton sampling routine. From May 2008, every 4th bag in the RAS-500 sampler contained formaldehyde as a preservative.

Formaldehyde had to be tested for its suitability as a long term fixative for samples that were to be used to distinguish autotrophic, mixotrophic, and heterotrophic organisms, especially micro-flagellates. An experiment was carried out to test how long the auto-fluorescence of micro-flagellates lasted when preserved in 1% formaldehyde.

A 5 L water sample was collected from the western Irish Sea and returned to the laboratory on July 20th, 2008. It was fixed with 1% formaldehyde (final concentration) and divided into eleven sub-samples. Each sub-sample was stored in a 250 mL amber glass bottle in darkness at room temperature. The first sub-sample was set up immediately after fixation and three replicates were counted using the Nikon Diaphot inverted microscope with the epi-fluorescence attachment. The other sub-samples were analysed every three days after preservation (23rd, 25th, 28th, 31st) until August 2nd. Then three weeks after preservation (August 10th), four (14th),

and six weeks (24th). After two months (September 19th) another set of sub-sample was analysed and the last set was examined after two and a half months (October 1st). For each sub-sample three replicate 50 mL aliquots were analysed to ensure sufficient replication for statistical analysis.

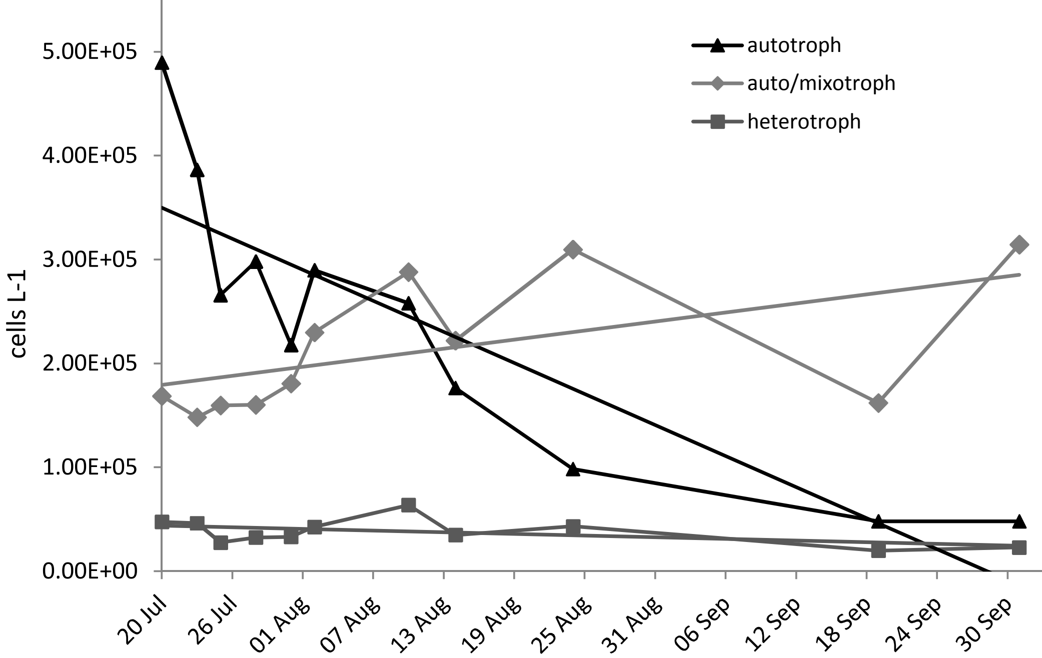


Figure 3.21: Temporal changes in auto-fluorescence of autotrophic microplankton (red), autotrophic/mixotrophic microplankton (orange), heterotrophic microplankton(green).

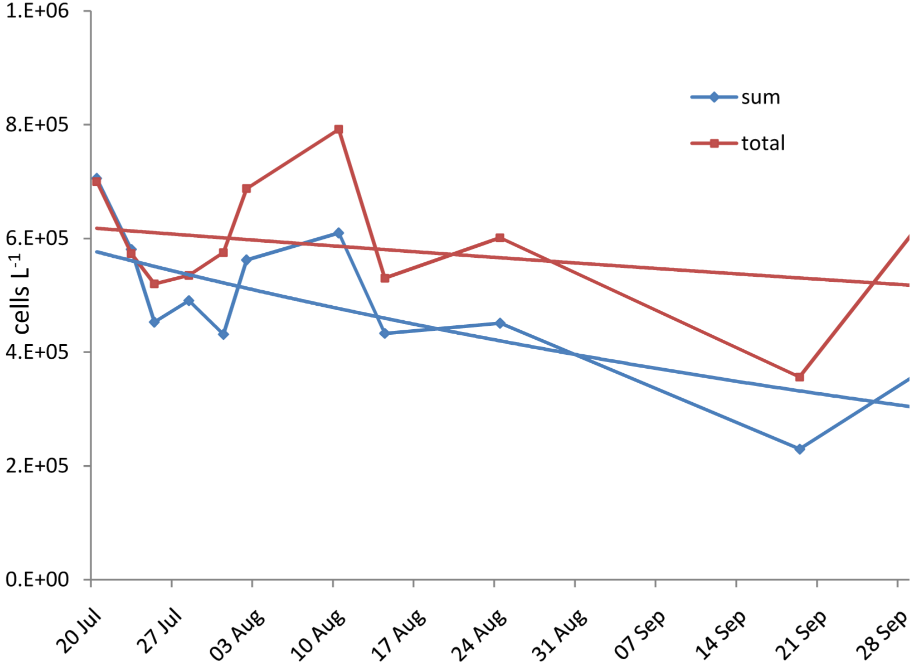


Figure 3.22: The difference in cell abundance estimated in transmitted and fluorescence light. Where "total" is the total number of flagellates counted in transmission light and "sum" represents the sum of all flagellates that reflected auto-fluorescence.

Results

There was a marked overall decrease with time in the auto-fluorescence of the autotrophic organisms (red) while there was an overall increase in the orange signal over time (Figure 3.21). The signal of the heterotrophic organisms (green) was consistently low. Figure 3.22 shows the total number of micro-flagellates counted in transmission light and the sum of all micro-flagellates that reflected auto-fluorescence. There was agreement over the first twenty days and thereafter the discrepancy between the "sum" (sum of all flagellates that reflected auto-fluorescence) and the "total" (total number of flagellates counted in transmission light) increased over time. Regression analysis showed that the slope for "total" did not decrease significantly over time ($p > 0.05$), whereas the regression slope for "sum" of all micro-flagellates that reflected auto-fluorescence decreased significantly ($p < 0.05$) over the two and a half month period. However, for the first twenty days the decrease was not significant ($p > 0.05$). Applying a paired t-test to the two datasets a significant difference between them ($p < 0.05$) was found.

Discussion and Conclusion

One explanation for the overall decrease in the autotrophic signal together with the increase of the "orange" signal leads to the conclusion that the "orange" signal derived from fading autotrophic organisms rather than mixotrophic microplankters. This was taken into consideration when the micro-flagellate fluorescence counts were analysed in chapter 4 (Observational results).

Some of the variability noticeable in Figures 3.21 and 3.22 can be explained by subsampling variability (approximately 26%). The significant decrease ($p < 0.05$) of the "sum" over a period of two and a half months in Figure 3.22 can be explained by the loss of auto-fluorescence in cells. The results lead to the conclusion that within the first twenty days of preservation the auto-fluorescence of micro-flagellates does not decrease significantly and some conclusions about their nutritional mode can be made. Beyond this time period, auto-fluorescence decreases and cannot be reliably used to detect and discriminate the nutritional mode of micro-flagellates.

An experiment similar to that described above was performed in 2009 because it was still not possible to fully distinguish between mixotrophic and autotrophic microflagellates which was an initial objective. The experiment was also performed to find out exactly where the "cut off" time of detection for auto-fluorescence was and whether detection and reliability of results could be improved by means of a camera with long exposure time.

This experiment was carried out in collaboration with Dr. George McKerr at Ulster University, Coleraine in July 28th 2009. For the experiment, samples dated March 9th to July 7th 2009 were observed and analysed with a non inverted Olympus

microscope with epi-fluorescence attachments and a camera attached directly to a computer. The set up entailed a few difficulties. Not many cells were found within the limited focus of the microscope as the Utermöhl sedimentation chamber was too deep. None of the cells found were flagellates, so detecting their auto-fluorescence was not possible.

The option of filtering the sample and therefore separating the micro-flagellates from the larger sized organisms was ruled out as no appropriate filter equipment was available and the risk of damaging the cells was too high.

The approach to gently centrifuge the samples to get a higher density and prepare a slide with the concentrated sample from the bottom of the centrifuge tube did not lead to any success in finding flagellates either.

However, small centric diatoms cells were found on a few slides from different samples and in the 21 day old samples (July 7th) the auto-fluorescence of organisms could be detected with no need for the camera. In a six week old sample (June 6th) small centric diatom cells were found and their auto-fluorescence could be detected without a camera light. Auto-fluorescence of cells in ten week old samples was still detectable with a maximum light exposure of 4 sec but resulted in a very weak image. In four month old samples (March 9th) none of the auto-fluorescent cells could be detected, and even with the camera set on its highest exposure time of 4 sec there was no signal.

The experiment shows that the "cut off" time for auto-fluorescence detection in organisms that are fixed and stored, was somewhere between ten weeks and four months. For samples preserved longer than that, the detection of the nutritional mode of organisms is not possible. Better results were obtained with samples that had been preserved for less than ten weeks. Although the "cut off" point was found, the results of this experiment should be treated with caution as there was a limited number of cells and the experimental set-up experienced difficulties. Despite these difficulties, the experiment showed that accurate distinction of nutrition modes in microplankton was possible when a camera was used and the preserved samples are not older than ten weeks. The "cut off" point for samples analysed by microscopy without camera light exposure was assumed to be six weeks. Better results were achieved in samples preserved less than six weeks.

Experiment 3: DAPI experiment

The reason for introducing DAPI stain to the sampling procedure was because during training at SAMS in April 2008, it was found that when DAPI stain was added to formaldehyde preserved samples the auto-fluorescence of microplankton was stronger and more intense over a longer period of time. An experiment was undertaken to find

out whether DAPI stain could be introduced to the routine sampling and therefore help to distinguish the nutritional mode of micro-flagellates.

In July 2008, two 250 mL water samples were collected from a depth of 14 m from station 38A using the rosette water sampler on board the *RV Corystes*. The samples were stored in 250 mL amber glass bottles. The depth of 14m corresponded to the notional depth of the RAS-500 which collected the high frequency samples. The samples collected for the DAPI experiment were fixed with a final concentration of 1% formaldehyde. Replicates from each sample were kept for comparison without DAPI stain and five replicates of each sample were filtered and DAPI stained shortly after the samples were taken. A blank slide was prepared with filtered seawater and stain as a control. The prepared slides were then frozen and stored in the dark until analysed.

Results

The intensity of fluorescence in the DAPI stained samples was greater than the intensity of fluorescence with formaldehyde alone. The outline of the organism's shapes were clear and the resulting colours representing the different nutrition of the organisms (red = autotrophic, orange = mixotrophic, greenish-blue = heterotrophic) were easily identifiable. The intensity of colour did not decrease as quickly as in the samples preserved without DAPI. After a further two weeks, the intensity and quality of the prepared slides did not appear to be visibly reduced. This method looked promising as a method for identifying the nutrition of micro-flagellates.

Discussion and Conclusion

The results of the DAPI experiment clearly showed that this stain made the distinction of the different nutrition modes of micro-flagellates easier. With these promising results it was decided to prepared further slides. However, this method was time consuming. It took between three and four hours to stain five samples and prepare the slides. A further one to two hours was needed to identify the organisms in each sample using epi-fluorescent microscopy. Thirty-six to 48 routinely samples were processed after every survey which would have meant that an additional 200 hours (nine days) would have been required for the DAPI method every six weeks. This was not practical and the method could not be integrated into the high frequency sampling because DAPI had to be removed after staining the sample and this was not possible with the applied remote access sampling method. Thus DAPI staining procedure was not used on a regular basis. As an alternative, during the second year, water samples were collected from approximately 14 m during each mooring service and five sub samples were fixed and stained before being stored at -20 °C and analysed back in the laboratory. Although this resulted in less-frequent sampling it was hoped that it would provide a backup for the routine epi-fluorescence sample

analysis.

While trying to count and identify the microplankters on the prepared DAPI stained slides it became clear that identification was difficult. The filters used were wrong in the sense that they reflected too much light to identify species in transmitted light which made it impossible to categorise the organisms into species, groups or families. My skills to identify species only by their fluorescence characteristics and shapes were not advanced enough. Both difficulties prevented precise analysis. A microscope filter for DAPI stain (G 365 FT 395 nm, LP 420) and an oil objective (magnification x100) would have made it possible to solve the filter problem, but neither additions were available. A workshop for identifying species by fluorescence characteristics could have also helped. By the time this problem was identified, the project was too advanced and the DAPI staining approach was dismissed and not used.

Even though formaldehyde preserved samples stained with DAPI promised good results for the discrimination of the nutritional mode of micro-flagellates the stain was not used in further analysis. With the right filters (white cellulose backing filters 0.8 μm pore), more time, a microscope equipped with DAPI stain filters (G 365 FT 395 nm, LP 420) and a x100 oil objective this procedure could be a useful method.

Flow cytometry

Instruments such as flow cytometers can be used to enumerate micro-flagellates and pigment analysis by high pressure liquid chromatography (HPLC) provide information on taxonomic composition to discriminate their nutritional modes. Neither instrument was available for routine use. Nevertheless, an attempt was made to discriminate micro-flagellates using flow cytometry. Dr. Rodney Forster (Cefas) analysed some formaldehyde preserved samples from the western Irish Sea using this method. The samples were fixed and stored for six to 16 weeks before analysis. None of the resulting signals was clear and strong enough to draw reliable conclusions about the organisms and their nutrition. Neither was it possible to compare the results from preserved samples with those from fresh samples as the degradation of organisms auto-fluorescence was too advanced. Flow cytometry was therefore dismissed as a potential method for the discrimination of micro-flagellate nutrition in long-term preserved samples. HPLC instrumentation was not available within the budget of this PhD project.

Summary of Methods

Table 3.4: A summary of the methods tested in the experiments to find a suitable method for the distinction of nutritional modes of microplankton

Preservative	Method	Result	Applied
Lugol's	epi-fluorescence	masks auto-chlorophyll	NO
Formalin	epi-fluorescence	good up to six weeks	YES
DAPI	epi-fluorescence	suitable with right equipment/time	NO
—	Flow cytometry	not suitable for remote sampling	NO

3.4.7 Conclusion of Experiments

The results show that Lugol's acidified iodine could not be used for the discrimination of microplankton nutrition as it masks chlorophyll auto-fluorescence. The DAPI staining method seemed promising but the right equipment and time were not available. If these difficulties were overcome it could provide a solution for the identification of auto-fluorescence of microplankton in long-term preserved samples. Flow cytometry analysis was not suitable for the distinction of nutritional modes of microplankton sampled remotely. However, if fresh samples were available, this method could be an option. Formaldehyde was an appropriate preservative for the purpose of identifying the nutritional mode of microplankters by epi-fluorescence and can be a substitute for the more harmful glutaraldehyde. When samples were preserved in formaldehyde and processed within six weeks (ideally twenty days for best results), it was possible to distinguish between heterotrophic and autotrophic microplankters by epi-fluorescence. Formaldehyde was therefore used for routine sampling. It was ensured that formaldehyde samples were always processed prior to Lugol's preserved samples. For mixotrophic micro-flagellates no reliable method was found within the time allocated during this study.

To count and identify autotrophic and heterotrophic micro-flagellates using the epi-fluorescence technique involved a slightly different method to that used for light microscopy (Figure 3.18).

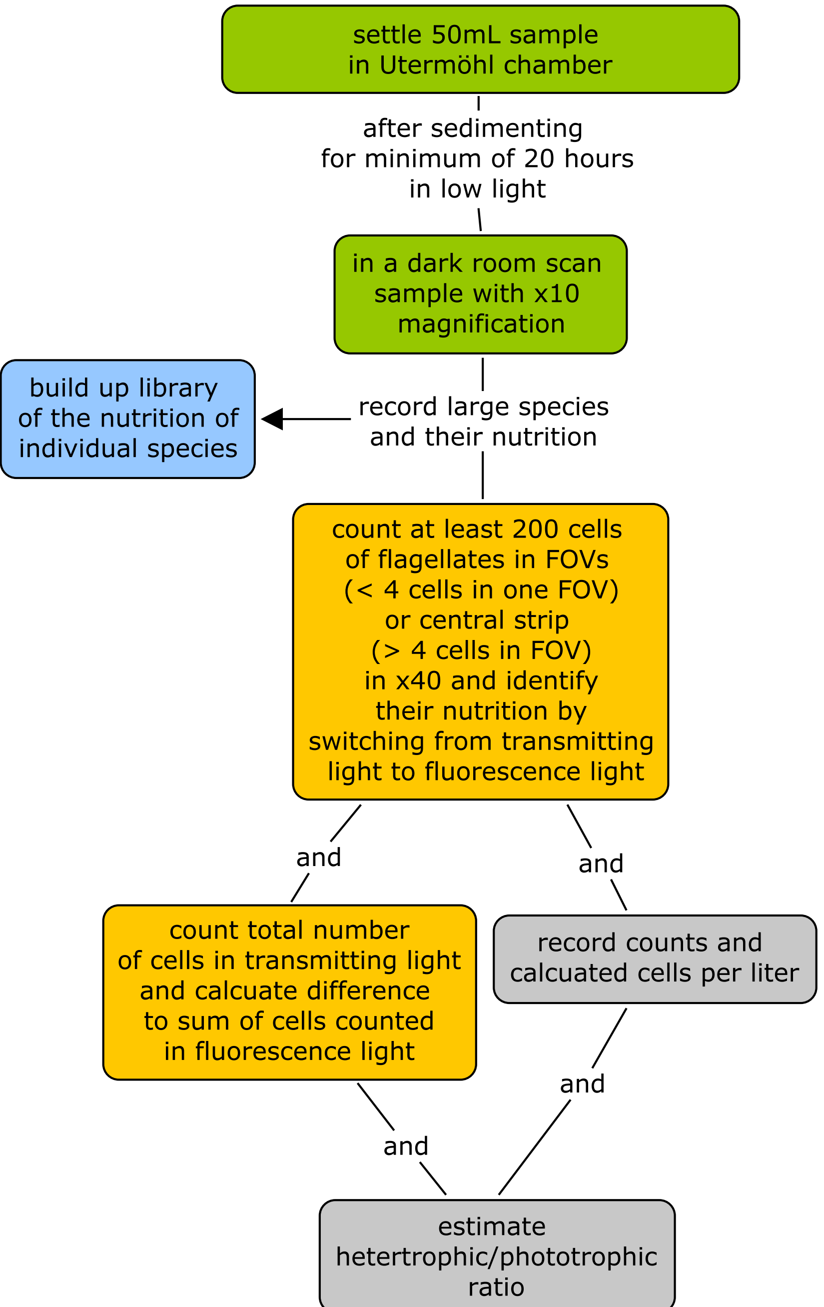


Figure 3.23: A flow diagram illustrating the procedure for the identification and enumeration of the nutritional mode of microflagellates using epi-fluorescence microscopy.

3.4.8 Summary of Applied Methods

On the basis of the results from the experiments on methods to identify and enumerate microplankton and their nutritional mode the following methods were chosen to process the samples:

- For the identification and enumeration of general microplankton samples acidified Lugol's iodine was used as the preservative and light microscopy used for analysis following the Standard Operation Procedure developed for this study. (appendix A.3.1)
- To discriminate the nutritional mode of dinoflagellates a library was established from the scientific literature and observed fresh samples collected during winter, spring, summer, and autumn 2008 to refer to. (appendix A.4, A.5)
- To discriminate micro-flagellate nutrition, formaldehyde was used as the preservative and epi-fluorescence was used to analyse samples within six weeks of the sampling date. The SOP developed for this is given in the appendix A.3.3.

3.5 Statistical Analysis

All of the statistical analyses presented in Chapters 3 and 4 were performed using Minitab 15. A paired sample t-test was used to determine whether there were significant differences between the two sampling years (2008 and 2009) in normally distributed data (e.g. temperature, salinity). Non parametric Mann-Whitney tests were used to examine differences in the microplankton data. This statistic was also used for the nutrient data because they were also not normally distributed (even after log transformation). Least squares regression analysis was used to assess the relationship between salinity and nutrients. And analysis of variance was performed to asses the assimilation of nutrients by microplankton.

Most plots and graphs presented in Chapter 4 were created in windows vista excel. Contour plots to display the seasonal pattern of water column temperature, salinity and chlorophyll at station 38A, were created using Surfer 8.

Chapter 4

Observational results and discussion

4.1 Introduction

The data presented here were collected from three sampling sites: 38A in the western Irish Sea (instrumented mooring operated by AFBI, Belfast), LBay in Liverpool Bay, eastern Irish Sea, and WGabb in the West Gabbard, Outer Thames estuary, southern North Sea (the latter two instrumented moorings were operated by Cefas, Lowestoft).

Data were obtained using two different sampling methods (detailed in chapter 3). High frequency data were obtained for all three sites using instruments and samplers attached to the moorings (thermistors, ctd, nutrient sampler, biological sampler) at a constant (tide dependent) water depth. Regular periodic sampling was undertaken at 38A every six to eight weeks during surveys in the Irish Sea and provided 18 vertical profiles (90 m) of temperature, salinity, chlorophyll, and nutrients. Sampling at 38A began in February 2008 and ended in December 2009. In Liverpool Bay (LBay) data for twenty-five vertical profiles (30 m) (on six days two data points were provided) of temperature, salinity, chlorophyll and nutrients were collected. In the southern North Sea (WGabb) ten vertical temperature, salinity and fluorescence profiles (35 m) were available. Data from the Cefas stations were extracted for a time period from January 2008 to December 2009 or alternatively to whatever time data were available (see WGabb site in detail in chapter 3).

4.1.1 Hypotheses

The characteristics and hydrodynamics of the three mooring sites are hypothesised to be:

1. station 38A: site with low nutrient enrichment (close to pristine conditions) at which the microplankton biomass season is closely coupled to the seasonal pattern of stratification.
2. station LBay: highly nutrient enriched and intermittently stratified site that is located in a ROFI with restricted coupling of the microplankton biomass season to the stratification pattern.
3. station WGabb: medium high nutrient enriched and intermittently stratified site that is influenced by freshwater with restricted coupling of the microplankton biomass season to the intermittent stratification pattern.

It was further hypothesised that the microplankton biomass and composition are different at the three sites. At station 38A this was hypothesized to be due to the different hydrodynamics and nutrient loadings. At station LBay and WGabb, both are hypothesised to be similar in their hydrodynamics; but the difference in microplankton communities at these two sites is assumed to be due to the different nutrient loadings.

Results for station 38A are presented first and in more detail than the other two sites as this was the site used to develop the MCI. High frequency physical and chemical data are presented in days of the year (DOY) if not otherwise mentioned, followed by the survey data and the microplankton results. A data summary for all three sites follows the results and leads into the discussion with the conclusion of the end of this chapter.

4.2 Station 38A

4.2.1 Temperature and Salinity Data

In general, near surface temperatures increased from winter 2008 (7.8° C) to summer and autumn months (16.3° C) and decreased toward the end of the year (7.5°C) (Figure 4.1). The near surface temperature values in 2008 ranged from a minimum of 7.8° C reached on day 89 (29th March) to a maximum of 15.9° C on day 217 (4th August). For 2009, the temperature values ranged from 7.5° C between day 63 and 70 (4th and 11th March) to a maximum of 16.3° C on day 153 (2nd June). The difference between the annual mean values of the near surface temperature 11.5° C in 2008 and 11.8° C in 2009 was significant (paired t-test p = 0.00; n = 2250).

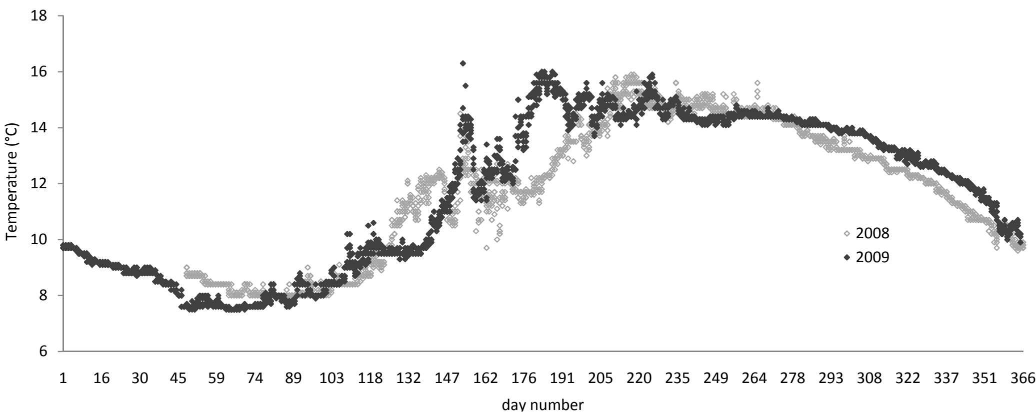


Figure 4.1: Thermistor data showing the seasonal cycle of near surface temperature (° C) at station 38A in 2008 and 2009.

The seasonal temperature pattern of the water column at station 38A is shown as a contour plot in Figure 4.2. The black dots (making up a line) in the graph indicate the depths of the five thermistors that were attached to the mooring wire and delivered 3 hourly temperature values throughout the year. In 2009, temperature was lower (9.5° C) in the beginning of the year and near surface water warmed up faster than in 2008. It also looked like warm temperatures (14.5° C) in 2009 stayed longer (until late October; day 300) and reached deeper (90 m) than in 2008. By the end of autumn in both years the water column was isothermal and remained isothermal until the end of the year.

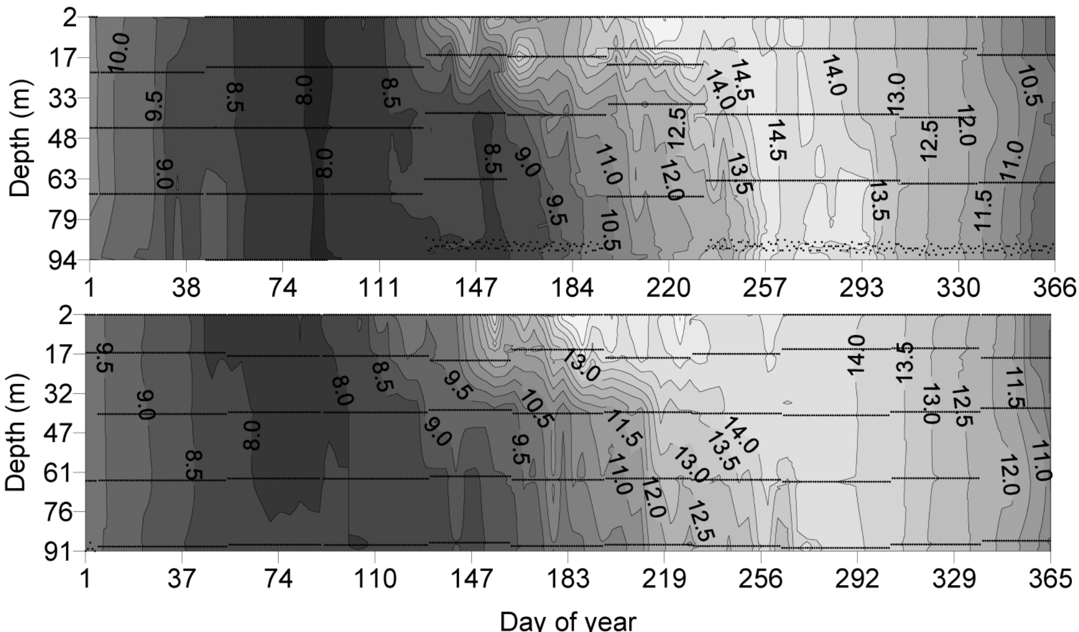


Figure 4.2: A contour plot of the seasonal development and erosion of thermal stratification at station 38A during 2008 and 2009. The dotted lines indicate the position of the five thermistors that delivered the data for this plot. The contour intervals are 0.5° C.

In 2008, Δt (the near surface to near bottom differences in temperature) had increased beyond 0.5 by mid April and stayed higher than 0.5 throughout the summer period (Figure 4.3). A maximum Δt (5.7) was recorded on day 161 (9th June). After the summer, it had dropped below 0.5 in mid September. In October, Δt was greater than 0.5 for 20 days before it dropped again below 0.5 after day 298 (24th October). In 2009, a similar pattern was observed. Δt was highest (6.1) on day 186 (5th July). However, no values above 0.5 were obvious in October. In the beginning of the year Δt increased beyond 0.5 for a period of 22 days (from day 48 (17th February) to day 71 (12th March)).

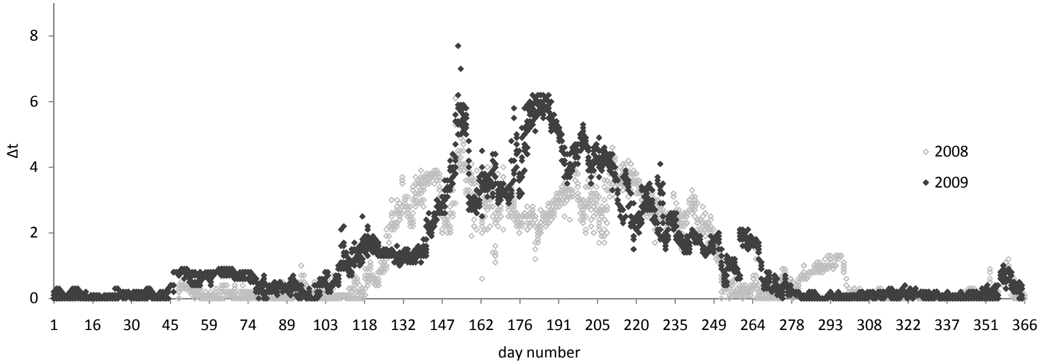


Figure 4.3: Seasonal variation in the near surface to near bottom differences in temperature (Δt) from 17th February 2008 to 31st December 2009 at station 38A.

Examples of vertical temperature profiles on a specific day representing each season in 2009 are given in Figure 4.4. The plots display the seasonal differences in water column structure. In winter (day 54; 23rd February) the difference in the near surface temperature to near bottom temperature of 0.6 suggested a slight stratification of the water column. In spring (day 129; 9th May), the water column was 1.6° C warmer than in February. In summer, a warm surface mixed layer (mean t = 14.9° C) lay on top of colder water (10.8° C) with a pronounced thermocline at approximately 40 m. In late autumn (day 304; 31st October), the water column was completely isothermal with a decreased near surface temperature of 13.8° C compared to 14.9° C in summer.

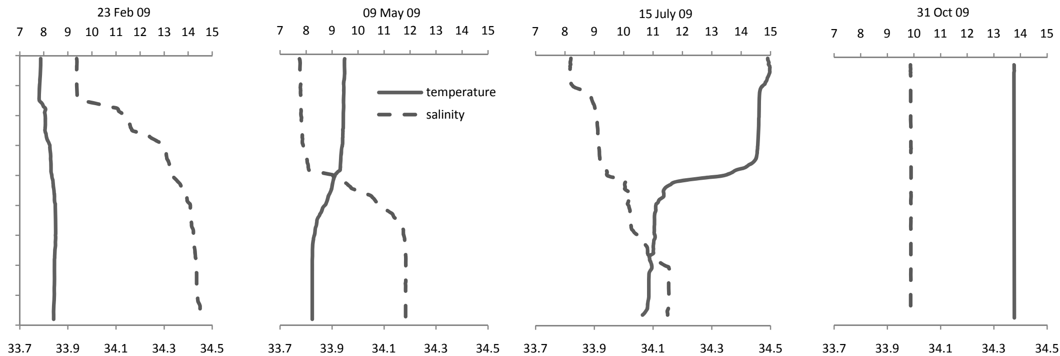


Figure 4.4: Examples of vertical temperature and salinity profiles for particular days in winter, spring, summer, and autumn in 2009. Top axis: temperature (° C), bottom axis: salinity

The seasonal cycle for the near surface salinity from 1st January 2008 to 9th December 2009 is displayed in Figure 4.5. In 2008, near surface salinity generally varied between a minimum of 33.38 on day 158 (6th June) and a maximum of 34.42 on day 165 (13th June). The mean salinity in January 2008 was 34.14 whereas the mean salinity in July 2008 was 33.86. The difference of 0.28 was significant (paired t-test $p = 0.00$, $n = 2951$). In 2009, the near surface salinity varied between 33.25 on day 157 (6th June) and 34.85 on day 208 (27th July). The mean salinity for January 2009 was 33.91 whereas the mean value in July 2009 was 33.78 resulting in a statistically significant difference (paired t-test $p = 0.00$, $n = 2950$).

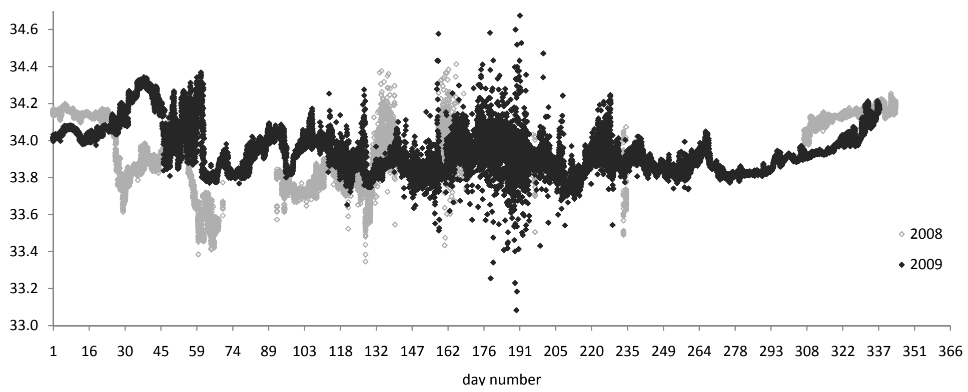


Figure 4.5: The seasonal cycle of near surface salinity from 1st January 2008 to 9th December 2009 at station 38A.

A weak seasonal pattern from salty waters in the winter to fresher waters toward spring was observed in both years (see Figure 4.6). The contour plots also show that fresher water was present in the surface layers during summer in both years while the heavier, saltier waters lay underneath. Measurements indicate that the water column in the beginning of 2009 was saltier than in 2008. By the end of both years salinity was the same.

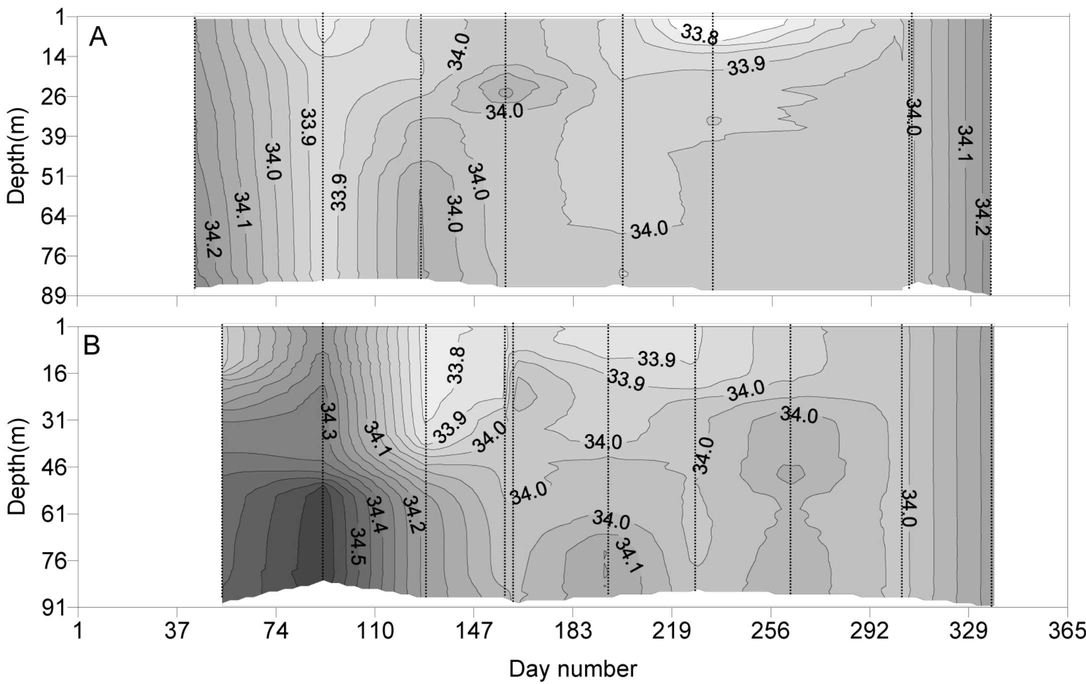


Figure 4.6: A contour plot of the seasonal variation in salinity at station 38A in 2008 and 2009. The dotted lines denote the days on which measurements were made. The contour intervals are 0.1.

The examples of vertical profiles given for temperature in Figure 4.4 display the seasonal differences in water column structure for salinity. A halocline was apparent in winter (day 54; 23rd February) at a depth of about 14 m with a near surface to near bottom salinity difference of 0.51. Near surface salinity was lower (33.73) in May (day 129; 9th May) compared to 33.93 in February. A marked halocline was apparent at a depth of approximately 40 m. Salinity stratification on day 196 (15th July) was small with a near surface to near bottom difference of 0.35. In late autumn (day 304; 31st October), the water column was completely isohaline.

A calculated Δt+s expressed the general pattern of the overall stratification in a climatology (Figure 4.7). Fourteen out of 18 data points exceeded 0.5 that indicated when stratification was considered to be established. Although vertical temperature data were available consistently throughout the year from the thermistors, vertical salinity data were only retrieved during surveys to the mooring site.

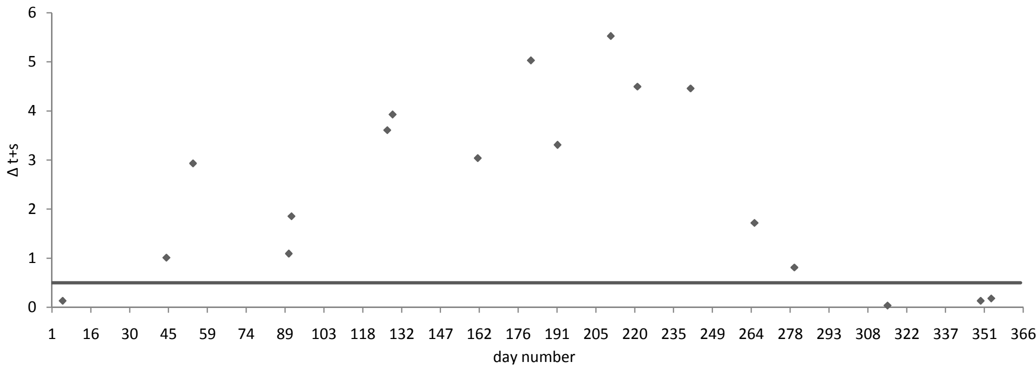


Figure 4.7: Climatology for a calculated Δt+s (Δt and Δs were added assuming that 0.1 salinity unit has the same density effect as 0.5° C). Profile data for station 38A from 2008 and 2009 were merged together to display a general pattern of overall stratification. The solid line at 0.5 indicates the value above which the water column was considered to be stratified.

4.2.2 Nutrient data

All three nutrients (nitrogen as TOxN, silicate as SiO_2 and phosphate as $PO_4{}^{3-}$) exhibited the same seasonal cycle with high levels in winter and early spring, low levels during late spring and summer and increasing levels in autumn (Figure 4.8).

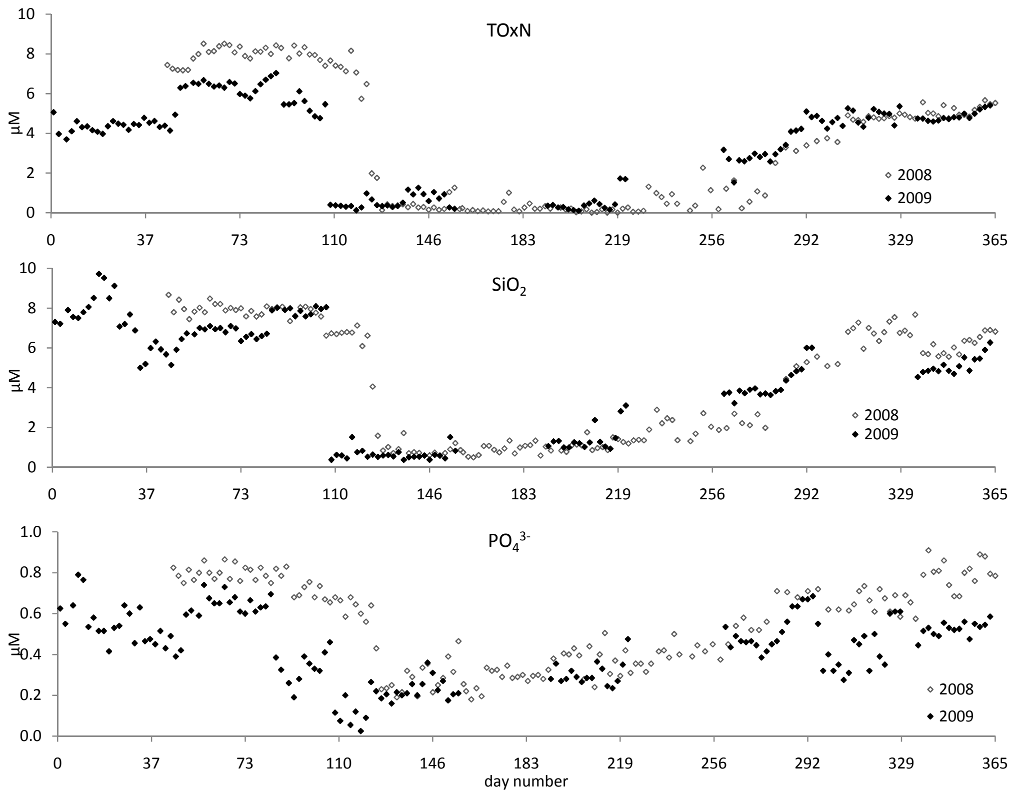


Figure 4.8: Seasonal cycles of near surface concentrations of total oxidised nitrogen (TOxN), silicate (SiO_2) and phosphate, ($PO_4{}^{3-}$) at 38A in 2008 and 2009.

Maximum concentrations measured in 2008 were 8.52 μM TOxN, 8.68 μM SiO_2 and 0.91 μM $PO_4{}^{3-}$ during mid February and early March. There was a rapid draw down of all three nutrients at the end of April and early May. For example, TOxN dropped from 6.49 μM on day 120 (29th April) to 1.99 μM on day 122 (1st May). After this period and throughout the summer concentrations of all three nutrients remained low (0.27 μM TOxN, 1.20 μM SiO_2 and 0.34 μM $PO_4{}^{3-}$). In autumn concentrations increased to 3.48 μM TOxN, 5.09 μM SiO_2 and 0.63 μM $PO_4{}^{3-}$, respectively. Maximum concentrations measured in 2009 were 7.04 μM TOxN on day 87 (28th March), 14.11 μM SiO_2 from day 93 - 99 (2nd to 8th April) and 0.79 μM $PO_4{}^{3-}$ day 8 (8th January). (The SiO_2 value seemed unreasonably high. An assessment in error analysis related this high value that remained for six days to an instrumental failure. These values were not included in further analysis, instead a mean value of SiO_2 concentration based on measurements ten days before the 2nd and ten days after the 8th April ranging from 6.10 to 8.10 μM was taken to replace these data. The mean late winter SiO_2 concentration in 2009 was then 7.31 μM.) There was a rapid draw down of all three nutrients during mid and the end of April. For example, TOxN dropped from 5.47 μM on day 109 (18th April) to 0.40 μM on day 111 (20th April). After this period and throughout the summer concentrations of all three nutrients remained low (0.53 μM TOxN, 1.26 μM SiO_2 and 0.29 μM $PO_4{}^{3-}$). During autumn concentrations increased (3.44 μM TOxN, 5.09 μM SiO_2 and 0.62 μM $PO_4{}^{3-}$).

In 2009, TOxN concentrations were not significantly different from TOxN concentrations in 2008 (Mann-Whitney test; $p>0.05$; $n = 145$). Similar statistical results were found for silicate (Mann-Whitney test; $p>0.05$; $n = 126$). For phosphate concentrations, the data from the two years were significantly different (Mann-Whitney test; $p<0.05$; $n = 143$) with a median of 0.13 lower for 2009.

Vertical nutrient profiles for a specific day in winter, spring, summer, and autumn in 2009 (Figures 4.9) showed that the shape of the three main nutrients in winter (day 54; 23rd February) had the same characteristics. There was a main nutrient distribution in sub-surface water most obvious for SiO_2 (6.92 μM) at approximately 8 m followed by a minimum (3.49 μM) in 12 m depth. In spring (day 129; 9th May), all three nutrients decreased in the upper 40 m of the water column with elevated levels from > 40m to near bottom waters. In summer (day 196; 15th July) all nutrients were depleted in the upper 40 m depth (mean TOxN 0.07, SiO_2 0.12 μM and $PO_4{}^{3-}$ 0.09 μM) and only beyond 40 m depth elevated levels of TOxN, SiO_2 and $PO_4{}^{3-}$ were measured. In late autumn (day 304; 31st October) nutrient levels were uniformly distributed throughout the water column.

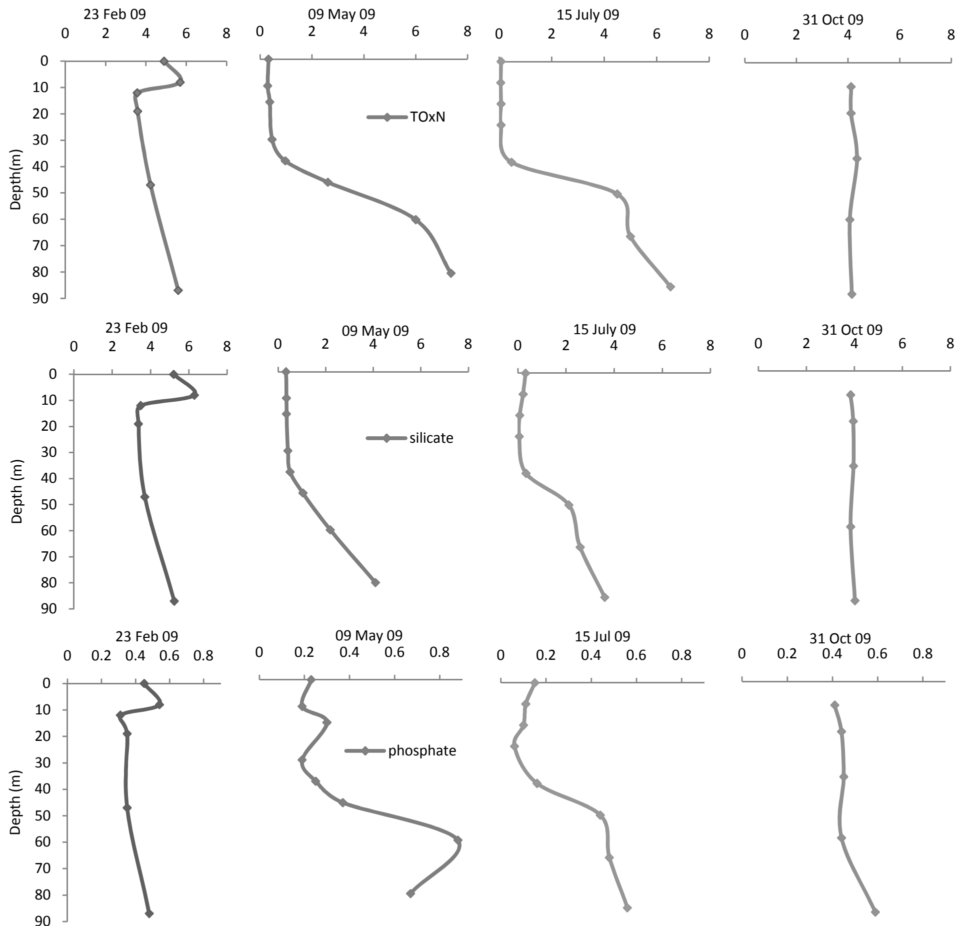


Figure 4.9: Vertical profiles of TOxN, SiO_2 and PO_4^{3-} in μM for specific days in winter, spring, summer, and autumn 2009 for station 38A in the western Irish Sea. The profiles for PO_4^{3-} are displayed on a scale from 0 to 0.9 μM, while the scales for TOxN and SiO_2 reach up to 8 μM.

The temporal pattern in the TOxN:SiO_2 ratio (N:Si) and the TOxN:PO_4^{3-} ratio (N:P) is displayed in Figure 4.10 and varied between 0.16 (July 2008) and 1.21 (May 2009).

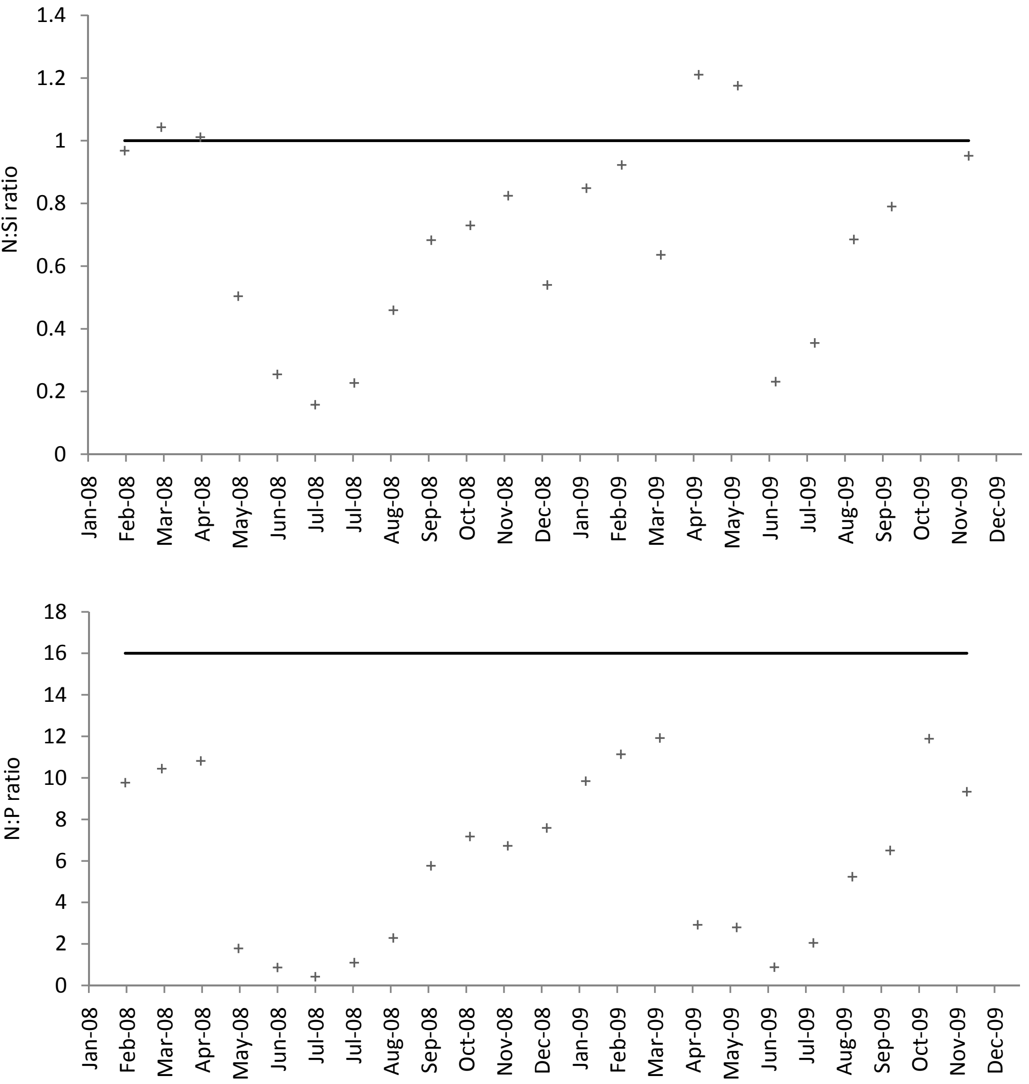


Figure 4.10: The temporal pattern of the TOxN:SiO_2 ratio (top) and the TOxN:PO_4^{3-} ratio (bottom) for near surface nutrients at 38A between January 2008 and December 2009. The black line in each plot indicates the Redfield ratio of 1:1 for N:Si and 16:1 for N:P, respectively.

The linear regressions in Figure 4.11 show the nutrient assimilation by microplankton during spring (April - May). Regressions were calculated using nutrient concentrations from spring 2008 and 2009 together to increase the number of data for each plot. The regressions were both statistically significant (analysis of variance, $p < 0.05$) for both plots, but the intercept was only significantly different from 0 in the regression of TOxN and phosphate (PO_4^{3-}) ($p < 0.05$). The regression of TOxN and silicate (SiO_2) gave an N:Si uptake ratio of 0.9 while the N:P draw down rate was 13.2 for TOxN and phosphate.

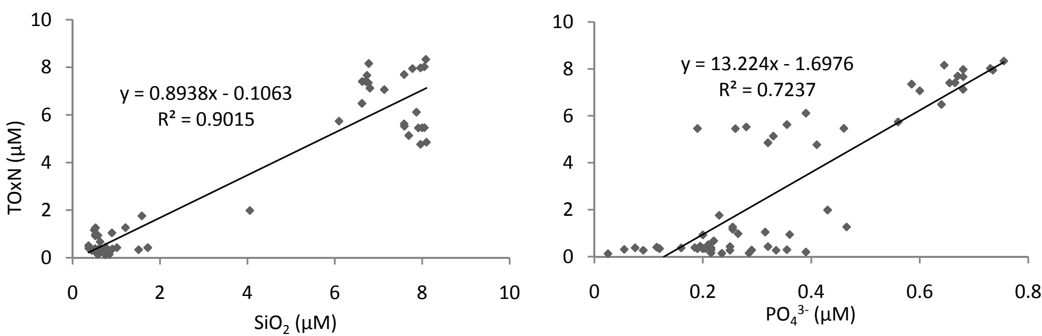


Figure 4.11: Linear regression analyses between TOxN and silicate (SiO_2) (left plot) and TOxN and phosphate ($PO_4{}^{3-}$) (right plot) for accumulated data in spring (April-May) 2008 and 2009 at station 38A. Number of observations, n = 59 for both plots. Both regressions were statistically significant ($p < 0.05$), but only for the TOxN vs phosphate ($PO_4{}^{3-}$) plot the intercept was significantly different from 0 ($p < 0.05$).

In 2009, near surface winter nutrient concentrations were not significantly related to near surface salinity (least square linear regression analysis, p>0.05), indicating no relationships between salinity and all three nutrients (Figure 4.12). For 2008 this could not be tested as sufficient (salinity) data were not available.

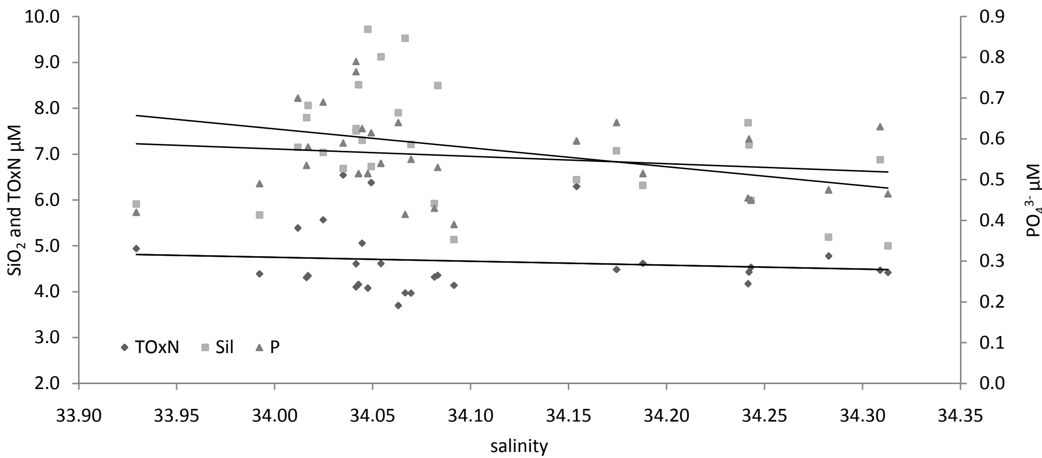


Figure 4.12: Near surface salinity, TOxN (regression y = -0.8555x + 33.836, R^2 = 0.0147), SiO_2 (regression y = -4.1171x + 147.53, R^2 = 0.01167) and $PO_4{}^{3-}$ (regression y = -0.1811x + 6.7331, R^2 = 0.0325) plot for winter 2009 data from station 38A in the western Irish Sea. The relationship was not significant; least square linear regression analysis, p>0.05

4.2.3 Chlorophyll data

The seasonal cycle of chlorophyll standing stock integrated to the base of the euphotic zone at station 38A is illustrated for data from 2008 and 2009 in Figure 4.13. In both years, there was a peak in chlorophyll standing stock in May (2008: 174.2 mg Chl m^{-2} day 127; 6th; May 2009: 212.5 mg Chl m^{-2} day 129; 9th May). The contour plots in Figure 4.14 show that there was a peak in chlorophyll on the same day and that the bulk of the standing stock was found in the upper 30 m of the water column. In 2008, the peak in spring was associated with a sub-surface chlorophyll maximum (10.0 mg m^{-3} day 127; 6th May), but in May 2009, the standing stock was more evenly spread (4.23 mg m^{-3}) over the upper 40 m of the water column (Figure 4.15). In July, the chlorophyll patterns were similar to the ones observed in May in both years (Figure 4.15) with generally lower concentrations (1.76 mg m^{-3} and 1.09 mg m^{-3}, respectively) but a distinct sub-surface chlorophyll maximum in 2008 (day 201; 19th July) and more evenly spread chlorophyll in 2009 (day 197; 15th July). In late summer, early autumn, elevated chlorophyll was apparent in both years (Figure 4.14), although it is more evident in 2008 on day 234 (22nd August) (3.4 mg chl m^{-3}; 60.85 mg chl m^{-2}) than in 2009 on day 264 (21st September) (1.6 mg chl m^{-3}; 49.84 mg chl m^{-2}). A chlorophyll concentration of 2 mg m^{-3} at a depth of 50 m was recorded for the spring period in both years.

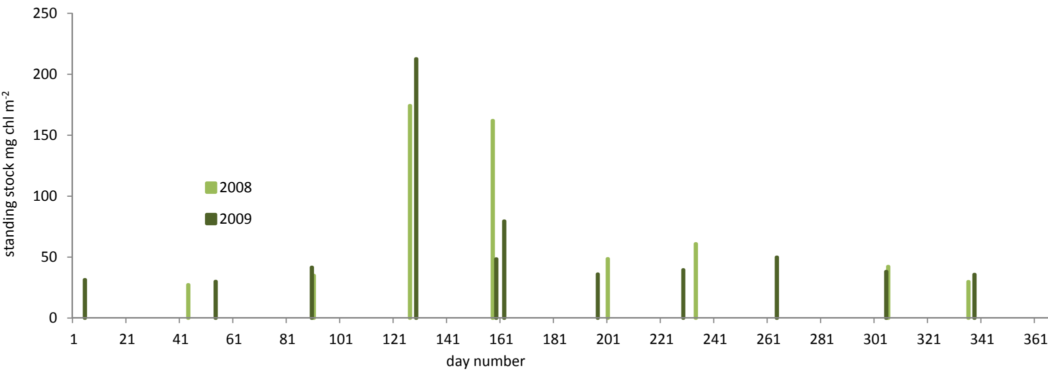


Figure 4.13: The general seasonal cycle of chlorophyll standing stock (mg m^{-2}) for 2008 and 2009, given for station 38A in the western Irish Sea.

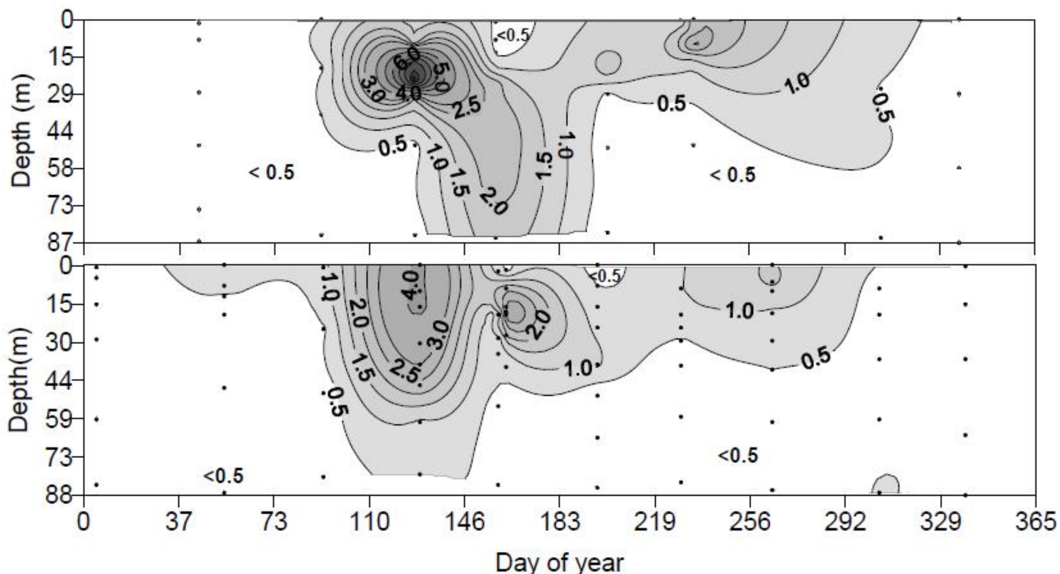


Figure 4.14: Contour plots of the seasonal cycle of chlorophyll (mg m^{-3}) at station 38A in 2008 and 2009 in the western Irish Sea. The contour levels are 0.5 mg m^{-3}. The dots indicate the measurement points along the vertical profile.

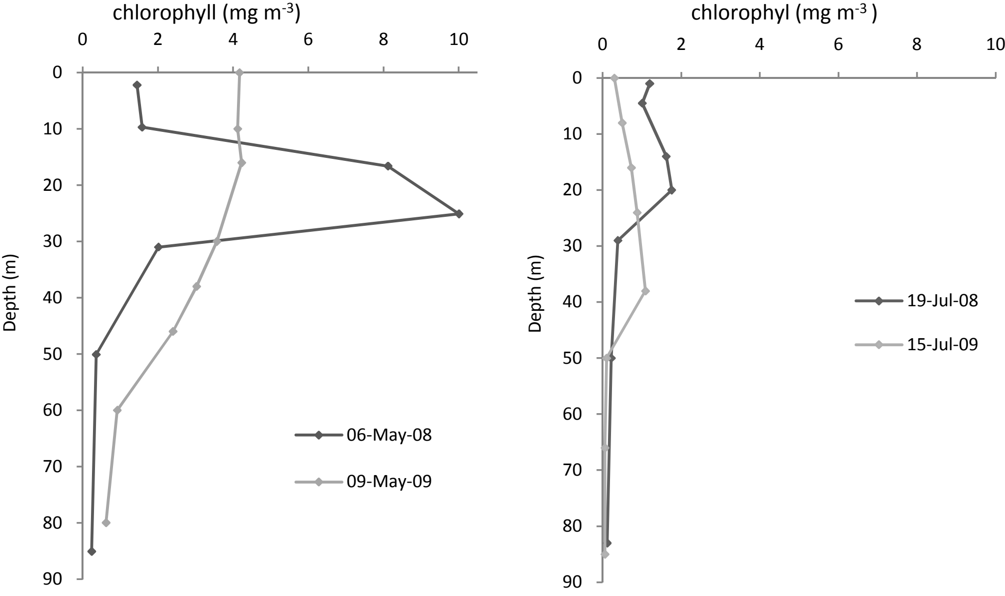


Figure 4.15: Vertical chlorophyll profiles for station 38A in the western Irish Sea for a day in spring 2008 (day 127; 6th May) and for a day in spring 2009 (day 129; 9th May) on the left hand side; for a day in summer 2008 (day 201; 19th July) and for a day in summer 2009 (day 197; 15th July) on the right hand side.

4.2.4 Microplankton data

Between February 2008 and December 2009, a total of 105 species/taxa were identified and enumerated at station 38A. This included 52 diatoms belonging to 29 genera (categorised into centric and pennate diatoms), 37 dinoflagellates comprising 17 genera, (grouped as naked and armoured dinoflagellates), the silicoflagellates *Dictyocha speculum* and *Dictyocha fibulum*, six categories of flagellates (divided into nano-flagellates, *Cryptophytes*, *Prasinophytes*, *Euglenophytes*, *Prymnesiophytes* (*Phaeocystis spp.*) and heterotrophic flagellates, and ciliates belonging to eight genera (*Laboea* sp., *Lohmaniella* sp., *Leegardia* sp., *Strombidium* sp., *Mesodinium* sp., *Dididium* sp., tintinnids and unidentified ciliates). A complete list of all species identified in 2008 and 2009 is given in the appendix (A.1).

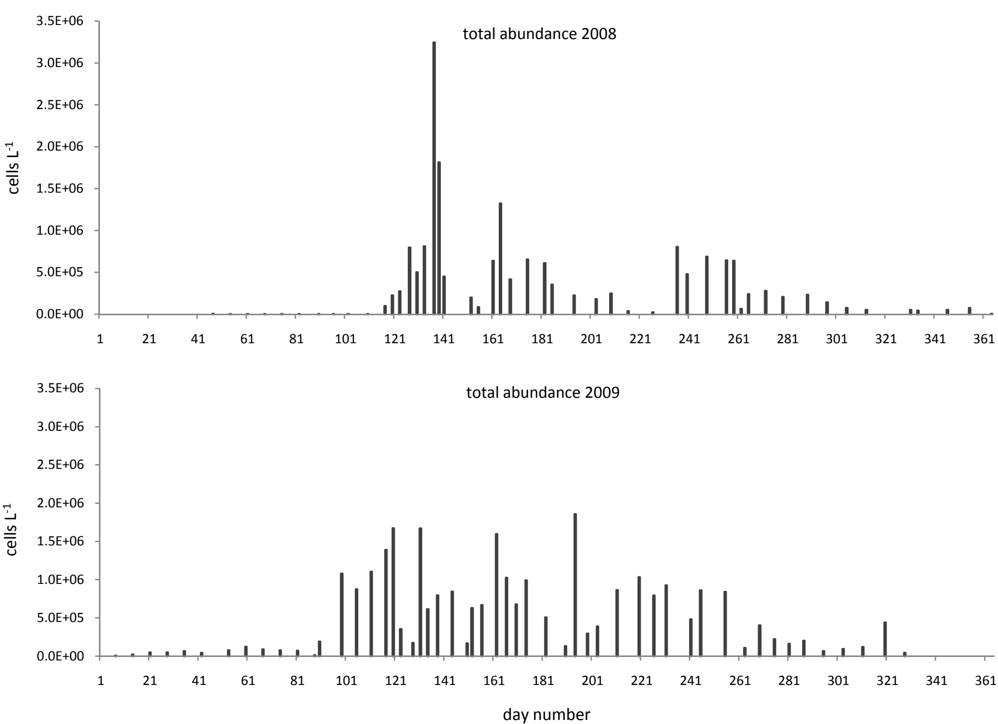


Figure 4.16: Seasonal variation in total cell abundance (cells L^{-1}) at station 38A in 2008 and 2009.

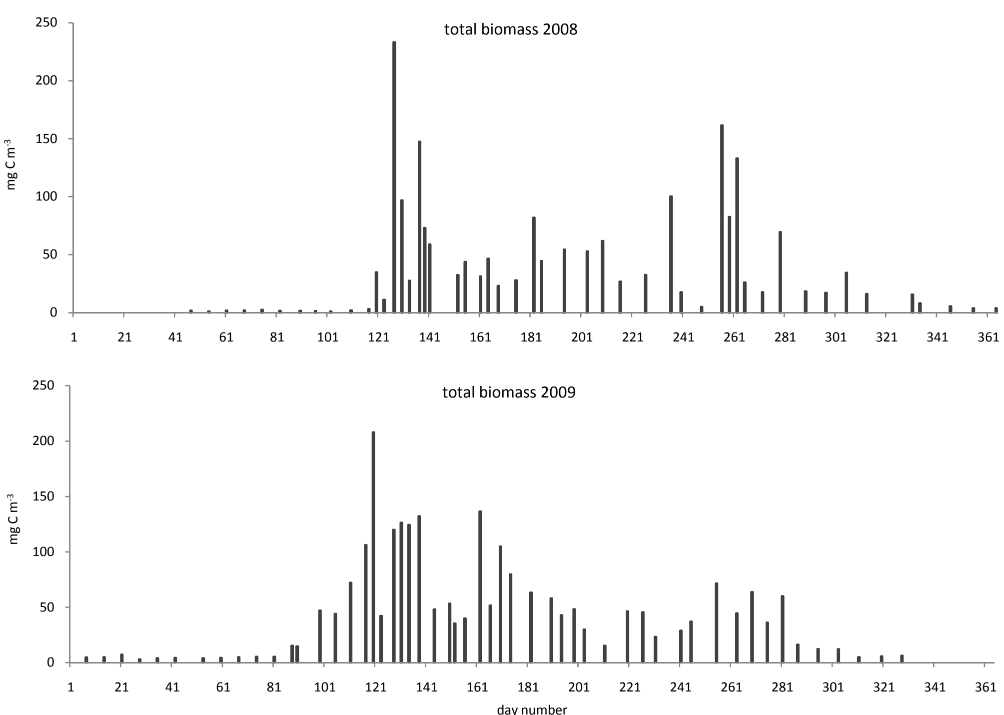


Figure 4.17: Seasonal variation in total carbon biomass (mg C m^{-3}) at station 38A in 2008 and 2009.

Over the 22 months sampling period the most frequently occurring group was 'unidentified ciliates' that occurred in 92% of the samples. Species of *Cryptophytes* and nano-flagellates occurred in 91% and 90% of the samples respectively. The most frequently occurring diatoms were *Pseudonitzschia* species (belonging to the *seriata* group) and *Cylindrotheca closterium* that were present in 85% and 84% of the samples, respectively. Medium sized species of *Gymnodinium* and the armoured dinoflagellate *Prorocentrum micans* were the most consistent dinoflagellates and occurred in 80% and 76% of the samples, respectively.

There was a pronounced seasonal cycle in total microplankton cell abundance (Figure 4.16) and carbon biomass (Figure 4.17). In both years, cell abundance began to increase in mid April although the increase began a few days earlier in 2009. The average cell abundance was 32.1 x 10^3 cells L^{-1} for winter, 684.9 x 10^3 cells L^{-1} for spring, 447.1 x 10^3 cells L^{-1} for summer, and 184.5 x 10^3 cells L^{-1} for autumn in 2008 (table 4.1). In 2009, the average abundance was 56.2 x 10^3 cells L^{-1} for winter, 896.2 x 10^3 cells L^{-1} for spring, 765.6 x 10^3 cells L^{-1} for summer, and 296.5 x 10^3 cells L^{-1} for autumn. Maximum peaks (3,244.3 x 10^3 cells L^{-1}, 1,854.6 x 10^3 cells L^{-1}) were recorded on day 136 (16th May) in 2008 and on day 196 (15th July) in 2009. Spring peaks of carbon biomass (233.1 mg C m^{-3}, 207.9 mg C m^{-3}) were recorded on day 125 (5th May) in 2008 and on day 121 (2nd May) in 2009, respectively. Cell abundance and biomass were decreased in the summer period in 2008, but showed increased values toward the early autumn months. Maximum cell

abundance in autumn was 638.2 x 10^3 cells L^{-1} on day 260 (5th September) and carbon biomass was high (161.5 mg C m^{-3}) on day (30th August). In 2009, a pronounced growth period in late summer/early autumn was not as apparent as it was in 2008. In the summer period, the values stayed higher than they did in 2008. The highest peak for cell abundance (855.9 x 10^3 cells L^{-1}) in autumn 2009 was recorded on day 246 (4th September). The highest carbon biomass value (71.5 mg C m^{-3}) in the autumn period in 2009 was recorded on day 256 (14th September).

A significant difference in cell abundance was recorded (Mann-Whitney test; $p<0.05$) between the two years, however biomass did not differ significantly between 2008 and 2009 (Mann-Whitney test; $p>0.05$).

Table 4.1 displays the five most abundant species for the four taxonomic functional groups diatoms, dinoflagellates, micro-flagellates, and ciliates in abundance (cells L^{-1}) and carbon biomass (mg C m^{-3}) for winter 2008 to autumn 2009. The average cell abundance and biomass for each season is given in the top line of every table and in brackets behind each time period a number is given for taxa that were identified out of a total of 105. The seasons were defined on the basis of the TOxN concentration pattern (see chapter 3). Winter was from December to March, spring from April to May, summer from June to August and autumn was defined from September to November.

Table 4.1: The tables display the dominating species for each functional group (diatoms, dinoflagellates, micro-flagellates and ciliates). The top five species/taxa for each lifeform are displayed for the average cell abundance and the average carbon biomass for winter, spring, summer and autumn in 2008 and 2009 at station 38A. The number of identified species/taxa for each season is given in brackets at the top of each table. A total number of 105 identified species/taxa was recorded for this station.% represents the amount of the total biomass of all species identified.

winter 08 (51)

average biomass [mg C m^{-3}]	**2.56**	average cell abundance [cells L^{-1}]	**32.1 x 10^3**
Thalassiosira cf. *rotula*	0.29 (12.0%)	*Paralia sulcata*	636 (3.2%)
Navicula medium (50-150µm)	0.13 (5.3%)	small centric diatoms (<25µm)	296 (1.5%)
Ditylum brightwellii	0.11 (4.4%)	*Cylindrotheca closterium*	204 (1.0%)
Paralia sulcata	0.08 (3.4%)	*Navicula* small (<50µm)	196 (1.0%)
Rhizosolenia styliformis/imbricata	0.05 (1.9%)	*Pseudo-nitzschia seriata* group	188 (0.9%)
Gymnodinium med (25-40µm)	0.1 (4.0%)	*Gymnodinium* small (<25µm)	122 (0.6%)
Gyrodinium medium (25-40µm)	0.05 (1.9%)	small naked dinos (<25µm)	46 (0.2%)
Protoperidinium crassipes/divergens	0.04 (1.5%)	*Gyrodinium* medium (25-40µm)	40 (0.2%)
Gonyaulax sp.	0.03 (1.3%)	small armoured dinos (<25µm)	24 (0.1%)
small armoured dinos (<25µm)	0.02 (1.0%)	*Amphidinium* sp.	20 (0.1%)
nano flagellates	0.28 (11.4%)	**nano-flagellates**	**6772 (33.9%)**
Cryptophytes	0.14 (5.9%)	*Cryptophytes*	4770 (24%)
Prasinophytes	0.07 (3.0%)	*Rhodomonads*	178 (0.9%)
Euglenophytes	0.06 (2.4%)	*Prasinophytes*	4680 (23.6%)
Rhodomonads	0.02 (0.7%)	*Prymnesiophytes (Phaeocystis)*	70 (0.4%)
unidentified ciliates	**0.34 (14.1%)**	unidentified ciliates	103 (0.5%)
Strombidium sp.	0.06 (2.5%)	Tintinnids	10 (0.1%)
Laboea sp.	0.04 (1.5%)		
Tintinnids	0.02 (0.9%)		

spring 08 (92)

average biomass [mg C m^{-3}]	**57.53**	average cell abundance [cells L^{-1}]	**684.9 x10^3**
Thalassiosira* cf. *rotula	**21.29 (36.6%)**	***Chaetoceros* small (<25µm)**	**258862 (37.8%)**
Chaetoceros small (<25µm)	9.15 (15.9%)	*Chaetoceros* medium (25-40µm)	16259 (2.4%)
Rhizosolenia styliformis/imbricata	2.99 (5.2%)	*Thalassiosira* cf. *rotula*	12002 (1.8%)
Melosira nummuloides	2.53 (4.4%)	*Thalassiosira angulata*	3167 (0.5%)
Laudaria annulata	1.90 (3.3%)	*Pseudo-nitzschia seriata* group	1330 (0.2%)
Gymnodinium medium (25-45µm)	0.52 (0.9%)	*Gymnodinium* medium (25-45µm)	386 (0.1%)
Ceratium furca	0.35 (0.6%)	*Ceratium lineatum*	220 (0.05%)
Gyrodinium medium (25-45µm)	0.21 (0.4%)		
Protoperidinium spp.	0.2 (0.4%)		
Protoperidinium micans	0.16 (0.3%)		
Cryptophytes	2.47 (4.3%)	*Prymnesiophytes (Phaeocystis)*	210099 (30.7%)
Prymnesiophytes (Phaeocystis)	1.78 (3.1%)	*Cryptophytes*	94268 (13.8%)
nano flagellates	1.55 (2.7%)	nano-flagellates	45177 (6.6%)
Prasinophytes	0.81 (1.4%)	*Prasinophytes*	24943 (3.6%)
Euglenophytes	0.80 (1.4%)	*Euglenophytes*	4982 (0.7%)
unidentified ciliates	0.92 (1.6%)	unidentified ciliates	445 (0.1%)
Laboea sp.	0.78 (1.4%)	*Strombidium* sp.	369 (0.1%)
Strombidium sp.	0.57 (1.0%)	*Laboea* sp.	167 (0.03%)

summer 08 (94)

average biomass [mg C m^{-3}]	**49.65**	average cell abundance [cells L^{-1}]	**447.1 x10^3**
Rhizosolenia styliformis/imbricata	3.62 (7.3%)	*Pseudo-nitzschia seriata* group	17537 (3.9%)
Pseudo-nitzschia seriata group	3.28 (6.6%)	*Leptocylindrus danicus*	7440 (1.7%)
Proboscia alata	3.13 (6.3%)	*Skeletonema* spp.	6023 (1.3%)
Rhizosolenia setigera	2.2 (4.5%)	*Leptocylindrus minimus*	2481 (0.6%)
Leptocylindrus danicus	1.64 (3.3%)	*Rhizosolenia setigera*	2475 (0.6%)
***Gymnodinium* medium (25-45µm)**	**7.6 (15.3%)**	*Gymnodinium* medium (25-45µm)	8382 (1.9%)
Prorocentrum micans	0.79 (1.6%)	*Gymnodinium* small (<25um)	3208 (0.7%)
small armoured dinos (<25µm)	0.4 (0.9%)	small armoured dinos (<25µm)	1465 (0.3%)
Protoperidinium spp.	0.39 (0.8%)	*Protoperidinium bipes*	1451 (0.3%)
Gyrodinium medium (25-45µm)	0.35 (0.7%)	*Gyrodinium* medium (25-45µm)	1086 (0.2%)
nano flagellates	7.25 (14.6%)	**nano-flagellates**	**175920 (39.4%)**
Cryptophytes	2.43 (4.9%)	*Cryptophytes*	96746 (21.7%)
Prasinophytes	2.13 (4.3%)	*Prasinophytes*	76176 (17.%)
Euglenophytes	0.5 (1.0%)	*Prymnesiophytes (Phaeocystis)*	12904 (2.9%)
Prymnesiophytes (Phaeocystis)	0.1 (0.1%)	*Euglenophytes*	4287 (1.0%)
Strombidium sp.	1.34 (2.7%)	unidentified ciliates	797 (0.2%)
unidentified ciliates	1.29 (2.6%)	*Strombidium* sp.	652 (0.1%)
Laboea sp.	0.79 (1.6%)	*Lohmaniella strobilidium*	234 (0.1%)

autumn 08 (92)

average biomass [mg C m^{-3}]	**39.86**	average cell abundance [cells L^{-1}]	**184.5 x10^3**
Rhizosolenia setigera	**7.29 (18.3%)**	*Leptocylindrus danicus*	1683 (0.9%)
Proboscia alata	1.79 (4.5%)	*Paralia sulcata*	959 (0.5%)
Stephanopyxis turris	1.08 (2.7%)	*Proboscia alata*	819 (0.4%)
Rhizosolenia pungens	0.6 (1.5%)	small centric diatoms (<25µm)	780 (0.4%)
Leptocylindrus danicus	0.56 (1.4%)	*Pseudo-nitzschia seriata* group	678 (0.4%)
Gymnodinium medium (25-45µm)	3.63 (9.1%)	*Gymnodinium* medium (25-45µm)	3798 (2.0%)
small naked dinos (<25µm)	2.67 (6.7%)	*Gymnodinium* small (<25µm)	2193 (1.2%)
Prorocentrum micans	0.51 (1.4%)	*Ceratium minutum*	444 (0.2%)
Ceratium minutum	0.36 (0.9%)	*Gyrodinium* medium (25-45µm)	267 (0.1%)
Ceratium tripos	0.32 (0.8%)	*Gyrodinium* small (<25µm)	136 (0.1%)
nano flagellates	7.17 (18.0%)	**nano-flagellates**	**75699 (39.9%)**
Cryptophytes	3.55 (8.9%)	*Prasinophytes*	43667 (23.0%)
Prasinophytes	3.15 (7.9%)	*Cryptophytes*	33311 (17.6%)
Rhodomonas sp.	0.34 (0.9%)	*Rhodomonas* sp.	5000 (2.6%)
Euglenophytes	0.28 (0.7%)	*Euglenophytes*	1309 (0.7%)
unidentified ciliates	0.52 (1.3%)	unidentified ciliates	196 (0.1%)
Dididium sp.	0.49 (1.2%)	Tintinnids	58 (0.02%)

winter 09 (76)

average biomass [mg C m^{-3}]	**5.59**	average cell abundance [cells L^{-1}]	**56.2 x10^3**
Thalassiosira cf. *rotula*	0.28 (5.0%)	*Paralia sulcata*	1360 (2.4%)
Coscinodiscus spp.	0.26 (4.6%)	*Thalassiosira angulata*	327 (0.6%)
Paralia sulcata	0.16 (2.9%)	*Pseudo-nitzschia delicatissima* group	287 (0.5%)
Dithlum brightwellii	0.14 (2.5%)	*Pseudo-nitzschia seriata* group	257 (0.5%)
Navicula medium (50-150µm)	0.11 (1.9%)	small centric diatoms (<25µm)	235 (0.4%)
Gymnodinium medium (25-45µm)	0.17 (3.1%)	*Gymnodinium* small (<25µm)	450 (0.8%)
Gyrodinium medium (25-45µm)	0.08 (1.5%)	*Gymnodinium* medium (25-45µm)	210 (0.4%)
Protoperidinium spp.	0.08 (1.4%)	small armoured dinos (<25µm)	132 (0.2%)
Prorocentrum micans	0.06 (1.0%)	*Gyrodinium* medium (25-45µm)	115 (0.2%)
small armoured dinos (<25µm)	0.04 (0.7%)	*Gyrodinium* small (<25µm)	67 (0.1%)
nano flagellates	1.01 (18.1%)	**nano-flagellates**	**24058 (42.8%)**
Cryptophytes	0.6 (10.8%)	*Cryptophytes*	21183 (37.7%)
Euglenophytes	0.28 (5.0%)	*Prasinophytes*	3600 (6.4%)
Prasinophytes	0.13 (2.2%)	*Euglenophytes*	1500 (2.7%)
Rhodomonas sp.	0.01 (0.2%)	*Rhodomonas* sp.	150 (0.3%)
unidentified ciliates	**1.02 (18.2%)**	unidentified ciliates	470 (0.8%)
Laboea sp.	0.11 (2.0%)	*Strombidium* sp.	55 (0.1%)
Strombidium sp.	0.1 (1.7%)	*Lohmaniella strobilidium*	48 (0.1%)
Lohmaniella strobilidium	0.05 (0.9%)	*Laboea* sp.	28 (0.1%)

spring 09 (89)

average biomass [mg C m^{-3}]	**90.41**	average cell abundance [cells L^{-1}]	**896.2 x10^3**
***Chaetoceros* small (<25µm)**	**14.19 (15.7%)**	***Chaetoceros* small (<25µm)**	**455973 (50.9%)**
Guinardia delicatula	14.1 (15.6%)	*Guinardia delicatula*	41260 (4.6%)
Rhizosolenia styliformis/imbricata	10.94 (12.1%)	*Pseudo-nitzschia seriata* group	14233 (1.6%)
Thalassiosira cf. *rotula*	9.04 (10.0%)	*Chaetoceros* medium (25-40µm)	7290 (0.8%)
Laudaria annulata	3.53 (3.9%)	*Cylindrotheca closterium*	7063 (0.8%)
Protoperidinium spp.	1.54 (1.7%)	*Gymnodinium* small (<25µm)	11325 (1.3%)
Gymnodinium medium (25-45µm)	0.99 (1.1%)	small armoured dinos (<25µm)	1890 (0.2%)
Gymnodinium small (<25µm)	0.72 (0.8%)	*Gymnodinium* medium (25-45µm)	1232 (0.1%)
Gyrodinium large (>45µm)	0.63 (0.7%)	*Gyrodinium* medium (25-45µm)	1232 (0.1%)
Prorocentrum micans	0.54 (0.6%)	*Protoperidinium* spp.	770 (0.1%)
nano flagellates	6.87 (7.6%)	nano-flagellates	167325 (18.7%)
Cryptophytes	2.26 (2.5%)	*Cryptophytes*	81000 (9.0%)
Prasinophytes	1.36 (1.5%)	*Prasinophytes*	41513 (4.6%)
Prymnesiophytes (Phaeocystis)	0.09 (0.1%)	*Prymnesiophytes (Phaeocystis)*	12344 (1.4%)
Euglenophytes	0.09 (0.1%)	*Euglenophytes*	488 (0.1%)
unidentified ciliates	2.53 (2.8%)	unidentified ciliates	1175 (0.2%)
Laboea sp.	1.36 (1.5%)	*Laboea* sp.	345 (0.04%)
Strombidium sp.	0.54 (0.6%)	*Strombidium* sp.	3900 (0.04%)

summer 09 (91)

average biomass [mg C m^{-3}]	**53.07**	average cell abundance [cells L^{-1}]	**765.6 x10^3**
Rhizosolenia styliformis/imbricata	5.1 (9.6%)	*Leptocylindrus danicus*	23612 (3.1%)
Leptocylindrus danicus	4.4 (8.3%)	*Leptocylindrus minimus*	22540 (2.9%)
Guinardia striata	1.59 (3.0%)	*Chaetoceros* small (<25µm)	15885 (2.1%)
Pseudo-nitzschia seriata group	1 .06 (2.0%)	*Cylindrotheca closterium*	9380 (1.2%)
Chaetoceros medium (25-40µm)	0.96 (1.8%)	*Chaetoceros* medium (25-40µm)	8006 (1.0%)
Gymnodinium medium (25-45µm)	4.09 (7.7%)	*Gymnodinium* small (<25µm)	21514 (2.8%)
Prorocentrum micans	1.96 (3.7%)	*Gymnodinium* medium (25-45µm)	4502 (0.6%)
Gymnodinium small (<25µm)	1.49 (2.8%)	*Ceratium minutum*	1667 (0.2%)
Gyrodinium medium (25-45µm)	0.85 (1.6%)	*Gyrodinium* medium (25-45µm)	1105 (0.1%)
Ceratium minutum	0.74 (1.4%)	*Prorocentrum micans*	1056 (0.1%)
Prymnesiophytes (Phaeocystis)	**5.73 (10.8%)**	***Prymnesiophyceae (Phaeocystis)***	**293754 (38.4%)**
Euglenophytes	3.87 (7.3%)	nano-flagellates	123702 (16.2%)
Cryptophytes	3.77 (7.1%)	*Cryptophytes*	120451 (15.7%)
nano-flagellates	3.08 (5.8%)	*Prasinophytes*	78429 (10.2%)
Prasinophytes	2.92 (5.5%)	*Euglenophytes*	19006 (2.5%)
Strombidium sp.	0.53 (1.0%)	*Strombidium* sp.	272 (0.04%)
unidentified ciliates	0.42 (0.8%)	unidentified ciliates	188 (0.02%)

autumn 09 (88)

average biomass [mg C m^{-3}]	**30.89**	average cell abundance [cells L^{-1}]	**296.5 x10^3**
Leptocylindrus danicus	3.27 (10.6%)	*Chaetoceros* small (<25µm)	19620 (6.6%)
Eucampia zoodiacus	1.32 (4.3%)	*Leptocylindrus danicus*	14877 (5.0%)
Laudaria annulata	0.77 (2.5%)	*Pseudo-nitzschia seriata* group	2527 (0.9%)
Thalassiosira cf. *rotula*	0.65 (2.1%)	*Eucampia zoodiacus*	1653 (0.6%)
Pseudo-nitzschia seriata group	0.62 (2.0%)	*Paralia sulcata*	1053 (0.4%)
Gymnodinium medium (25-45µm)	1.2 (3.9%)	*Gymnodinium* small (<25µm)	9042 (3.0%)
Gymnodinium small (<25µm)	0.74 (2.4%)	*Gymnodinium* medium (25-45µm)	1117 (0.4%)
Gyrodinium medium (25-45µm)	0.28 (0.9%)	small armoured dinos (<25µm)	407 (0.1%)
Ceratium minutum	0.22 (0.7%)	*Ceratium minutum*	403 (0.1%)
small armoured dinos (<25µm)	0.15 (0.5%)	*Gyrodinium* medium (25-45µm)	292 (0.1%)
Euglenophytes	**7.32 (23.7%)**	***Cryptophytes***	**57375 (19.4%)**
nano flagellates	2.72 (8.8%)	*Prymnesiophytes (Phaeocystis)*	55027 (18.6%)
Cryptophytes	2.13 (6.9%)	nano-flagellates	49650 (16.7%)
Prasinophytes	1.92 (6.2%)	*Prasinophytes*	43575 (14.7%)
Prymnesiophytes (Phaeocystis)	0.7 (2.2%)	*Euglenophytes*	30450 (10.3%)
unidentified ciliates	1.14 (3.7%)	unidentified ciliates	407 (0.1%)
Strombidium sp.	0.86 (2.8%)	*Strombidium* sp.	387 (0.1%)
Dididium sp.	0.41 (1.3%)	*Dididium* sp.	87 (0.03%)

In winter 2008, when average cell abundance (32.1 x 10^3 cells L^{-1}) and carbon biomass (2.6 mg C m^{-3}) were low, large centric and pennate diatoms represented 1.1 mg C m^{-3} of the microplankton. The spring bloom appeared to have started on day 123 (2nd May) in 2008 (Figure 4.16) with small sized *Chaetoceros spp.* (258.9 x 10^3 cells L^{-1}) as the most abundant species followed by *Phaeocystis spp.* (210.1 x 10^3 cells L^{-1}) (Table 4.1). The dominating species in respect of carbon biomass was *Thalassiosira rotula* (21.3 mg C m^{-3}) followed by small *Chaetoceros spp.* (9.2 mg C m^{-3}).

Ninety two out of a total of 105 species/taxa identified were present during the spring period. Those were represented by 44 diatom species, 30 dinoflagellate species, both silicoflagellates species (*Dictyocha speculum* and *Dictyocha fibulum*), five micro-flagellate taxa, and eight ciliate taxa.

The average summer assemblage in 2008 was dominated by nano-flagellates (175.9 x 10^3 cells L^{-1}), followed by *Cryptophytes* (96.7 x 10^3 cells L^{-1}). Considering the average carbon biomass of the summer period, naked dinoflagellates like medium sized *Gymnodinium spp.* dominated the field (7.6 mg C m^{-3}) (Table 4.1). Microplankton abundance in the autumn months consisted mainly of micro-flagellates (158.9 x 10^3 cells L^{-1}) in general and nano-flagellates (75.7x 10^3 cells L^{-1}) in particular while diatoms represented the highest average carbon biomass (11.3 mg C m^{-3}) with species like *Rhizosolenia setigera*, *Probosia alata* and *Stephanopyxis turris*. In winter 2009, average cell abundance and biomass were low (56.2 x 10^3 cells L^{-1} and 5.6 mg C m^{-3}, respectively). 2.0 mg C m^{-3} of the composition were micro-flagellates (specifically nano-flagellates), diatoms contributed 1.8 mg C m^{-3} (specifically *Paralia sulcata*), ciliates contributed 1.3 mg C m^{-3}, and 0.4 mg C m^{-3} of the biomass were dinoflagellates. 'Unidentified ciliates' were the dominant single category with an average biomass of 1.0 mg C m^{-3}. In spring 2009, increased cell abundance and biomass were apparent from the 10th April (day 100). The peak in cell abundance (1,077.3 x 10^3 cells L^{-1}) and biomass (47.0 mg C m^{-3}) on that day was mainly due to the high abundance of small *Chaetoceros* species that contributed 836.0 x 10^3 (78%) to the cell abundance and 30.6 mg C m^{-3} (65%) to the microplankton biomass. After the peak on day 100 (10th April), biomass and cell abundance decreased until another peak was recorded on day 112 (22th April). The highest cell abundance peak (1,668.5 x 10^3 cells L^{-1}) was recorded on days 121 (2nd May) and 132 (13th May) with small *Chaetoceros* species (886.5 x 10^3 cells L^{-1}) contributing the largest amount. The highest total biomass (207.9 mg C m^{-3}) was recorded on day 121 (2nd May). In respect to the average spring biomass (90.4 mg C m^{-3}), the bloom was dominated by small *Chaetoceros* followed by *Guinardia delicatula* and *Rhizosolenia styliformis/imbricata* (Table 4.1). Eighty nine out of a total 105 species/taxa identified were present during the spring period in 2009. This was made up of 46 diatom taxa, 29 dinoflagellate species, both silicoflagellates species

(*Dictyocha speculum* and *Dictyocha fibulum*), five flagellate taxa, and eight ciliate taxa. During the summer 2009, an average cell abundance of 765.6 x 10^3 cells L^{-1} was dominated by *Phaeocystis* spp. (293.8 x 10^3 cells L^{-1}) that also contributed the highest single carbon biomass (5.7 mg C m^{-3}) to a total carbon biomass of 55.5 mg C m^{-3}. However, all together diatoms were the dominant taxonomic functional group and represented 19.7 mg C m^{-3} of the carbon biomass with species like *Rhizosolenia styliformis/imbricate* and *Leptocylindrus danicus*. In autumn 2009, micro-flagellates (272.4 x 10^3 cells L^{-1}) represented the highest average abundance of all with *Cryptophytes* (57.4 x 10^3 cells L^{-1}) as the most abundant taxa. The average carbon biomass (27.3 mg C m^{-3}) was dominated by micro-flagellates (10.5 mg C m^{-3}), while diatoms (9.5 mg C m^{-3}), dinoflagellates (8.4 mg C m^{-3}) and ciliates (2.4 mg C m^{-3}) represented lower concentrations (Table 4.1). The dominant diatom species in autumn were *Leptocylindrus danicus* and *Eucampia zodiacus*. The dinoflagellates were represented mostly by naked dinoflagellates (small and medium sized *Gymnodinium spp.*) and species of *Ceratium*. The ciliates were mainly represented by 'unidentified ciliates'.

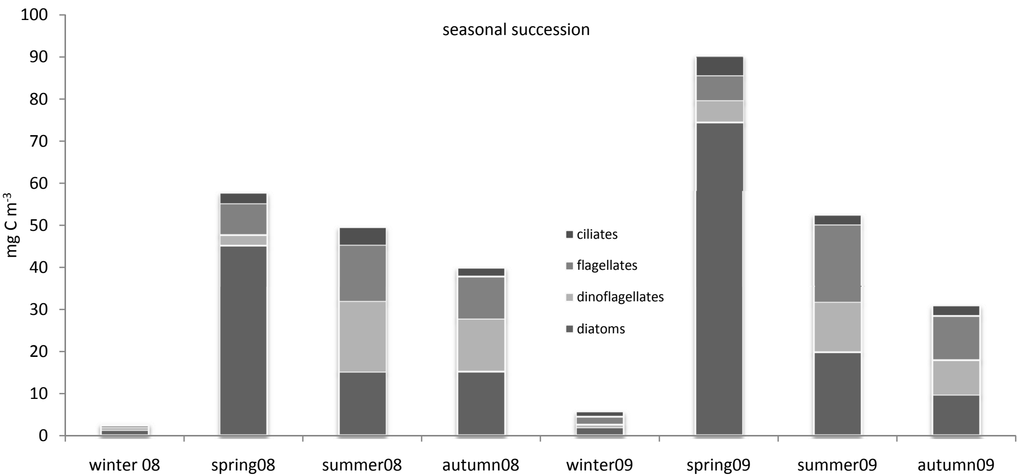


Figure 4.18: The succession of microplankton categorised into four taxonomic functional groups at 38A for winter, spring, summer, and autumn in 2008 and 2009.

Categorising the microplankton into the four functional groups diatoms, dinoflagellates, micro-flagellates, and ciliates, a seasonal succession was apparent in both sampling years (Figure 4.18). Diatoms dominated the spring periods in both years (44.80 mg C m^{-3} (78%) of the total spring average in 2008 and 74.51 mg C m^{-3} (84%) of the total spring average in 2009). The highest average carbon biomass of dinoflagellates was recorded in summer in both years (16.90 mg C m^{-3} (34%) of the total summer average in 2008 and 11.91 mg C m^{-3} (22%) of the total summer average in 2009). Ciliates contributed between 0.34 mg C m^{-3} (12%) in winter 08 and 4.67 mg C m^{-3} (5%) in spring 2009 to the average carbon biomass. Micro-flagellates

were present in no pronounced pattern contributing, for example, 0.52 mg C m^{-3} (17%) to the total winter carbon biomass, 7.51 mg C m^{-3} (13%) to the spring, 13.07 mg C m^{-3} (26%) to the summer, and 9.88 mg C m^{-3} (25%) to the total carbon biomass average in autumn 2008.

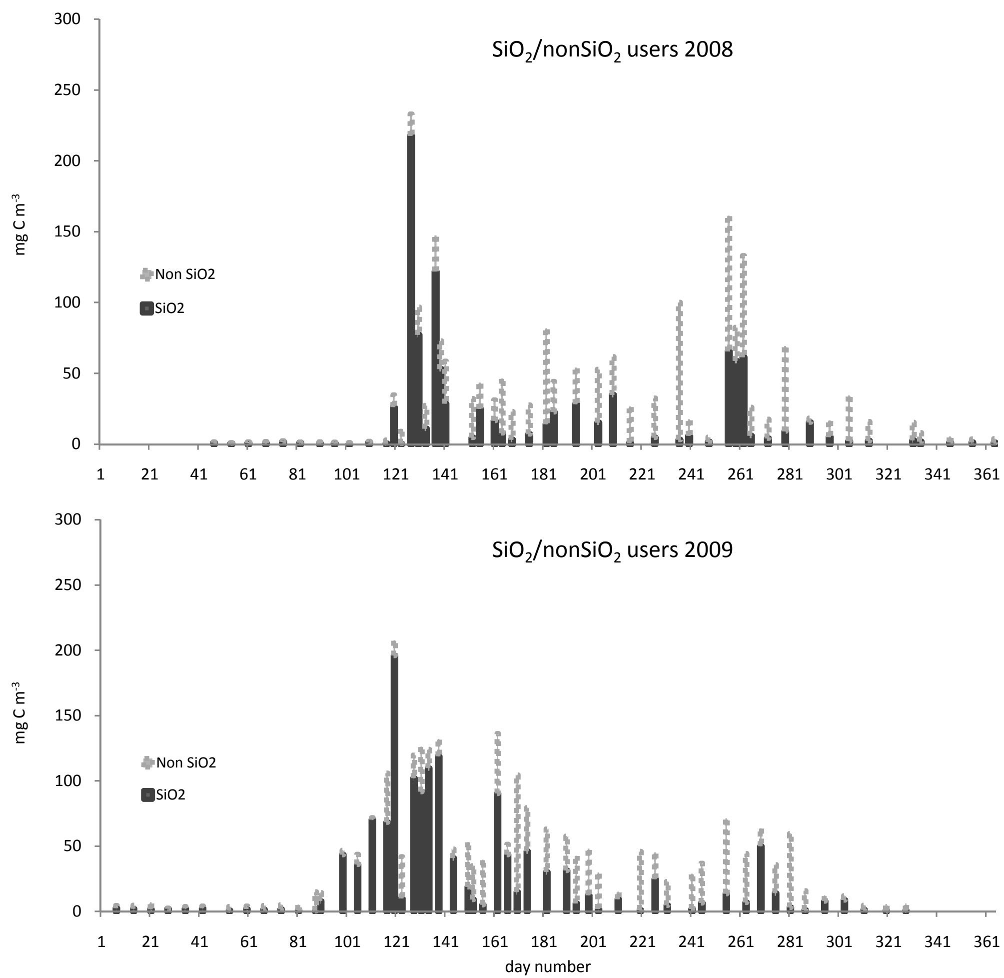


Figure 4.19: The seasonal variation in carbon biomass of silicate users and non-silicate users during 2008 and 2009 at station 38A in the western Irish Sea.

The seasonal succession of the silicate and non silicate users was displayed in seasonal average carbon biomass (mg C m^{-3}). Silicate users represented higher carbon biomass than non-silicate users (Figure 4.19) throughout both sampling years. From spring to summer a significant decrease (Mann- Whitney $p < 0.05$; $n = 50$) in the biomass of silicate users (median 2008 = 38.5 to 9.3; median 2009 = 68.5 to 10.9) and a significant increase (Mann- Whitney $p < 0.05$; $n = 50$) in the non-silicate users biomass (median 2008 = 10.6 to 26.0; median 2009 = 13.3 to 30.6) was observed.

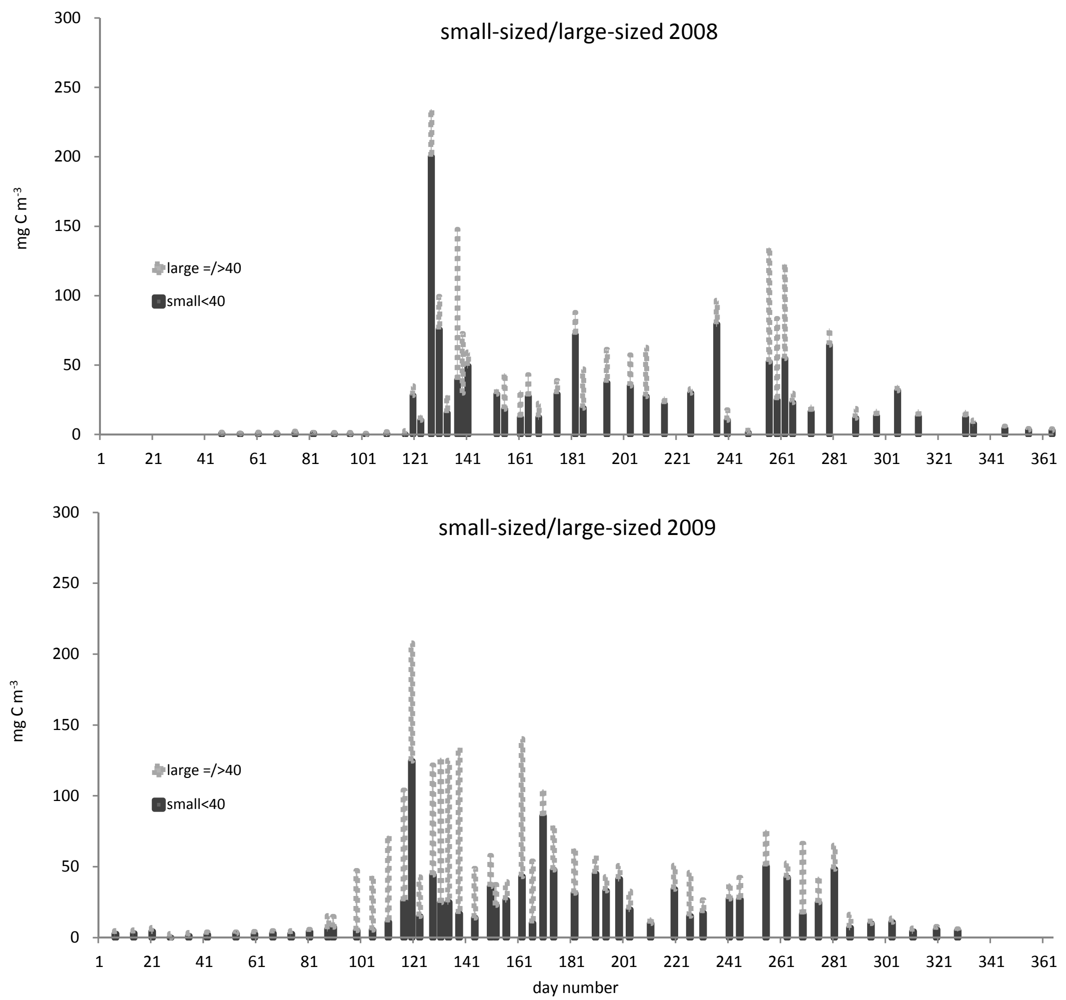


Figure 4.20: The seasonal distribution of large ($\geq$ 40 μm) and small ($<$ 40 μm) microplankters displayed in biomass during 2008 and 2009 at station 38A.

The median carbon biomass of small sized ($<$ 40 μm) microplankton in 2008 was higher in spring and summer (median spring = 15.4 mg C m^{-3}; median summer = 27.5 mg C m^{-3}) than the median carbon biomass of large sized ($\geq$ 40 μm) microplankton for these seasons (median spring = 7.1 mg C m^{-3}; median summer = 16.2 mg C m^{-3}) (Figure 4.20). However, the differences were statistically not significant (Mann-Whitney p $>$ 0.05). In 2009, the biomass of the large sized (=/$>$ 40μm) microplankton decreased significantly (Mann-Whitney test; p$<$ 0.05) from spring to summer (median spring = 61.3 mg C m^{-3}; median summer = 13.9 mg C m^{-3}) while the median biomass of small sized ($<$ 40μm) microplankton did not differ significantly (median spring = 16.8 mg C m^{-3}; median summer = 29.3 mg C m^{-3}) (Mann-Whitney test; p $>$ 0.05; n = 50).

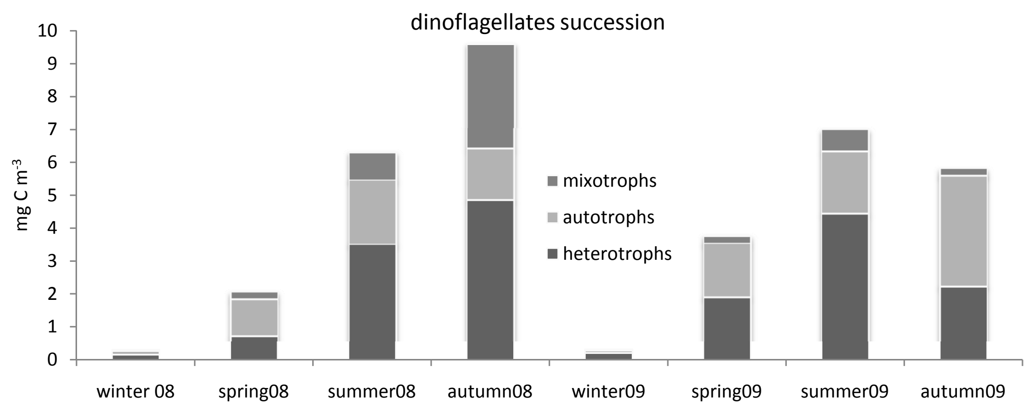


Figure 4.21: The seasonal distribution of heterotrophic, mixotrophic and autotrophic dinoflagellate carbon biomass in 2008 and 2009 at station 38A.

The seasonal variation in autotrophic, mixotrophic and heterotrophic dinoflagellate carbon biomass from winter to autumn showed that of the three nutritional types, heterotrophic species dominated all four seasons in both years, except for spring 2008 and autumn 2009 (Figure 4.21). In these two seasons, autotrophic dinoflagellates were the dominating species with 56% and 59% respectively. For example, heterotrophic dinoflagellates contributed 0.21 mg C m^{-3} (52%) to the total winter dinoflagellate carbon biomass in 2008, while autotrophs contributed 0.14 mg C m^{-3} (35%), and mixotrophs contributed 0.04 mg C m^{-3} (13%). In spring 2008, autotrophic dinoflagellates dominated (1.13 mg C m^{-3}) (56%) the carbon biomass. The contribution of mixotrophic dinoflagellates was highest in autumn 2008 with 3.21 mg C m^{-3} (33%) of the total biomass.

For micro-flagellates it was only possible to discriminate between autotrophic and heterotrophic organisms. In general, the autotrophic micro-flagellates dominated the micro-flagellate community in all four seasons in both years. For example, in winter 2009, the maximum biomass of heterotrophic micro-flagellates was 0.5 mg C m^{-3} contributing 22% to the total micro-flagellate carbon biomass (2.3 mg C m^{-3}). Toward spring heterotrophic micro-flagellate biomass decreased and autotrophic micro-flagellates dominated that period (5.37 mg C m^{-3}). In summer, autotrophic micro-flagellates contributed 18.1 mg C m^{-3} to the highest carbon biomass of micro-flagellates (18.2 mg C m^{-3}). By autumn, the contribution of hetertrophic micro-flagellates was 5% (0.5 mg C m^{-3}) to a total of 10.5 mg C m^{-3} (Figure 4.22).

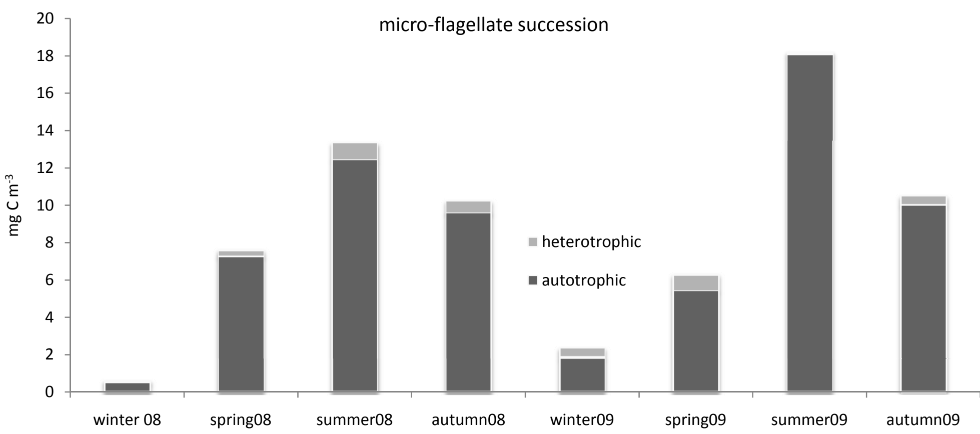


Figure 4.22: The seasonal distribution of heterotrophic and autotrophic micro-flagellates carbon biomass in 2008 and 2009 at station 38A in the western Irish Sea.

4.3 Station WGabb

4.3.1 Temperature and salinity data

In general, near surface temperature was low (approximately 7° C) during winter months (December to March) and early April and high (approximately 18° C) during the summer period with a decrease toward the end of the year (Figure 4.23). The near surface temperatures in 2008 ranged from 6.5° C (day 91; 31st March) to 18.5° C (day 224; 11th August) with an annual average of 12.5° C. In 2009, the near surface temperature ranged from 5.5° C (day 20; 20th January) to 18.9°C (day 237; 25th August) with an annual average of 12.8° C. The annual means were significantly different (paired t-test p = 0.00; n = 3214).

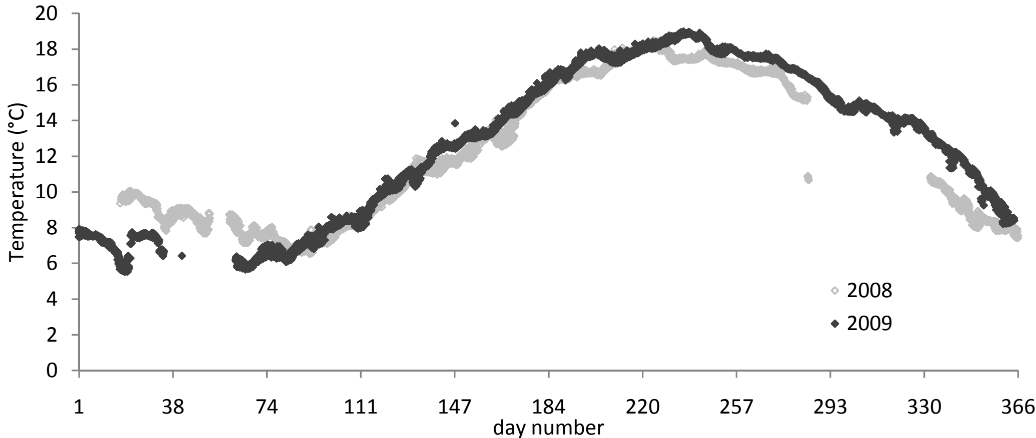


Figure 4.23: The seasonal cycle of near-surface temperature at the West Gabbard station in 2008 and 2009.

Examples of vertical temperature profiles for a specific day in 2009 representing winter, spring, summer and autumn are given in Figure 4.24. Warming of the water column was apparent in spring, summer and autumn, and the highest temperature (17.4° C) was recorded in autumn (day 266; 21st September). Thermal stratification was not apparent at any time period of the year illustrating an isothermal water column year round ($\Delta t < 0.5$).

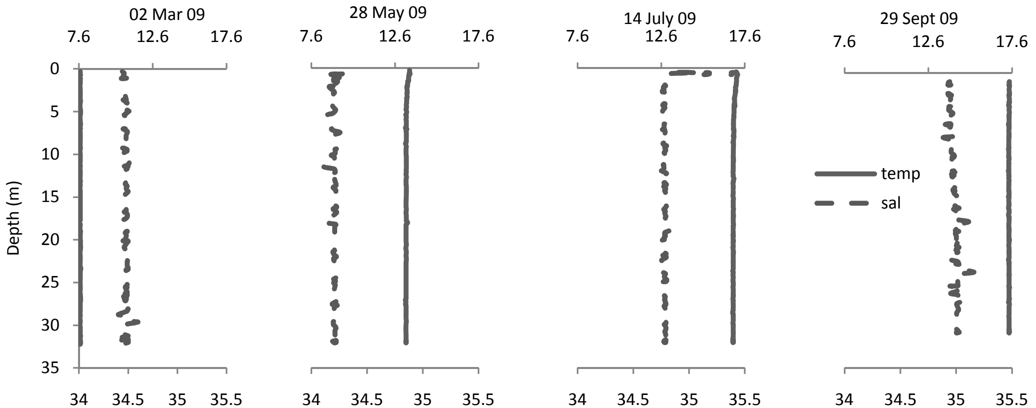


Figure 4.24: Examples of vertical profiles of temperature and salinity at station West Gabbard for a specific day in 2009 representing winter, spring, summer and autumn. Top axis: temperature (° C), bottom axis: salinity; these data are uncalibrated.

Salinity data ranged from 34.10 to 35.35 (Figure 4.25). In 2008, the minimum salinity (34.10) was reached on day 91 (31st March) and the maximum (35.35) was reached on day 40 (9th February). In 2009, the minimum salinity was 34.24 on day 138 (18th May) and an average maximum salinity was 35.25 on day 23 (23rd January). In both years, there was an indication of winter maximum (approximately 35.25) followed by a decrease later in the year. Salinity in both years was generally low in a period from day 70 to 180 (10th March - 29th June), followed by variable values in mid summer and autumn and an increase towards winter. The difference of 0.31 between the mean salinity in January (35.27) and July (34.96) 2008 was significant (paired t-test $p = 0.00$; $n = 3214$). The difference of 0.1 between the mean salinity in January (34.91) and July (34.81) 2009 was also significant (paired t-test $p = 0.00$; $n = 3214$).

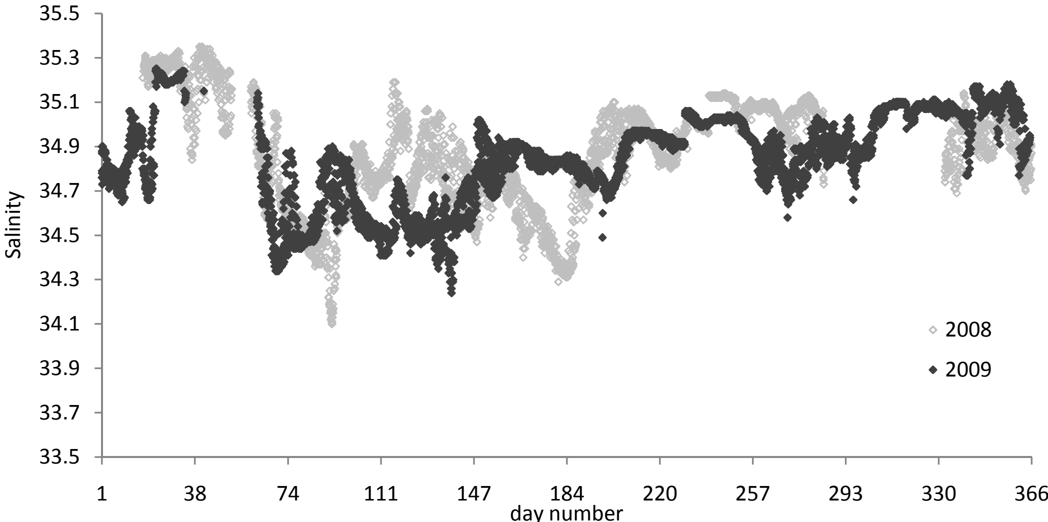


Figure 4.25: The seasonal cycle of near-surface salinity at the West Gabbard station in 2008 and 2009.

Examples of vertical salinity profiles for a specific day in 2009 representing winter, spring, summer and autumn (Figure 4.24). Haline stratification was not apparent in winter (day 61; 2nd March), spring (day 148; 28 th May) and autumn (day 272; 29th Sept) and the water column was isohaline. In summer (day 196; 14th July) near surface salinity was 0.45 greater than the near bottom salinity.

A total of ten temperature and salinity profiles were available for the two sampling years. Half of the profiles showed that the water column was stratified (Δt+s >0.5) although there was no evidence of a seasonal pattern to this (Figure 4.26).

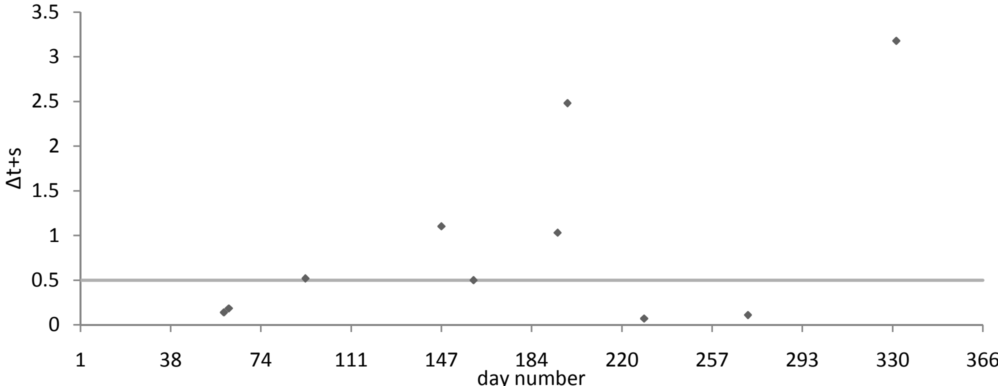


Figure 4.26: Climatology for a calculated Δ t+s (Δt and Δs were added assuming that 0.1 salinity unit has the same density effect as 0.5° C). Vertical temperature and salinity profiles from the WGabb station during 2008 and 2009, merged together to display a general pattern of overall stratification. The solid line at 0.5 indicates the value above which the water column was considered to be stratified.

4.3.2 Nutrient data

The seasonal cycles of TOxN and silicate at the WGabb station showed a similar pattern to the nutrients at station 38A in the western Irish Sea. These were high in late winter (approximately 15.0 μM TOxN and 8.0 μM SiO_2) and low in summer (approximately 2.5 μM TOxN and 0.5 μM SiO_2) (Figure 4.27).

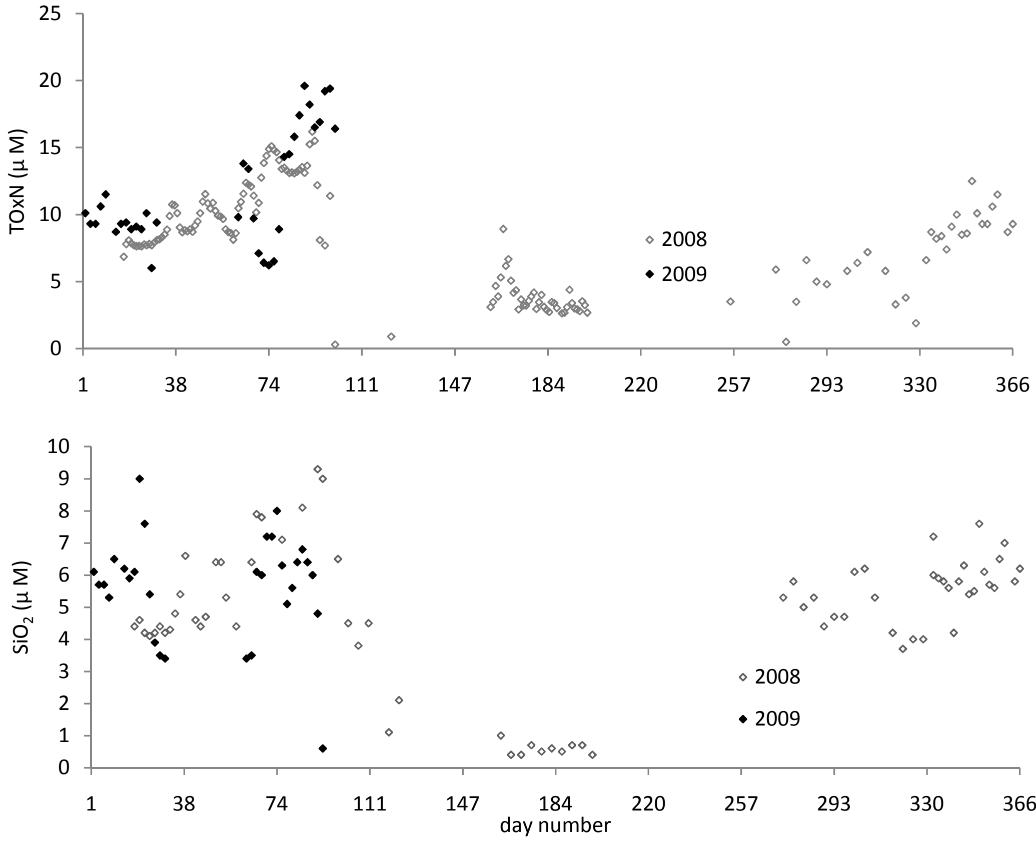


Figure 4.27: Seasonal cycles of near surface mean oxidised nitrogen (TOxN) and silicate (SiO_2) concentrations for 2008 and 2009 at station West Gabbard.

Maximum concentrations in 2008 were 16.19 μM TOxN on day 73 (13th March) and 9.30 μM SiO_2 on day 90 (31st March) with annual means of 8.07 μM and 4,77 μM, respectively. There was a rapid draw down of both nutrients during early April. For example in 2008, TOxN decreased from 11.4 μM on day 98 (7th April) to 0.3 μM on day 100 (9th April). Only limited data were available for the summer period, but the average concentrations from day 161 (10th June) to day 193 (12th July) were 3.89 μM TOxN and 0.59 μM SiO_2. In autumn, nutrient concentrations increased from values as low as 0.5 μM TOxN (day 277; 3rd October) to 6.6 μM TOxN. SiO_2 concentrations varied from 4.4 μM (day 289; 15 th October) to 6.1 μM (day 301; 27th October). Fewer data for TOxN and SiO_2 were available for 2009 (up to day 101; 11th of April for TOxN and up to day 90; 31st of March for SiO_2). For this period maximum concentrations were 19.6 TOxN μM and 9.0 μM SiO_2. The available data were consistent with the seasonal pattern observed in 2008.

The temporal pattern in the TOxN:SiO_2 ratio (N:Si) is displayed in Figure 4.28 and varied between 0.43 (May 2008) and 9.00 (June 2008).

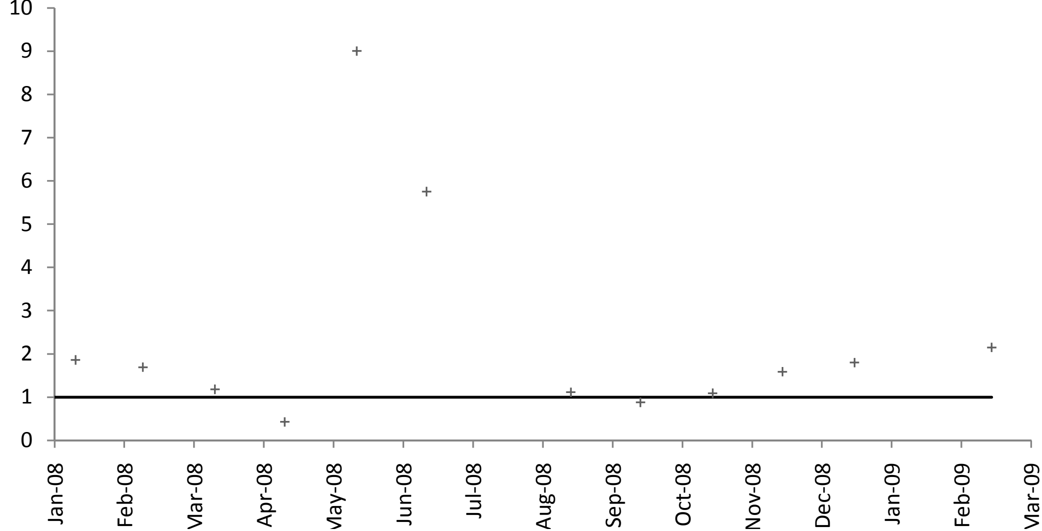


Figure 4.28: The temporal pattern of the TOxN:SiO_2 ratio for near surface nutrients at the West Gabbard station between January 2008 and March 2009. The solid line indicates the Redfield ratio of 1:1 for N:Si.

The linear regressions in Figure 4.29 show the nutrient assimilation by microplankton during spring (March - May). The regression was calculated using nutrient concentrations from spring 2008 and 2009 together to increase the number of data for each plot. The regression was not statistically significant and the draw down rate by microplankton was minimal. When data from the early summer period (June and July, marked red) were included in the analysis as data for most days in May were missing, the regression was statistically significant (analysis of variance, $p < 0.05$) and the intercept differed significantly from 0 ($p < 0.05$). The TOxN:SiO_2 uptake rate was 0.35 strictly for the spring period and 1.09 for the period between March and July.

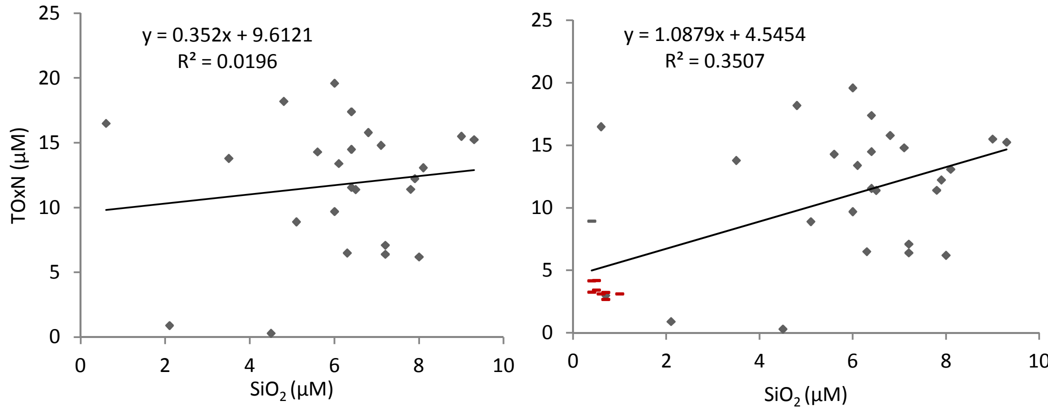


Figure 4.29: Linear regression analyses between TOxN and silicate (SiO_2) for accumulated data in spring (March-May) 2008 and 2009 (left) and for accumulated data from March to July (right) at the West Gabbard station. Number of observations, n = 25 for spring and n = 35 for spring and summer. The regression was statistically significant ($p < 0.05$) for the time period between March and July, not however, for the spring (March-May). Both intercepts were significantly different from 0 ($p < 0.05$).

Winter salinity values from station WGabb were plotted against the winter 2008 nutrient data (TOxN and SiO_2) in Figure 4.48. Nutrient concentrations were significantly related to salinity. Least square linear regression analysis ($p < 0.05$) indicating that higher nutrient concentrations were associated with lower salinity water.

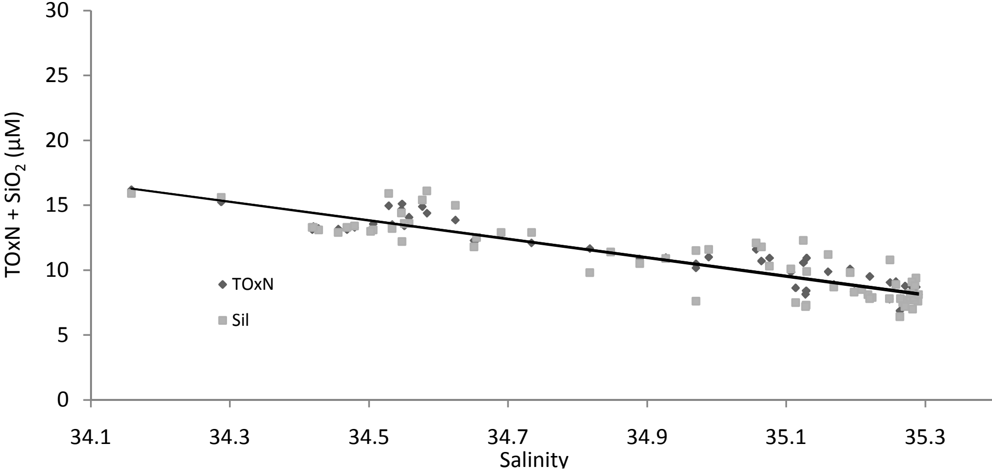


Figure 4.30: The relation between near surface salinity and nutrients TOxN and SiO_2 concentrations at station WGabb in winter 2008. Regression equations y = -7.1243x + 259.64 R^2 = 0.8989 and y = -7.2203x + 262.91 R^2 = 0.789. The least square linear regression analysis ($p < 0.05$) indicated an inverse relationship.

4.3.3 Light data

A climatology was created for the euphotic zone depth (z_{eu}) for 2008 and 2009 that was calculated with K_d retrieved from PAR measurements delivered by light sensors in 1 and 2 meter water depths (Figure 4.31). A seasonal pattern in the euphotic zone depth was apparent and z_{eu} ranged from an average 6 m in the winter to an average of 11 m in the summer. The shallowest euphotic zone depth was 3.7 m on day 334 (30th November) and the deepest euphotic zone depth was 14.0 m on day 131 (11th May).

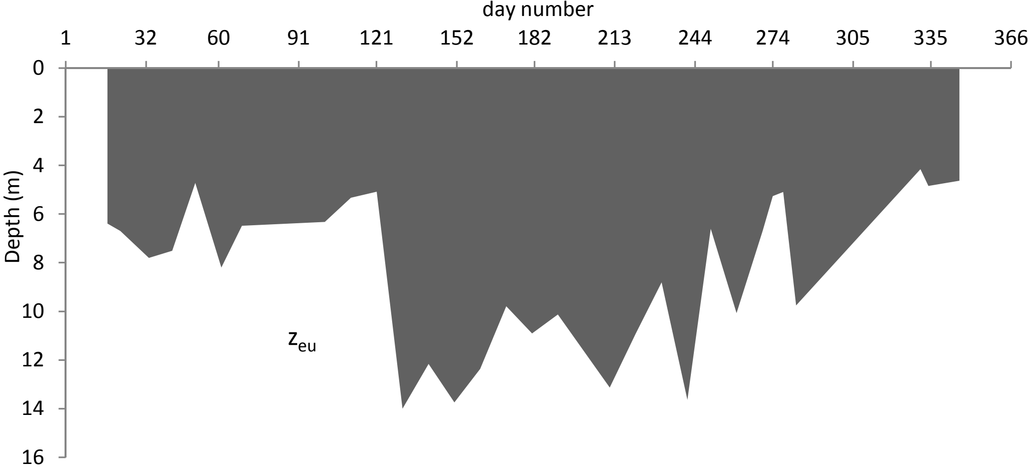


Figure 4.31: A climatology of euphotic zone depth at station WGabb during 2008 and 2009. z_{eu} was calculated with K_d retrieved from PAR measurements delivered by light sensors in 1 and 2 meter water depths.

4.3.4 Chlorophyll data

Near surface chlorophyll data were available for 2008 and are shown in Figure 4.32. The average levels were low in winter (0.8 mg m^{-3}), high in spring (12.6 mg m^{-3}), elevated in summer (3.0 mg m^{-3}) and low in late autumn (0.9 mg m^{-3}). The maximum near surface chlorophyll concentration was 19.9 mg m^{-3} on day 122 (1st May) and the lowest was 0.2 mg m^{-3} on day 282 (8th October). The average chlorophyll concentrations in spring (12.6 mg m^{-3}) decreased rapidly (0.4 mg m^{-3}) after day 141 (20th May) and increased (up to 4.8 mg m^{-3} on day 184; 2nd July) after day 153 (1st June). Until day 256 (12th September) chlorophyll varied between 3.0 mg m^{-3} and 1.9 mg m^{-3} and then decreased to winter levels.

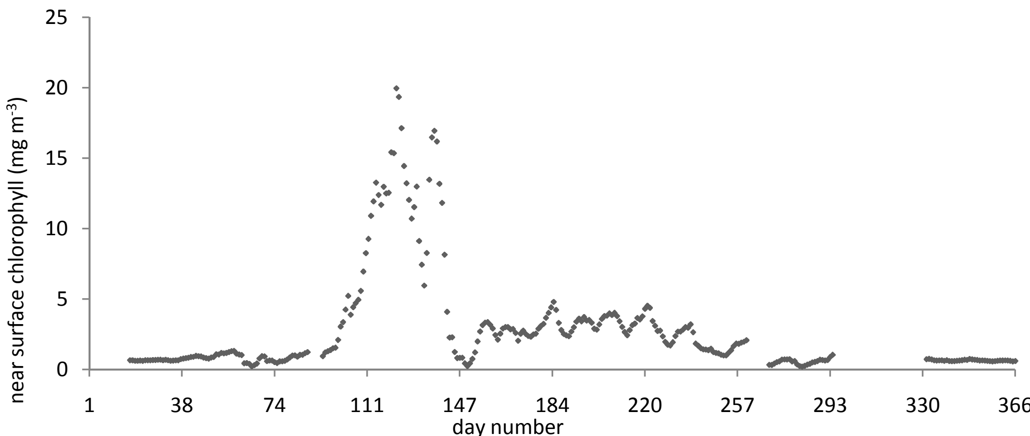


Figure 4.32: The seasonal variation of near surface chlorophyll in 2008 at the West Gabbard station.

No extracted chlorophyll profiles were available for this station. Instead ten vertical profiles of chlorophyll fluorescence presented in relative fluorescence units (RFU) averaged over the euphotic zone depth were used for the sampling period at this mooring site. The data provide an indication of the seasonality in chlorophyll at this station. Generally, fluorescence was low in winter and high in spring showing a decrease in early summer and an increase toward mid summer before decreasing again in the autumn (Figure 4.33). The highest average RFU (1.3) was recorded on day 198 (17th July). The lowest average value (0.3) was observed on day 331 (26th November).

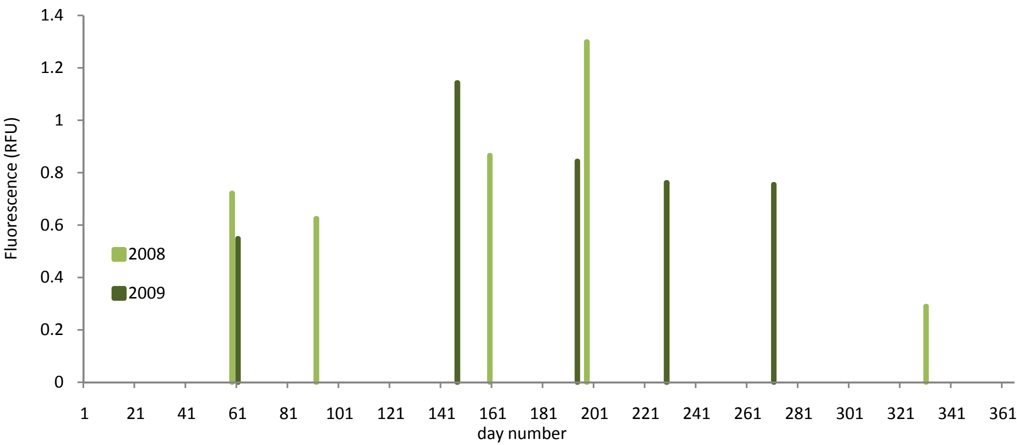


Figure 4.33: Average fluorescence given in relative fluorescence units (RFU) for 2008 and 2009 displayed in a climatology for station West Gabbard.

Vertical profiles of fluorescence for a specific day in winter, spring, summer and autumn 2009 show that in winter (day 63; 2nd March) fluorescence was uniformly distributed throughout the water column (Figure 4.34). In spring (day 148; 28th

May) and summer (day 195; 14th July) reduced fluorescence was apparent in near surface water. In autumn (day 273; 29th September) fluorescence was elevated near the surface and low (0.6 RFU) in near bottom waters (27 m).

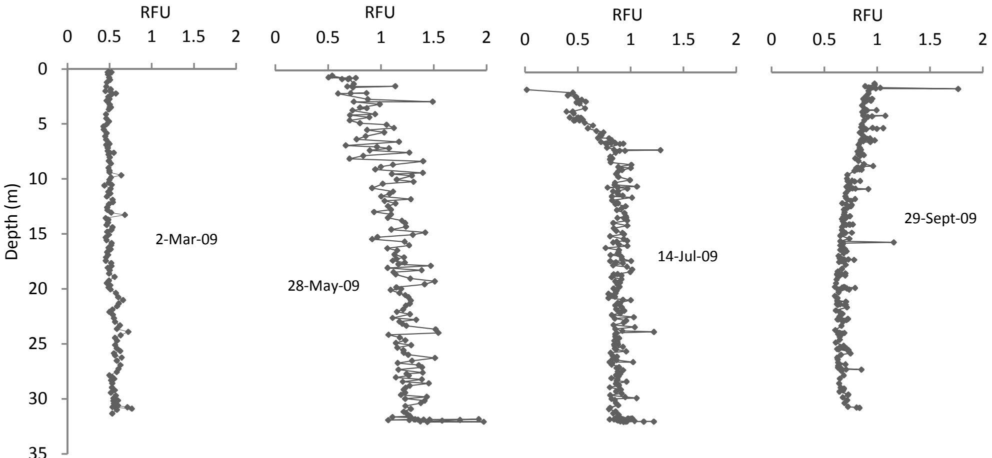


Figure 4.34: Vertical profiles of chlorophyll fluorescence (RFU) for a specific day in winter, spring, summer and autumn 2009 at station West Gabbard. These data are uncalibrated.

4.3.5 Microplankton data

Monthly microplankton data from January 2008 to December 2008 were provided by Cefas from their instrumented mooring in the West Gabbard. From January to November 2009, two samples per month were provided by Cefas and were analysed by me as part of this study. A total of thirty-four samples from this site were analysed and the microplankton in each sample was identified and enumerated. A total of 110 taxa were found and comprised of 58 diatoms belonging to 38 genera and categorised into centric and pennate diatoms; 33 dinoflagellates comprising 15 genera, grouped into naked and armoured dinoflagellates; the silicoflagellate *Dictyocha speculum*; eight groups of micro-flagellates divided into nano-flagellates, *Cryptophytes* (monad flagellates), *Prasinophytes*, *Euglenophytes*, *Phaeocystis spp.*, heterotrophic micro-flagellates, *Cyclotella*, and *Scenedesmus spp.*; ciliates belonging to six genera.

The most frequently occurring species over the sampling period was *Paralia sulcata* that was present in 91% of the samples. The species *Cylindrotheca closterium* occurred in 88% of all analysed samples. The dinoflagellate *Prorocentrum micans* and micro-flagellates belonging to *Cryptophytes* were found in 62% of the samples respectively while "unidentified ciliates" represented the most frequently occurring ciliate category, present in 41% of the samples.

In 2008, average cell abundance and carbon biomass were 1.9 x 10^3 cells L^{-1} and 1.0 mg C m^{-3} for winter, 57.8 x 10^3 cells L^{-1} and 56.3 mg C m^{-3} for spring, 44.2

x 10^3 cells L^{-1} and 31.7 mg C m^{-3} for summer, and 8.4 x 10^3 cells L^{-1} and 8.1 mg C m^{-3} for autumn. A maximum peak in carbon biomass (108.4 mg C m^{-3}) was recorded on day 114 (23rd April), while the maximum peak in cell abundance (122.9 x 10^3 cells L^{-1}) was recorded a few days later (day 121; 1st May)(Figure 4.35 and 4.36). In 2009, average cell abundance and carbon biomass were 72.3 x 10^3 cells L^{-1} and 6.7 mg C m^{-3} for winter, 102.3 x 10^3 cells L^{-1} and 43.6 mg C m^{-3} for spring, 104.3 x 10^3 cells L^{-1} and 36.1 mg C m^{-3} for summer, and 102.1 x 10^3 cells L^{-1} and 14.8 mg C m^{-3} for autumn. The highest peaks in cell abundance (226 x 10^3 cells L^{-1}) and in carbon biomass (97.8 mg C m^{-3}) were both recorded in August (day 224, 12th August and day 234, 22nd August, respectively). Medium sized species of *Chaetoceros spp.* together with *Cylindrotheca closterium*, small sized *Gymnodinium* spp., monad micro-flagellates, and premnesiophyte micro-flagellates were the main contributors to the peak in cell abundance. *Guinardia* species contributed the main part to the peak in carbon biomass.

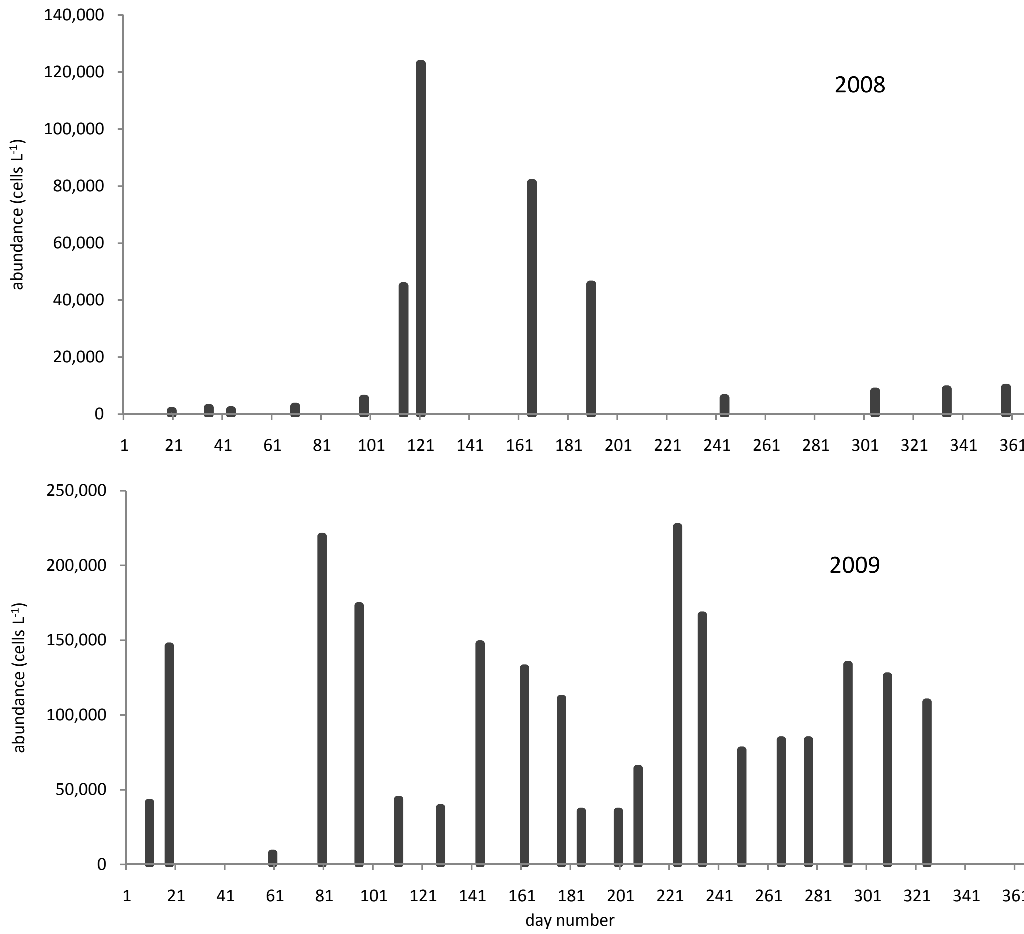


Figure 4.35: Temporal variation in total cell abundance (cells L^{-1}) from January 2008 to November 2009 at station West Gabbard.

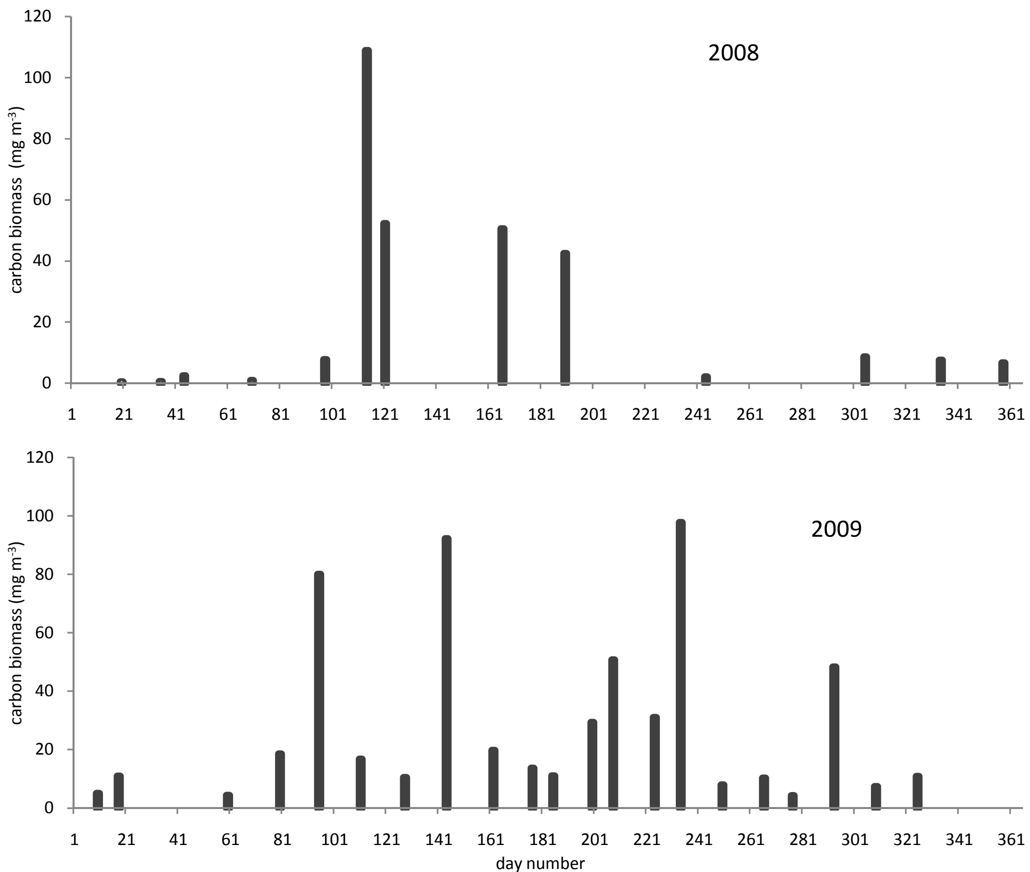


Figure 4.36: Temporal variation in total carbon biomass (mg C m^{-3}) from January 2008 to November 2009 at station West Gabbard.

An early peak in cell abundance was recorded on day 18 (18th January) in 2009 with a total of 146.2 x 10^3 cells L^{-1}. The presence of small *Gymnodinium* species (10.8 x 10^3 cells L^{-1}) and micro-flagellates (127.8 x 10^3 cells L^{-1}) were the main reason for this early peak. Another peak in cell abundance (219.0 x 10^3 cells L^{-1}) was recorded on day 80 (21st March). Biomass on that day was 18.52 mg C m^{-3}.

Table 4.2: The tables display the dominating species for each functional group (diatoms, dinoflagellates, micro-flagellates and ciliates). The top five species/taxa for each lifeform are displayed for the average cell abundance and the average carbon biomass for winter, spring, summer and autumn in 2008 and 2009 at the West Gabbard station. The number of identified species/taxa for each season is given in brackets at the top of each table. % represents the amount of the total biomass of all species identified.

total species identified 110

winter 08 (19)

average biomass [mg C m^{-3}]	**1.03**	average cell abundance [cells L^{-1}]	**1.9 x10^3**
***Coscinodiscus* spp.**	**0.31 (29.7%)**	***Paralia sulcata***	**650 (34.2%)**
Navicula medium (50-150µm)	0.2 (19.6%)	*Bacillaria* cfr. *paxillifera*	450 (23.7%)
Laudaria annulata	0.19 (18.5%)	*Cylindrotheca closterium*	250 (13.2%)
Bacillaria cfr. *paxillifera*	0.09 (8.7%)	*Navicula* medium (50-150µm)	190 (10.0%)
Paralia sulcata	0.09 (8.7%)	*Laudaria annulata*	130 (6.8%)
Prorocentrum micans	0.04 (3.7%)	*Prorocentrum micans*	20 (1.1%)
small naked dinos (<25µm)	0.01 (0.6%)	small naked dinos (<25µm)	20 (1.1%)
Prorocentrum cordatum	0.01 (0.5%)	*Triceratium* spp.	10 (0.5%)
		Prorocentrum cordatum	10 (0.5%)
Cryptophytes	0.003 (0.1%)	*Cryptophytes*	10 (0.5%)

spring 08 (33)

average biomass [mg C m^{-3}]	**56.25**	average cell abundance [cells L^{-1}]	**57.8 x10^3**
Guinardia flaccida	5.34 (9.5%)	*Pseudoguinardia recta*	5773 (10.0%)
Rhizosolenia pungens	5.12 (9.1%)	*Rhizosolenia pungens*	5280 (9.1%)
Pseudoguinardia recta	3.94 (7.0%)	*Rhizosolenia imbricata/styliformis*	2707 (4.7%)
Rhizosolenia imbricata/styliformis	2.03 (3.6%)	*Guinardia* sp.	2293 (4.0%)
Guinardia sp.	1.46 (2.6%)	*Guinardia flaccida*	1773 (3.1%)
Gyrodinium spirale	0.02 (0.02%)	*Pyrophacus horologium*	80 (1.4%)
Prorocentrum micans	0.02 (0.02%)	*Gyrodinium spirale*	27 (0.5%)
Protoperidinium steinii	0.02 (0.02%)	small armoured dinos (<25µm)	27 (0.5%)
Ceratium spp.	0.01 (0.01%)	*Prorocentrum micans*	13 (0.2%)
Gonyaulax spp.	0.01 (0.01%)	*Alexandrium* spp.	13 (0.2%)
Phaeocystis globosa	**9.5 (35.7%)**	***Phaeocystis globosa***	**35720 (61.8%)**

summer 08 (48)

average biomass [mg C m^{-3}]	**31.67**	average cell abundance [cells L^{-1}]	**44.12 x10^3**
Rhizosolenia pungens	**12.39 (39.1%)**	***Guinardia delicatula***	**8600 (19.5%)**
Guinardia sp.	7.23 (22.8%)	*Chaetoceros* spp.	7027 (15.9%)
Guinardia striata	3.12 (9.9%)	*Leptocylindrus minimus*	6520 (14.8%)
Guinardia delicatula	2.13 (6.7%)	*Rhizosolenia pungens*	5213 (11.8%)
Coscinodiscus spp.	0.82 (2.6%)	*Guinardia* sp.	4560 (10.3%)
Protoperidinium steinii	0.63 (1.9%)	*Protoperidinium steinii*	333 (0.8%)
Protoperidinium spp.	0.44 (1.4%)	small armoured dinos (<25µm)	133 (0.3%)
Prorocentrum micans	0.15 (0.5%)	*Protoperidinium* spp.	133 (0.3%)
Prorocentrum gracile	0.11 (0.3%)	*Gyrodinium spirale*	80 (0.2%)
Gyrodinium spirale	0.08 (0.3%)	*Prorocentrum micans*	80 (0.2%)
Cryptophytes	0.03 (0.1%)	*Cryptophytes*	27 (0.06%)

autumn 08 (13)

average biomass [mg C m^{-3}]	**8.06**	average cell abundance [cells L^{-1}]	**8.4 x10^3**
***Coscinodiscus* spp.**	**6.14 (76.1%)**	***Paralia sulcata***	**4800 (57.1%)**
Paralia sulcata	0.66 (8.2%)	*Anabena* sp.	600 (7.1%)
Navicula medium (50-150µm)	0.59 (7.3%)	*Navicula* medium (50-150µm)	550 (6.6%)
Fragilaria spp.	0.18 (2.2%)	*Plagiogrammopsis* spp.	400 (4.8%)
Ditylum brightwellii	0.14 (1.8%)	*Fragilaria* spp.	350 (4.2%)
small armoured dinos (<25µm)	0.1 (1.2%)	small armoured dinos (<25µm)	300 (3.6%)
small naked dinos (<25µm)	0.03 (0.4%)	small naked dinos (<25µm)	100 (1.2%)
Cyclotella spp.	0.19 (2.3%)	*Cyclotella* spp.	550 (6.6%)

winter 09 (46)

average biomass [mg C m^{-3}]	**6.71**	average cell abundance [cells L^{-1}]	**72.3 x10^3**
***Coscinodiscus* spp.**	**2.46 (36.6%)**	*Skeletonema* spp.	8430 (11.7%)
Paralia sulcata	0.53 (8.0%)	*Paralia sulcata*	5520 (7.6%)
Pleurosigma spp.	0.53 (2.2%)	small centric diatoms (<25µm)	2350 (3.3%)
Odontella sinensis	0.14 (2.1%)	*Cylindrotheca closterium*	730 (1.0%)
Odontella sp.	0.11 (1.7%)	*Pseudo-nitzschia delicatissima*	200 (0.3%)
Gonyaulax sp.	0.45 (6.7%)	*Gymnodinium* small (<25um)	4230 (5.8%)
Gymnodinium small (<25um)	0.36 (5.4%)	small naked dinos (<25µm)	1260 (1.8%)
Gymnodinium medium (25-45µm)	0.15 (2.3%)	*Gonyaulax* sp.	630 (0.6%)
Prorocentrum micans	0.08 (1.2%)	*Gymnodinium* medium (25-45µm)	430 (0.1%)
Prorocentrum sp.	0.03 (0.5%)	small armoured dinos (<25µm)	50 (0.04%)
nano-flagellates	0.94 (14.0%)	**nano-flagellates**	**31620 (43.7%)**
Cryptophytes	0.34 (5.1%)	*Cryptophytes*	12710 (17.6%)
Prasinophytes	0.09 (0.35%)	*Prasinophytes*	2410 (3.4%)
unidentified ciliates	0.22 (3.3%)	unidentified ciliates	70 (0.1%)
Laboea sp.	0.05 (0.7%)	Tintinnids	10 (0.01%)

spring 09 (60)

average biomass [mg C m^{-3}]	**43.59**	average cell abundance [cells L^{-1}]	**102.3 x10^3**
***Guinardia* spp.**	**8.55 (19.6%)**	***Thalassiosira* sp.**	**29860 (29.2%)**
Thalassiosira cf. *rotula*	7.64 (17.5%)	*Thalassiosira* cf. *rotula*	16790 (16.4%)
Thalassiosira sp.	6.76 (15.5%)	*Fragilaria* spp.	7480 (7.3%)
Guinardia flaccida	3.91 (9.0%)	*Skeletonema* spp.	6512 (6.4%)
Guinardia striata	3.11 (7.1%)	*Guinardia* sp.	4550 (4.4%)
Gymnodinium medium (25-45µm)	0.63 (1.5%)	*Gymnodinium* medium (25-45µm)	410 (0.4%)
Scrippsiella sp.	0.41 (0.9%)	*Scrippsiella* sp.	260 (0.3%)
small armoured dinos (<25µm)	0.12 (0.6%)	*Gymnodinium* small (<25µm)	250 (0.2%)
Protoperidinium spp.	0.1 (0.3%)	small armoured dinos (<25µm)	100 (0.1%)
Gymnodinium small (<25µm)	0.1 (0.3%)	*Prorocentrum micans*	60 (0.05%)
nano-flagellates	1.19 (2.7%)	nano-flagellates	16740 (16.3%)
Cryptophytes	0.58 (0.6%)	*Cryptophytes*	8700 (8.6%)
Prasinophytes	0.06 (0.1%)	*Euglenophytes*	360 (0.4%)
unidentified ciliates	0.72 (0.6%)	unidentified ciliates	110 (0.1%)
Laboea sp.	0.04 (0.004%)	*Strombidium* sp.	20 (0.02%)
		Lohmaniella strobilidium	10 (0.01%)

summer 09 (64)

average biomass [mg C m^{-3}]	**36.13**	average cell abundance [cells L^{-1}]	**104.3 x10^3**
***Guinardia* spp.**	**13.96 (38.6%)**	*Cylindrotheca closterium*	13350 (12.8%)
Guinardia striata	7.19 (19.9%)	*Guinardia* spp.	7890 (7.6%)
Guinardia flaccida	2.50 (6.9%)	*Guinardia striata*	6250 (6.0%)
Coscinodiscus spp.	1.14 (3.2%)	*Leptocylindrus danicus*	4220 (4.0%)
Cylindrotheca closterium	0.74 (2.0%)	*Paralia sulcata*	1670 (1.6%)
Prorocentrum micans	0.98 (2.7%)	*Gymnodinium* small (<25µm)	3700 (3.5%)
Protoperidinium spp.	0.67 (1.8%)	small armoured dinos (<25µm)	1210 (1.2%)
small armoured dinos (<25µm)	0.46 (1.3%)	*Heterocapsa triquetra*	1200 (1.1%)
Gymnodinium small (<25µm)	0.35 (1.0%)	small naked dinos (<25µm)	810 (0.8%)
Heterocapsa triquetra	0.34 (1.0%)	*Prorocentrum micans*	460 (0.4%)
nano-flagellates	1.08 (3.0%)	**nano-flagellates**	**36200 (34.7%)**
Cryptophytes	0.52 (1.5%)	*Cryptophytes*	14010 (13.4%)
Prasinophytes	0.14 (0.4%)	*Prasinophytes*	4600 (4.4%)
Euglenophytes	0.11 (0.3%)	hetero flagellates	600 (0.6%)
Laboea sp.	0.42 (1.2%)	unidentified ciliates	100 (0.1%)
		Laboea sp.	40 (0.04%)

autumn 09 (54)

average biomass [mg C m^{-3}]	**14.78**	average cell abundance [cells L^{-1}]	**102.1 x10^3**
Rhizosolenia imbricata/styliformis	**4.22 (28.6%)**	*Paralia sulcata*	3090 (3.0%)
Coscinodiscus spp.	2.05 (13.8%)	*Rhizosolenia imbricata/styliformis*	2320 (2.3%)
Guinardia spp.	0.43 (2.9%)	*Leptocylindrus minimus*	530 (0.5%)
Paralia sulcata	0.43 (2.9%)	*Chaetoceros* small (<25µm)	420 (0.4%)
Guinardia flaccida	0.15 (1.0%)	*Cylindrotheca closterium*	310 (0.3%)
Gymnodinium small (<25µm)	0.72 (4.9%)	*Gymnodinium* small (<25µm)	9900 (9.8%)
Gyrodinium medium (25-45µm)	0.42 (2.8%)	*Gyrodinium* medium (25-45µm)	510 (0.5%)
Protoperidinium brevipes	0.16 (1.1%)	*Protoperidinium brevipes*	150 (0.1%)
Prorocentrum micans	0.12 (0.8%)	*Gymnodinium* medium (25-45µm)	110 (0.1%)
Gymnodinium medium (25-45µm)	0.08 (0.6%)	*Prorocentrum micans*	60 (0.05%)
nano-flagellates	1.39 (9.4%)	**nano-flagellates**	**37500 (37.2%)**
Cryptophytes	0.79 (5.4%)	*Cryptophytes*	24300 (24.2%)
Prasinophytes	0.29 (1.9%)	*Prasinophytes*	11400 (11.3%)
hetero flagellates	0.04 (0.2%)	hetero flagellates	8400 (8.3%)
unidentified ciliates	2.29 (15.5%)	*Strombidium* sp.	30 (0.3%)
Laboea sp.	0.19 (1.3%)	*Laboea* sp.	20 (0.2%)
Strombidium sp.	0.07 (0.5%)		

The microplankters identified at station WGabb were divided into the same taxonomic functional groups as those used to explore seasonal succession at station 38A (Figure 4.37). In general, diatoms were the dominant functional group at the West Gabbard station (Figure 4.37). In winter 2008, the total biomass was comprised of diatoms (0.98 mg C m^{-3}; 95%) and dinoflagellates (0.05 mg C m^{-3}; 5%). Microflagellate and ciliates were not identified. In spring 2008, diatoms contributed the main part (47.06 mg C m^{-3}; 84%) of the total biomass, micro-flagellates contributed 9.05 mg C m^{-3} (16%) and dinoflagellates 0.14 mg C m^{-3} (0.2%). In summer, 29.93 mg C m^{-3} (95%) of the total carbon biomass were diatoms, dinoflagellates contributed 1.74 mg C m^{-3} (5%). The total carbon biomass in autumn was comprised of diatoms (7.94 mg C m^{-3}; 98%) and dinoflagellates (0.13 mg C m^{-3}; 2%). In 2009, the total microplankton carbon biomass in all four seasons was dominated by diatoms: diatoms contributed 3.98 mg C m^{-3} (59%) in winter, 39.97 mg C m^{-3} (92%) in spring, 29.71 mg C m^{-3} (82%) in summer, and 8.03 mg C m^{-3} (54%) in autumn. However, dinoflagellates, micro-flagellates, and ciliates were present in all four seasons and micro-flagellates contributed for example 2.56 mg C m^{-3} (19%) to the total biomass in autumn.

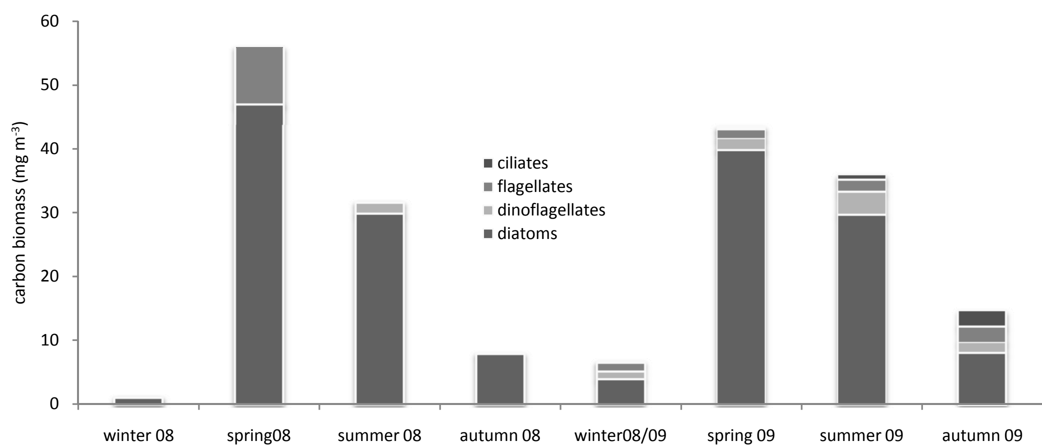


Figure 4.37: The succession of microplankters categorised into four taxonomic functional groups at station West Gabbard for winter 2008 to autumn 2009.

With one exception silicate users were dominant and represented a higher biomass than non-silicate users throughout both sampling years (Figure 4.38). On day 121, (1st May 2008) the biomass of non silicate users (27.48 mg C m^{-3}) was higher than the biomass of silicate users (24.77 mg C m^{-3}). A significant shift from silicate users in spring to non-silicate users in summer was not apparent (Mann-Whitney $p > 0.05$; $n = 13$) in either of the two sampling years.

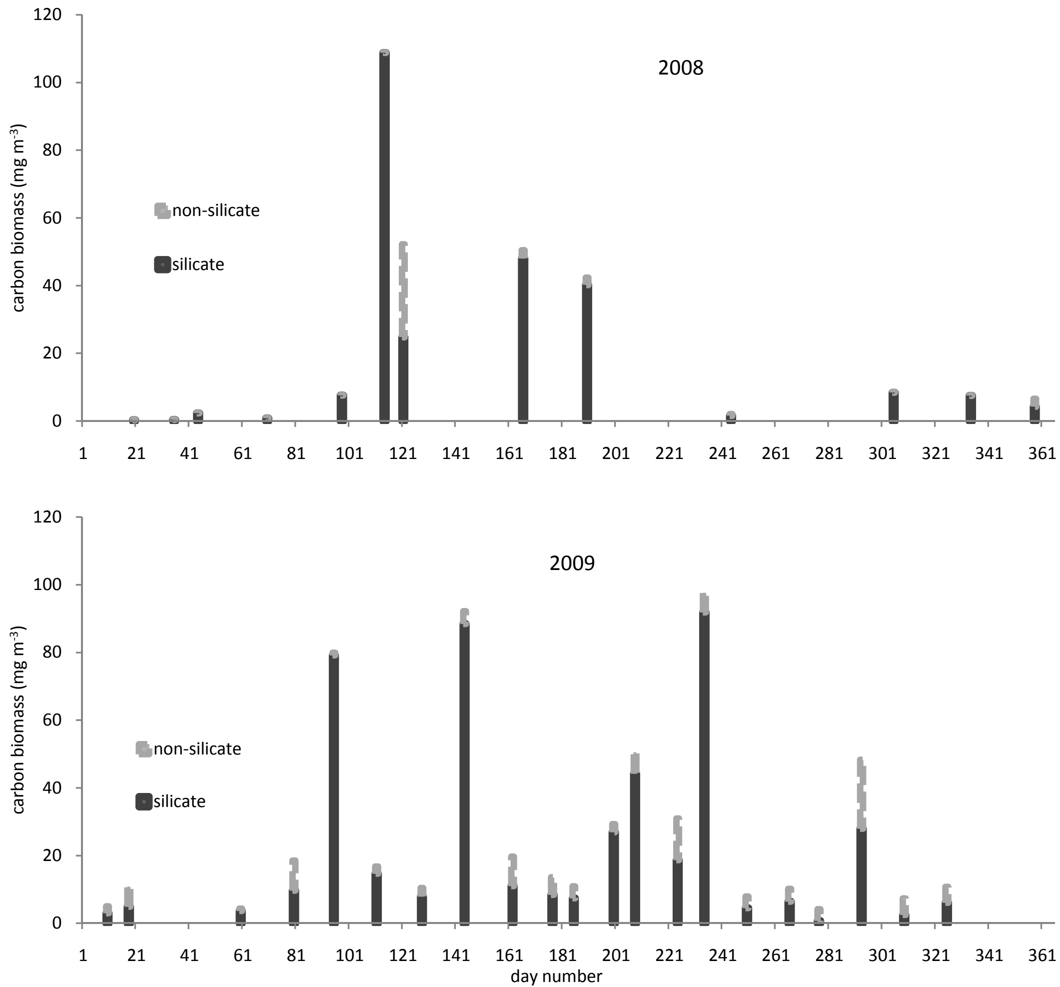


Figure 4.38: The seasonal distribution of SiO_2 users and non- SiO_2 users displayed in carbon biomass for 2008 and 2009 at station West Gabbard.

Large sized ($\geq 40\mu$m) species of microplankton dominated the biomass in both years (Figure 4.39). In one occasion (1st May 2008, day 121) smaller sized ($< 40\mu$m) species (27.30 mg C m^{-3}) dominated the microplankton biomass compared to the larger sized organisms (24.95 mg C m^{-3}). Statistical analyses (Mann Whitney; $p>0.05$; $n = 13$) resulted in no significant difference in the biomass of small and large microplankters in 2008 and 2009.

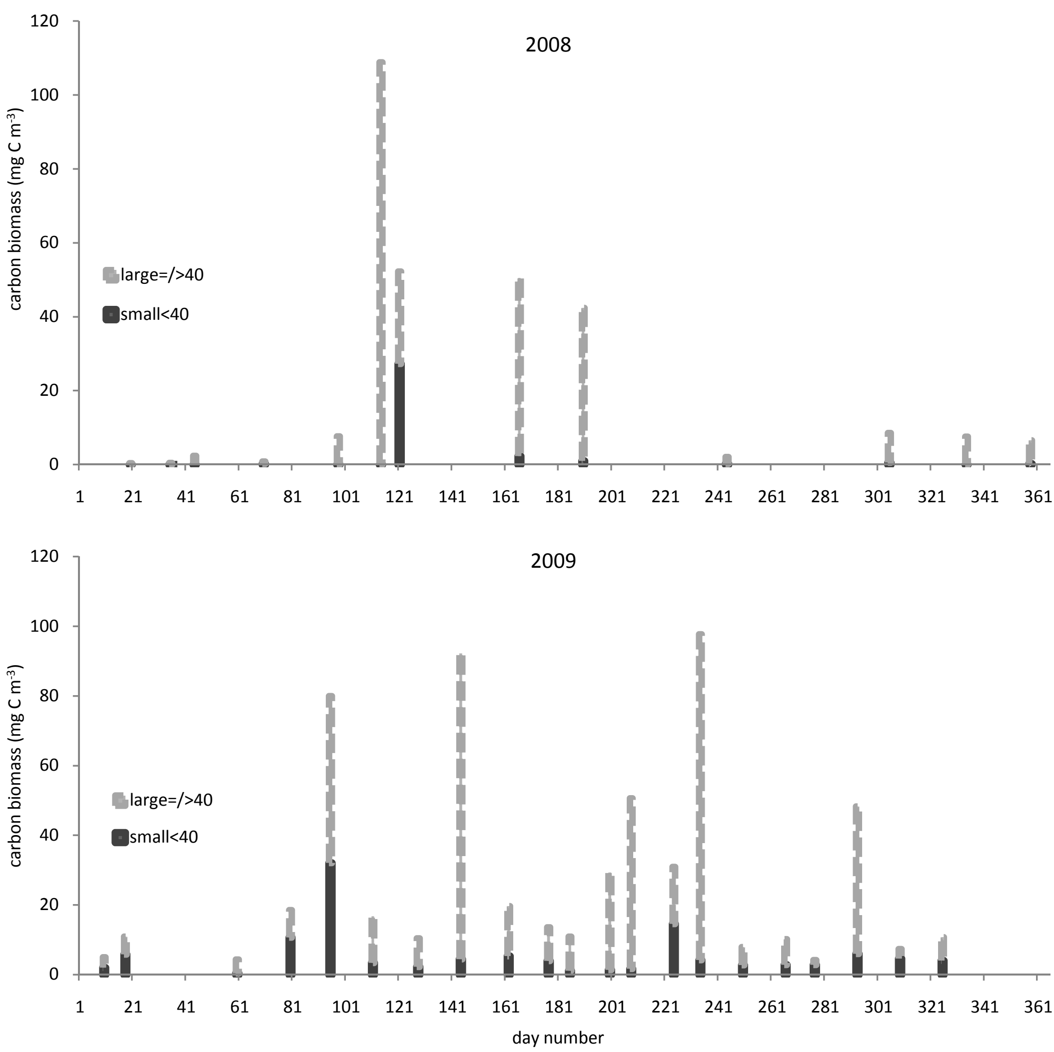


Figure 4.39: The seasonal distribution of the biomass of large ($\geq 40\mu$m) and small ($< 40\mu$m) microplankters during 2008 and 2009 at station West Gabbard.

4.4 Station LBay

4.4.1 Temperature and salinity data

Near surface temperature data were recorded from 1st January 2008 to 31st December 2009 by a CTD (approximately 4 m depth) attached to the Cefas smartbuoy. Salinity data were derived for the same frequency from conductivity and temperature recorded by the same CTD.

In general, temperatures increased from approximately 5° C in winter to approximately 18° C in summer and autumn months and decreased toward the end of the year (Figure 4.40). The near surface temperatures in 2008, ranged from 4.6° C recorded on day 99 (8th April) to 18.6° C recorded on day 221 (8th August) and the average temperature was 10.9° C. In 2009, the near surface temperatures ranged from 4.4° C recorded on day 6 (6th January) to 19.6° C recorded on day 220 (8th August) and the average temperature was 10.8° C. The difference between the annual mean near surface temperatures in 2008 and 2009 was significant (paired t-test p = 0.00; n = 4304).

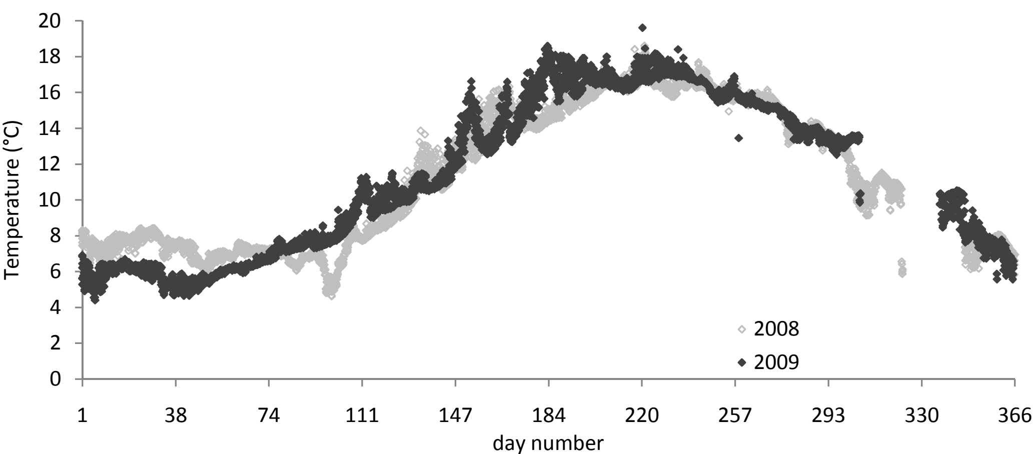


Figure 4.40: The seasonal cycle of near-surface temperature at the LBay station for 2008 and 2009.

Examples of vertical temperature and salinity profiles on a specific day representing winter, spring, summer, and autumn in 2009 are given in Figure 4.41. The plots display the seasonal differences in water column structure. In winter (day 55; 24th February) the water column was isothermal at 5.5° C. Haline stratification was apparent on this day at a depth of approximately 14 m (dashed line) with a salinity difference of 0.6 compared to the surface. For the other three profiles (representing spring, summer, and autumn) the water column was isothermal and isohaline. Of the 25 profiles recorded however, vertical gradients in temperature and salinity (Δ t+s) were evident in thirteen measurements (Figure 4.42).

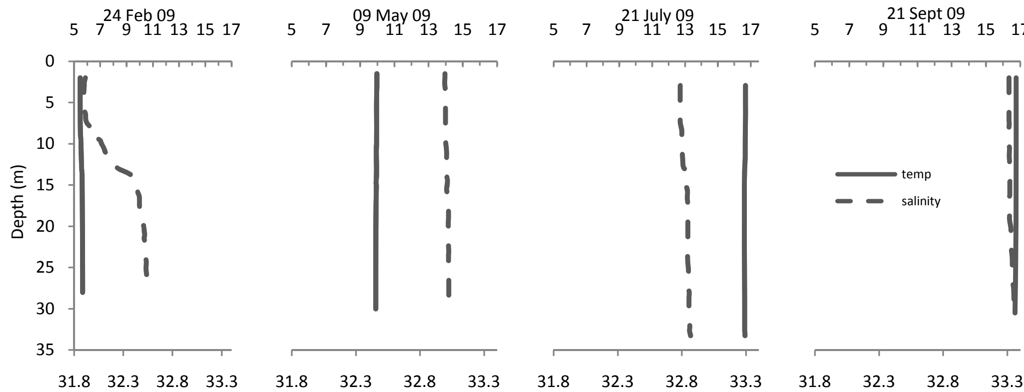


Figure 4.41: Examples of vertical temperature and salinity profiles for a particular day in winter, spring, summer, and autumn in 2009. Top axis: temperature (° C), bottom axis: salinity

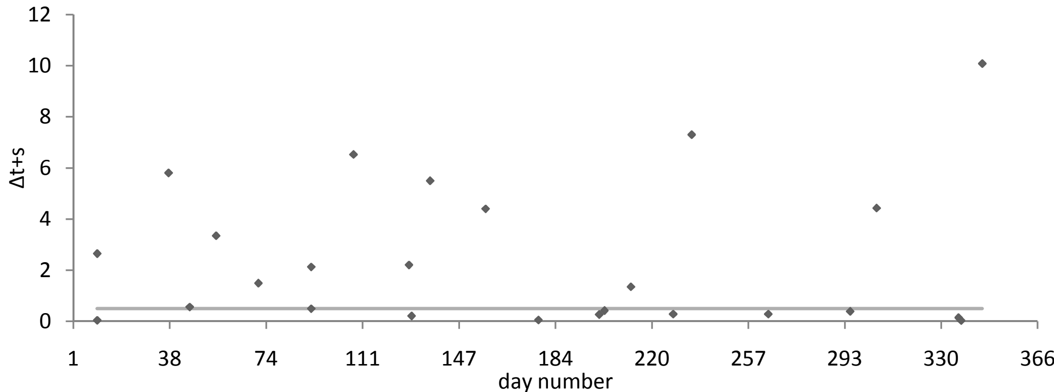


Figure 4.42: Climatology for a calculated Δ t+s (Δt and Δs were added assuming that 0.1 salinity unit has the same density effect as 0.5° C). Profile data from station LBay from 2008 and 2009 were merged together to display a general pattern of overall stratification. The solid line at 0.5 indicates the value above which the water column was considered to be stratified.

In general, near surface salinity in 2008 varied between 27.51 recorded on day 309 (4th November) and 35.51 recorded on day 98 (7th April). In 2009, the salinity variation was between 28.52 on day 258 (15th September) and 33.80 on day 345 (11th December) and the annual average value was 32.43 (Figure 4.43). The average salinity in January 2008 (32.96) was compared to the average salinity in July (31.71). The difference of 1.25 was significant (paired t-test p = 0.00). In 2009, the average salinity in January was 31.79 and in July 2009 32.22. The difference of 0.43 was also statistically significant (paired t-test p = 0.00).

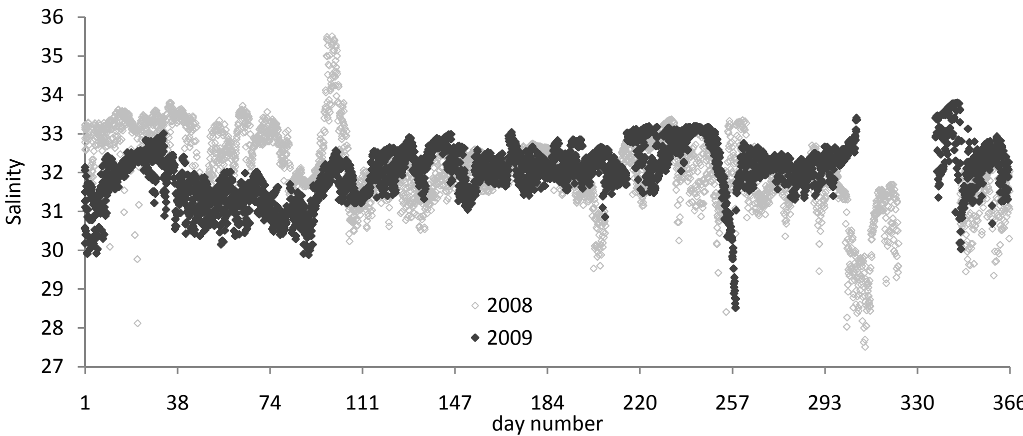


Figure 4.43: The seasonal cycle of near-surface salinity at the LBay station for 2008 and 2009.

4.4.2 Nutrient data

In general, the seasonal cycles of TOxN and SiO_2 at the mooring site LBay in Liverpool Bay showed a similar pattern to that of the nutrients at station 38A in the western Irish Sea and station WGabb in the southern North Sea. There were high average winter concentrations (approximately 25 μM TOxN and approximately 11 μM SiO_2) and low average summer concentrations (1.0 μM TOxN and 1.5 μM SiO_2) that increased again toward the end of the year (21.0 μM TOxN and 10.0 μM SiO_2) (Figure 4.44).

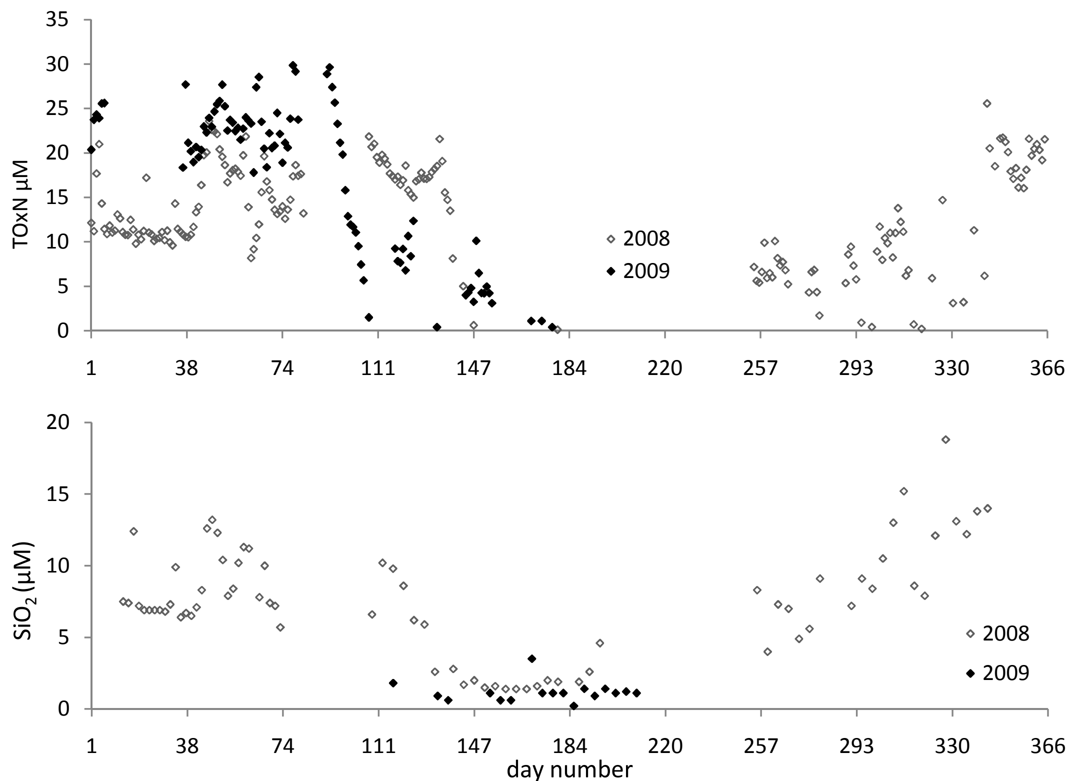


Figure 4.44: Seasonal cycles of near surface average total oxidised nitrogen (TOxN) and silicate (SiO_2) concentrations for 2008 and 2009 for station LBay in Liverpool Bay displayed in day of year.

Maximum concentrations of TOxN (25.57 μM) were measured in 2008 on day 344 (9th December) and for SiO_2 (18.80 μM) on day 306 (2nd November). There was a rapid draw down of both nutrients during mid May. For example, TOxN decreased from 18.18 μM on day 132 (12th May) to 8.13 μM on day 139 (18th May). After this period and throughout the summer, average concentrations of both nutrients decreased further and remained low (approximately 2.5 μM TOxN and 2.0 μM SiO_2). During autumn, concentrations increased slowly back to winter levels. Maximum concentrations measured in 2009 were 29.88 μM TOxN on day 92 (2nd April). High frequency sampling silicate data in 2009 were only available from day 117 to day 205 (27th April to 24th July) with the highest recording of 3.50 μM on day 169 (18th June). TOxN data for 2009 were infrequent which made it difficult to detect a detailed seasonal pattern. However, it was apparent that the TOxN concentration decreased from the maximum value 29.88 μM on day 92 (2nd April) to 1.50 μM on day 107 (17th April). There was then an increase to a maximum of 12.37 μM TOxN at the end of April and early May, before the concentration fell to low summer levels around 1 μM.

Examples of vertical TOxN, SiO_2 and ${PO_4}^{3-}$ profile are given in Figure 4.45 for a specific day in winter, spring, summer, and autumn in 2009. These data were retrieved from the AFBI oceanography surveys as the Liverpool Bay mooring site

was a station in the transect routinely surveyed on those research cruises.

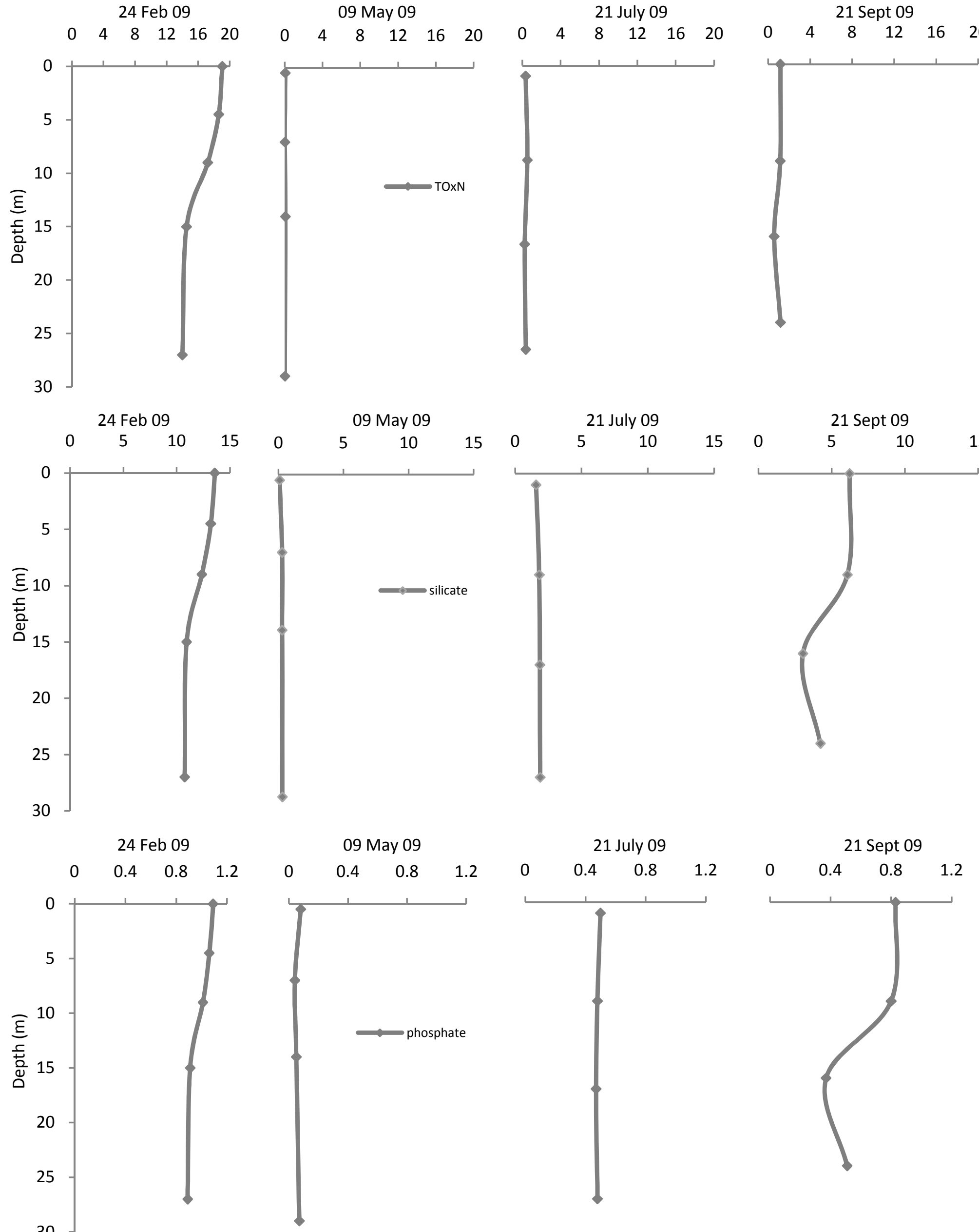


Figure 4.45: Vertical profiles of TOxN, SiO_2 and $PO_4{}^{3-}$ at station LBay for specific days in winter, spring, summer, and autumn 2009. The profiles for TOxN are displayed on a scale from 0 to 20 μM, while the scale for silicate reaches up to 15 μM and $PO_4{}^{3-}$ is plotted on a scale up to 1.2 μM.

In winter (day 55; 24th February), all three nutrients showed the same pattern. Near surface concentrations were higher than near bottom concentrations (e.g. TOxN 19.06 μM and 14.0 μM). In spring (day 129; 9th May), all three nutrients were depleted throughout the water column (TOxN 0.04 μM, SiO_2 0.25 μM and $PO_4{}^{3-}$ 0.06 μM). In summer (day 197; 21st July), concentrations of all three nutrients were

low throughout the water column but slightly higher than in spring (TOxN 0.38 μM, SiO_2 1.78 μM and $PO_4{}^{3-}$ 0.48 μM). In autumn (day ; 21st September), increased levels of all three nutrients were measured. All three nutrients showed a similar 'shoulder'-pattern at a depth of approximately 16 m indicating lower concentrations (e.g. TOxN 0.6 μM) at this depth than in the upper 15 m (TOxN 1.2 μM) and the water below this depth (1.18 μM).

The temporal pattern in the TOxN:SiO_2 ratio (N:Si) is displayed in Figure 4.46 and varied between 0.56 (June 2009) and 4.18 (May 2008).

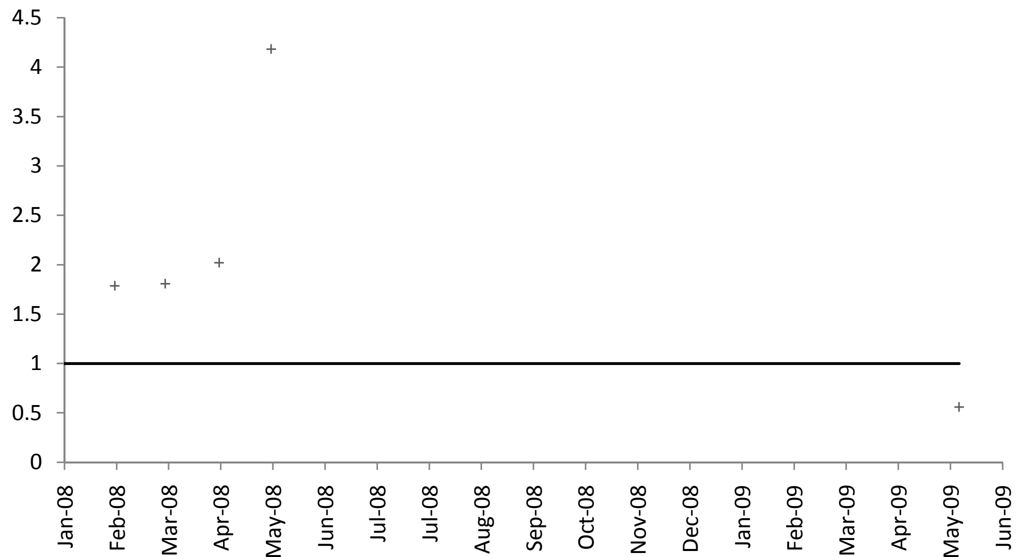


Figure 4.46: The temporal pattern of the TOxN:SiO_2 ratio for near surface nutrients at the Liverpool Bay station between January 2008 and March 2009. The black line in each plot indicated the Redfield ratio of 1:1 for N:Si.

The linear regressions in Figure 4.47 show the nutrient assimilation by microplankton during spring (March - May). The regression was calculated using nutrient concentrations from spring 2008 and 2009 together to increase the number of data for each plot. The regression was not statistically significant. When data from the early summer period (June, marked red) were included in the analysis, the regression was statistically significant (analysis of variance, $p < 0.05$) and the intercept differed significantly from 0 ($p < 0.05$). The TOxN:SiO_2 uptake rate was 1.15 strictly for the spring period and 1.43 for the period between March and June.

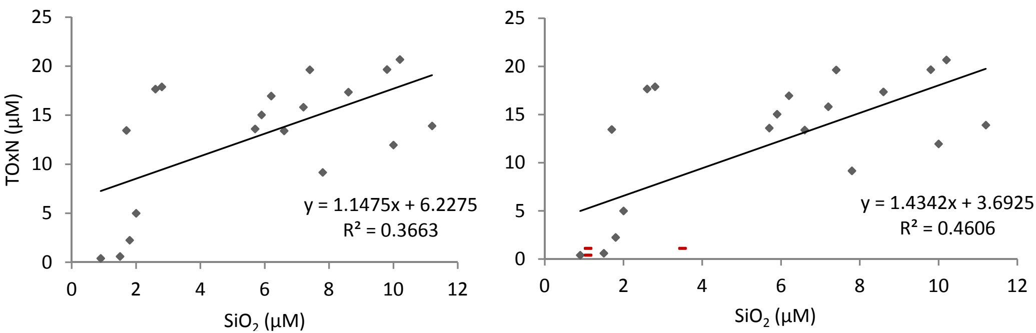


Figure 4.47: Linear regression analyses between TOxN and silicate (SiO_2) for accumulated data in spring (March-May) 2008 and 2009 (left) and for accumulated data from March to June (right) at the Liverpool Bay station. Number of observations, n = 19 for spring and n = 22 for spring and summer. The regression was statistically significant ($p < 0.05$) for the time period between March and June, not however, for the spring (March-May). Both intercepts were significantly different from 0 ($p < 0.05$).

In 2008, near surface winter nutrient concentrations were significantly related to near surface salinity (least square linear regression analysis, $p < 0.05$), indicating a negative relationship between salinity and both nutrients (Figure 4.48).

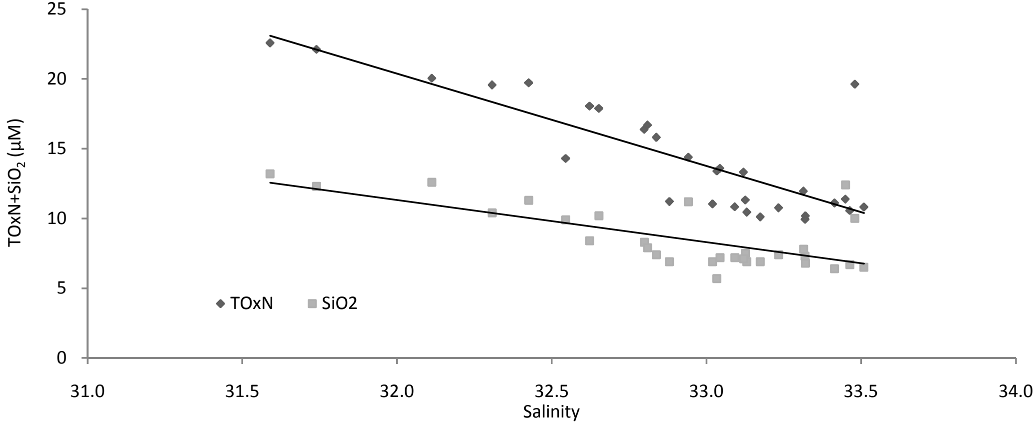


Figure 4.48: Near surface salinity, TOxN and SiO_2 (μM) plots for winter data 2008 from station LBay. There were insufficient data for 2009. Regression equations y = -6.618x + 232.15 $R^2 = 0.6699$ for TOxN and y = -3.0224x + 108.04 $R^2 = 0.4686$ for SiO_2. The least square linear regression analysis ($p < 0.05$) indicated a negative relationship between salinity and both nutrients.

4.4.3 Light data

A climatology was created for the euphotic zone (z_{eu}) from values calculated for 2008 and 2009 for station LBay (Figure 4.49). A seasonal pattern was apparent and z_{eu} ranged from an average depth of 4 m in the winter to an average depth of 12 m in the summer. The lowest depth (2.5 m) was reached in autumn (day 292; 18th October) and the maximum depth (21.2 m) was reached in summer (day 179; 28th June).

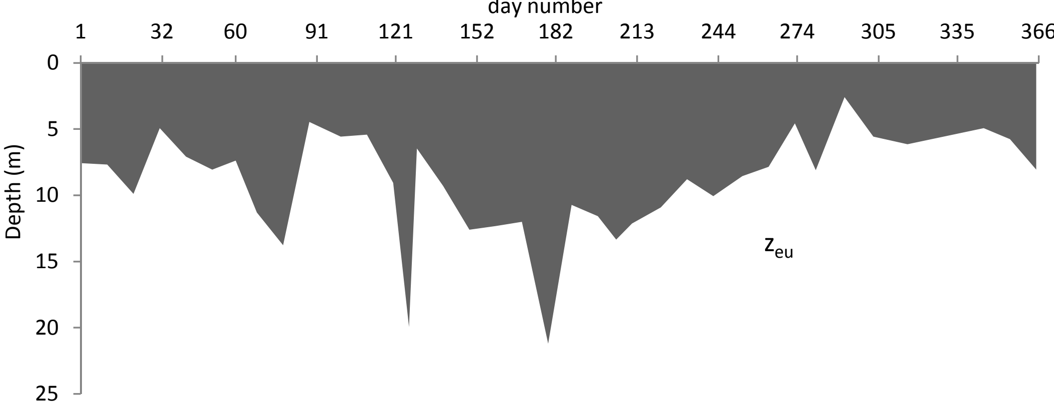


Figure 4.49: Climatology of the euphotic zone depth at station LBay for 2008 and 2009. z_{eu} was calculated with K_d retrieved from PAR measurements delivered by light sensors attached to the smartbuoy mooring in 1 and 2 meter water depths.

4.4.4 Chlorophyll data

In general, chlorophyll standing stock (column chlorophyll), integrated from the surface to the base of the euphotic zone, was low in winter, showed the highest concentrations in mid spring, decreased after that and showed another smaller increase in late summer before decreasing toward autumn and winter (Figure 4.50). The highest chlorophyll standing stock (141.10 mg m^{-2}) for 2008 was recorded on day 235 (22nd August). In 2009, the highest value (211.51 mg m^{-2}) was recorded on day 129 (9th May 2009). In 2008, the highest spring value (108.13 mg m^{-2}) was reached on day 128 (7th May). The lowest standing stock (11.70 mg m^{-2}) was recorded for the beginning of the year 2009 on day 10 (10th January).

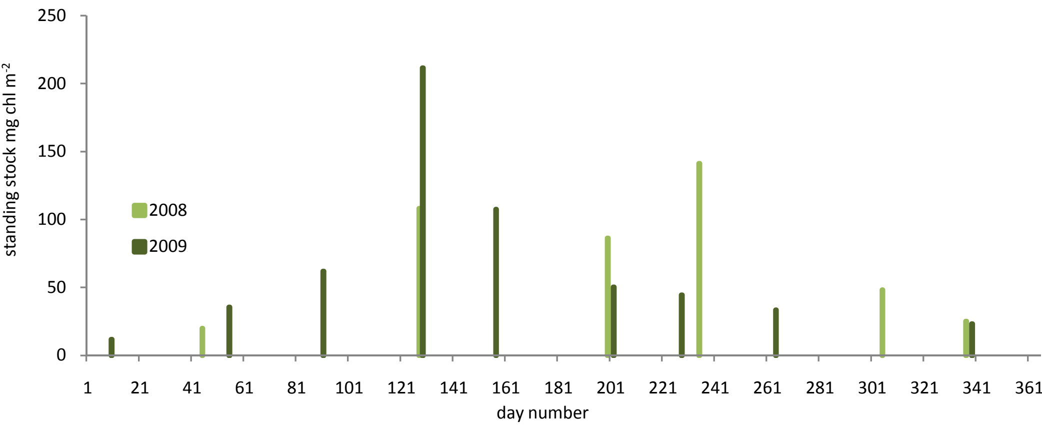


Figure 4.50: The seasonal cycle of chlorophyll standing stock for 2008 and 2009 given as a climatology at station LBay.

Figure 4.51 shows the vertical distribution of chlorophyll for a specific day in winter, spring, summer and autumn 2009. The data were retrieved during AFBI surveys to the Cefas mooring site. The profiles show that in general chlorophyll was equally distributed throughout the water column in all four seasons. For example, in spring (day 129; 9th May) chlorophyll was 7.66 mg m^{-3} at the near surface water and 6.58 mg m^{-3} at a depth of 7 m and 7.81 mg m^{-3} in near bottom waters. The plots further show, that highest chlorophyll concentrations were recorded in spring followed by summer and autumn. The loweste levels were recorded in winter.

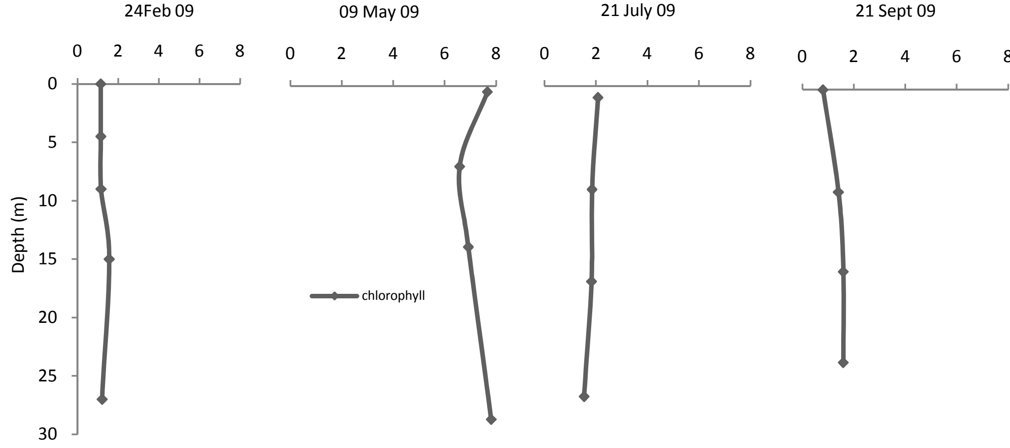


Figure 4.51: Vertical profiles of chlorophyll for a specific day in winter, spring, summer and autumn 2009 at station LBay.

4.4.5 Microplankton data

Monthly microplankton data from January 2008 to August 2009 were provided from the monitoring programme based around the Cefas mooring in Liverpool Bay. A total of 17 samples were analysed and 84 species and taxa were identified and enumerated. This included 44 diatoms belonging to 33 genera and categorised into centric and pennate diatoms. Thirty six dinoflagellates comprised 17 genera, grouped into naked and armoured dinoflagellates. The silicoflagellates *Dictyocha speculum* and *Dictyocha fibulum* were identified, and *Euglenophytes*, monate micro-flagellates, and *Phaeocystis globosa* made up the micro-flagellate composition. Ciliates were not counted at this site. A complete species list is given in the appendix listing A.3. Total cell abundance and total carbon biomass for 2008 and 2009 are presented in Figure 4.52 and 4.53.

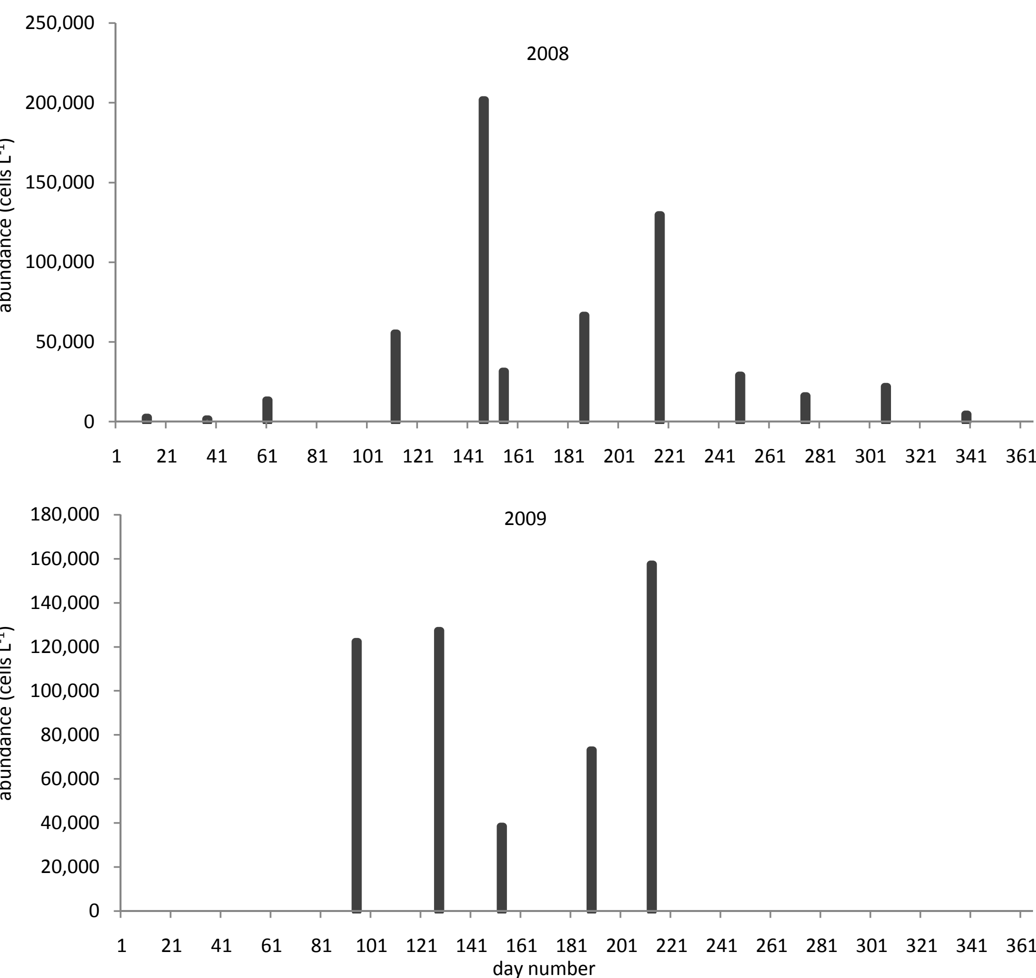


Figure 4.52: The temporal variation in total cell abundance (cells L^{-1}) at station LBay during 2008 and 2009. In 2009, only five samples were counted and identified.

The average cell abundance in 2008 was 47.8 x 10^3 cells L^{-1} and the highest peak (201.8 x 10^3 cells L^{-1}) was recorded on day 147 (26th May) with diatoms contributing

the major part to the abundance. The average carbon biomass in 2008 was 27.72 mg C m^{-3} with the maximum peak (109.64 mg C m^{-3}) recorded on day 187 (5th July). In 2009, average cell abundance was 103.7 x 10^3 cells L^{-1} with the highest peak (157.5 x 10^3 cells L^{-1}) on day 213 (1st August). The average carbon biomass was high with 226.13 mg C m^{-3} and the maximum peak (276.85 mg C m^{-3}) was recorded on day 190 (8th July). The mean carbon biomass and cell abundance for winter, spring, summer and autumn 2008 and winter, spring, and summer 2009 are displayed in table 4.3.

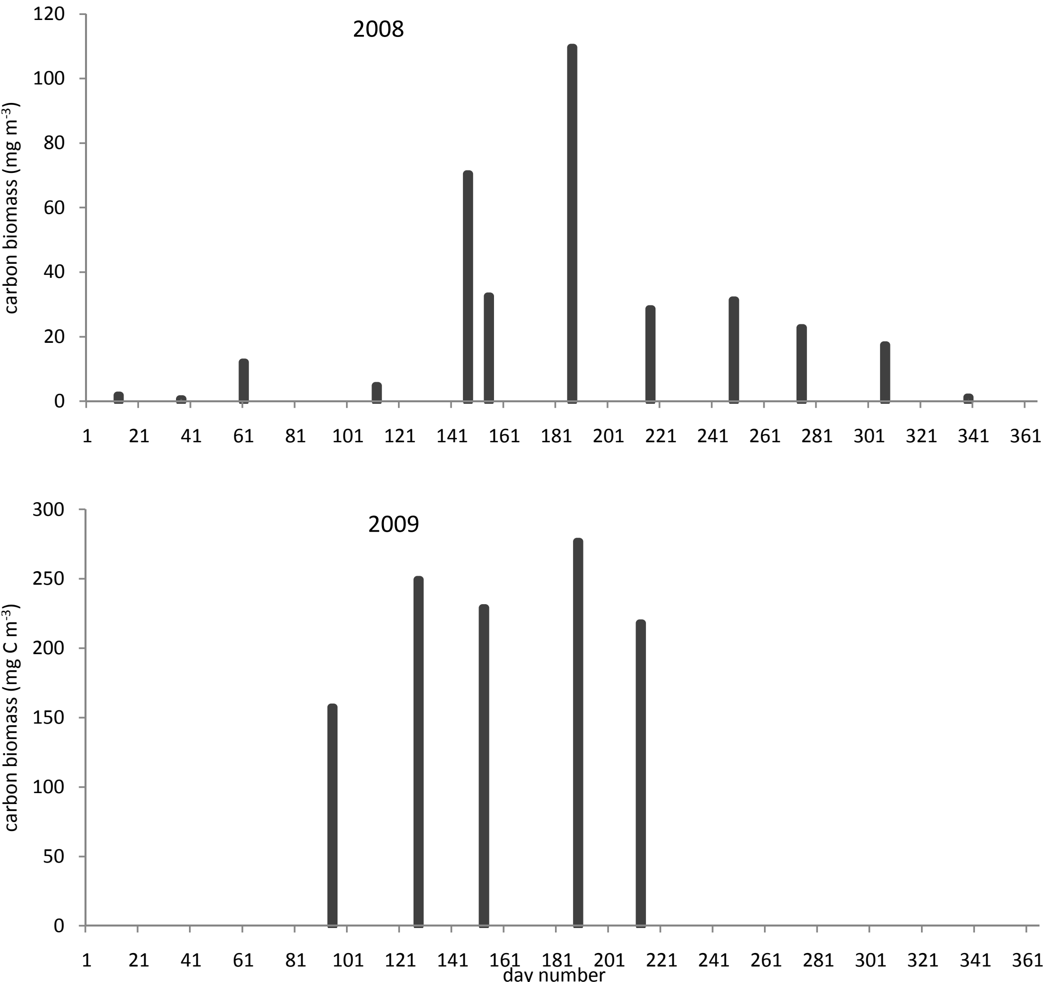


Figure 4.53: The temporal variation in total carbon biomass (mg C m^{-3}) at station LBay during 2008 and 2009. In 2009, only five samples were counted and identified.

To illustrate seasonal succession the microplankters identified and counted at LBay were divided into the functional taxonomic groups of diatoms, dinoflagellates and micro-flagellates (see Figure 4.54). In general, diatoms were the dominant group. For example, in 2008, diatoms contributed 4.78 mg C m^{-3} to the total microplankton biomass in winter, 37.35 mg C m^{-3} in spring and 56.62 mg C m^{-3} in summer all accounting for 99% of the total average microplankton carbon biomass. In autumn, diatoms represented 16.24 mg C m^{-3} (68%) of the total average microplankton

biomass while dinoflagellates contributed 7.64 mg C m^{-3} (32%). Micro-flagellates contributed only in spring 2008 more than 0.01 mg C m^{-3} (0.5%) to the average carbon biomass. In 2009, diatoms contributed 202.33 mg C m^{-3} (99%) to the average spring carbon biomass, and 106.47 mg C m^{-3} (44%) to the summer biomass, respectively. Dinoflagellates (134.97 mg C m^{-3}) (56%) dominated the summer period and micro-flagellates contributed 0.03 mg C m^{-3} (0.5%) to the total microplankton carbon biomass during that time.

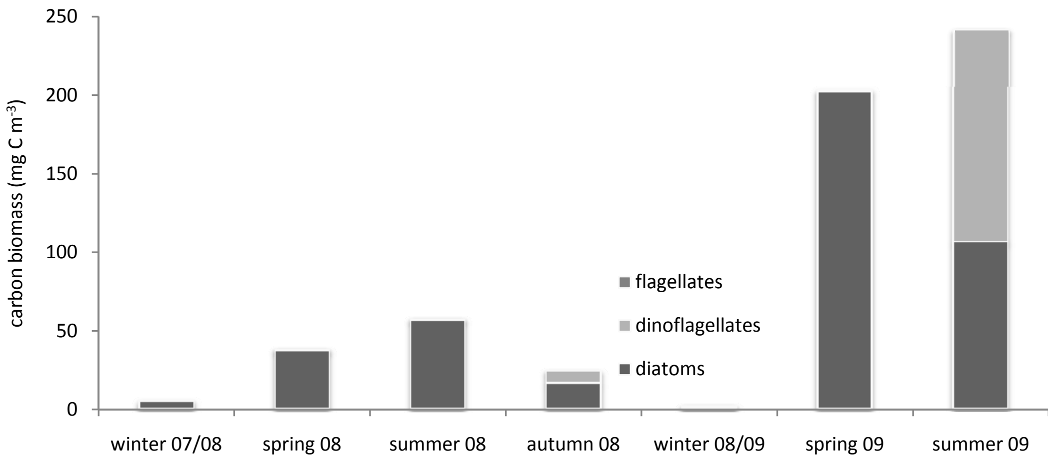


Figure 4.54: An illustration of the succession of microplankters categorised into three taxonomic functional groups for 2008 and 2009 at LBay.

Details about the top five dominant species in terms of abundance and carbon biomass are given in Table 4.3 for each season over this study.

Table 4.3: The tables display the dominating species for each functional group (diatoms, dinoflagellates, micro-flagellates) at station LBay. The top five species/taxa for each lifeform are displayed for the average cell abundance and the average carbon biomass for winter, spring, summer and autumn in 2008 and 2009. The number of identified species/taxa for each season is given in brackets at the top of each table. % represents the amount of the total biomass of all species identified.

total species identified 84

winter 08 (17)

average biomass [mg C m^{-3}]	**4.84**	average cell abundance [cells L^{-1}]	**5.75 x10^{3}**
***Coscinodiscus* spp.**	**2.43 (50.3%)**	***Thalassiosira* spp.**	**2373 (40.6%)**
Thalassiosira spp.	1.68 (34.7%)	*Paralia sulcata*	1387 (23.7%)
Paralia sulcata	0.27 (5.7%)	*Cylindrotheca* spp.	653 (11.2%)
Dithlum brightwellii	0.22 (4.6%)	*Bacillaria sp.*	427 (7.3%)
Bacillaria sp.	0.09 (1.8%)	*Skeletonema* spp.	240 (4.1%)
Cyclotella	0.03 (0.7%)	*Cyclotella*	93 (1.0%)
Amylax sp.	0.03 (0.6%)	*Amylax* sp.	33 (0.6%)
Ceratium furca	0.02 (0.5%)	*Ceratium furca*	13 (0.2%)
small naked dinos (<25µm)	0.01 (0.1%)	small naked dinos (<25µm)	13 (0.2%)

spring 08 (30)

average biomass [mg C m^{-3}]	**37.6**	average cell abundance [cells L^{-1}]	**128.5 x10^{3}**
Rhizosolenia imbricata/styliformis	**11.31 (30.1%)**	*Pseudo-nitzschia* spp.	26100 (20.3%)
Pseudo-nitzschia spp.	3.93 (10.5%)	*Skeletonema*	19250 (14.9%)
Pseudoguinardia recta	3.86 (9.8%)	*Lectrocylindrus danicus*	19000 (14.8%)
Ceratulina spp.	3.31 (8.8%)	*Guinardia delicaltula*	8300 (6.5%)
Rhizosolenia setigera	2.22 (5.9%)	*Chaetoceros* spp.	4900 (3.8%)
Scrippsiella sp.	0.02 (0.04%)	*Scrippsiella* sp.	150 (0.12%)
Phaeocystis globosa	0.19 (0.5%)	***Phaeocystis globosa***	**28100 (21.8%)**
Euglenophytes	0.03 (0.1%)	monad flagellates	200 (0.15%)
monad flagellates	0.01 (0.04%)	*Euglenophytes*	150 (0.12%)

summer 08 (47)

average biomass [mg C m^{-3}]	**56.91**	average cell abundance [cells L^{-1}]	**57.88 x10^{3}**
Pseudoguinardia recta	**30.62 (53.8%)**	***Cylindrotheca* spp.**	**30920 (40.7%)**
Rhizoselenia imbricata/styliformis	7.45 (13.1%)	*Pseudoguinardia recta*	18240 (24.0%)
Rhizoselenia spp.	5.54 (9.7%)	*Leptocylindrus* spp.	8400 (11.1%)
Guinardia spp.	2.68 (4.7%)	*Chaetoceros* spp.	5893 (7.8%)
Leptocylindrus spp.	1.63 (2.9%)	*Rhizosolenia* spp.	2640 (3.5%)
Ceratium furca	0.07 (0.12%)	small armoured dinos (<25µm)	160 (0.2%)
Dinophysis norvegica	0.05 (0.1%)	*Ceratium furca*	40 (0.5%)
Prorocentrum micans	0.05 (0.1%)	*Ceratium fusus*	40 (0.5%)
small armoured dinos (<25µm)	0.05 (0.1%)	*Prorocentrum micans*	40 (0.5%)
Ceratium fusus	0.09 (0.05%)	*Dinophysis norvegica*	27 (0.2%)
		Phaeocystis globosa	2360 (1.04%)
		monad flagellates	160 (0.07%)

autumn 08 (42)

average biomass [mg C m^{-3}]	**23.75**	average cell abundance [cells L^{-1}]	**22.32 x10^{3}**
Coscinodiscus spp.	4.06 (17.1%)	***Leptocylindrus* spp.**	**5333 (23.9%)**
Eucampia zodiacus	2.85 (11.9%)	*Eucampia zodiacus*	2347 (10.5%)
Rhizoselenia imbricata/styliformis	2.32 (9.8%)	*Chaetoceros* spp.	1560 (6.9%)
Pseudoguinardia recta	1.61 (6.8%)	*Guinardia delicatula*	1547 (6.2%)
Lauderia sp.	1.13 (4.8%)	*Paralia sulcata*	1373 (4.3%)
Cyclotella	0.22 (0.9%)	*Cyclotella*	640 (2.8%)
***Akashiwo* sp.**	**4.71 (19.8%)**	*Akashiwo* sp.	2760 (12.4%)
Prorocentrum gracile	1.09 (4.6%)	*Prorocentrum gracile*	267 (1.2%)
Ceratium tripos	0.64 (2.7%)	*Ceratium furca*	213 (1.0%)
Ceratium furca	0.37 (1.6%)	*Prorocentrum micans*	213 (1.0%)
Prorocentrum micans	0.25 (1.0%)	small armoured dinos (<25µm)	173 (0.8%)
monad flagellates	0.01 (0.1%)	monad flagellates	160 (0.1%)

winter 09 (15)

average biomass [mg C m^{-3}]	**1.23**	average cell abundance [cells L^{-1}]	**4.64 x10^3**
Paralia sulcata	0.2 (15.99%)	*Paralia sulcata*	1000 (21.6%)
Leptocylindrus	0.09 (6.91%)	*Leptocylindrus* spp.	440 (9.5%)
Rhizoselenia spp.	0.08 (6.81%)	*Chaetoceros* spp.	360 (7.8%)
Ditylum brightwellii	0.08 (6.79%)	*Asterionellopsis* spp.	280 (6.1%)
***Cyclotella* spp.**	**0.52 (41.94%)**	***Cyclotella* spp.**	**1520 (32.8%)**
Prorocentrum micans	0.1 (7.48%)	*Prorocentrum micans*	80 (1.7%)
small armoured dinos (<25µm)	0.01 (0.92%)	small armoured dinos (<25µm)	40 (0.9%)
monad flagellates	0.003 (0.24%)	monad flagellates	600 (8.6%)

spring 09 (31)

average biomass [mg C m^{-3}]	**203.28**	average cell abundance [cells L^{-1}]	**124.7 x10^3**
***Guinardia* spp.**	**173.82 (85.5%)**	***Guinardia* spp.**	**88100 (71.0%)**
Ditylum brightwellii	11.81 (5.8%)	*Chaetoceros* spp.	17650 (14.1%)
Coscinodiscus	6.08 (3.0%)	*Ditylum brightwellii*	5650 (4.5%)
Chaetoceros spp.	3.29 (1.6%)	*Pseudo-nitzschia* spp.	5200 (4.2%)
Guinardia flaccida	3.26 (1.6%)	*Cylindrotheca closterium*	1500 (1.2%)
Protoperidinium depressum	0.41 (0.2%)	small armoured dinos (<25µm)	150 (0.1%)
Gyrodinium spp.	0.12 (0.1%)	small naked dinos (<25µm)	100 (0.1%)
Protoperidinium spp.	0.08 (0.04%)	*Ceratium lineatum*	100 (0.1%)
non existent		non existend	

summer 09 (47)

average biomass [mg C m^{-3}]	**241.47**	average cell abundance [cells L^{-1}]	**896.33 x10^3**
Rhizosolenia spp.	49.66 (20.6%)	***Eucampia zodiacus***	**25100 (28.0%)**
Eucampia zodiacus	30.43 (12.6%)	*Rhizosolenia* spp.	23667 (26.4%)
Lauderia spp.	16.99 (7.0%)	*Leptocylindrus danicus*	10100 (11.3%)
Leptocylindrus danicus	2.39 (1.0%)	*Chaetoceros* spp.	7500 (8.4%)
Chaetoceros spp.	1.4 (0.6%)	*Naviculaceae*	6667 (7.4%)
Noctiluca scintillans	**131.36 (54.4%)**	small armoured dinos (<25µm)	833 (0.9%)
Prorocentrum micans	0.69 (0.3%)	*Prorocentrum micans*	600 (0.7%)
Prorocentrum gracile	0.68 (0.3%)	*Ceratium fusus*	233 (0.3%)
Prorocentrum spp.	0.32 (0.1%)	small naked dinos (<25µm)	233 (0.3%)
Ceratium spp.	0.3 (0.1%)	*Prorocentrum* spp.	200 (0.2%)

Silicate users were more abundant and represented a higher proportion of the annual mean microplankton carbon biomass (25.7 mg C m^{-3} for 2008 and 144.9 mg C m^{-3} for 2009) than the non-silicate users (2.1 mg C m^{-3} and 81.4 mg C m^{-3} in 2008 and 2009, respectively) (Figure 4.55). The carbon biomass for silicate users in spring (37.4 mg C m^{-3}) and summer (56.6 mg C m^{-3}) was higher than in autumn (16.3 mg C m^{-3}) 2008. In 2009, silicate users (202.2 mg C m^{-3}) contributed the main part (99%) to the total carbon biomass while their contribution to the total carbon biomass in the summer was 106.6 mg C m^{-3} (44%). Non-silicate user biomass was low in spring (0.3 mg C m^{-3}) and summer (0.3 mg C m^{-3}) 2008 and high (7.7 mg C m^{-3}) in autumn 2008. In spring 2009, carbon biomass of non-slicate users was low (0.9 mg C m^{-3}) and high in summer (134.9 mg C m^{-3}). Statistical analysis were not possible due to the small data set.

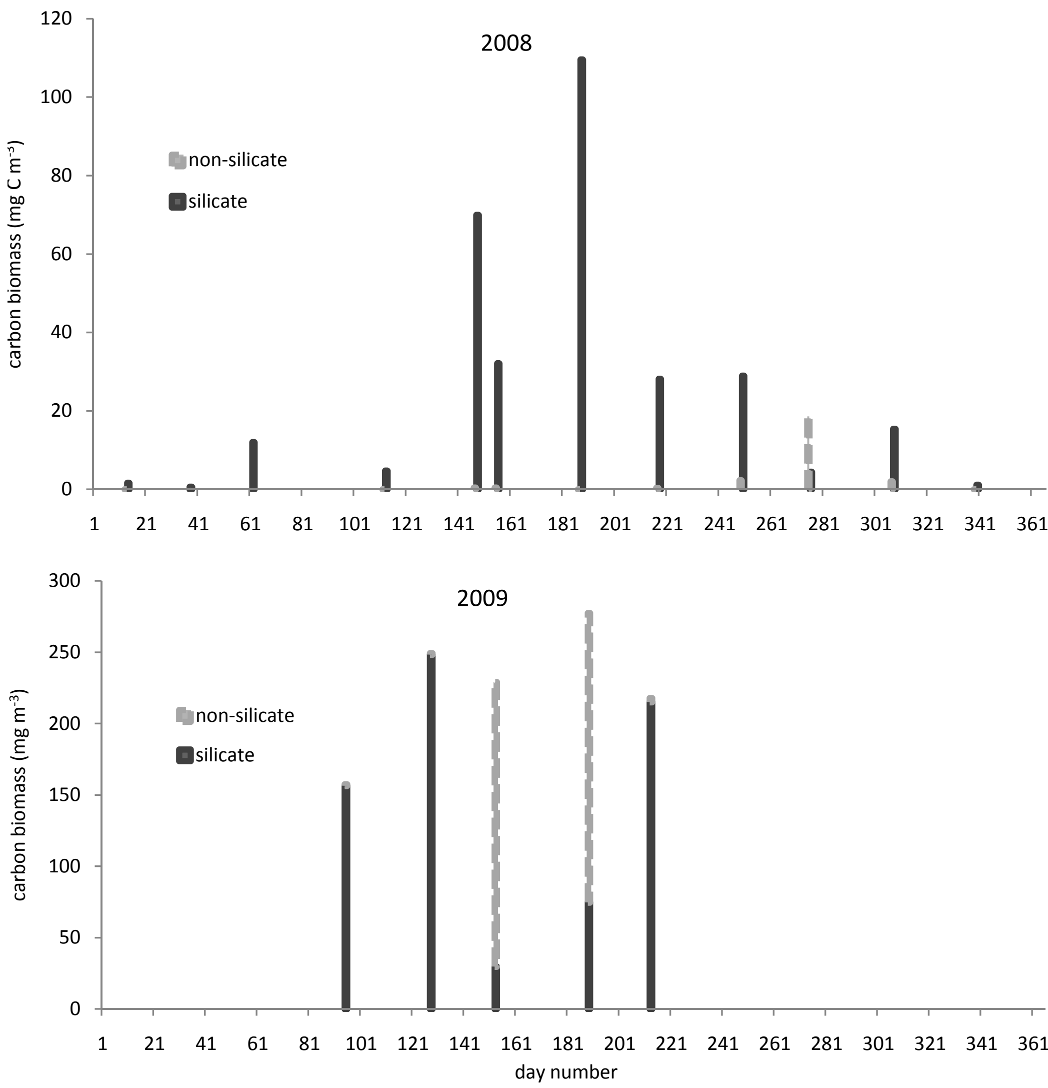


Figure 4.55: The seasonal distribution of silicate users and non-silicate users in carbon biomass for 2008 and 2009 at station LBay.

Large sized microplankton ($\geq 40\mu$m) dominated the microplankton carbon biomass in both years (Figure 4.56). In one occasion (day 275, 1st October 2008) the biomass of the small sized microplankters ($< 40\mu$m) was higher (14.52 mg C m^{-3}) than the biomass of the large sized microplankton (8.16 mg C m^{-3}).

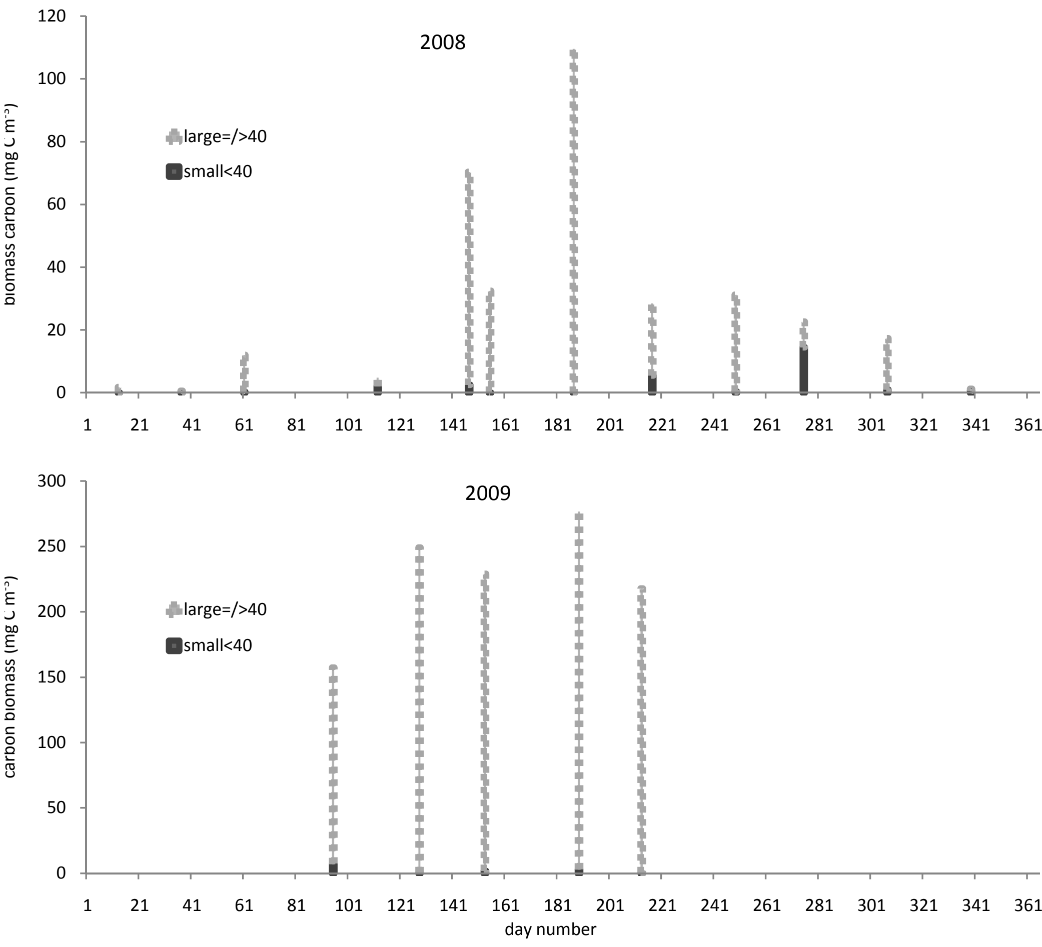


Figure 4.56: The seasonal distribution of large ($\geq 40\mu$m) and small ($< 40\mu$m) sized microplankton in carbon biomass for 2008 and 2009 station at LBay.

4.5 Data summary

In this summary section the physical, chemical, biological and microplankton data from the three sampling sites are briefly compared and summary plots are presented.

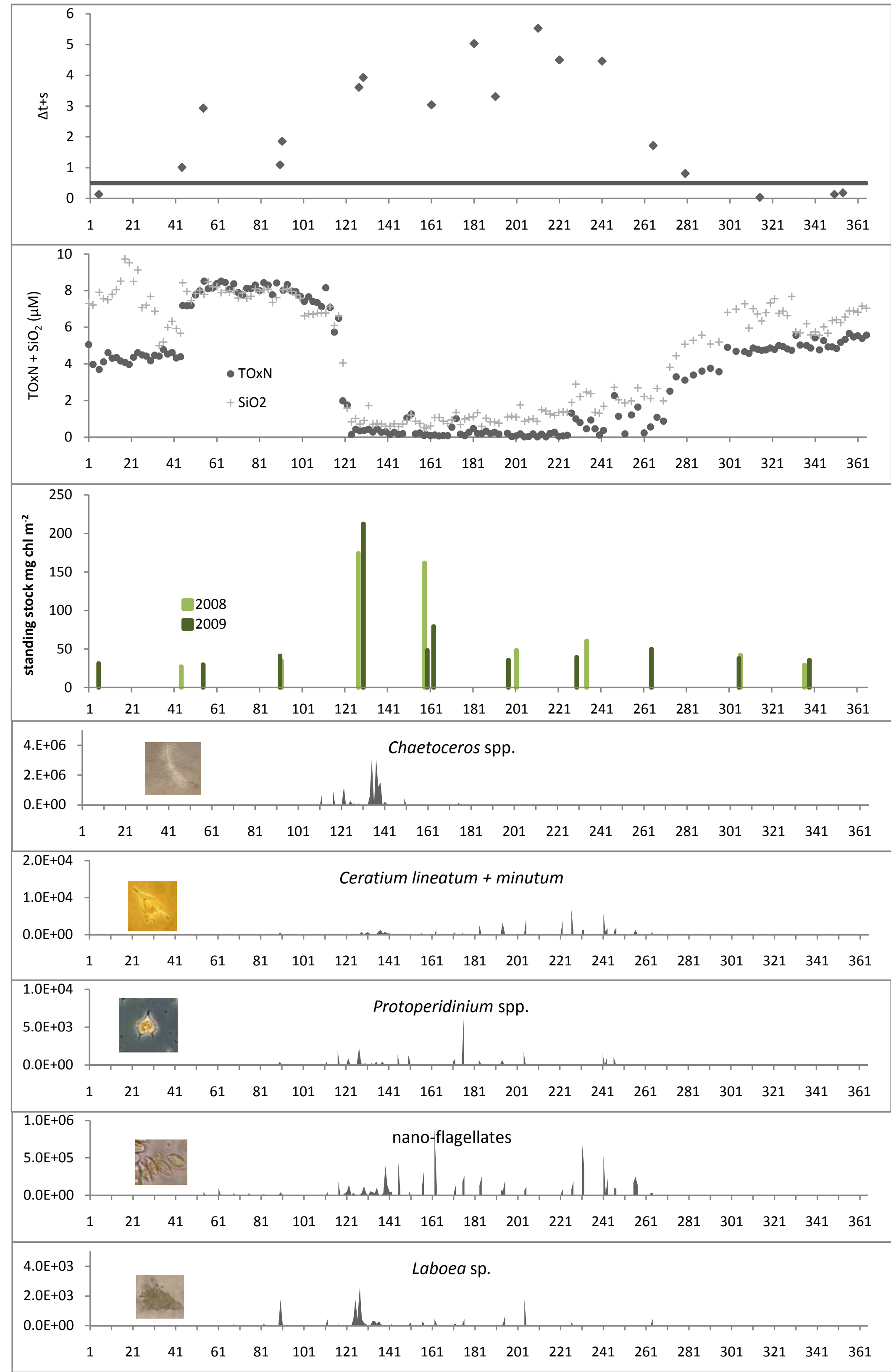


Figure 4.57: A climatology for station 38A. Data for the two sampling years were merged together to show the composite seasonal cycle of Δ t+s, TOxN and SiO_2, column chlorophyll and characteristic microplankton species (cells L^{-1}) for the mooring site in the western Irish Sea. The images of the microplankton were taken during microplankton analysis.

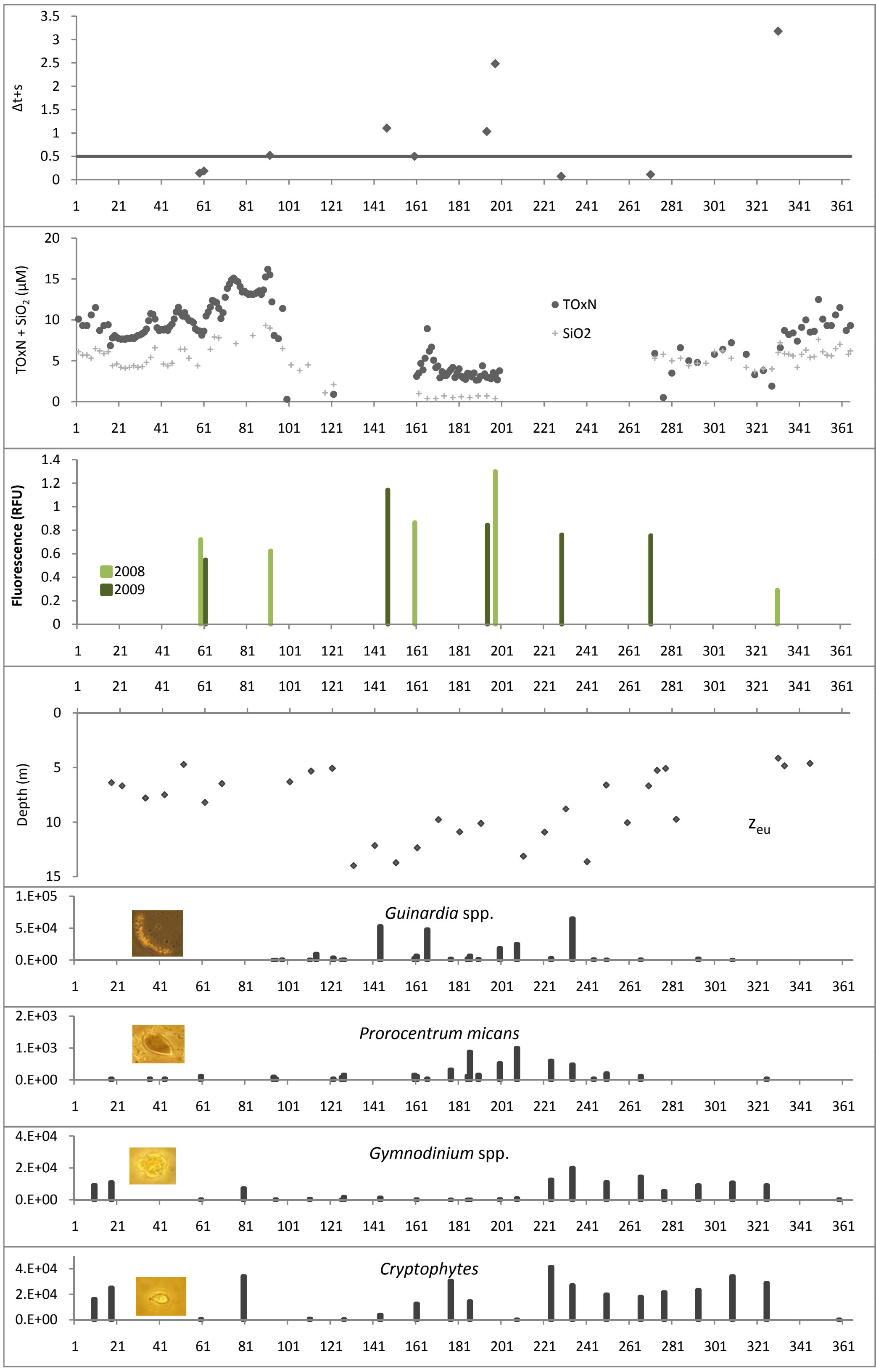


Figure 4.58: A climatology for station WGabb in the southern North Sea. Data for the two sampling years were merged together to show the composite seasonal cycle of Δ t+s, TOxN and SiO_2, column fluorescence and characteristic microplankton species (cells L^{-1}) for the site. The images of the microplankton were taken during microplankton analysis.

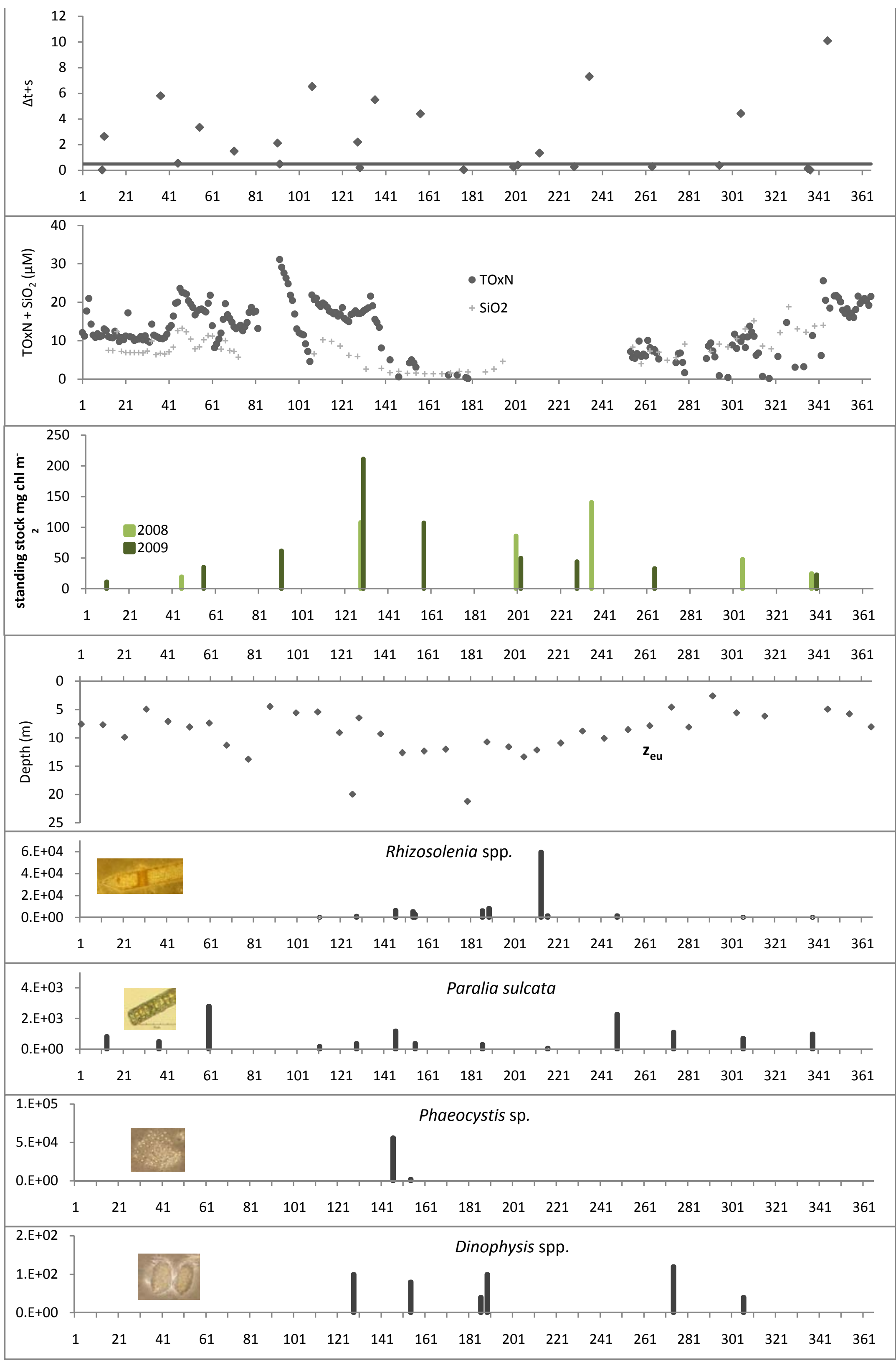


Figure 4.59: A climatology for station LBay in Liverpool Bay. Data for the two sampling years were merged together to show the composite seasonal cycle of Δ t+s, TOxN and SiO_2, column chlorophyll and characteristic microplankton species (cells L^{-1}) for thismooring site. The images of the microplankton were taken from the image collection established during analysis at station 38A.

Physical data

The annual temperature range at the coastal Cefas stations LBay (14.0° C in 2008 and 15.2° C in 2009) and WGabb (12.0° C in 2008 and 13.5° C in 2009) were larger than the range of the offshore AFBI station 38A (8.1° C and 8.8° C, respectively). Vertical temperature gradients were small at both Cefas mooring sites considering the mean value for all data points available (LBay 2008 mean Δt = 0.35° C, n = 25; WGabb mean = 0.14° C, n = 10). Compared to that the mean vertical temperature gradient at the AFBI mooring site 38A was 1.53° C, n = 18. Surface salinity was highest at the West Gabbard station in both years (mean = 34.88 and 34.83, respectively), followed by 38A (mean = 33.95 and 33.97, respectively) and LBay (mean = 32.43 and 31.73) (Tables 4.4 - 4.9). The temperature and salinity range as well as Δt and Δs in spring and summer for both sampling years for all three sites are given in tables 4.10, 4.11, 4.12. Descriptive statistics are given in tables 4.4, 4.5, 4.6, 4.7, 4.8, 4.9.

Seasonal stratification at station 38A was apparent from February to September (Figure 4.57) while it was intermittently apparent at stations LBay and WGabb with no obvious seasonality (Figure 4.59 and 4.58).

Irradiance data for the western Irish Sea site were taken from publications (Gowen, 2000) and more recent measurements in July 2010 (Gowen *et al.* unpublished data) and gave a euphotic zone depth of 28 - 30 meters. The average summer euphotic zone depth at stations LBay and WGabb over the two sampling years were 12 m and 11 m, respectively.

Table 4.4: The following tables display the descriptive statistics for temperature (°C), salinity, nutrients (μM), cell abundance (cells L^{-1}), carbon biomass (mg m^{-3}), and chlorophyll standing stock (mg m^{-2}) at the three sampling site. Station 38A in the western Irish Sea in 2008 and 2009 was presented first, the West Gabbard station in the southern North Sea for 2008 and 2009 followed, here fluorescence was presented in relative fluorescence units (RFU) instead of chlorophyll standing stock, and the Liverpool Bay station in the eastern Irish Sea for 2008 and 2009 was displayed last.
Station 38A in 2008

38A	Temperature	Salinity	TOxN	Silicate	Phosphate	cell abundance	carbon biomass	standing stock
N	2528	18554	145	145	145	50	50	8
mean	11.5	33.95	3.45	4.21	0.55	360.0 x 10^3	39.97	72.39
median	12.1	33.94	3.30	5.08	0.59	202.4 x 10^3	26.44	45.35
minimum	7.8	33.35	0.01	0.50	0.18	1.2 x 10^3	1.00	27.10
maximum	15.9	34.41	8.52	8.49	0.91	3,244.3 x 10^3	233.08	174.00
StDev	2.4	0.17	3.18	2.95	0.21	555.2 x 10^3	48.06	60.07

Table 4.5: Descriptive statistics for station 38A in 2009.

38A	Temperature	Salinity	TOxN	Silicate	Phosphate	cell abundance	carbon biomass	standing stock
N	2528	18554	143	126	143	53	53	10
mean	11.8	33.97	3.57	4.50	0.43	528.2 x 10^3	45.75	55.57
median	12.9	33.96	4.38	4.89	0.46	388.1 x 10^3	39.79	36.97
minimum	7.5	33.08	0.10	0.37	0.03	6.9 x 10^3	2.85	29.90
maximum	16.3	34.85	7.04	6.73	0.79	1,854.6 x 10^3	207.89	212.54
StDev	2.6	0.13	2.15	2.80	0.17	502.9 x 10^3	44.20	55.54

Table 4.6: Descriptive statistics for station WGabb in 2008.

38A	Temperature	Salinity	TOxN	Silicate	cell abundance	carbon biomass	fluorescence
N	3820	3474	151	73	13	13	5
mean	12.5	34.88	8.07	4.77	26.1 x 10^3	22.36	0.76
median	10.3	34.90	8.40	5.00	8.0 x 10^3	7.55	0.72
minimum	6.5	34.10	0.30	0.40	1.2 x 10^3	0.40	0.29
maximum	18.5	35.35	16.19	9.30	122.9 x 10^3	108.85	1.30
StDev	3.8	0.24	3.79	2.15	37.9 x 10^3	32.50	0.37

Table 4.7: Descriptive statistics for station WGabb in 2009.

38A	Temperature	Salinity	TOxN	Silicate	cell abundance	carbon biomass	fluorescence
N	3820	3474	90	54	21	21	5
mean	12.8	34.83	12.39	5.63	104.8 x 10^3	27.61	0.81
median	13.9	34.85	10.35	6.00	108.8 x 10^3	13.63	0.76
minimum	5.5	34.24	6.00	0.60	7.6 x 10^3	4.19	0.55
maximum	19.0	35.25	19.60	9.00	226.0 x 10^3	97.79	1.14
StDev	4.1	0.20	4.44	1.72	61.9 x 10^3	29.25	0.22

Table 4.8: Descriptive statistics for station LBay in 2008.

38A	Temperature	Salinity	TOxN	Silicate	cell abundance	carbon biomass	standing stock
N	4304	1956	186	72	12	12	6
mean	11.0	32.43	13.43	7.57	47.9 x 10^3	27.72	71.44
median	10.1	32.34	13.45	7.30	25.4 x 10^3	20.0	67.21
minimum	4.6	28.12	0.10	1.40	1.5 x 10^3	0.67	19.97
maximum	18.6	35.51	25.57	18.80	201.8 x 10^3	109.64	141.10
StDev	3.8	0.84	5.60	3.86	60.6 x 10^3	32.41	48.51

Table 4.9: Descriptive statistics for station LBay in 2009.

38A	Temperature	Salinity	TOxN	Silicate	cell abundance	carbon biomass	standing stock
N	4304	1956	90	17[1]	5[1]	5[1]	9
mean	10.9	31.73	17.43	1.16	103.7 x 10^3	226.13	64.39
median	10.3	31.76	20.65	1.10	122.3 x 10^3	229.12	44.36
minimum	4.4	29.88	0.40	0.20	38.4 x 10^3	157.45	11.70
maximum	19.6	33.01	29.88	3.50	157.5 x 10^3	276.85	211.51
StDev	4.4	0.65	8.66	0.71	47.4 x 10^3	44.42	61.60

Table 4.10: Overview of temperature (° C), salinity, Δt and Δs for the spring (April - May) and summer (June - August) periods during 2008 and 2009 for all three sampling stations starting with 38A, then WGabb, and LBay.

Variable	Spring'08	Summer'08	Spring'09	Summer'09
Temperature	6.6-12.7	11.9-18.5	6.9-13.8	12.9-19.0
Salinity	34.13-35.19	34.29-35.13	34.29-35.02	34.49-35.06
Δt	0.2-2.7	2.9-3.0	0.7-1.9	2.8-4.7
Δs	0.05	0.07-0.4	0.21	0.01-0.19

Table 4.11: Station WGabb

Variable	Spring'08	Summer'08	Spring'09	Summer'09
Temperature	4.6-14.6	12.2-18.6	7.3-15.5	12.6-19.6
Salinity	30.23-35.51	29.53-33.37	30.15-32.99	30.86-33.19
Δt	0.5-0.6	0.2-1.4	0.0-0.1	0.00-1.0
Δs	0.16-1.34	0.05-1.73	0.01-0.07	0.00-0.97

Table 4.12: Station LBay

Variable	Spring'08	Summer'08	Spring'09	Summer'09
Temperature	8.0-14.5	9.7-15.9	7.9-12.9	11.4-16.3
Salinity	33.35-34.38	33.43-34.41	33.65-34.28	33.62-34.58
Δt	0.3	0.2-0.5	0.1	0.0-0.1
Δs	0.07-0.33	0.01-0.51	0.00-0.01	0.02-0.34

Chemical data

Near surface nutrient (TOxN and SiO_2) concentrations showed similar patterns at all three sampling sites with winter maxima and spring/summer minima (Figures 4.57 4.58 and 4.59). Highest winter concentrations at station 38A for 2008 (TOxN = 8.5 μM and SiO_2 = 8.5 μM), and 2009 (TOxN = 7.0 μM and SiO_2 = 6.7 μM) were low compared to station WGabb (TOxN = 16.2 μM and SiO_2 = 9.3μM in 2008; TOxN = 19.6 μM and SiO_2 = 9.0 μM in 2009) and LBay (TOxN = 25.6 μM and SiO_2 = 18.8 μM in 2008; TOxN = 29.9 μM and SiO_2 = 3.5 μM (SiO_2 summer values only) in 2009). The concentration of both nutrients during April and May decreased rapidly at all three sites.

The estimation of the nutrient assimilation by microplankton was made by taking the N:P and N:Si ratio from April to May in 2008 and 2009 cumulatively for station 38A. For the West Gabbard and Liverpool Bay stations N:Si ratios were estimated in the same way with data from March to May. Additional data from June and July in the case of WGabb and June in the case of LBay were taken into consideration to provide more information as data during the critical draw down period were not available. The nutrient draw down at station 38A was between 23rd April and 21st May in 2008, and 10th April and 2nd May in 2009 with an N:P uptake ratio of 13.2 while the intercept was significantly different from 0 ($p < 0.05$) and an N:Si ratio of 0.9 with the intercept not significantly different from 0. At the West Gabbard station the N:Si draw down was observed between the first growth period between 1st April and 14th June 2008. Considering only the spring period (March-May) the uptake ratio was 0.35. Taking June and July into account, the draw down ratio was 1.09. In both cases the intercept was significantly different from 0 ($p < 0.05$). For LBay, N:Si draw down was observed first on 17th April 2009 with a ratio of 1.15 considering only the spring period. When data from June were accounted for, the uptake ratio increased to 1.43. The intercepts were significantly different from 0 in both cases ($p < 0.05$).

Microplankton biomass

At all three stations, a well-defined seasonal cycle of chlorophyll was observed with a pronounced spring peak during mid May and a less pronounced but recognisable peak in autumn. Maximum chlorophyll standing stock for station 38A was 212.54 mg m^{-2} and for station LBay 211.51 mg m^{-2} both measured in 2009. For station WGabb chlorophyll standing stock data were not available. However, the highest near surface chlorophyll concentration was 19.95 mg m^{-3} on day 122 (1st May) that compared to the highest near surface chlorophyll (4.17 mg m^{-3}) at station 38A and 7.66 mg m^{-3} at station LBay on day 129 (9th May). Maximum carbon biomass for station 38A was 233.08 mg m^{-3} in 2008 and 207.89 mg m^{-3} in 2009, 108.85 mg m^{-3} in 2008 and 97.76 mg m^{-3} in 2009 for station WGabb, and 109.64 mg m^{-3} in 2008

and 276.85 mg m^{-3} in 2009 for station LBay. The mean carbon biomass at station 38A in spring 2008 and 2009, respectively was 57.53 mg m^{-3} and 90.41 mg m^{-3} compared to 56.25 mg m^{-3} and 43.59 mg m^{-3} at WGabb and 37.60 mg m^{-3} and 203.28 mg m^{-3} at the LBay station.

Liverpool Bay showed the highest winter nutrients (TOxN 29.88 μM and Si 18.80 μM) and the highest carbon biomass (276.85 mg C m^{-3}), but only the second highest chlorophyll standing stock (211.51 mg chl m^{-2}). At station 38A, where lowest winter nutrient concentrations were recorded (TOxN 7.04 μM and Si 6.73 μM), the highest chlorophyll standing stock (212.54 mg chl m^{-2}) was higher than at Liverpool Bay and biomass (233.08 mg C m^{-3}) was lower than for Liverpool Bay. However, these value were higher than the values for the WGabb station (max. biomass 108.85 mg C m^{-3}) where maximum winter nutrient levels were TOxN 19.60 μM and Si 9.30 μM.

Microplankton succession

At station 38A, silicate users were dominant in spring while non-silicate user carbon biomass was high in summer. In autumn, the microplankton biomass for silicate users was higher than it was for non-silicate users. There was a statistically significant shift from silicate users in the spring to non-silicate users in the summer (Mann-Whitney non-paramentric test). In 2009, there was also a significant shift from large sized microplankton in spring to small sized members in summer. At the LBay station large sized silicate users were dominant except for one occasion in October 2008 when small sized microplankton carbon biomass was higher. In summer 2009, non-silicate users were the dominant functional group. At the WGabb station, the microplankton biomass was dominated by diatoms and a statistical significant shift from diatoms to dinoflagellates was not apparent. In spring, small sized non-silicate users contributed an increased amount to the microplankton carbon biomass.

In order to introduce the concept of different lifeforms (taxonomic functional groups) on which the microplankton community index (MCI) is based, characteristic species genera were displayed as representatives of these lifeforms (Figure 4.57, 4.58, 4.59). At the mooring site in the western Irish Sea the genus *Chaetoceros* was chosen to represent autotrophic silicate users, because *Chaetoceros* species were strongly seasonal related to the spring bloom in both sampling years. *Ceratium lineatum* + *Ceratium minutum* were chosen to represent the autotrophic non-silicate users, because they were a pronounced feature after the spring bloom characterising the growing season in summer and autumn in both years. The genus *Protoperidinium* represented the heterotrophic non silicate users that were only present in elevated numbers well after the spring bloom and in the summer characterising a typical heterotrophic organism. Nano-flagellates (<10 μM) represented the small sized microplankton, because

they were always present in high abundance with no obvious pattern and the ciliate *Laboea* sp. was chosen as a representative of a large, non-flagellate heterotrophic microplankton. The appearance of the genus *Chaetoceros* was strongly related to early spring and occurred in high abundance. After day 167 (16th June) the genus was reduced. The two *Ceratium* species had a similar strong seasonal pattern. They occurred in high numbers in mid summer (day 194; 13th July) until mid autumn (day 252; 9th September). While the nano-flagellates were present in high cell abundance with no obvious pattern throughout the year, the ciliate *Laboea sp.* appeared in low cell abundance, had its maximum in early spring and was present during the summer period.

At the mooring site WGabb, the genus *Guinardia* was chosen to represent the autotrophic silicate user, because it was strongly related to the spring and the autumn peaks in both years. *Prorocentrum micans* and *Gymnodinium* species were representing the typical autotrophic and heterotrophic non-silicate users partly showing atypical characteristics at this station. *Cryptophytes* represented the small sized microplankton at this site, because they were the most abundant and most consistent genus and with no obvious pattern. Apart from the micro-flagellates that generally appeared throughout the whole sampling period with the exception of the early spring period, the other three lifeforms showed a strong seasonal pattern in their appearance. *Guinardia* peaked in spring and in mid summer and disappeared while species of the *Gymnodinium* were abundant in the beginning of the year and with maximum numbers in the late summer and autumn period from day 224 to 325. The dinoflagellate *Prorocentrum micans* appeared in low numbers compared to the other three microplankters with highest cell numbers in the summer period.

The genus *Rhizosolenia* and the species *Paralia sulcata* were chosen to be the characteristic autotrophic silicate users at station LBay because they followed a strong seasonal pattern typically occurring in autumn and in winter. *Phaeocystis* spp. was the representative for the small sized microplankton because it has been reported to be a nuisance alga in European coastal areas and because it is often used as an indicator species occurring in mid spring. The genus *Dinophysis* was sued to represent the mixotrophic non-silicate user. Data for non-flagellate heterotrophic microplankton such as ciliates were not available. The genera of *Rhizosolenia* and *Phaeocystis* followed a strong seasonal pattern occurring in high numbers in mid summer and mid spring, respectively, whereas *Paralia sulcata* generally occurred in lower numbers throughout the year with higher abundances in the beginning and in the end of the year. The dinoflagellate *Dinophysis* appeared in low abundance and with no obvious pattern during spring, summer and late autumn.

4.6 Discussion

4.6.1 Annual cycle of microplankton in temperate shelf seas

It is generally accepted that in temperate coastal shelf seas and coastal waters tidal mixing, the sub-surface light climate and nutrient supply have important influence on the seasonal cycle, biomass and composition of the microplankton (Gran & Braarud, 1935; Sverdrup, 1953; Pingree *et al.*, 1978; Smetacek *et al.*, 1990; Tett, 1990).

The characteristics and hydrodynamics of the three mooring sites involved in this study were hypothesised to be:

1. station 38A: pristine (little nutrient enriched) site at which the microplankton biomass season is closely coupled to the seasonal pattern of stratification.

2. station WGabb: medium high nutrient enriched and intermittently stratified site that is influenced by freshwater with restricted coupling of the microplankton biomass season to the intermittent stratification pattern.

3. station LBay: highly nutrient enriched and intermittently stratified site that is located in a ROFI with restricted coupling of the microplankton biomass season to the stratification pattern.

It was further hypothesised that the microplankton biomass and composition are different at the three sites. At station 38A this was hypothesized to be due to the different hydrodynamics and nutrient loadings while for station LBay and WGabb this was hypothesized to be due to the different nutrient loadings only.

The hypotheses of the hydrodynamical differences are examined first by discussing the physical and chemical environment at all three site. Then the seasonal cycle of the microplankton is considered in this context. The dynamics of the microplankton community structure at the three sites is discussed in a subsequent section.

Hydrodynamics

Station 38A

Station 38A was located in the deep trough that runs south-north through the western Irish Sea. The mooring was located in a region of weak tidal flows and this feature together with the depths meant that the water column in this area was subject to seasonal stratification (Pingree *et al.*, 1976). Gowen *et al.* (1995) described the seasonal pattern of stratification and Horsburgh *et al.* (2000) demonstrated that this is a re-occurring annual event. Using the surface to bottom difference in

temperature it is evident from Figures 4.2 and 4.3 that solar radiation began to warm the surface layers in April. Considering the stratification index according to Talling (1971) who defined the boundary of the surface mixed layer as the depth with a temperature 0.5°C less than the temperature at 2 m, thermal stratification at station 38A resulted from a rapid increase in near surface temperature during May 2008 and April 2009. The period of stratification lasted for six months in 2008 and five and a half months in 2009, and this is consistent with findings by Gowen *et al.* (1995) and Horsburgh *et al.* (2000). Observations have shown that annual temperature differences of 2 °C and more have resulted in increased copepod populations that were shown to have an effect on microplankton biomass, size and composition (Wiltshire & Manly, 2004; Sommer *et al.*, 2007; Sommer & Lewandowska, 2011). However, the significant difference of 0.3 °C observed between the two sampling years was assumed to have no influence on biological factors. The low Δt at the end of June/beginning of July (Figures 4.3 and 4.2) related first to an increase and then a decrease in nutrients (Figure 4.8) and with a few days delay elevated microplankton biomass (Figure 4.17), confirming that the cooling of the surface temperature (Figures 4.2 and 4.3) could have caused mixing of the water column that made isolated near bottom nutrients available for microplankton growth. It is possible that the increase of surface temperature and therefore an increase of stratification (Figure 4.3) in October 2008 (day 298; 23rd October) led to elevated microplankton carbon biomass (34.5 mg m^{-3}) (Figure 4.17) and chlorophyll standing stock (38.0 mg m^{-2}) (Figure 4.13) on day 305 (31st October). The expected decrease in nutrients for that period was apparent, but not strong enough to confirm a relationship between decreasing nutrients and elevated growth (Figure 4.8). The increased Δ t observed in February and March 2009 (day 44 - 75; 13 th February - 16th March) had no obvious effect on the microplankton chlorophyll and biomass (Figures 4.13 and 4.17) and suggested that the euphotic zone depth was not deep enough at this time of the year to coincide with the surface mixed layer and therefore create favourable conditions for microplankton growth. In their work, Gowen *et al.* (1995) observed an apparent relationship between the depth of the surface mixed layer (SML), I_{sml} which is the threshold of irradiance and the onset of the production season at a sampling station close to sampling station 38A. As long as SML depth exceeded 50 m and I_{sml} was <168 Wh m^{-2}, chlorophyll concentrations remained below or 1mg m^{-3}. As soon as there was a marked reduction in the depth of the SML and an increase in I_{sml}, an increase in chlorophyll concentrations was recorded. Detailed information about the threshold of irradiance was not available for 2008 and 2009. However, a relationship between the reduction of the SML depth beyond 40 m to approximately 30 m and increasing biomass was observed by the end of April 2009 (Figures 4.4 and 4.2) and suggested that the relationship observed by Gowen *et al.* (1995) could also be true for station 38A in 2008 and 2009.

Generally, thermal stratification is the main and more important stratification to initiate microplankton growth however, haline stratification can contribute an important feature in the early period of the year. Slinn (1974) and Gowen *et al.* (1995) demonstrated the importance of haline stratification for the offshore region of the western Irish Sea. At station 38A, early haline stratification was evident in both years (Figures 4.4 and 4.6). However, as vertical salinity data were not available in high temporal resolution it can only be assumed that the overall stratification (Δt+s) was apparent from February to October. The seasonal pattern of salinity (high in winter, low in summer) more obvious in 2009 than in 2008, was consistent with findings by others (Pingree *et al.*, 1978; Gowen & Stewart, 2005). A tongue of more saline water extending northwards through the western Irish Sea appears to be a consistent winter feature (Matthews 1914 and Lee 1960 as cited in (Gowen *et al.*, 2002). Low salinity water was possibly trapped in a near surface gyre that is driven by a dome of cold bottom water (Hill *et al.*, 1994) during summer and was released when mixing set in toward the end of the year. The reason why winter salinity was higher in 2009 than in 2008 is unclear and cannot be explained by high rainfalls or other freshwater influence in 2008 during this period. It might be that the influence of salty Atlantic water that provides the source water for the Irish Sea (Bassett, 1908) had more influence in 2009 than it had in 2008. The saltier bottom water in the beginning of 2009 (Figure 4.6) together with an increased surface to bottom difference in temperature (Δ t) in February (Figure 4.3) could explain the early stratification in 2009 and therefore the early nutrient draw down and the beginning of the microplankton growth in the spring.

Stations West Gabbard and Liverpool Bay

In areas with tidally strong mixing stratification only occurs rarely. It can be intermittent when these areas are located in coastal regions influenced by freshwater (so called ROFIs as defined in Tett *et al.* (2003) causing haline stratification (e.g. Sanders *et al.*, 2001). Confirmation of freshwater influence was given for the LBay station by apparent haline stratification at the end of February (Figure 4.41) and by a significant winter salinity - nutrient relationship (least square regression; $p < 0.05$) (Figure 4.48). Although it seemed that the West Gabbard mooring site was not a freshwater influenced region (ROFI), considering the relatively high salinity values (34.88 and 34.85, respectively) and missing haline stratification (Figure 4.24), a significant relationship of decreasing salinity and increasing TOxN and SiO_2 was observed ($p<0.01$) for winter 2009 (Figure 4.30) and confirmed ROFI characteristics of the site. These findings were consistent with work by Sanders *et al.* (2001), who found a winter relationship of salinity and TOxN in the Outer Thames plume. The 'up and down' pattern (noisy background) observed in the winter near-surface salinity (Figure 4.25) and the winter nutrients range (TOxN 9.1 μM and Si 4.9 μM) at station WGabb from mid January to early April (Figure 4.27) indicated pulsed

(tidal) freshwater input. At station WGabb four out of ten data points for Δt+s exceeded 0.5°C with no obvious pattern (see Figure 4.26) and the vertical temperature and salinity profiles showed no evidence of any stratification (thermal or haline) (Figure 4.24). No published data were available to compare the hydrodynamics of the WGabb station as the mooring was deployed only recently (2006). According to Howarth *et al.* (1993) the southern North Sea is well mixed all year round due to its shallow depth (approximately 40m) and strong tidal currents opposing summer stratification. Learning from a publication by Sanders *et al.* (2001) and references therein, estuarine plume zones on the east of the UK are generally shallow and well mixed with weak, but significant, horizontal salinity gradients and relatively high turbidity. Mills *et al.* (2005) confirmed that thermal stratification is typically missing in the Thames plume due to a strong tidally mixed regime. The available data points for Δ t+s from 2008 and 2009 (n = 10) showed indication of a mixed, maybe intermittently stratified trend although significant vertical salinity gradients were not apparent in 2009 (Figure 4.26). The significant temperature difference at the West Gabbard station (0.3° C) between the two sampling years was assumed to have no influence on the biological factors and showed no effect on the nutrient pattern (Figure 4.27).

A broad winter salinity (5.68) and winter nutrient range (24.2 μM TOxN and 7.5 μM Si) in Liverpool Bay reflected the degree of mixing between freshwater and seawater. The data for Δt+s strengthened the argument that the water column at station LBay intermittently stratified with no obvious pattern (Figure 4.42). Thirteen out of 25 data points increased beyond 0.5°C at the station LBay. The vertical profiles (Figure 4.41) suggested that haline stratification was apparent at a water depth of approximately 13 m by the end of February in 2009. Judging by the data available thermal stratification was not apparent at any time of the year. Work by Gowen *et al.* (2000) showed similar findings for Liverpool Bay in 1997 with intermittent stratification and freshwater influence. Low temperatures in early April 2008 coincided with increased salinity for the same period, suggesting that saltier water had entered the region or freshwater input (by rivers) was reduced. This condition could be explained by the argument that the Cefas mooring was located at the edge of a salinity front and was pushed in and out of it by tidal movement. Gowen *et al.* (2005) showed that a salinity range in January 2000 in Liverpool Bay was 1.5. The significant difference 1.25 ($p = 0.00$) in salinity between 2008 and 2009 could have had an effect on the microplankton growth, composition, and size but was impossible to examine during this study.

Light

Conditions in which decreasing mixed layer depths coincide with the euphotic zone are normally absent in areas with tidally strong mixing (Mills *et al.* 2005). However,

microplankton growth and production is apparent. According to Gowen & Stewart (2005) the influence of mixing could likely be limited by the shallow depth of coastal areas (e.g. Liverpool Bay) and with sufficient light microplankton growth and production in such conditions can be possible. Gowen *et al.* (1995) found that a daily light threshold of 183 to 245 Wh m^{-2} was needed for net production of phytoplankton in the western Irish Sea. According to Gowen *et al.* (2000) such light regimes were evident in the eastern Irish Sea in late spring and summer 1997 and supported net photosynthesis throughout the water column. Daily irradiance data for the water column for 2008 and 2009 were not available for any of the three stations. However, K_d for station LBay and WGabb was calculated from PAR (μE m^{-2} s^{-1}) at 1 m and 2 m depth indicating that the average euphotic zone depth (z_{eu}) in the summer was 12 m for station LBay and 11 m for station WGabb (Figures 4.49 and 4.31). These results suggested that less than half of the water column at both stations Liverpool Bay (total depth 30 m) and West Gabbard (total depth 35 m) was illuminated. According to Sanders *et al.* (2001) the subsurface irradiance regime is likely to be influenced by tidal re-suspension and surface irradiance levels. The high turbidity may be an important control over phytoplankton production, as a consequence of its role in controlling subsurface irradiance levels. Vertical chlorophyll (LBay) (Figure 4.51) and fluorescence (WGabb) profiles (Figure 4.34) showed consistent levels down the water column with no decrease below the calculated euphotic zone. There were several possible reasons why the calculation of z_{eu} resulted in such low euphotic zone depth. Shading of the surface buoy could have effected measurements of PAR in 1 and 2 meters as well as waves that could have caused bubbles interfering with measurements. One should also keep in mind that the light absorption in the first few meters is highest throughout the water column (Tett, 1990). Evaluation has shown that a K_d estimated with 1 and 2 meter PAR measurements is approximately twice as high as a K_d estimated with a vertical light profile (E. Capuzzo, person. comm.) consequently leading to lower z_{eu} values. Calculating the euphotic zone for Liverpool Bay with an estimated K_d by Gowen *et al.* (2000) for a vertical light profile for this area gave an average depth of 12.5 m. This result rejects the possible reasons for a wrong K_d estimation by PAR sensors at 1 and 2 meter depths. Therefore, it can be assumed that the values for mean water column irradiance were similar to the ones observed between late spring and summer 1997 (149 - 324 Wh m^{-2} d^{-1}) by Gowen *et al.* (2000), giving evidence for sufficient light supporting photosynthesis throughout the water column in this area. Permanent strong mixing could be an explanation for the homogeneous distribution of chlorophyll. It suggests that even if photosynthesis took place only in the first 12 m of the water column, the strong mixing distributed phytoplankters throughout the water column. For the mooring site in the West Gabbard, there were no published irradiance or euphotic zone data with which to compare my data. I assume that the light regime for 2008 and 2009

was similar to the light regime found in Liverpool Bay and allowed photosynthesis. The mean depth of the euphotic zone at both test sites was similar for the summer, there was no decrease in the vertical summer profile of fluorescence at the WGabb station, microplankton biomass was observed and physical data (temperature range and Δt+s) suggested that the hydrodynamics at both sites were similar.

Nutrients

In addition to the influence of light and mixing, microplankton growth and production is also influenced by nutrients. The availability of nutrients is to some extend affected by mixing. Coastal shelf seas receive nutrients from a range of sources such as oceans, rivers, atmosphere and local water-column and sea-bed-recycling of biologically produced organic compounds. Generally, nutrient concentrations are highest in late winter while growth and production of microplankton is minimal and lowest during summer, reflecting the seasonality of biological production and breakdown (Tett, 2003). Toward spring, when light initiated favourable conditions for the development of a spring bloom, nutrient supply rapidly decreases and determines the level of production during the growing season (Gibson *et al.*, 1997; Sanders *et al.*, 2001; Mills *et al.*, 2005; Gowen *et al.*, 2008). It is normally a few days after an observed decrease in nutrients that an increase of biomass is apparent (response time of microplankton).

Station 38A

The pronounced seasonal cycles of all three main nutrients with near surface late winter (March/early April) maximum and summer (June-September) minimum concentrations observed in 2008 and 2009 for station 38A (Figure 4.8) were found to be typical and characteristic for the western Irish Sea (Gowen & Stewart, 2005; Gowen *et al.*, 2008). According to Gowen & Stewart (2005) measurements of winter concentrations (March/early April) of the three nutrients in offshore near-surface waters in the western Irish Sea measured between 1998 and 2002 were 8.3 μM DIN (dissolved inorganic nitrogen), 6.6 μM Si and 0.7 μM DIP (dissolved inorganic phosphorus). For the years 2000 to 2004, Gowen *et al.* (2008) showed that late winter near surface concentrations for DAIN (dissolved available inorganic carbon) concentrations decreased with the years (7.7 μM), and Si and DAIP (dissolved available inorganic phosphorus) stayed the same (6.7 μM and 0.72 μM respectively). Winter concentrations (March/early April) of the three nutrients in 2008 were 7.92 μM TOxN, 7.69 μM SiO_2, and 0.75 μM $PO_4{}^{-3}$. For 2009, they were 5.55 μM TOxN, 7.79 μM SiO_2, and 0.58 μM $PO_4{}^{-3}$, respectively. Estimating the average winter concentrations for N, Si, and P over the two year sampling period, the values were 6.5 μM TOxN, 7.36 μM SiO_2 and 0.71 μM $PO_4{}^{-3}$, indicating that in a time period of four to nine years previously, winter concentration of TOxN (total oxidised nitrogen) values have de-

creased, SiO_2 increased and P values stayed the same. According to Hartnoll (2002), winter concentrations in the central Irish Sea were 7.5 μM TOxN, 6.3 μM silicate and 0.6 μM soluble reactive phosphate. The measuring station these data derived from in central Irish Sea was regarded to be away from major anthropogenic input and should be representative of background levels in the Irish Sea (Hartnoll *et al.* 2002).

Significant relationships between salinity and the three main nutrients were not obvious (least square regression $p > 0.05$) for station 38A and was consistent with findings by Gowen *et al.* (2002). The 'nose'-shaped pattern that was observed in all three nutrients (Figure 4.9) in winter (day 54; 23rd February) may be explained by a coinciding weak haline stratification in 12 m depth (Figure 4.4). However, this would have meant that the nutrients in the near surface water was elevated by freshwater influence that would have also resulted in lower temperatures and a negative relationship of salinity and nutrients. These were not observed. The source of the vertical pattern remained unclear because an instrumental failure was also excluded. The 'shoulder'-pattern apparent in all three nutrients (Figure 4.9) in the summer 2009 (day 197; 15th July) correlated with the thermocline observed on that day (Figure 4.4) at a depth of 38 m. No further investigation could be made about this characteristic, whether it was a consistent feature in the summer period could also not be investigated as high frequency sampling data for vertical measurements were not available. The nutrient depletion of the upper 40 m was reflected in low chlorophyll (Figure 4.14) and microplankton biomass (Figure 4.17). The 'shoulder' was assumed to be created by diapycnal mixing which explained that the nutrient levels right below the cline were less than in deeper waters. The increase of near surface nutrient toward the end of both years (Figure 4.8) can be separated into two phases. In the first phase, nutrients increase was steep, because all un-used bottom water nutrients were mixed into the upper water column after stratification collapsed and wind picked up. In the second phase, a slower increase was apparent reflecting re-mineralisation from sediment.

Stations West Gabbard and Liverpool Bay

Nutrient levels in coastal areas can be majorly affected by high nutrient concentrations in river and effluent waters (see Boelens *et al.*, 1999; Gowen *et al.*, 2000; Sanders *et al.*, 2001; Mills *et al.* 2003) and are therefore generally higher than nutrient levels in offshore waters.

Average winter nutrient levels for the Outer Thames were reported to be 12 μM nitrate, > 6.0 μM silicate and 0.7 μM phosphate by Sander *et al.* (2001) who also reported that the Outer Thames experienced enhanced and continuous nutrient loads. Concentrations for TOxN were 11.12 μM for 2008 and 12.14 μM for 2009, respectively and SiO_2 concentrations were 5.82 μM for both years indicating that

the average winter levels for nitrogen and silicate in 2008 and 2009 were similar to those measured in 1996 and 1997. The nutrient input through freshwater (the river Thames) was demonstrated by a significant negative relationship of salinity and nutrients ($p < 0.01$) for winter 2009 (Figure 4.30). These findings were consistent with work by Sanders *et al.* (2001), who found a conservative winter relationship of salinity and TOxN in the Outer Thames plume.

Winter nutrient levels for Liverpool Bay were reported to be 30.3 μM DIN, 13.6 μM Si and 1.6 μM DIP by Gowen *et al.* (2002). Gowen & Stewart (2005) reported values of $> 20\mu$M for DIN, > 12 μM for Si and > 1.5 μM for DIP for January 2000. Mills *et al.* (2005) recorded maximum winter concentrations of TOxN in 2003 of 45 μ M for Liverpool Bay. Compared to those data, average late winter measurements (March/early April) of 18.16 μM TOxN, 8.56 μM Si and 0.93 μM P were recorded for the sampling period (2008 and 2009), suggesting that all three nutrients showed lower values than in former years. A significant salinity nutrient relationship ($p<0.01$) for TOxN and silicate was evident at station LBay in Liverpool Bay in 2008 and 2009 (Figure 4.48), confirming the influence of freshwater. These findings were consistent with others (Gowen *et al.*, 2000; Mills *et al.*, 2005). The elevated levels of nitrate and silicate in Liverpool Bay are suggested to be the result of anthropogenic enrichment (Gowen *et al.*, 2000; Mills *et al.*, 2003; Gowen & Stewart 2005; Gowen *et al.*, 2008).

Nutrient uptake

Plotting nitrogen and phosphate concentration ratios (TOxN:$PO_4{}^{-3}$) and nitrogen and silicate concentrations ratios (TOxN:SiO_2) against time, provides a static picture of the dissolved nutrients in the water column and their relative accumulation ratios throughout the year. The regressions of the same ratios for the spring period provide a dynamic picture about the rate microplankton assimilates these nutrients (e.g. Gowen *et al.*, 2000).

Redfield (1958) reported that the C:N:P atomic ratio of particulate matter in seawater is 106:16:1 and that these nutrients appear to be depleted in a similar proportion during microplankton growth. For autotrophs, carbon is rarely limiting (Parsons & Takahashi, 1973; Schindler, 1977) and thus only the ratio of N: P is of concern. According to literature (Redfield *et al.*, 1963) the molar proportions of total oxidised nitrogen to silicate are 1:1 (N:Si) in marine systems. If the N:P ratio is less than 16, N is considered to be limiting, if the ratio is larger, P is limiting. The same is true for N: Si in a ratio of 1:1. Menzel *et al.* (1963) found a wide variation for N:P ratios of 5.4 - 17 in the open sea. And Rhee (1982) supported this statement pointing out that it is rare to find an N:P ratio of 16:1 in marine systems, except in upwelling areas, where nutrient-rich deep water mixes with surface water. Ryther & Duunstan

(1971) recorded that when the utilization of N and P was closely examined in surface waters, N was found to be depleted first with a significant amount of P left. Other authors agreed (Tett & Droop, 1988) that nitrogen is assumed to be the nutrient most likely to limit microplankton growth in marine systems and phosphorus may be important in some low salinity environments (Tett *et al.*, 2003a; Blomqvist *et al.*, 2004). Gowen & Bloomfield (1996) suggested that in seasonal stratified waters in the western Irish Sea nutrient is the limiting factor of microplankton production during summer and nitrogen is most likely to be the limiting nutrient which is supported by Beardall *et al.*, 1978; Gibson *et al.* (1997); Gowen & Stewart (2005).

Station 38A

At station 38A, the temporal pattern of the TOxN:SiO_2 ratio (N:Si) between 0.16 (July 2008) and 1.21 (May 2009) and the TOxN:$PO_4{}^{3-}$ ratio (N:P) between 0.42 (July 2008) and 11.92 (April 2009), respectively (Figure 4.10) suggested that the western Irish Sea site was not enriched in nitrogen relative to silicate and phosphate. In May 2009, the N:Si ratio was highest 1.21 indicating that more TOxN than silicate was available and suggesting that the draw down of silicate was higher in the spring period. Looking at the linear regression plot of TOxN:SiO_2 during spring (Figure 4.11) provided a draw down ratio of 0.9 with an intercept not significantly different from 0 ($p > 0.05$). Comparing this value with the Redfield ratio of 1:1 indicated that the uptake ratio in the western Irish Sea followed Redfield's prediction and both nutrients were taken up to a similar proportion during the growth period. However, some species appear to have a threshold concentration of nutrients below which no growth can take place (Rhee, 1982). Escaravage & Prins (2002) suggested that SiO_2 becomes limited when it reaches concentrations of 2 μM. Therefore, it can be assumed that silicate was the limiting factor during spring rather than nitrogen. The linear regression of TOxN:$PO_4{}^{3-}$ during the spring period delivered an N:P ratio of 13.2 with an intercept significantly different from 0 ($p < 0.05$), indicating that nitrogen assimilation by microplankton was lower than expected (Redfield ratio 16:1) and showing a clear limitation of N. The findings are consistent with published work in this area (Gibson *et al.* 1997, Gowen *et al.* 2008).

Gowen & Bloomfield (1996) suggested that in the seasonal stratified regions of the Irish Sea nutrients may limit the summer growth of phytoplankton. Concentrations of TOxN near detection limit (0.01μM on 27th July in 2008 and 0.1μM on 2nd August 2009) confirmed that. The overall phytoplankton production however, was suggested to be controlled by subsurface light climate as a function of global radiation and the depth of the surface mixed layer rather than nutrients (Gowen *et al.* 1995). This was also true for station 38A in both sampling years as microplankton biomass (Figure 4.17) and cell abundance (Figure 4.16) decreased when stratification collapsed in September (Figures 4.3 and 4.7) and nutrients were available in

elevated concentrations (Figure 4.8).

Stations West Gabbard and Liverpool Bay

Nitrate concentrations for the summer period in 1996 and 1997 presented by Sanders *et al.* (2001) in the Outer Thames plume were close to detection limit (0.1 μM) and low concentrations of silicate (approximately 1-2 μM) indicated that phytoplankton production in summer in this area could be nutrient controlled. In 2008 and 2009, nitrate minimum concentrations (0.3 μM) were observed early in the year (day 100; 9th April) while silicate levels were still elevated (4.5 μM) during that time. Silicate minimum concentrations (0.4 μM) were observed in early summer (day 170; 18th June) while TOxN levels were elevated (4.2 μM). For the southern North Sea, Hydes *et al.* (1999) reported an average late winter (March) N:P ratio of 10 with a corresponding nitrate concentration of 12 μM. Sanders *et al.* (2001) reported the production in the Outer Thames estuary to be nitrogen and possibly silicate limited during summer. The linear regression for the West Gabbard station during spring was not statistically significant (analysis of variance, $p < 0.05$) (Figure 4.29) and the draw down ratio by microplankton was minimal (0.35). However, data in May were missing and prevented a full investigation of the spring period. When data from June and July were added, the draw down ratio was more apparent (1.09) and the regression was statistically significant (analysis of variance $p < 0.05$) with the intercept significantly different from 0 ($p < 0.05$). Looking at the temporal pattern (Figure 4.28) confirmed the indication of the draw down plot, suggesting silicate limitation during summer. Judging by the data available for the summer period in 2008 from day 161 (10th June) to 199 (18th July) microplankton growth could have been limited by silicate as the average concentration level for this nutrient was below 0.5 μM whereas TOxN average concentrations for this period were 3.78 μM. This confirmed the uptake ratio and is consists with the suggestion by Sanders *et al.* (2001) that the Outer Thames estuary is possibly silicate limited and that nutrients therefore controlled microplankton growth during the growing season in 2008 and 2009.

Short term studies have shown that eastern Irish Sea waters in Liverpool Bay are enriched with nitrogen and phosphate (Jones & Folkard, 1971; Beardall *et al.*, 1978; Foster, 1984; Gillooly *et al.*, 1992; Gowen *et al.* 1995, and Gowen *et al.* 2002) and microplankton growth is therefore likely to be limited by light (Mills *et al.* 2005, Gowen *et al.* 2000). High frequency near surface phosphate data were not available for this study in 2008 and 2009 and also nitrogen data were inconsistent. However, looking at the temporal pattern of the TOxN:silicate from February to May in 2008 and in June 2009, the plot could indicate that nitrogen was not a limiting factor to microplankton growth. The linear regression (Figure 4.47) that showed the nutrient assimilation by microplankton during spring (March - May) was not statistically

significant and the TOxN:SiO_2 uptake ratio was 1.15. When data from the early summer period (June, marked red) were included in the analysis, the regression was statistically significant (analysis of variance, $p < 0.05$) with a draw down ratio of 1.43 and an intercept significantly different from 0 ($p < 0.05$). The ratios both suggested that the growing period was limited by silicate. Compared to a N:Si ratio of 4.8 found by Gowen *et al.* (2000) this value seemed low. However, the ratio of 1.43 was probably more comparable to the mean N:Si ratio of 0.89 for marine diatoms given by Brzezinski (1985) considering that nitrogen values in 2008 and 2009 were lower and silicate values were higher than the values recorded for 1996 and 1997 by Gowen *et al.* (2000). An N:P ratio could not be estimated during this study as data were not sufficient, however Gowen *et al.* (2000) recorded a molar N:P ratio of 17.0 μM in 1997 indicating that nitrogen was not a limiting factor for microplankton growth.

Chlorophyll and microplankton carbon biomass

Station 38A

Trimmer *et al.* (1999) reported that the initiation of the spring bloom in the western Irish Sea coincided with the onset of thermal stratification at the end of April 1998 with highest chlorophyll levels (6.48 mg chl m^{-3}) and chlorophyll standing stock (145.0 mg chl m^{-2}) recorded for 11th May with the bloom lasting for one month. Considering Gowen *et al.* (1995) who stated that the start of the production season is when chlorophyll concentrations exceeded 1 mg m^{-3}, spring bloom at station 38A started by the end of April 2008 and by mid April 2009 (Figure 4.13), shortly after a rapid draw down of nutrients was recorded as stratification developed. The start of the spring bloom and the duration of the production period was generally similar to the chlorophyll pattern observed. For example, a peak in biomass (97 mg C m^{-3}) on day 127 (6th May) 2008 related to the highest chlorophyll standing stock 174.2 mg m^{-2} on the same day. In 2009, high microplankton biomass (120 mg C m^{-3}) related to the maximum chlorophyll standing stock (212 mg m^{-2}) on day 129 (9th May). That production followed a similar seasonal trend to chlorophyll was expected, given the relationship of chlorophyll and microplankton biomass. However, sometimes it was not possible to compare values for chlorophyll concentrations and biomass simultaneously for depth and time as the microplankton sampler was fixed to a notional depth of approx. 14 m and the vertical chlorophyll profiles were not synchronised to the sampler. Therefore, continuous comparisons were not possible and discrepancies between data were not avoidable. In 2009, an early peak in cell abundance (1077.34 x10^3 cells L^{-1}) and biomass (47.02 mg C m^{-3}) of mainly small *Chaetoceros* species on day 101 (11th April) coincided with a prior decrease in TOxN (7.1 μM to 5.5 μM) and thermal stratification (Δ t = 0.8). An expected decrease in

silicate, however, was not detected. Gowen and Bloomfield (1996) recorded elevated chlorophyll standing stock by the end of May (> 64 mg m^{-2}) with single values of up to 16 mg m^{-3} and a deep chlorophyll maximum reached in July. Gowen *et al.* (2008) reported chlorophyll levels from > 0.1 to 16.4 mg chl m^{-3} with a summer mean of 2.2 mg chl m^{-3}. Observations for this study gave highest chlorophyll standing stock in May in both sampling years (174.2 mg chl m^{-2} and 212.5 mg chl m^{-2}, respectively) with single maximum values of 10.01 mg chl m^{-3} and 4.23 mg chl m^{-3} Chl in early May 2008 and 2009, respectively. A deep chlorophyll maximum (10.01 mg m^{-3}) in 2008 was apparent at a depth of 24 m in early May (Figure 4.15). In 2009, such pattern was not obvious (Figure 4.15) and the chlorophyll distribution was spatially homogeneous extending from the surface (4.17 mg chl m^{-3}) to a depth of 30 meters (3.57 mg chl m^{-3}). Comparing the contour plots for temperature and salinity of both years showed that the thermocline and halocline in 2009 were apparent in deeper waters (approx. 40m) than in 2008 (approx. 30m) (Figure 4.2) which could explain the more even distribution in 2009. However, it could also be possible that a deep chlorophyll maximum in the spring 2009 was missed due to low frequency vertical sampling (n = 18). The high chlorophyll levels in early summer at a depth of approx. 50 m did not reflect growth but were believed to be microplankton sinking as the euphotic zone depth was estimated to be 28 - 30 m and it was assumed that light beyond such depth was not sufficient for growth. The concentrated chlorophyll (approximately 4 mg chl m^{-3}) in June (day 161; 10th June) 2009, at a depth of 19 m, led to the assumption that an early summer peak was present around that time (Figure 4.14). Plotting the vertical chlorophyll profile for the two measurements in June (Figure 4.60) resulted in evenly distributed low chlorophyll concentrations (0.82 - 0.66 mg m^{-3}) on day 158 (7th June) and a sub-surface chlorophyll maximum (3.77 mg m^{-3}) at 19 m on day 161 (10th June).

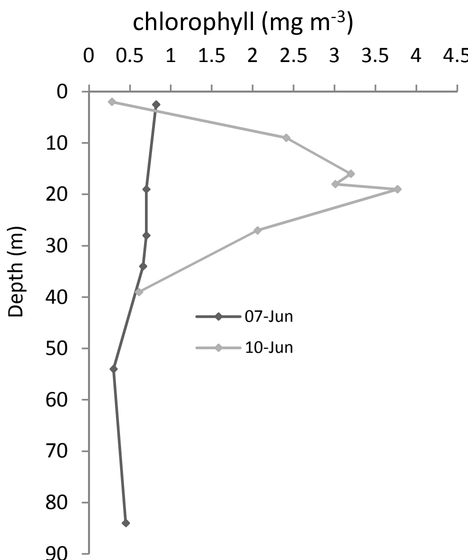


Figure 4.60: A vertical chlorophyll profile for two days in June 2009 at station 38A.

The microplankton carbon biomass for this period showed a similar pattern (39.8 mg m^{-2}) for the beginning of June (day 156) and high (136.4 mg m^{-2}) for mid June (day 161). A related decrease in nutrients was apparent a few days before (day 154; 3rd June) confirming the assumption of an early summer peak and underlining the highly inherent variability and patchiness of microplankton (Tett *et al.*, 2007). The depletion of nutrients in the upper approx. 40 m (Figure 4.15) in May and July 2009 and the correlated low microplankton cell abundance and carbon biomass (Figures 4.16 and 4.17) indicated that growth was nutrient limited during the summer. This was consistent with work by Gowen & Stewart (2005). In 2008, a pronounced autumn peak in chlorophyll was apparent that was also obvious in 2009, but to a lesser extend (Figure 4.14). This was reflected in the microplankton carbon biomass in both years (Figure 4.17), not however, in cell abundance (Figure 4.16). A pronounced autumn bloom may or may not develop in this region and depends on the strength of wind mixing. The wind often initiates re-mineralisation supply processes due to water column mixing and 'new' nutrients are released from the bottom water into the euphotic zone exceeding the nutrient uptake of the ambient microplankton population. The availability of the released nutrients often triggers an autumn bloom. In coastal areas, this bloom is usually dominated by diatoms as sufficient silica is normally present. This was observed during the sampling years. It seemed that in 2009, wind mixing was apparent, but not strong enough to create favourable conditions to trigger a pronounced autumn bloom. The decline of the autumn growth is usually caused by light limitation (Mills *et al.*, 2003; Tett & Mills, 1991) consequently stratification collapses which leads to mixing of the whole water column.

Biomass was not significantly different in both years, but cell abundance was significantly higher in 2009 than in 2008 (Mann-Whitney $p = 0.05$). The difference could be down to the inter annual variability of species and to some extend my improvement in counting small sized microplankton in the second year had to be included (error estimation for small sized microplankton was 16%).

Stations West Gabbard and Liverpool Bay

The development of a seasonal stratification was not apparent at any stage at LBay and stratification was absent at station WGabb. However, judging by the rapidly decreasing nutrients and by the light availability prior to the spring bloom, conditions were suitable for elevated biomass by mid/end of April 2008 and early April 2009. Sanders *et al.* (2001) observed generally low chlorophyll concentrations in the southern North Sea reaching concentrations of 6 mg m^{-3} during spring (May 1996). The rapid fluctuations in chlorophyll concentrations as observed by Sanders *et al.* (2001) that can occur at sites in the southern North Sea cannot be strictly interpreted to reflect seasonal variations in chlorophyll concentrations (Mills *et al.*,

1994). The near surface chlorophyll data for 2008 showed elevated (1.22 mg m^{-3}) and maximum (19.95 mg m^{-3}) chlorophyll concentrations between day 94 (3rd April) and 143 (22nd May) (Figure 4.32) relating to the highest microplankton biomass (108.85 mg C m^{-3}) on day 114 (23rd April) and reflecting the spring bloom. After day 145 (24th May) chlorophyll concentration were minimal (0.44 mg m^{-3}) and increased to a summer concentrations of 4.80 mg m^{-3} (day 184; 2nd July) without an obvious autumn bloom. The microplankton carbon biomass was related to the chlorophyll pattern, also showing no evidence of a microplankton bloom toward the end of the summer (42.42 mg C m^{-3}) (Figure 4.36). Nutrient data were incomplete, but low chlorophyll concentrations (0.4 mg m^{-3}) in summer 2008 and microplankton biomass (10.9 mg C m^{-3}) in summer 2009 indicated that microplankton growth was limited during those periods. Tett (1993) proposed that the primary control of phytoplankton growth in the southern North Sea was through light availability and that anthropogenic nutrients would only influence biomass levels when light was not limiting. McQuatters-Gollop (2007) argued that the level of phytoplankton biomass in the North Sea found today is more closely related to climatic variability via sea surface temperature and water transparency than nutrient concentrations. The latter cannot be resolved in this study, but the argument by Tett *et al.* (1993) seemed partly confirmed by the observations made in 2008 and 2009. The observations in nutrient draw down (Figure 4.29) indicated that summer growth was limited by silicate which was also found by Mills *et al.* (2005). The wave-like pattern observed in the near surface chlorophyll during summer could reflect the tidal influence of the area. This was underlined by the rapid fluctuation observed in fluorescence (Figure 4.33) and the randomly distributed microplankton (Figures 4.36 and 4.35). By interpreting these data it has to be taken into consideration whether the sampling frequency was adequate to resolve the seasonal variability known to occur in coastal shelf environments such as the southern North Sea. Even the microplankton data in 2009 (analysed every fortnight) showed high variability in microplankton biomass and cell abundance. The vertical temperature and salinity profiles (Figure 4.24) showed complete mixing down the water column for all four season. The Δt+s data that indicated no obvious stratification pattern (see Figure 4.26) and the mixing characteristics indicated that station WGabb was located in an area of high turbulence presumably causing mixing of microplankton and thus its high variability in carbon biomass and cell abundance.

Similar to the observation in the West Gabbard were the observations for the mooring site in Liverpool Bay. However, a rapid draw down of TOxN and silicate by the end of April was observed prior to rapid growth in spring. From the microplankton data it was difficult to determine when the spring bloom actually started as only monthly samples were provided. However, decreasing nutrients (Figure 4.44) and increasing chlorophyll (Figure 4.50) suggested that spring bloom started around day

133 (13 th May) in 2008, a similar time was assumed for 2009. Highly enriched with nutrients, the concentrations in Liverpool Bay have been shown to support enhanced microplankton production and chlorophyll standing stock (Allen *et al.*, 1998; Mills *et al.*, 2005; Gowen *et al.*, 2008). Maximum chlorophyll levels for Liverpool Bay were reported to be up to 43.9 mg chl m^{-3} during spring (Gowen *et al.* 2008) and with a summer mean of 8.8 mg chl m^{-3} in 1996 and 1997 (Gowen *et al.* 2000) as a consequence of nutrient enrichment (Gowen *et al.* 2008). Such high values were not observed in 2008 or 2009. Highest chlorophyll levels were 7.81 mg C m^{-3} observed in May 2009. Chlorophyll standing stock was found to be 141.10 mg chl m^{-2} in 2008 and 211.51 mg chl m^{-2} in 2009, respectively. The microplankton carbon biomass values were in comparison 109.64 mg C m^{-3} and 276.85 mg C m^{-3} for 2008 and 2009, respectively and related well to the chlorophyll data. Generally, these data seemed low compared to the findings by Gowen *et al.* (2000, 2008). However, average winter nutrient concentrations for the sampling period (TOxN 18.2 μM, Si 8.6 μM, phosphate 0.9 μM) were also lower than in previous years (DIN 29.2 μM, Si $>$12 μM, DIP 1.6 μM) (Gowen & Stewart 2005). Further, it is possible that the highest levels of nutrients and microplankton chlorophyll were missed due to low frequency sampling. Whether the nutrients and microplankton carbon biomass and therefore chlorophyll levels were indeed lower in 2008 and 2009 and thus indicated a decrease of nutrient enrichment can not be reported with confidence as there were too little data to support this. A big part of the samples collected were impossible to analyse due to too much sediment in the sample, consequently resulting in low sampling data. The low numbers and therefore infrequency of microplantkon samples also made it difficult to state whether growth and production was at any stage limited by nutrients. The depleted nutrients (TOxN: 0.04 μM, SiO_2: 0.24 μM, PO_4^{3-}: 0.06 μM) in May 2009 (Figure 4.45) could indicate nutrient limitation.

4.6.2 Microplankton composition

It is generally accepted that the variation in the supply of external energy in the form of light, turbulence and nutrients are the main factors controlling the seasonal composition and succession of microplankton in the sea (Margalef, 1978; Smayda, 1980; Reynolds, 1996; Peperzak *et al.*, 1998; Escaravage *et al.*, 1999; Smayda & Reynolds, 2001; Tett *et al.*, 2008). According to Smayda, (1980) evidence has grown that species successions are of major significance to microplankton dynamics and in coupling the microplankton community to higher trophic levels. In 1978, Margalef proposed a model (Margalef's Mandala) to describe marine microplankton composition. This conceptual model combines the interactive effects of mixing and nutrient regimes on selection of phylogenetic morphotypes and their seasonal succession occurring along a template of r versus K growth strategies. Tilman *et*

al. (1982) proposed three broad factors influencing microplankton succession: 1. physics: utilisation of differences in the capacity of species or lifeforms to grow in physical environments that differ especially in their vertical mixing intensity; 2. nutrient ratios: the relationship between the ratio of nutrient elements needed for growth and the ambient ratio of these elements; 3. grazing: variable loss rate due to grazing by protozoans or zooplankton that preferentially take some species or lifeforms rather than others. Officer & Ryther (1980) stated that nutrients play an important role in microplankton succession because re-mineralisation returns N, P and Si to solution at different rates and thus processes separate these nutrients. Silicate is slowest therefore it is supposed to be most limiting.

In the seasonally stratified (Gowen & Bloomfield, 1996) western Irish Sea, Beardall *et al.* (1978) found that diatoms dominated the spring bloom in 1977 while micro-flagellates were apparent in high numbers. In 2008 and 2009 a similar pattern was observed with diatom dominating the spring carbon biomass and micro-flagellates dominating the cell abundance. McKinney *et al.* (1997) undertook a detailed study of diatom abundance for station 38A in 1995 (from April to August) and identified a total of 39 diatom species. The microplankton analysis during this study in 2008 and 2009 resulted in 53 identified diatom species. McKinney *et al.* (1997) found *Skeletonema costatum* to be the most abundant species in the spring bloom while species of *Chaetoceros*, *Pseudonitzschia*, and *Thalassiosira* formed an important part of the composition. *Skeletonema costatum* was not a dominant species in 2008 or 2009 and only occurred in low numbers. But *Chaetoceros* species and the diatom *Thalassiosira rotula* dominated the spring bloom in 2008. Species of the genus *Pseudonitzschia* were also present in high numbers in 2008 and 2009. *Guinardia delicatula* and *Guinardia striata* were consistent species in both years and *Guinardia delicatula* dominated the spring bloom in 2009 (see Table 4.1). The dominance of *Guinardia delicatula* was consistent with findings by Gowen *et al.* (2000) for spring 1997. Gowen *et al.* (in press) submitted a table of diatom variability during spring bloom over the last decades and observed that there has been considerable inter-annual variability in bloom composition and that although diatoms were dominating the spring bloom most years, micro-flagellates represented an important component or even dominated the bloom. Gowen *et al.* (2000) observed *Phaeocystis* in the western Irish Sea and Gowen & Stewart (2005) recorded micro-flagellates dominating the spring bloom in 2001. In this study micro-flagellates like *Phaeocystis* and nano-flagellates were the most abundant species in spring, summer and autumn but due to their small size never dominated the microplankton carbon biomass.

No detailed study has been undertaken on dinoflagellate abundance in the western Irish Sea. According to Gowen *et al.* (2008) there have been no further reports of *Karenia mikimotoi* blooms in the Irish Sea since those observed in 1971 by Helm *et al.* (1974) and in 1975 by Evans (1976) as cited in Ayres *et al.* (1982). Gowen

et al. (2008) stated that there was some evidence that the abundance of *Noctiluca scintillans* has increased in the 1990s compared to the 1980s. Edwards (2005) argued that the higher abundance of this large, heterotrophic dinoflagellate is due to climate change rather than anthropogenic nutrient enrichment directly. In the western Irish Sea *Noctilua scintillans* was observed during 2008 and 2009 in low numbers and on rare occasions. In Liverpool Bay it was observed in summer 2009 having a significant impact on the summer microplankton composition and being responsible for a shift from diatom to dinoflagellate dominance during the summer period. At the West Gabbard station *Noctilua scintillans* appearances were not observed during 2008 and 2009. In general, dinoflagellates were present in low numbers, this could be explained by the fact that dinoflagellates grow slowly (K-selected species) (Margalef 1978; Reynolds 1996) and are often out competed for nutrients by faster growing (r-selected) species like diatoms. Dinoflagellates at station 38A were most abundant and with highest biomass in summer and autumn. This was when stratification was most stable, the water column was well illuminated and depleted silicate limited the diatom growth. Large dinoflagellates can be successful by maintaining position near the pyconcline to alternately move to the eutrophic zone obtaining light and to the nitracline obtaining nutrients (Cullen, 1982). Large species of *Gymnodinium* and *Gyrodinium* as well as *Protoperidinium crassipes* and *Protoperidinium depressum* occurred in elevated numbers in summer and autumn of both years in the western Irish Sea. In winter, large heterotrophic dinoflagellates contributed the main part to the low dinoflagellate biomass (0.59 mg C m^{-3}) which was expected because light climate and stratification were not sufficient to support autotrophic growth. In spring, lowest heterotrophic dinoflagellates were recorded confirming that a sufficient light climate favoured autotrophic and mixotrophic dinoflagellates. Highest heterotrophic carbon biomass was recorded in summer and autumn coinciding with stable and strong stratification (maximum Δt = 6.1), sufficient light and food supply. Mixotrophic dinoflagellates were probably underestimated because I only identified them as such when they reflected the dedicated light under epi-fluorescence microscopy, not however, by their identification in the literature. This might have resulted in wrong estimates for mixotrophic dinoflagellates and it might be worth re-evaluating the results considering the library established and literature on this topic.

The micro-flagellate succession resulted in the dominance of autotrophs and an increase of heterotrophic micro-flagellates in winter. One reason for that could be that the autotrophs out competed the heterotrophs in summer, when nutrients and light availability was high. Another reason could be that the light climate in the western Irish Sea favoured the autotrophic micro-flagellates more than the heterotrophic ones. Not so much is known about heterotrophic micro-flagellates and their diet. One suggestion is that the food supply mainly composed of diatoms is too large for

micro-flagellates to feed on. In this study it was not possible to analyse the small fraction of the microplankton or bacteria at the study sites, so the nutritional mode of micro-flagellates could not be differentiated in detail. The increase of heterotrophs in winter could simply be the result of a decrease in autotrophs due to insufficient light.

In the intermittently stratified (Gowen & Bloomfield, 1996) eastern Irish Sea, Beardall *et al.* (1978) found that in 1977 the spring bloom was dominated by large diatoms like *Navicula* while a *Phaeocystis* bloom was also recorded. *Navicula* species were frequently present in samples of 2008 and 2009, but never dominated the carbon biomass of the microplankton. Large diatom species like *Eucampia zoodiacus*, *Guinardia flaccida* and *Rhizosolenia styliformis* were prominent during spring bloom in Liverpool Bay in 2008 and 2009. *Phaeocystis* blooms are argued to be an indication of nutrient enrichment (Riegman *et al.*, 1992; Cadée & Hegeman, 2002; Lancelot *et al.*, 2006) in coastal waters and are therefore widely regarded as nuisance algae. However, they have also been reported to be a component of the spring bloom in unenriched coastal waters (Wood *et al.*, 1973) and some other authors argue that *Phaeocystis* blooms occur naturally or are linked to climate change or pollution in general (Gieskes *et al.*, 2007; Tett *et al.*, 2007; Gowen *et al.*, 2008). The genus has been present in the eastern Irish Sea since the 1950s (Williamson, 1956; Jones & Haq, 1963) and is still present in most years (Foster *et al.*, 1982; Kennington *et al.*, 1998; Gowen *et al.* 2000). Some authors have hypothesized that silicate concentrations and supply rates are the controlling factor of the phytoplankton composition (Officer & Ryther, 1980; Egge & Aksnes, 1992), others suggest that light levels control the species composition (Peperzak *et al.*, 1998). Peperzak *et al.* (1998) specifically suggest that *Phaeocystis* sp. has a higher light threshold for colony formation to occur than diatoms have.

At the WGabb station the phytoplankton composition was dominated by diatoms consistently from winter 2008 through to autumn 2009 with one exception. In spring 2008 (day 121; 1st May), the carbon biomass of monad flagellates was higher (27.5 mg C m^{-3}) than the biomass of diatoms 24.8 mg C m^{-3}. The flagellate peak was also the only occasion when small sized organisms dominated over large sized organisms. Minimum TOxN concentration (0.3 μM) for 2008 was recorded on day 100 (9th April) while silicate was still high (4.5 μM). High abundance or carbon biomass of non-silicate users was not apparent during this time which could have explain the strong draw down of nitrogen observed. However, the event could have been missed as high temporal resolution sampling was not performed. Samples analysed close to the TOxN draw down in April did not present high carbon biomass or cell abundance in micro-flagellates or any other non-silicate users. The stage that was reached during summer 1996 (Sanders *et al.* 2001) with high light and low turbidity levels to allow non-silicate user growth and dominance was not present during 2008 and 2009 at

the mooring site West Gabbard. Even during the late autumn and winter months, pennate diatoms like species of *Fragilaria* and the species *Paralia sulcata* dominated the microplankton composition. The observations lead to the hypothesis that West Gabbard could be influenced by the low light and high turbidity regime dominant in the inner Thames plume (as observed by Sanders *et al.* 2001) which would explain the diatom dominant microplankton composition. Although the N:Si ratio uptake was 1.79 and indicated a Si limitation, diatoms were found all year round. Large macro tidal and well mixed estuarine waters like the Thames embayment were identified at little risk from eutrophic conditions due to light limiting conditions of the water type (Devlin *et al.*, 2008). Inshore and near-shore environments may severely be restricted to light availability due to high levels of suspended particulate materials (Mills *et al.* 2005). Shallow estuaries have high sediment surface area to water volume ratios, frequent water re-suspension of sediments and low pelagic and high benthic primary productivity because most of the sediment surface is in the photic zone (Sand-Jensen & Borum, 1991). This suggests that sediment re-suspension and not increased pelagic productivity could be the controlling factor on light limitation in UK coastal waters (Bowers, 2005) and this could also explain why the study site in the West Gabbard showed low biomass of diatoms during summer in 2008 (95 mg C m^{-3}) and the depletion of silicate (0.04 μM) while TOxN levels were elevated (4.2 μM). The Si-limitation hypothesis by Officer and Ryther (1980) that re-mineralisation rates of Si are slower than the ones for N and P could also explain the results. Benthic silicate mineralisation (Del Amo *et al.*, 1997b) along with the tidally mixed conditions could explain part of the diatom abundance throughout the year.

In 1996 and 1997 Gowen *et al.* (2000) reported blooms of micro-flagellates (mainly *Phaeocystis* spp.) in Liverpool Bay prior to the spring bloom that was dominated by *Guinardia delicatula*. In terms of carbon biomass diatoms dominated both spring blooms (2008 and 2009) with species of *Rhizosolenia*, *Guinardia*, *Thalassiosira*, *Chaetoceros* and *Skeletonema*. In spring 2008, Gowen's findings (2000) could be confirmed with *Phaeocystis* showing the highest cell abundance of all present microplankton. In 2009 however, no micro-flagellate peak was apparent. Frequently missing data for micro-flagellates suggested that the analysts in charge did not include micro-flagellates in routine sample analysis. The argument that the appearance of *Phaeocystis* sp. in coastal eastern Irish Sea waters is due to enrichment is weakened by the occurrence of these algae during the spring bloom in offshore Irish Sea waters (Gowen *et al.* 2008) and unenriched Atlantic waters (Gieskes *et al.* 2007). Gowen *et al.* (1999, 2000) concluded that although anthropogenic nutrient enrichment in Liverpool Bay had caused elevated production and biomass, there was no obvious increase in non-silicate users and hence no evidence of a shift in microplankton community structure, here from the dominant diatoms to another

lifeform. This was also found during 2008 and 2009 although the dinoflagellate *Noctiluca scintillans* had a high impact to the summer growth in 2009 and resulted in a shift from diatoms during that period. Though in low numbers (100 cells L^{-1}), the biovolume of *Noctiluca scintillans* is large and it therefore elevated the biomass in that particular period. However, it has to be questioned whether the low sample frequency (n = 5) can give a representative resolution of the seasonal variation in microplankton during 2009. Further, the succession from diatoms to dinoflagellates resulted from the high impact in carbon biomass by 100 cells of *Noctiluca scintillans* in one sample. In general, dinoflagellates never reached high numbers. Dinoflagellates that were found frequently, though in low numbers, were mainly species belonging to *Dinophysis*, *Ceratium*, and *Prorocentrum*. The findings of the main microplankton species was consistent with findings by Mills *et al.* (2005). The diatom dominance throughout the spring and summer with little evidence of succession to dinoflagellates or flagellates in 2008 is consistent with events reported by Gowen *et al.* (2000) (see Figure 4.54). Tett (2003b) suggested that the N:Si ratio must at least exceed 2:1 and even 4:1 for micro-flagellates to become dominant in the sea. The N:Si ratio in 2008 was 1.88 for station LBay and 1.79 for the WGabb station and argue against a shift to micro-flagellate or dinoflagellate dominance which is consistent with findings by Gowen *et al.* (2008).

4.6.3 Microplankton Succession

Margalef (1963, 1967) identified four stages of succession in the Mediterranean Sea. Within the four stages of succession Margalef typically found the following organisms dominating the community: 1. small, colony forming flagellates and diatoms like *Skeletonema* and *Chaetoceros*; 2. medium to large sized chains of diatoms and small to medium sized dinoflagellates like *Ceratium* and *Prorocentrum*; 3. large, cylindrical cells like *Rhizosolenia* and an increasing dinoflagellate population; 4. large motile dinoflagellates dominating the biomass and micro-flagellates representing the highest abundance.

Station 38A

At station 38A in the western Irish Sea three of these four stages of succession could be identified starting with elevated abundance of small *Chaetoceros* before the maximum peaks of medium to large diatoms such as *Guinardia delicatula* and *Thalassiosia rotula* in spring. A recognisable shift from diatoms to small and medium dinoflagellates (e.g. *Ceratium* spp., *Protoperidinium bipes*, *Gymnodinium* species) was observed from spring to summer and underlined a shift from silicate users to non-silicate users. Toward autumn large, cylindrical cells of diatom species such as *Rhizosolenia imbricata/styliformis* and *Probosia alata* were apparent in high abun-

dances along with mainly heterotrophic dinoflagellates in 2008. In autumn 2009, microplankton composition was made up of large cylindrical diatoms as observed in 2008 but dinoflagellates were mainly recorded to be autotrophic. In 2009, micro-flagellates contributed over one third to the autumn composition. Although large motile dinoflagellates such as *Protoperidinium divergens* and large sized *Gymnodinium* species were present during late summer, and autumn, they never dominated the microplankton biomass. There was little evidence that the last stage of Margalef's observed succession was reached at the station in the western Irish Sea. It is assumed that decreasing light and mixing of the water column due to decreasing temperature and increasing wind were the reasons that prevented this last stage. According to Kilham & Kilham (1980) the fourth stage of Margalef's succession is never reached in coastal and estuarine temperate waters. Micro-flagellates at station 38A were apparent in high cell abundance year round confirming the suggestion of Kilham & Kilham (1980) that flagellates are numerically always important, but only recognised when the total phytoplankton population decreases.

Station West Gabbard and Liverpool Bay

At station LBay in the eastern Irish Sea and station WGabb in the southern North Sea two of the four stages of succession could be identified. Small diatoms such as *Skelentonema* (in the case of Liverpool Bay) and colonial flagellates such as *Phaeocystis globosa* (in the case of the southern North Sea) were recorded prior the spring bloom in 2008 and 2009 dominated by large diatoms such as *Guinardia* spp., *Dithylum brightwellii* and *Rhizosolenia styliformis.* While chain forming diatoms such as *Pseudoguinardia recta* and *Eucampia zoodiacus* and large solitary centric and pennate diatom species such as *Coscinodiscus* and *Navicula* were prominent during summer and autumn small to medium sized dinoflagellates (e.g. *Prorocentrum micans*, *Protoperidinium steinii*, *Ceratium* spp., *Gymnodinium* spp., *Dinophysis* spp.) were also recorded. However, an increased dinoflagellate population was never apparent and large motile dinoflagellates were absent from both sampling sites. One exception was found in summer 2009 at station LBay when 100 cells of *Noctilua scintillans* were identified and influenced the microplankton biomass.

Pingree *et al.* (1978) explained microplankton distribution by the strength of stratification (Δt), suggesting that:

- Δ t $< 3°C$ = mixture of diatoms and dinoflagellates,
- Δ t 3 -6°C = dinoflagellates,
- Δ t $> 6°C$ = micro-flagellates

This observation could also be partly applied to the microplankton during this study. At station 38A a mixture of diatoms and dinoflagellates was observed while Δ t was between 0.5°C and 3°C with dominance of diatoms. When stratification grew

stronger and Δ t was $> 3°$ C a shift from diatoms to dinoflagellates was observed with both lifeforms contributing equally to the biomass (in summer 08 dinoflagellates dominated over diatoms, although thermal stratification was stronger in summer 09). Micro-flagellates were always present in high numbers while they dominated over dinoflagellates and contributed the same amount to the total biomass as diatoms in summer 09 when Δ t increased over 6°C over a 10 day period.

At the intermittently stratified mooring sites (LBay and WGabb) where stratification never grew strong, a mixture of diatoms and dinoflagellates was observed with diatoms being the dominant lifeform throughout the year. One exception was recorded for summer 2009 in Liverpool Bay when dinoflagellates dominated the biomass due to *Noctiluca scintillans* occurrence, however there was no obvious relation between the dinoflagellate occurrence and an increased Δ t. Micro-flagellates had very little impact on the biomass at both stations except for spring 2008 when a micro-flagellate bloom of *Phaeocystis globosa* occurred in the West Gabbard. There was no evidence that this micro-flagellate bloom was related to an increased Δ t. It was not certain that this micro-flagellate bloom was exceptional as the responsible analysts for the Cefas microplankton samples did not account for micro-flagellates on a regular basis and recorded only very obvious blooms.

4.7 Conclusion

The central hypotheses proposed in the beginning of this chapter were confirmed.

With the results of this study, the observations by Hartnoll *et al.* (2002) and the recent work of Gowen & Stewart (2005) and Gowen *et al.* (2008) who reported the waters of the western Irish Sea to be little nutrient enriched, a near pristine condition could be concluded for station 38A in 2008 and 2009. Station 38A as the development site for the MCI was shown to be seasonally stratified and with little evidence of nutrient enrichment. Average winter concentrations of the two year sampling period were 6.5 μM TOxN, 7.36 μM SiO_2 and 0.71 μM $PO_4{}^{-3}$. A stratification period of approximately six to seven months allowed a microplankton growing season of four to five months indicating that TOxN was the limiting nutrient during summer and light was the limiting factor to microplankton growth in autumn. Si was assummedly the limiting factor of the spring bloom which was dominated by diatoms in respect of carbon biomass. The succession from diatoms in the spring to heterotrophic microplankton and autotrophic dinoflagellates associated with stronger stratification and a well illuminated euphotic zone and the occurrence of another, weaker (autumn) bloom dominated by diatoms is a classical pattern (Chang *et al.*, 2003) for off shore waters in temperate shelf seas. Three succession stages were identified following Margalef's stages of succession (1963, 1967).

Stations WGabb and LBay were both intermittently stratified and similar in their hydrodynamical characteristics. Nutrient concentrations at both sites were associated with more variability ('background noise') than at sampling site 38A due to the strong tidal mixing throughout the year and freshwater influence. The average winter nutrient concentrations at Liverpool Bay were nearly double the average concentrations of the West Gabbard in the Outer Thames estuary and confirmed the argument that this area is nutrient enriched (Jones & Folkard, 1971; Foster, 1984; Gillooly *et al.*, 1992, Gowen *et al.* 1995; Gowen *et al.* 2002). However, in 2008 and 2009 lower nutrient levels (TOxN 18.2 μM, Si 8.6 μM, phosphate 0.9 μM) were observed than in earlier years and minimum nutrients in 2008 and reduced growth in early summer could suggest nutrient limitation during this period. The microplankton growth at the WGabb station seemed limited by silicate for a short period in the summer and low values in carbon biomass and cell abundance could have been a result of low light conditions due to sediment mixing in the water column. A pronounced shift in the dominance of lifeforms (from diatoms to dinoflagellates or micro-flagellates) was not obvious at either station and two of Margalef's succession stages (1963, 1967) were identified with diatoms remaining the dominant lifeform. However, there was succession from small to large diatoms in spring.

Chapter 5

Indicators & Models

5.1 Introduction

This chapter is a discussion about indicators and models, what they are and how they are used in helping us understand marine ecosystem structure and functioning. Different indicators and models developed for the assessment of ecosystem health are discussed against the background of the need to detect and diagnose eutrophication and other human driven pressures in temperate shelf seas. In the second part, the theory and the idea behind the model developed during this study and the resulting microplankton community index are presented.

5.2 Indicators and Models

Monitoring the structure and function of marine ecosystems is needed to assess their status in the context of eutrophication and is of vital importance as it is indeed a legal requirement. Policy drivers need better quantitative information about ecosystem health and so it is a key challenge for scientists to develop indicators and models that are robust, integrated, sufficiently sensitive, comparable and with recognised scientific merit to help us gain insight into the complexity of marine ecosystems. The Oxford dictionary (1990) defines an indicator "as a measurable variable or characteristic that can be used to determine the degree of adherence to a standard or the level of quality achieved" (Oxford dictionary 1990). According to the ICES workshop on Evaluation of OSPAR comprehensive procedure in 2001 (ICES, 2001), an indicator should be:

- easy to understand by non-scientist users
- based on existing data to help set objectives

- measurable over the area where they may be used
- easily and accurately measured with a low error rate
- sensitive to a manageable anthropogenic activity
- tightly linked in space and time to that activity

To develop such indicators for the assessment of marine ecosystems, models can help understand and simplify the complexity of interactions between and amongst species. In this sense, models are defined as a simplified abstract view of the complex reality, representing processes in a logical way. Generally, models can perform two fundamentally different representational functions. They can be a representation of a selected part of the world and depending on the nature of the target, such models are either phenomena models or models of data. In the sense of theory, a model can interpret the laws and axioms of that theory. These two notions do not necessarily exclude each other as scientific models can be representations in both senses at the same time (Frigg and Hartmann, 2009). In practice, modellers seek functional groups, recognising for example taxa or trophic levels (Tett & Wilson, 2000). The question is how simple can models be while still reflecting and explaining parts of the dynamics of real ecosystems? Some scientists argue (e.g. Nihoul, 1998) that it is neither possible nor desirable to capture the whole complexity of marine ecosystems in a model. Models do not have to be highly complex and complicated to display and predict some of the dynamics of ecosystems. Tett & Wilson (2000) argued that models should be at least biogeochemical in the attempt to simulate key features of marine pelagic systems.

5.3 Multivariate Approach to assess Ecosystem Health

Compared to the chemical and physicochemical methods that have been developed to assess the environmental status of an ecosystem over a long period of time, the methodologies for the ecosystem approach are recent and may therefore give the impression not to be as reliable as the well established chemical and physicochemical procedures. Our understanding of the process of nutrient enrichment and its causative influence on eutrophication symptoms is an important component of any eutrophication assessment of marine waters. Changes in the conceptual understanding of eutrophication (Cloern, 1999; Costanza and Mageau, 1999; Tett *et al.*, 2007) suggest that the responses to anthropogenic nutrient enrichment (Nixon, 1995) are complex and can be direct and indirect. Over the last two decades scientists have paid increasing attention to the ecosystem approach to evaluate the status of marine

systems. The Convention on Biological Diversity (CBD, 1993) defined the ecosystem approach as *a strategy for the integrated management of land, water and living resources that promotes conservation and sustainable use in an equitable way.* It is believed that a multivariate approach like a measure of "community structure and functioning" is often a more robust method of detecting environmental stress or change and gives more insight into the ecosystem state than univariate measurements. One of the challenges in developing such multivariate indicators is to differentiate a change or shift due to anthropogenic pressure from the considerable natural variability in community structure.

5.3.1 Benthos Indicators

The developments of indicators for the benthos based on the ecosystem approach are more advanced than the attempts in the pelagic realm. The impact of organic matter on the macrobenthos in temperate shelf seas is well studied (Pearson and Rosenberg, 1978; Rosenberg, 2001). Indicators to assess the resulting change in community structure in response to organic loading (e.g. Infaunal Trophic Index (ITI)) (Word, 1990) have been developed. A marine biotic index (AMBI) was developed (Borja *et al.*, 2000) to determine the impacts and the quality status in soft-bottom marine benthic communities. Subsequently, it has been applied, tested and evaluated under different impact sources (Bolam *et al.*, 2002; Biles *et al.*, 2003; Borja *et al.*, 2003a), demonstrating its usefulness in detecting specific localized impacts as well as diffuse pollution (Borja *et al.* 2003a). Bolam *et al.* (2002) and Biles *et al.* (2003) support the hypothesis that marine shallow-water benthos only functions well when all expected 'guilds' are present although each guild needs flourishing populations of only a few species. A 'guild' is a group of species, not necessarily closely related, that have similar ecosystem functions. The AMBI has been assessed against several sources of disturbance, but it may prove insensitive to low-level, wide-area, organic enrichment that may be expected to occur during eutrophication (Tett *et al.*, 2007). So far as biodiversity is concerned, a balance amongst guilds or 'lifeforms' is thought to be more important for ecosystem health than the presence of many species (Loreau *et al.*, 2002; Hooper *et al.*, 2005) which supports the benthic hypothesis for guilds by Biles *et al.* (2003) and Bolam (2002). This means that as long as the resilience of that ecosystem is strong and all lifeforms are represented, the ecosystem is in a healthy condition (all interactions work well, the resilience is high and the ecosystem is balanced). Laine *et al.* (1997) and Karlson *et al.* (2002) found the same with benthic ecosystems in the Baltic Sea. But despite discounting species richness in relation to community organisation and function, species diversity within lifeforms or guilds may be important in aiding resistance and resilience, contributing a variety of detailed strategies and genotypes. This might increase the probability

that some species will survive increased pressure (Tett *et al.* 2007).

It has proven easy to establish structural indicators for the benthos, because the benthos is restricted by physical boundaries and already has a physical structure. Structure is often given by physical aspects like macro algae, corals, rocks and sand. Detecting a change or alteration in microplankton community structure caused by human influence or climate change is a more difficult task as physical structure in this sense is absent. Any approach has to be capable of quantifying the inherent dynamic variability of microplankton and incorporate the seasonal succession of the species.

5.3.2 Biodiversity Indices

The diversity indices are well-known in the ecological literature and have been applied broadly. Simple counts of the number of species and measures of species numbers in relation to total abundance of organisms are such indices. Quantifications based on the amount of information contained in a list of taxons and their abundances were performed by scientists like Margalef (1958). The Shannon index, (also referred to as the Shannon-Wiener Index) (Shannon, 1948) is one of the most commonly used diversity indices measuring diversity in categorical data. It treats species as symbols and their relative population sizes as the probability and so it takes into account the number of species and the evenness of the species. The index is increased by either having additional unique species, or by having a greater species evenness (Krebs, 1989). Orfanidis *et al.* (2001) developed a diversity index, the ecological evaluation index (EEI), that is based on the concept of morphological and functional groups of marine benthic macrophytes (seaweed and seagrasses). The macrophytes were used as bioindicators of ecosystem shifts due to anthropogenic stress, comparing a pristine with a degraded status (Orfanidis *et al.*, 2003). The EEI quantifies these shifts in the structure and function of transitional and coastal waters at different spatial and temporal scales by using non-linear and linear relationships (Orfanidis *et al.*, 2003). Using an approach like the EEI disregards information about the particular contributions of each species to the functioning of the pelagic community. Recent studies (Emmerson and Huxham, 2002) showed only weak correlations between generalized biodiversity and ecosystem function and thus it seems vital to retain such information. Diversity indices would not be appropriate in the context of a phytoplankton bloom event when there might be a natural reduction in diversity. Further, resistance does not appear to become stronger when a great number of species belonging to one genus or lifeform are present as long as the genus or lifeform is represented (Loreau *et al.*, 2002; Hooper *et al.*, 2005). However, species richness may be important in aiding resilience of an ecosystem increasing the probability that some species will survive increased pressure (Tett *et al.* 2007).

5.3.3 Ratios

In freshwater phytoplankton, shifts from desmids, chrysophytes or diatoms to cyanobacteria are known to be associated with nutrient enrichment (Hutchinson, 1969; Talling and Heaney, 1988). In coastal waters, increases in the ratio of N to Si may cause increases in the proportion of non-silicified algae (Gillbricht, 1988; Tett *et al.*, 2003b) which has led to the idea of using indicators based on the ratio of diatoms to dinoflagellates. Caution must be taken by using these diatom/dinoflagellates ratios (Tett *et al.*, 2003b; Tett *et al.*, 2007) as they tend to reflect the view of diatoms being 'good' and dinoflagellates being 'bad' when setting Ecological Quality Standards (EQSs). This misunderstands the multiple roles that each group plays in marine ecosystems and that dinoflagellate/diatom ratios will naturally fluctuate (Tett *et al.*, 2003b). Dickson (1992) suggested that for continental waters, an annual time-series of the seasonal diatom/dinoflagellate ratio would provide an early warning signal for both regional environmental change, such as eutrophication, and global change, such as global warming. The idea was based on the consistent patterns of ecological succession in marine phytoplankton (Margalef, 1978; Pingree *et al.*, 1978) which are observed as a result of the degree of vertical stability of the water column, consequently influencing nutrient ratios and life strategies adopted by specific groups of microplankton. The transition from a turbulent to a stable environment is associated with a microplankton succession from diatoms through flagellates to dinoflagellates. Anthropogenic increase in nutrients is thought to bring an increase in N and P but not Si, which means that changes in the N:Si and P:Si ratios will change to conditions unfavourable for diatoms and favourable for flagellates. However, dinoflagellate abundance and phenology have been related to temperature (Edwards and Richardson, 2004) and thus any change in temperature will always affect the dinoflagellate/diatom ratio (Edwards, 2005).

5.3.4 Species Lists

An alternative to the indicators mentioned above is to list the abundances of all species present in the considered ecosystem (Gowen *et al.* 2011). But this exposes some practical difficulties, especially when dealing with microplankton. The list might comprise hundreds of species, with numbers and biomasses fluctuating in time and space. Furthermore, there is no unique fixed assemblage of species each with its own unique abundance that is characteristic of any particular region. Neither is there a single species and its abundance, or taxa that can be used as an indicator species that signifies good status of the plankton communities.

5.3.5 Other Indices

Devlin *et al.* (2007) proposed three different indices to classify and assess the UK marine waters under the requirements of the WFD. These were (i) phytoplankton biomass measured as chlorophyll(I_{chl}), (ii) the frequency of elevated phytoplankton counts measuring individual species and total cell counts (I_E) and (iii) seasonal succession of phytoplankton functional groups through the year (I_{ss}). The three indices were compared to predetermined reference conditions developed for OSPAR (Malcolm *et al.*, 2002) and CSTT within the UWWTD (CSTT, 1994; 1997). The results were used to set boundary classes (thresholds) in the context of the WFD requirements. The idea is useful, because it combines univariate indices like chlorophyll that deliver the first indication of risk in eutrophication with indicators that give insight into the structure of microplankton (seasonal succession pattern). Other authors like Revilla *et al.* (2010) made a similar approach to assess the Basque estuaries (northern Spain) within the WFD. The established indices are simple and easy to apply to phytoplankton datasets. However, the phytoplankton index (I_E) proposed by Devlin *et al.* (2007) includes the chlorophyll index (I_{chl}) that is already an individual index in their overall approach and it therefore is then accounted for twice in the overall index. With the weighting of data and the ranking into a high, good, moderate, poor and bad status by setting boundaries we claim to know in which condition the marine ecosystem is, which suggests, that we fully understand the interactions of it. But we do not. Thus these assumptions should be avoided when developing indices to assess ecosystem health. It is further doubtful that thresholds of abundances of individual species can adequately distinguish between natural variability and anthropogenic pressure driven change (Tett *et al.*, 2007; Edwards, 2009).

5.3.6 Model Based Indicators

Hardy (1924) gave an impression of the complexity of trophic relationships amongst plankton and nekton for the North Sea presenting the relation of Herring to plankton community as a whole with twelve or more genera of herbivores and as many phytoplankton. The discovery of the microbial loop linking heterotrophic and photosynthetic bacteria and small eukaryotic algae with protistan grazers (William, 1981; Azam *et al.* 1983) increased the number of species and must be taken into consideration when developing models for marine ecosystems (Tett & Wilson, 2000). Models can complement observations of change in the microplankton community due to anthropogenic influence (e.g. Gillbricht, 1988; Hickel *et al.*, 1993). Tett (1990) for example, proposed a model under the aspect of the discovery of the 'microbial loop' linking heterotrophic and photosynthetic bacteria and small eukaryotic algae with

protistan grazers. The MicroPlankton (MP) model consists of three pelagic compartments and six independent state variables with the constant value of η (the ratio of microheterotrophs to total microplankton) being the key feature. It is important that an ecological model incorporates the microplankton models that distinguish between microplankton compartments and also simulate the key features of the physical environment as well as relevant chemical and biological processes (Tett *et al.*, 2005). A synthesising model was proposed by Tett & Wilson (2000) and later Lee *et al.* (2003) who became aware of the importance of η and formalising the calculation of microplankton parameters using this η. The resulting indicator is supposed to provide a simple parametrisation of pelagic autotrophic and micro-heterotrophic processes with respect to the different trophic levels representing the microplankton and exemplifying a broadly trophic taxonomic-functional approach.

5.3.7 Multivariate Statistical Analysis (MVA)

Multivariate statistical analysis (MVA) provides tools for simplifying the plenitude of information that a microplankton sample delivers. It is often possible to reduce the immense data information to a small number of components. One of the most common ways of measuring the concept of community structure between different samples is to measure the samples similarity in terms of their biological communities and this is normally done by using a similarity coefficient (Edwards, 2005). The similarity coefficient is conventionally defined to take the value in the range 0 - 100% where 0% indicates that the samples are totally dissimilar and 100% means that they are totally similar in terms of their biological community. A common method of measuring the multivariate response to community stress starts with measuring the similarity coefficient amongst samples followed by clustering the samples in the form of a dendrogram. The advantage of using a multidimensional scaling (MDS) plot is that the MDS preserves the distances in low dimensional space and also provides a measure of statistical confidence for the ordination. Pointing out the importance of non-subjective classification of species into lifeforms as it can result in complications when interpreting results (Edwards, 2005) the author suggests a thorough analysis of individual species niche requirements before constructing robust functional groups. However, even though the principle component analysis (PCA) might be a good technique for interpreting what the data signify we claim to know that it will remain valid in the future. Edwards' Defra report 2005 presents results that show the capability of this multivariate response method and the MVA has proven to be a powerful tool in analysing historical datasets and in suggesting testable hypotheses about the nature and causes of change in marine ecosystems. Edwards' proposed MDS model is able to show the expected results in the context of 'undesirable disturbance' (Edwards, 2005) and can be used as an indicator of

change in microplankton community structure (Figure 5.1).

Figure 6: MDS plot of community structure per month through time 1958-2003 with mean community and 2003 highlighted.

Figure 5.1: The MDS output as taken from Martin Edwards (SAHFOS) for Cefas/Defra PTI project in 2005. The multidimensional scaling (MDS) method tracks change in the plankton community.

5.4 Lifeforms in State Space

An alternative to MVA is a more theoretically-based approach to process and present the large amount of information that microplankton provides. That is the use of lifeforms as variables to define an ecosystem state space. Lifeforms are functional groups that are the equivalent to 'guilds' of species used by benthic ecologists as described above. These lifeforms do not necessarily need to be taxonomically related, but they carry out the same important functional role in the marine ecosystem. For example, silicate users (diatoms) as a group of species have a functional role related to silicon cycling as they require silicate for their cell walls.

The theory of state variable space derives the discipline of thermodynamics and the "system" theory. The state of a system can be described by a set of system state variables. These state variables change with time in response to each other and yet independently from each other and external conditions such as nutrient changes. Enough state variables need to be chosen to define all system variability describing the system state as a whole. State variables can be any physical, chemical or biological variables. In the case of nutrient supply for example it could be defined by nitrogen and phosphorus. In the pelagic environment it is difficult to describe the system's state with state variables as structure, as it is found in woodlands with scrubs, under stories and upper stories or in coral reefs, is absent. The idea of structure in the pelagic ecosystem instead is based on seasonal succession of

microplankton as observed by Margalef (1978) and many others.

5.4.1 A Phytoplankton Community Index

A phytoplankton community index (PCI) was advocated by Tett *et al.* (2007) and presented in detail by Tett *et al.* (2008). The index was developed by a collaboration of scientists coming from universities and government laboratories funded by DEFRA (Department of Environment, Food and Rural Affairs) and provides a means of detecting a change in phytoplankton community structure in response to environmental forcing such as anthropogenic nutrient enrichment or climate change. It follows a similar principle to the AMBI (Borja *et al.*, 2000) and ITI (Word 1990) designed for marine benthos and relates to the phytoplankton element of biological quality in the Water Framework Directive, Annex V. It was also used relevant to the MSFD quality descriptor 5, but more importantly to the UK's defence against alleged infraction of the UWWTD (Defra workshop on plankton indicators, June 2011, unpubl.). The PCI is based on the theory of lifeforms plotted into a multidimensional state variable space. The approach derived from thermodynamics and the general systems theory that requires a sufficient number of state variables to uniquely define a system state (Tett *et al.* 2008). However, it is desirable to minimise the list of state variables in the case of pelagic lifeforms in state space as practical and theoretical difficulties in estimating parameters generally increase with variable number (Tett & Wilson 2000). The visualization causes a problem as a multidimensional space can simply not be imagined or visualised. Illustrations of 3-D plots could have been an option, but it was concluded that sets of 2-D plots were illustrated best as one plot with two variables is the simplest combination and easiest to interpret.

There is an objection to state space as opposed to time-series graphs as it might be that a state-space plot results in a loss of information about the time-dependency of change in abundance. The main justification is that system state is not defined by time but by instantaneous values of state variables. This is, two systems that have the same pair of values of Y_1 and Y_2 are said to be in the same state. There are three main advantages of the lifeform concept: (i) state space plots are not as sensitive to defects in sampling regimes as statistics based on time-series graphs, although it is very important that sampling throughout the year is provided to characterise the microplankton regime completely; (ii) using lifeforms provides the chance to define state variables at any taxonomic or other level at which it is possible to distinguish kinds of organisms using morphology, life-history and biogeochemistry (Tett *et al.*, 2003b); (iii) plotting microplankton lifeforms into a state variable space takes into account the natural, especially seasonal and inter annual variability that is an essential part of phytoplankton ecology being the pelagic analogue of physical

structure.

An illustration of diatoms and dinoflagellates as examples for state variable 1 (Y_1) and state variable 2 (Y_2) over a time series of three years, plotted into the state space is given in Figure 5.2. The elliptical shape is a consequence of the seasonal succession and captures the inherent seasonal and inter annual variability of microplankton giving the impression of a "doughnut".

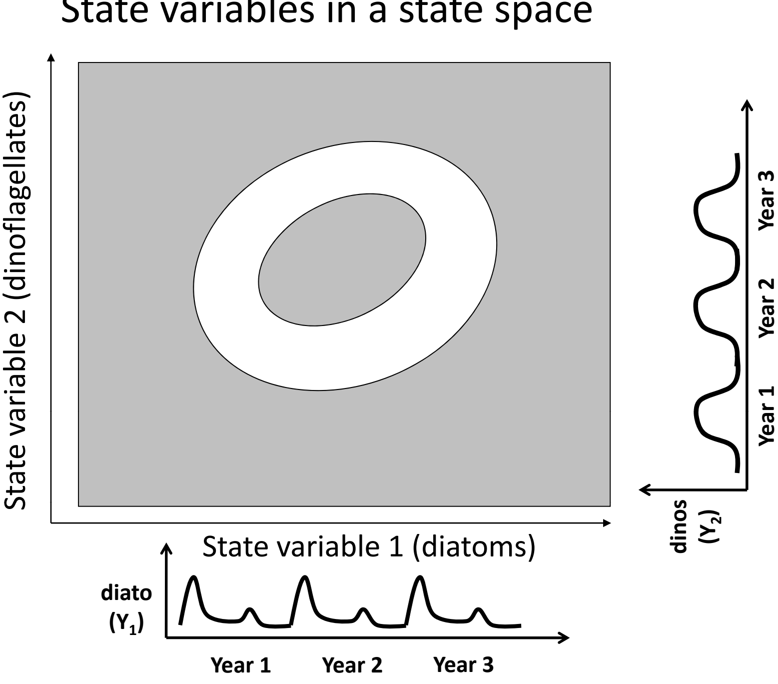
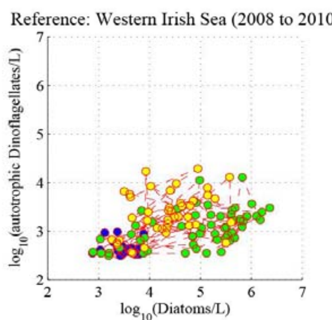


Figure 5.2: An illustration of an ecosystem state defined by two lifeforms i.e. diatoms and dinoflagellates as state variables in a two-dimensional 'state variable space' in the shape of a 'doughnut'.

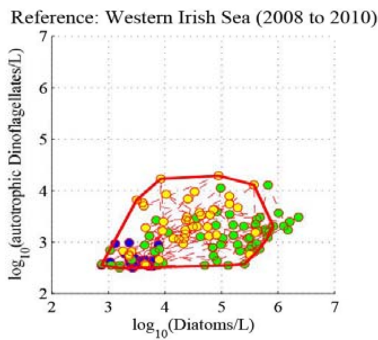
In Figure 5.3 the development of the ecosystem state is illustrated in three steps. First the data points of the state variables are plotted into the space plot for which the co-ordinates of each point is a pair of values of lifeform Y_1 and lifeform Y_2 from one sample taken on a particular day of the year. The state space plot can be considered as a map created by co-ordinates (Y_1, Y_2) but should not to be mistaken as an X-Y plot where Y is dependent on X. An elliptical shape like a 'doughnut' appears due to (natural) succession driven by nutrient depletion (mainly nitrate) that limits microplankton growth in the summer and light that limits microplankton growth in the beginning and by the end of the year. In order to calculate a PCI value, it is necessary to establish a reference condition. And so the next step is to define a reference envelope. To establish such an envelope, a geometric method known as Convex Hull (Sunday, 2004; Weisstein, 2006) is applied to the cloud of the data points with a certain data exclusion (in this study 90% of the data were

considered), drawing an outer envelope. According to Tett & Mills (2009) limitation theory suggests that the bundle of microplankton data points should have a hollow centre. To create this hollow centre, an inner envelope is established by applying the Convex Hull method to the centre points turning them inside-out and once the envelope is also drawn around them, they are re-inverted again. The envelopes define a domain in state space that contain a set of trajectories (points displaying the annual cycle) of two lifeforms (Y_1, Y_2) in the marine pelagic system and with that the prevailing regime during the reference period. Logarithmic transformations are commonly applied on plankton data (Barnes, 1952) because they allow more reliable statistical analysis and interpretation and also allow change at low abundance to be seen as clearly as change at high abundance. A given amount of change on a logarithmic axis shows the same proportionate increase or decrease, irrespective of abundance.

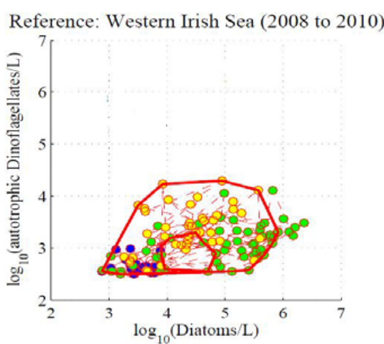
The PCI envelope in 3 steps



Step 1: every point is one observation (one sample) in that autotrophic dinoflagellates and diatoms occurred



Step 2: an envelope is drawn around the points by a convex hull function with a 10%-tile confidence outlier exclusion



Step 3: an inner envelope is created by a convex hull function for a more realistic representation of the microplankton seasonal succession

Figure 5.3: An illustration of the development of the state variable space plot in three steps fitting an outer and an inner envelope around the data points of the state variables (here: diatoms and autotrophic dinoflagellates from a sampling site in the western Irish Sea over a two year period).

Once the reference envelope (reference condition) is established, a second data set is plotted into the state space plot following the same procedure as described above, but without creating the envelope (Figure 5.4). The reference envelope is then plotted on top of that comparison regime and the PCI value is the proportion of the new points that fall inside the envelope or better, between the outer and inner envelope of the reference condition. The mathematical expression for the PCI value is:

$$PCI = \frac{\text{new data points inside the reference envelope}}{\text{total new points}} \tag{5.1}$$

All new data points that fall inside the reference envelope are divided by the total of the new data points. The PCI value is a number between 0 and 1, while 1 indicates no change from the reference envelope because all new data points lie inside the reference envelope while 0 indicates a complete change from one state into another as the new data points all lie outside the reference envelope. The envelope was made by excluding 10% of the points, so some new points are expected to fall outside the reference envelope. To test whether the points outside the reference envelope exceed the expected number and are significantly more, the exact probability is calculated using a binomial series expansion or more approximately, by a chi-square calculation (with 1 degree of freedom and a 1-tail test). These tests indicate whether a shift or a change in microplankton community is significant. An illustration in Figure 5.4 shows the end result of a comparison between two conditions.

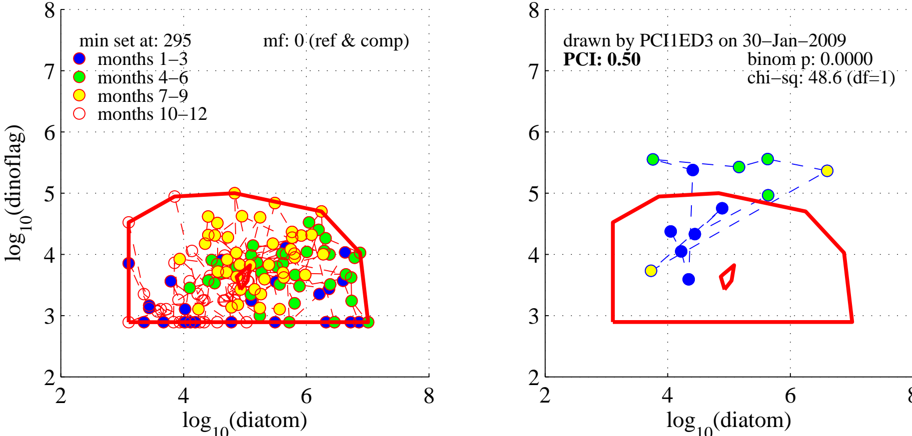


Figure 5.4: An illustration of the reference condition (left) and the comparison data plotted into the reference envelope (right) with the PCI value and the p value for statistical significance in the upper corner of the right plot. The different coloured points represent the four different seasons of the year (legend in the upper corner of left plot).

The PCI was tried out and evaluated for UK coastal waters in the northern North Sea (near Stonehaven), a Scottish fjord (Loch Creran), and the eastern Irish Sea

(Liverpool Bay) by Tett *et al.* (2008). A spatial comparison between enriched (inshore) and less- enriched (offshore) waters of the eastern Irish Sea showed little change in phytoplankton community structure, whereas the temporal comparison of Loch Creran showed that the sea loch has experienced a large change over the last 25 years at a time when nutrient levels appear unchanged (Tett *et al.* 2008). The index makes it possible to compare different sampling sites over several years (a 'pristine' site can be compared with a 'enriched' site) or one site in different years (displaying the shift from maybe one state to another) in order to identify shifts in microplankton community structure. The publication by Tett *et al.* (2008) demonstrated this. The index respects all key requirements that an indicator should incorporate as it is simple to understand, can be easily incorporated into existing monitoring schemes, it has proven to be sensitive to ecosystem stress and also incorporates natural variability in the microplankton community structure (seasonal and inter annual succession). It was further shown to be statistically reliable, accurate and robust. It is able to detect a regime shift and extreme events. With a theoretically based approach it should be able to distinguish between natural hydro-climatic variability and anthropogenic stress. In a few points the PCI needed some investigation and improvement to order to become an even more reliable, robust, and complete indicator of change. Those investigations were one objective of this study.

5.4.2 Outline of the MatLab program for the PCI

The program for the PCI was written in the programming language MatLab ^{TM}. It contains three main scripts and accessory functions written by Paul Tett that extract data, intersect them, and create the final PCI value and comparison plots. The first script `HPLP.m` selects phytoplankton data from data bases such as the Loch Creran data base and extracts the required lifeforms with the help of its control script `hcf.m` and its key function `extractlifeform.m`. The script `HPLP.m` outputs a time series plot of the extracted lifeforms and saves them in a 4 column matrix with the year, day no., cells/L and biovolume/L. The key element of the second script `HPLF.m` is the intersection or union of the saved data from script 1. The intersection creates a point (Y_1, Y_2) when a number for both lifeforms is apparent on the same day. The union option makes a data pair anytime it can find either Y_1 or Y_2 on the same day. If it can only find one of Y_1 or Y_2, it creates the pair with NaN (not a number) in place of the missing value. The function `Mwaves.m` fits sin-cos waves to the data and outputs a graph of the time series plot with fitted waves. The intersected or united data are saved as a 3 column matrix with the decimal year, Y_1, and Y_2. These files are read by the third and final script `PCI.m` that calculates the PCI value with the aid of its control script `cf.m`. The `PCI.m` script also calls its key function

`PCIplot.m` that outputs the graph of the PCI comparison and applies the necessary statistics.

5.4.3 A Microplankton Community Index

Theory and Concept

The current PCI has been shown to detect a statistically significant change in the proportion of two state variables (e.g. diatoms have decreased while dinoflagellates have increased) in the 'undesirable disturbance' context. The biggest change was observed in Loch Creran by Tett (2008), where diatoms have decreased with some obvious increase in dinoflagellates (data from 1979-1981 were compared with data from 2006-2007). Strictly speaking in the case of eutrophication an undesirable disturbance is a shift from one lifeform to another (diatoms replaced by dinoflagellates) and so the change observed in Loch Creran might not be expected to be one. However, the proportion of the two lifeforms changed significantly over the years and is, according to expert's opinion, indeed considered as an undesirable disturbance to the ecosystem in the Loch (P. Tett pers. comm.).

The main difficulty in developing a phytoplankton/microplankton community index is to identify the state variables (lifeforms) to indicate change in response to environmental forcing such as anthropogenic nutrient enrichment. In this study the interest lay in microplankton community structure and change of this structure by nutrient enrichment and so the ecosystem state was described by microplankton lifeforms. In order to reduce subjectivity in choosing these lifeform categories, 'expert opinion' was sought. Many approaches were performed to categorised microplankton into lifeforms. For example, Carreira (2006) considered shape (sphere, ellipsoid, cylindrical, etc.), the ability to colonise (e.g. chain forming, non-chain forming) and the motility of microplankton (e.g. motile, non-motile) in her Master project. Tett *et al.* (2008) distinguished between pelagic, tychopelagic and weed diatoms, while they discriminated the dinoflagellates into thecate and a-thecate, medium sized and large sized, autotrophs/mixotrophs and heterotrophs. Tett further made distinctions between flagellates, cyanobacteria and pico phytoplankton. During this project I investigated several lifeforms and identified a total number of twenty-one (as discussed in chapter 2). These were pelagic and tychopelagic (heavy diatoms such as *Pleurosigma*) diatoms, chain forming and solitary diatoms, naked and armoured dinoflagellates, micro-flagellates categorised into *Cryptophytes*, *Prasinophytes*, *Prymnesiophytes*, *Euglenophytes*, heterotrophic micro-flagellates and nano-flagellates. I further distinguished between ciliates and tintinnids, and more generally between silicate users and non-silicate users as well as autotrophic, heterotrophic, and mixotrophic, small ($< 40\mu$m) and large ($\geq 40\mu$m) sized microplank-

ton. There are many good reasons to split the microplankton into all these different lifeforms ("deep taxonomy", physical feature, biogeochemistry etc.), but in order to make a microplankton community index a tool that can be used and applied by non-experts in the context of detecting a change in the structure related to anthropogenic pressure, it seemed more desirable to keep it simple and easy to understand. Thus, I reduced the choice of lifeforms focusing on anthropogenic pressure in nutrient enrichment while aiming to extend the current PCI by incorporating the hetero- and mixotrophic components of the microplankton.

The PCI does not consider the distinction between the nutritional mode of the microplankton as lifeforms, nor does it include the ciliates and micro-flagellates as lifeforms in the microplankton community to define state variable space. But different nutritional modes of microplankton and the heterotrophic components are of vital importance as they bear directly on trophic interactions, especially the linkages between the microbial loop and the classical metazoan food web and help to explain ecosystem functioning. In my study I tried to find lifeform combinations that could be indicators or proxies for three of the eleven descriptors defined by the MSFD for the assessment of good environmental status of the marine environment. The concerning descriptors were biodiversity (QD1), food web (QD4), and eutrophication (QD5). Based on the PCI, I aimed to define lifeform state spaces that could link across each of the three descriptors and make the original PCI a more complete indicator of change while keeping it simple and easy to understand.

The resulting microplankton community index (MCI) distinguishes between the different nutritions (autotrophy, mixotrophy, heterotrophy) of microplankton as well as their size focused on ciliate and copepod prey-size and adding ciliates as a new lifeform to the 'non-silicate user' component. With the nutritional mode distinction, the idea was to indicate any change in the heterotrophic/autotrophic composition and consequently detect a change in the trophic status. The meaning of the trophic status here, was understood and dealt with in two different ways. The first considered the status of the food web, that is the effect of a shift in the balance of organisms amongst primary producers, with consequent effects on the type of grazer. If for example, the composition shifts towards the heterotrophic component, primary producers (as prey) would be reduced. If the changes happen towards the heterotrophic micro-flagellate component, no or little energy would be exported as it would end up in the microbial loop. It would also affect the primary production in the smaller size flagellates which can form an important part of the biomass (Joint and Pomroy, 1983). If on the other hand the shift would happen towards the autotrophic organisms, big blooms could occur leading to shading and light reduction as well as de-oxygenation. If the shift would happen in the smaller size flagellate range, more trophic levels would be present and energy export would be reduced. The other meaning of the trophic status considered the status in respect of eutrophica-

tion. Nixon (1995) followed the idea that eutrophication is excess of carbon, leading to excess of respiration, which could imply a change in the autotroph/heterotroph balance of organisms.

The conceptual idea of the MCI was to define state variable spaces that could provide insight into the process of eutrophication. Here, I present three state spaces (i.e. three pairs of state variables) that provide information on biogeochemistry and therefore biodiversity and energy flow and with that food webs equally holding information about the process of eutrophication. This is a demonstration of how an MCI could be used as a tool to indicate change in microplankton community structure contributing one part to the whole pelagic system.

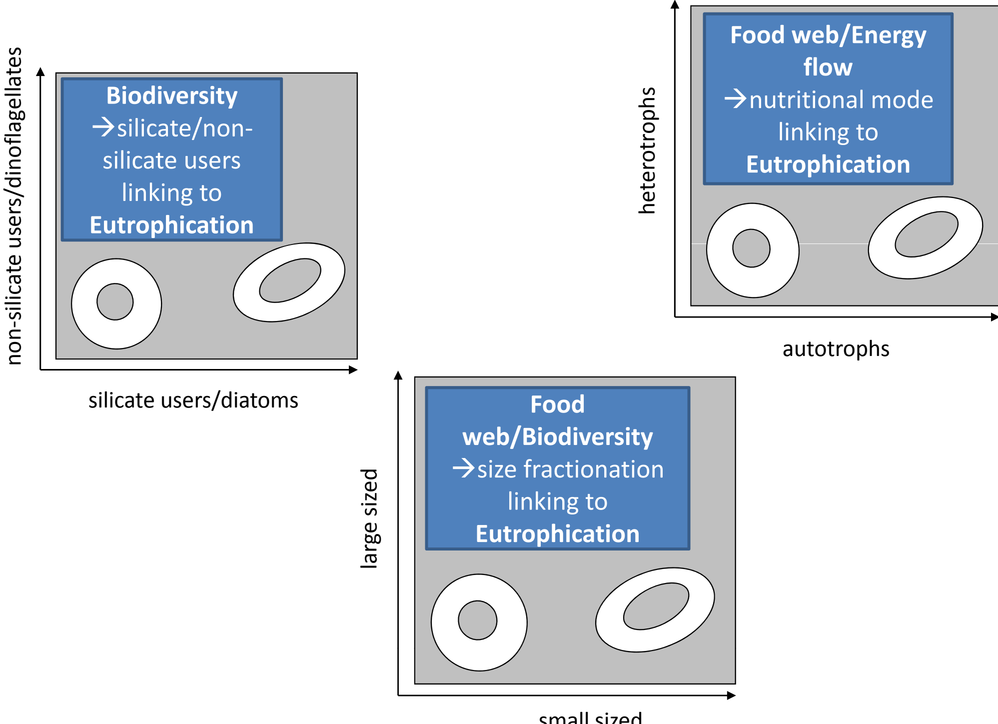


Figure 5.5: A presentation of my conceptional idea of lifeforms in three state variable spaces. The first concerns the taxonomy and the biogeochemistry distinguishing silicate and non-silicate users as proxies for biodiversity and eutrophication. The second concerns the particulate and dissolved nutrition matter and hence the nutritional mode of microplankton. Autotrophs and heterotrophs are proxies for the energy flow through the system and provide information about food web structure and functioning, and consequently for eutrophication. The third state variable space is described by size where small and large microplankton are supposed to be a proxy for energy flow/trophic levels also linking to food web, biodiversity and eutrophication.

To follow this conceptual idea seven lifeforms were decided upon. These were:

- silicate users (alternatively diatoms)
- non-silicate users (alternatively dinoflagellates)
- autotrophic microplankton
- mixotrophic microplankton
- heterotrophic microplankton
- small ($< 40\mu$m) sized microplankton
- large ($\geq 40\mu$m) sized microplankton

The combinations described in Figure 5.5 were deemed to provide insight into the three MSFD descriptors. A change of the state space defined by silicate and non-silicate users would be a proxy for the biogeochemistry and holds information about the N:Si ratio. It would also provide information about biodiversity because succession from silicate to non-silicate users would be expected to be found in a condition that represents Good Environmental Status (GES). A change toward non-silicate users would indicate a change in N:Si ratio and with that the first indication of eutrophication. The distinction of the nutritional mode would provide information about dissolved and particulate matter and therefore primary and secondary production which gives insight into energy flow (energy export) and therefore the structure of the food web also linking across eutrophication. Size distinction could be a proxy for biodiversity, because seasonal succession from large to small sized microplankton is believed to be characteristic of a pelagic ecosystem under seasonally stratifying hydrodynamic conditions when in (GES). It would also provide insight into different trophic levels and therefore energy flow (vigour) and food web which relate to eutrophication.

To combine and illustrate the lifeforms differently (e.g. small autotrophic silicate users compared to large heterotrophic non-silicate users) was decided against for two reasons. The first reason was the idea to combine all single MCI values to one overall MCI value and for this reason no lifeform was allowed to appear more than once (degree of freedom has to be 1). The second reason considered a practical idea aiming to keep this indicator approach simple and easy to use.

Harmful algae were also not considered as a lifeform here although they are a required indicator by the MSFD in assessing the good environmental status of the marine ecosystem. This decision was made because nothing groups the harmful algae together as a distinct lifeform considering the deep taxonomy and the origin of microplankton (e.g. some *Alexandrium* species are harmful and some are not, but all *Alexandrium* species descend from the same origin and have very similar taxonomic features).

The Matlab program for calculating the MCI

With the aid of the computer program MatLab^{TM}, scripts with accessory functions were written to process the datasets from the sampling sites and to illustrate and calculate the MCI value. Based on the PCI scripts and functions written by Paul Tett the system approach and operation procedure of the MCI scripts are similar. There is one main difference between the PCI and MCI model. While the PCI model consists of three main scripts (`ENV`, `WAVE`, `PCI`) and their related functions, in the MCI model I turned these scripts into functions that are operated by one main script (`MCI.m`) and a comprehensive control script (`cfx.m`). This step was done to reduce unnecessary interaction in the MatLab^{TM} program itself and allow efficient investigation of various lifeforms and combinations. Figure 5.6 shows a flow diagram of the operational MCI model. The illustration was inspired by Paul Tett and was created with cmap. The first function (`ENV.m`) selects the required microplankton data from data bases and converts them into files with columns displaying the year and day, the cells L^{-1} and biovolume pg C m^{-3} of selected species or lifeforms that are defined in the control script. These files are picked up by the next function (`WAVE.m`) where the key operation is to create an intersection or a union of the two output files from the first function with columns displaying the year and the two chosen species or lifeforms in either cells L^{-1} or biomass mg C m^{-3}. The third and last function (`PCI.m`) takes the output from the second function and creates the reference envelope and the comparison conditions. It calculates the MCI value and performs the required statistics, presenting everything in a two plot 2-D-illustration. A description of the script can be found in the appendix listing 2 B.1.

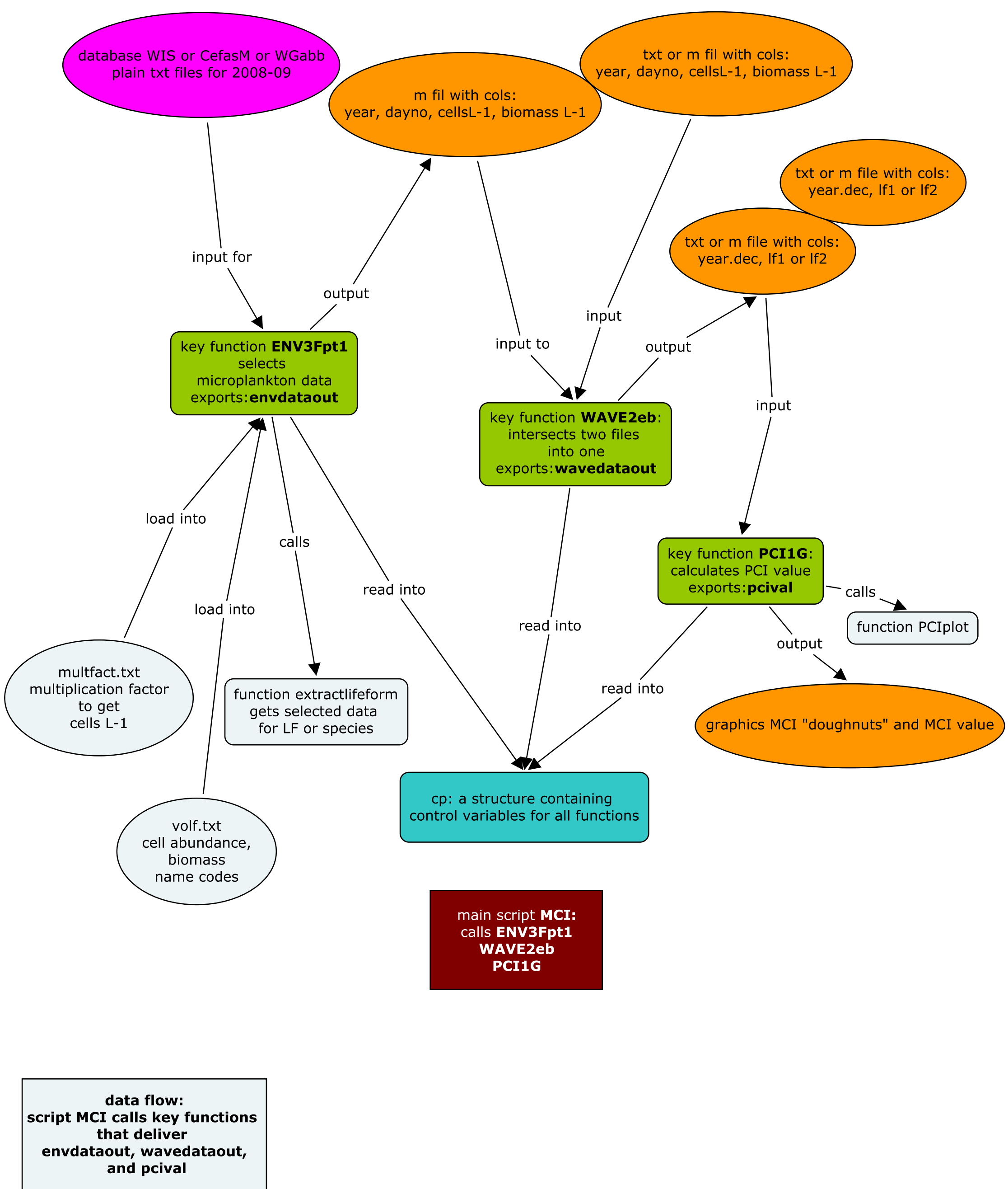


Figure 5.6: A flow diagram of the operational MCI model in May 2011. Inspired by Paul Tett and created with cmap.

Chapter 6

A Microplankton Community Index (MCI)

6.1 Introduction

Many lifeforms can describe the structure of the microplankton communities in state variable space. In this study, I focused on those lifeforms that can describe and link across biodiversity, food web, and eutrophication which are descriptors 1, 4 and 5 in the Marine Strategy Framework Directive (2008/56/EC). In respect to the statistical analyses (degree of freedom) I did not combine the lifeforms that I chose (e.g. small heterotrophic non-silicate users) to describe state variable space. Following my conceptual idea shown in chapter 5 Figure 5.5, I used silicate and non-silicate users, autotrophic, mixotrophic, and heterotrophic, and small and large sized microplankton. For the Liverpool Bay station diatoms and dinoflagellates were used instead of silicate and non-silicate users. The reason for this was that at this station non-silicate users apart from dinoflagellates such as ciliates and micro-flagellates were not included in the samples counts and so a distinction to the sites where they were included had to be given. For this reason all comparisons with Liverpool Bay for this lifeform category were performed with diatoms and dinoflagellates. The comparisons made here, were exclusively spatial between the development site (station 38A) with both test sites (WGabb and LBay) and between the two test sites. Temporal comparisons were not possible because a dataset large enough (comprising at least 4 years) was not available for the study sites.

6.2 Rationale

This chapter examines different methods of dealing with practical difficulties of the model, such as shortage of data and outlier exclusion. It presents the technical background of the model and describes and discusses the obtained results from the comparisons with the PCI. The compatibility of the MCI in detecting change in microplankton community structure is examined by applying the model to the three study sites that were located in the western Irish Sea (station 38A), the eastern Irish Sea (station Liverpool Bay), and the southern North Sea (station West Gabbard). The comparisons of the development site (station 38A) with the test sites (West Gabbard and Liverpool Bay) are examined first and then a comparison of the West Gabbard and Liverpool Bay stations in the context of anthropogenic nutrient enrichment is performed. The final part of the chapter incorporates the discussion of the performance of the MCI and some further thoughts concerning the development and application of the index.

6.2.1 Assumptions and Hypotheses

Some assumptions and expectations were made from the results in chapter 4 before analysing and testing the MCI tool. For example, it was expected that the MCI value significantly differed from 1 when the test sites in the West Gabbard and Liverpool Bay were compared to the development site in the western Irish Sea. Results and the categorisation into Margalef's succession scheme in chapter 4 showed that the different hydrodynamics accommodated different microplankton communities. If the MCI tool works correctly, this is expected to be detected and reflected in the MCI and its value. In particular, a significant difference was expected when silicate and non-silicate users were compared as the hydrodynamics at the seasonally stratified reference site (38A) favoured dinoflagellate (non-silicate user) growth during the strongly stratified season while the hydrodynamics at both test sites (station WGabb and LBay) favoured diatom (silicate user) growth being regions of consistently strong tidal influence that only stratify intermittently. The other expectation was that different nutrient loadings at the test sites (WGabb winter N approximately 16 μM, LBay winter N approximately 30μM) would be reflected in different microplankton biomass (higher in Liverpool Bay). From the results of chapter 4 it was evident that the biomass at Liverpool Bay was much higher than it was at West Gabbard and so the hypothesis was that this would be reflected in the MCI value (significantly different from 1) and a shift was expected. Different hypotheses and a priori reasons why the MCI value significantly differs from 1 and the shift is expected toward a certain direction mark the outline of this chapter and are given in Table 6.1 as an overview.

Table 6.1: Overview of the assumptions and hypotheses on the MCI results expected from the results of chapter 4. The first column expresses the statistically significant deviation of the MCI value from 1 while the second column indicates the lifeform described state space, the third column gives information about the study site comparison, the fourth column gives the predicted direction of a shift and the last column contains the reason why the difference is predicted.

Hypothesis	lifeform	comparison	shift	reason
MCI differs from 1	Si-/nonSi-users	38A with WG	south east	hydrodynamics, increased N+P
MCI differs from 1	autos/heteros	38A with WG	south west	hydrodynamics
MCI differs from 1	autos/mixos	38A with WG	south west	hydrodynamics
MCI differs from 1	mixos/heteros	38A with WG	south west	hydrodynamics
MCI differs from 1	small/large	38A with WG	north west	hydrodynamics, increased N+P
MCI differs from 1	diatoms/dinos	38A with LB	south east	hydrodynamics, increased N+P
MCI differs from 1	autos/heteros	38A with LB	south west	hydrodynamics
MCI differs from 1	autos/mixos	38A with LB	south west	hydrodynamics
MCI differs from 1	mixos/heteros	38A with LB	south west	hydrodynamics
MCI differs from 1	small/large	38A with LB	north west	hydrodynamics, increased N+P
MCI differs from 1	diatoms/dinos	WG with LB	east	increased N+P
MCI differs from 1	autos/heteros	WG with LB	east	increased N+P
MCI differs from 1	autos/mixos	WG with LB	east	increased N+P
MCI no difference	mixos/heteros	WG with LB	no shift	hydrodynamics
MCI differs from 1	small/large	WG with LB	north	increased N+P

6.3 Methods

6.3.1 General Description and Labelling

Published PCI results (Tett *et al.* 2008) showed that the PCI was able to detect changes in phytoplankton community structure. In the following the MCI is tested on whether it is able to indicate the expected changes. The plot area was divided into four quarters in order to make it easier to follow hypotheses and results. Hereafter, the directions were defined as south west referring to the left bottom corner, south east referring to the right bottom corner, north west referring to the top left corner and north east referring to the top right corner (see Figure 6.1). For example, in the comparison of diatoms and dinoflagellates between stations WGabb and LBay, it was hypothesised that the MCI value was significantly different from 1 and the shift was toward south east caused by a higher abundance in diatoms (horizontal

axis) and a lower abundance in dinoflagellates (vertical axis) at station LBay as an example.

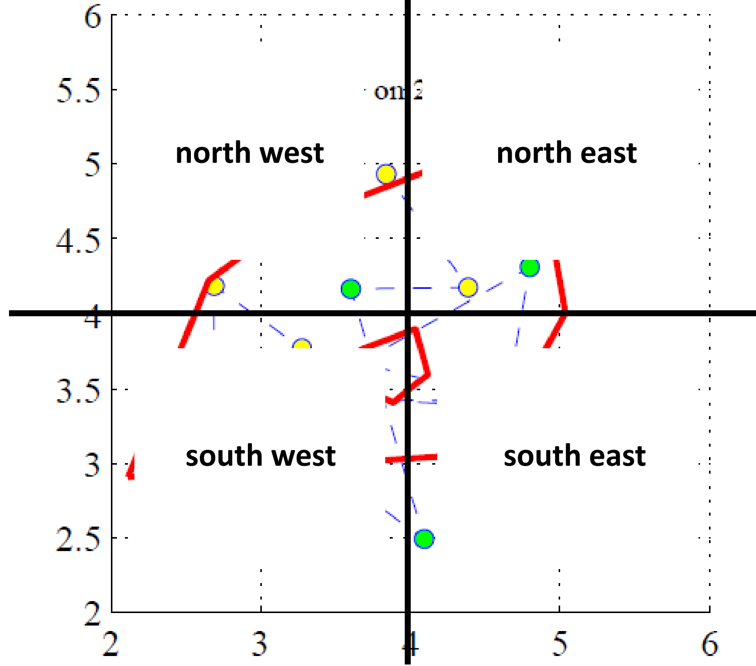


Figure 6.1: The plot area of the state space is divided into quarters. The left bottom part is referred to south west, the right bottom part to south east, the left top part is considered as north west and the right top part as north east.

Most MCI plots use a standard format (Figure 6.2). The top of each diagram gives the name of the reference site and the comparison site and the time period of examination (e.g. Reference: Western Irish Sea (2008-2009); Comparison: Liverpool Bay (2008-2009)). The results are plotted on a $\log_{10}$ scale and the lifeforms used label the axis. The left (reference) plot provides information about the %ile used to exclude outliers (p in %), the number of points examined and the number of sub-sampling (mf) which was set on 0 for all examinations during this study. In the left corner, the 'no-see' value is given (explained in subsection 6.3.5) as 'min set at' and the different coloured dots represent the four different seasons of the year given in months (legend). The right (comparison) plot gives information about the MatLab function that created the plots, the creation date, the actual MCI value, the data from the comparison ('new data'), the p value of statistical significance and the chi-square value with the degree of freedom (df) that gives the probability distribution of the new data points (Figure 6.2).

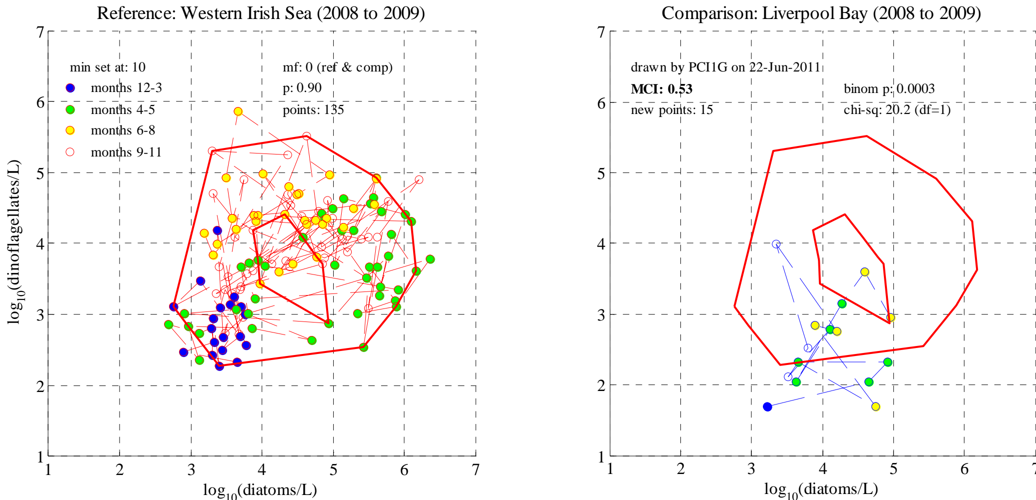


Figure 6.2: An illustration of the general diagrammatic conventions and legend of an MCI diagram as they are presented in the following sections.

6.3.2 Months Changes

The months that describe the four seasons were also changed in the MCI script and differed from the original months categorisation found in the PCI script (Figure 6.3). In the PCI, the months were colour coded and summed into the following: month 1 - 3 (winter) blue, 4 - 6 (spring) green, 7 - 9 (summer) yellow, and 10 - 12 (autumn) no colour. In the MCI, the categorisation was: 12 - 3 (blue) for winter, months 4 - 5 (green) for spring, months 6 - 8 (yellow) for summer, and months 9 - 11 (no colour) for autumn. This change was made to keep the consistency from chapter 4 where the seasons were defined upon the decrease and increase on the total oxidised nitrogen concentrations.

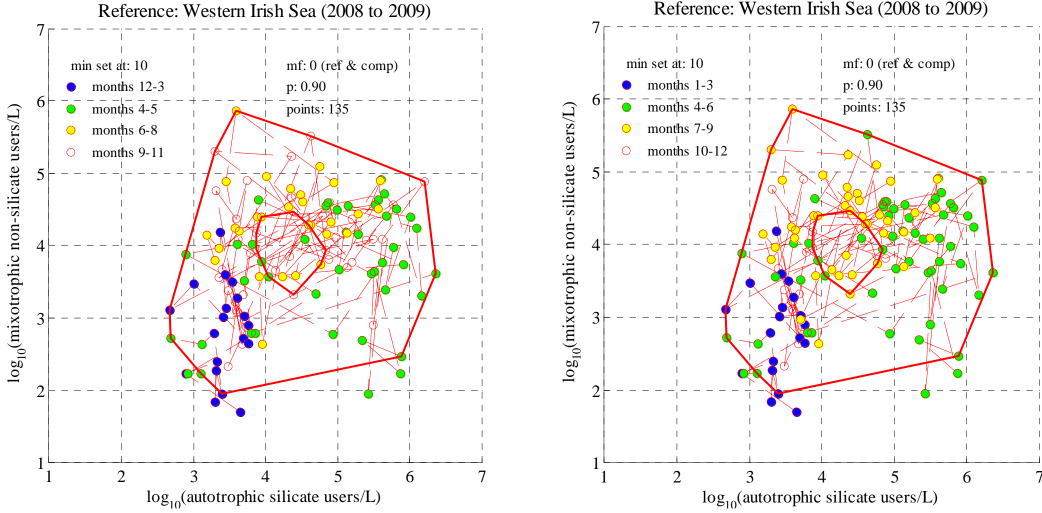


Figure 6.3: The difference of season (months) categorisation in the PCI (left) and the MCI (right) illustrated with data from station 38A.

Some differences in the colour of data points and therefore season categories were detected but the general pattern and shape of envelopes stayed the same.

6.3.3 Outlier Exclusion

To decide what %ile in terms of data exclusion was most appropriate for the reference envelope, three options to create a reference envelope were considered (Figure 6.4). This was done because it was recognised that a larger envelope was less sensitive to change in the distribution of points in state space. The greater variablilty evident in the envelopes with 100%ile and 95%ile data consideration resulted in more extensive envelopes, consequently, more new points fell within these envelopes, leading to a higher MCI value (0.60) (i.e. more agreement). The 90%ile data consideration reduced the variablilty for the reference envelope and excluded outliers, resulting in a less extensive envelope. Less new points fell within this envelope, consequently, resulting in lower MCI value (0.53). The 90%ile option seemed the most desirable and appropriate option not least because it agrees with the decision made by the OPSAR convention in considering the 90%ile of chlorophyll concentrations in the context of eutrophication. All following reference envelopes were therefore created with a 90%ile data consideration.

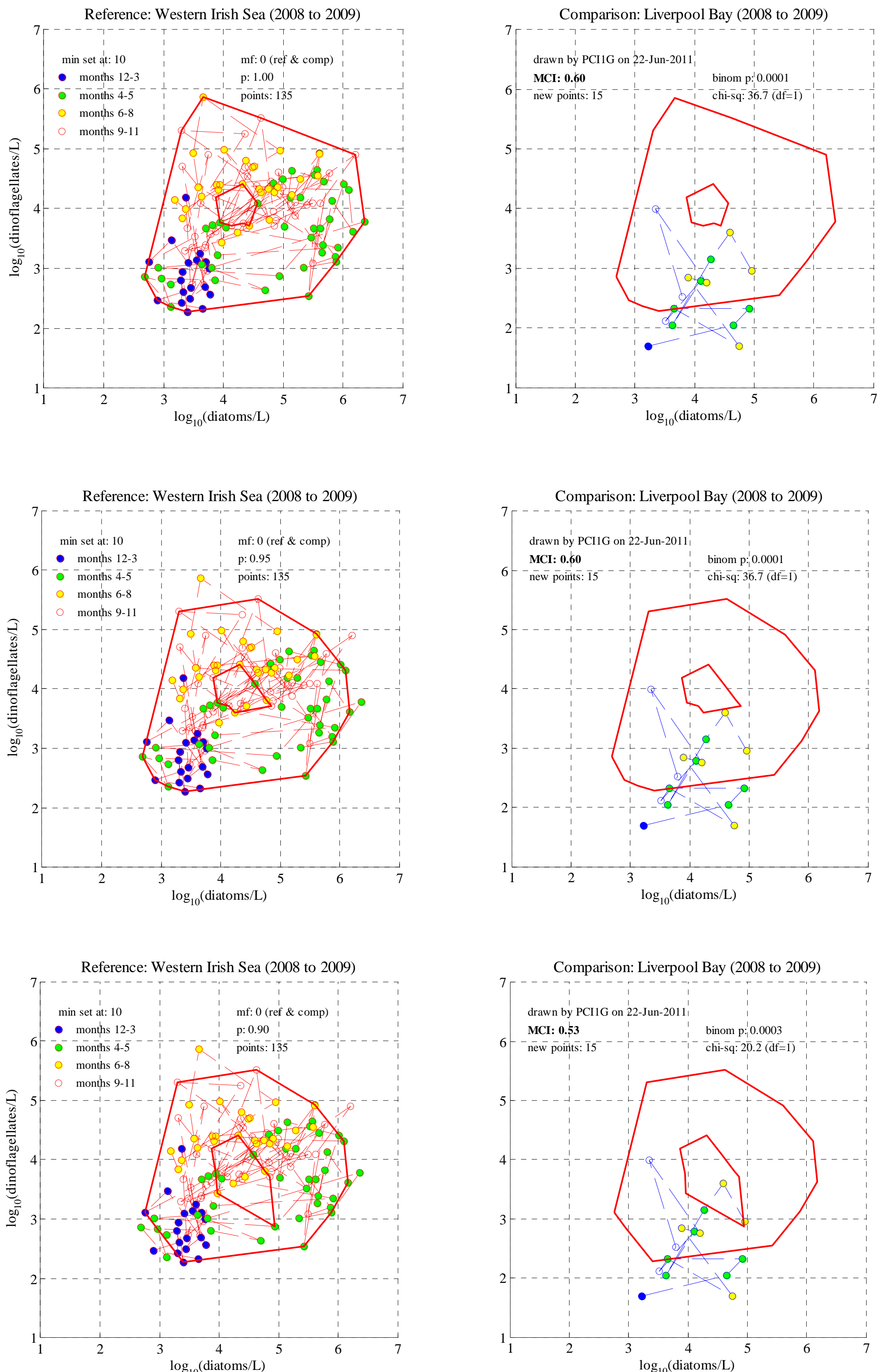
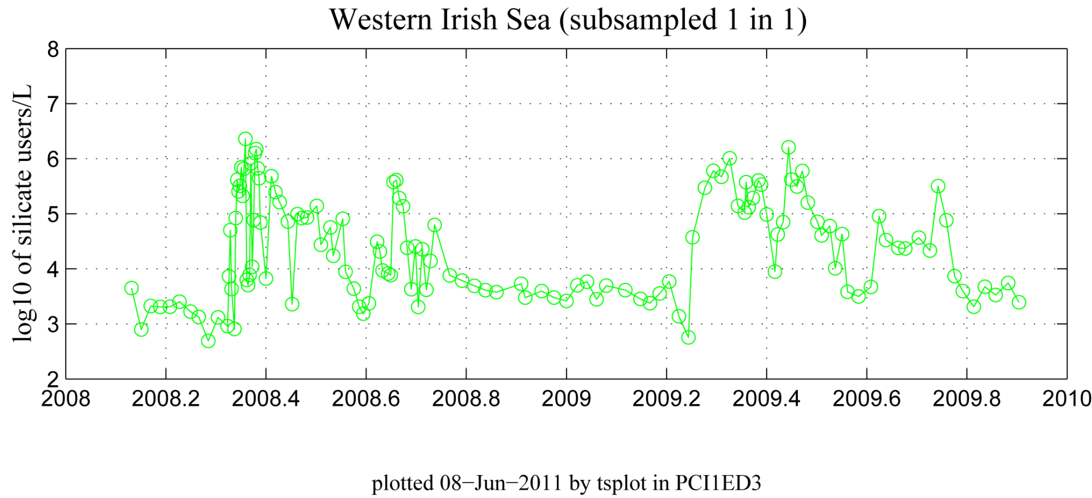


Figure 6.4: Illustrations of different data exclusions for diatoms and dinoflagellates between station 38A and Liverpool Bay. The top diagram includes all data (100%). The diagram in the middle represents a data exclusion of 5% and the bottom diagram shows 10% data exclusion. The different shapes of outer and inner envelopes with the different percentile of exclusion are apparent.

6.3.4 Minimum Datasets

Two issues concerning the minimum number of data points to define lifeform state space were addressed. One issue concerned the minimum number of datapoints for a reliable reference envelope, the other concerned the minimum number of datapoints for the comparison condition. Different time series were plotted using the data for silicate and non-silicate users from the western Irish Sea station 38A. The first time-series displays the pattern of all data available (i.e. all analysed samples, n = 135) and the actual daily to weekly resolution, the second time-series obtained the monthly average of the data. Time-series 3 - 7 showed several sub sampling options that resulted from picking one sample in two to one sample in ten at random (Figures 6.5 to 6.11).



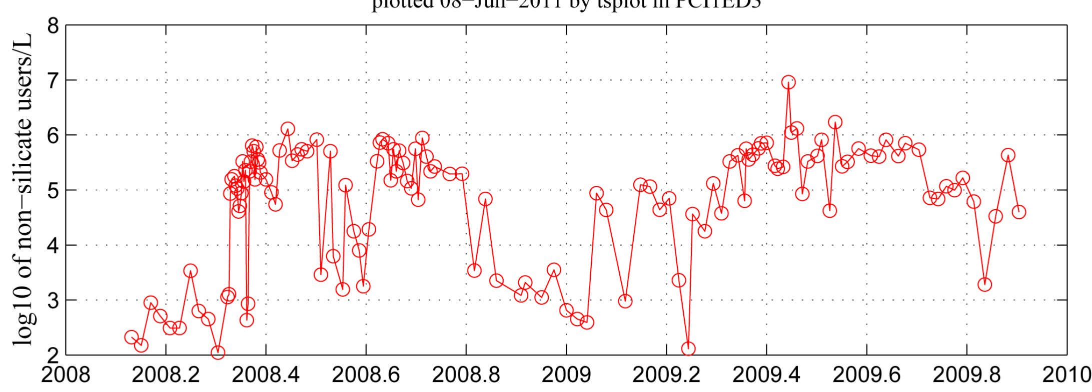


Figure 6.5: Time series of data analysed at station 38A considering all data. The top plot of the diagram displays the silicate users over the sampling period and the bottom plot showed the non-silicate users over the sampling period. Both lifeforms on a $\log_{10}$ scale.

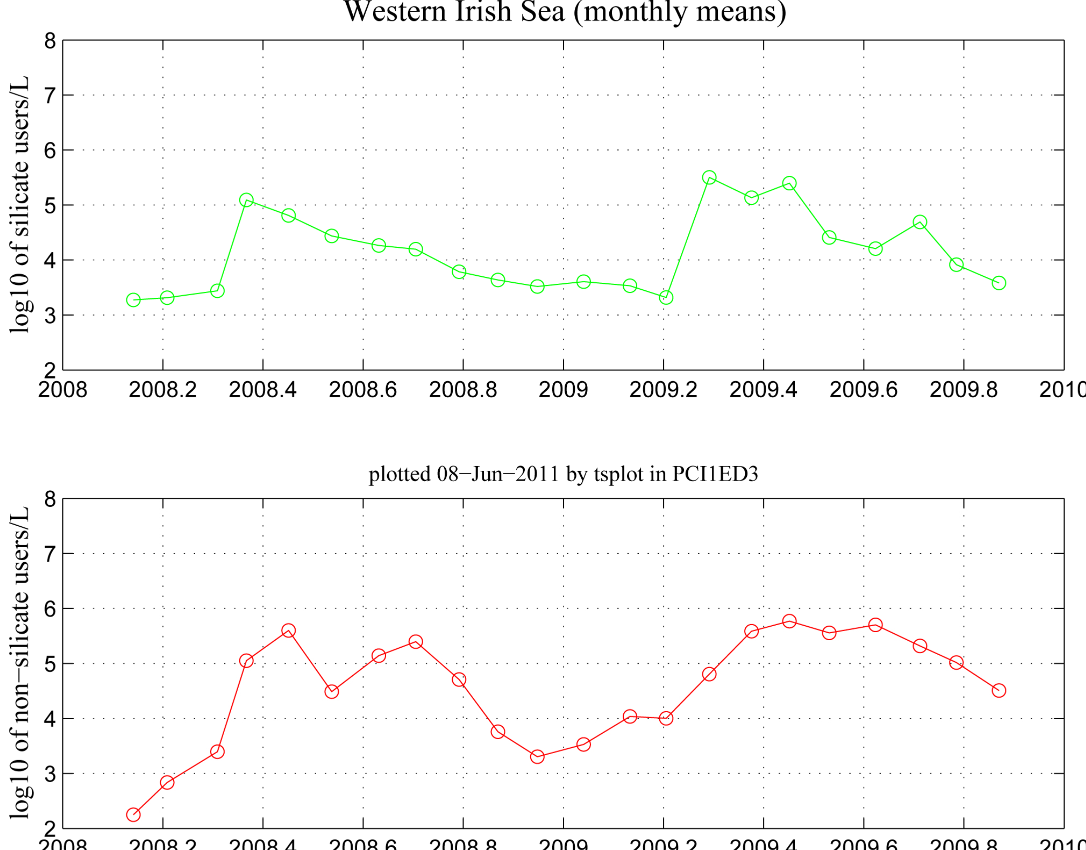


Figure 6.6: Time series of data analysed at station 38A considering the monthly average. The top plot of diagram displays the silicate users over the sampling period and the bottom plot showed the non-silicate users over the sampling period. Both lifeforms on a $\log_{10}$ scale.

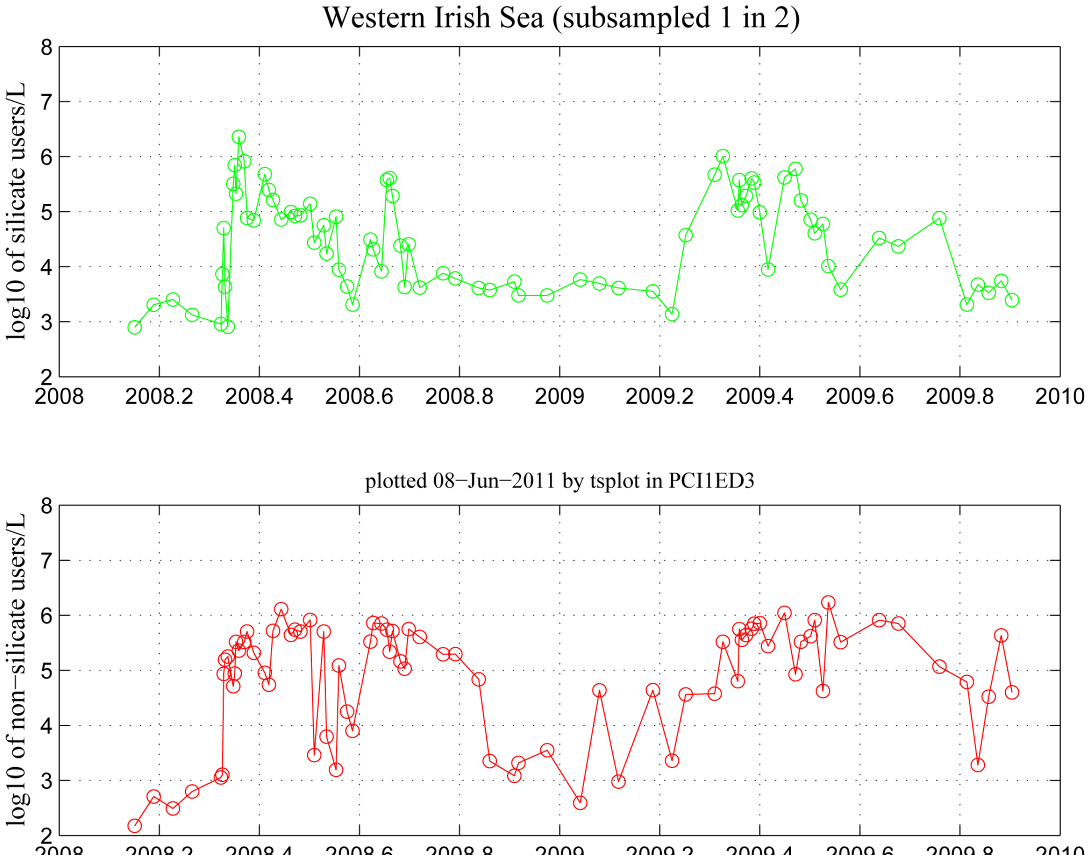


Figure 6.7: Time series of data analysed at station 38A considering considering one sample in two randomly chosen. The top plot of the diagram displays the silicate users over the sampling period and the bottom plot showed the non-silicate users over the sampling period. Both lifeforms on a $\log_{10}$ scale.

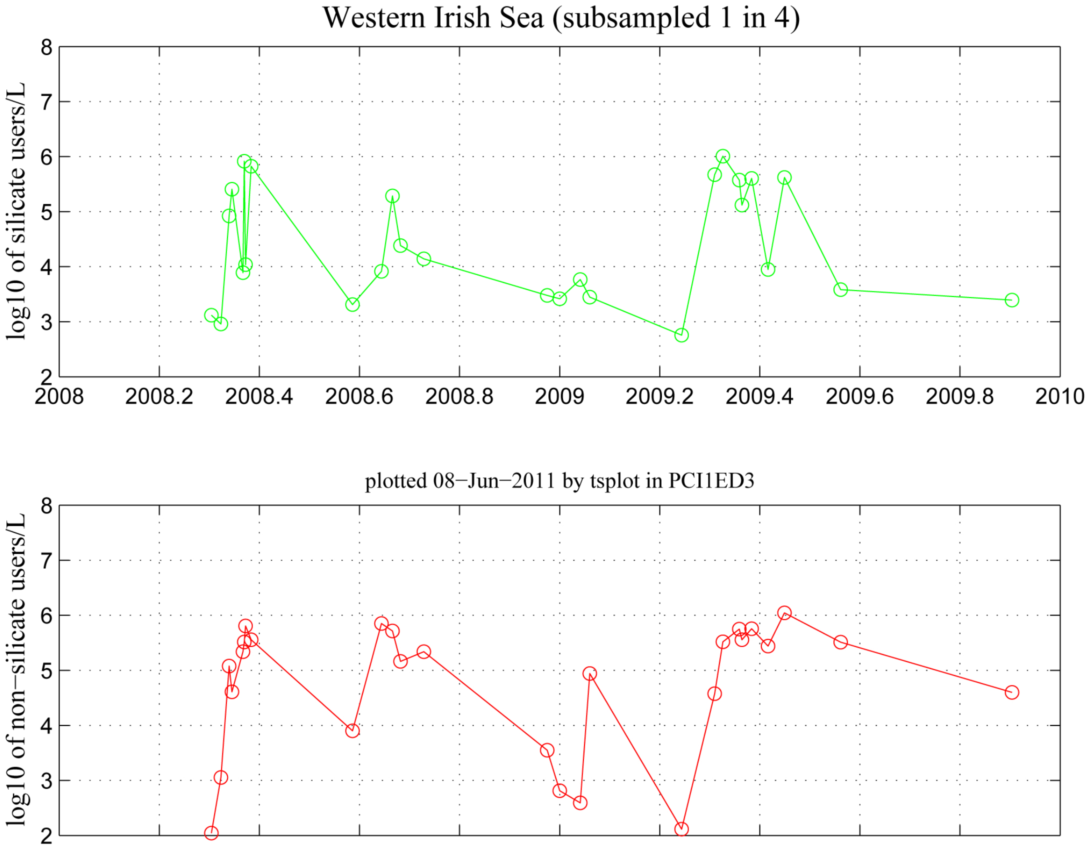


Figure 6.8: Time series of data analysed at station 38A considering considering one sample in four randomly chosen. The top plot of the diagram displays the silicate users over the sampling period and the bottom plot showed the non-silicate users over the sampling period. Both lifeforms on a $\log_{10}$ scale.

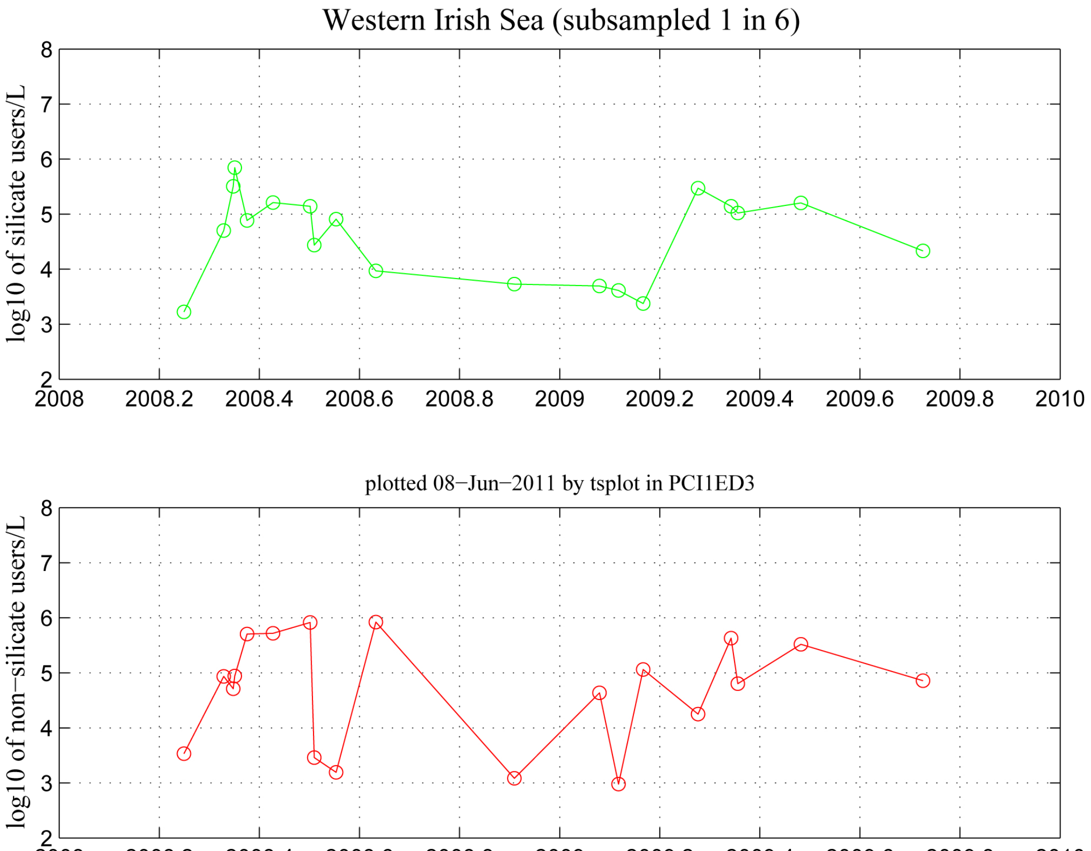


Figure 6.9: Time series of data analysed at station 38A considering considering one sample in six randomly chosen. The top plot of the diagram displays the silicate users over the sampling period and the bottom plot showed the non-silicate users over the sampling period. Both lifeforms on a $\log_{10}$ scale.

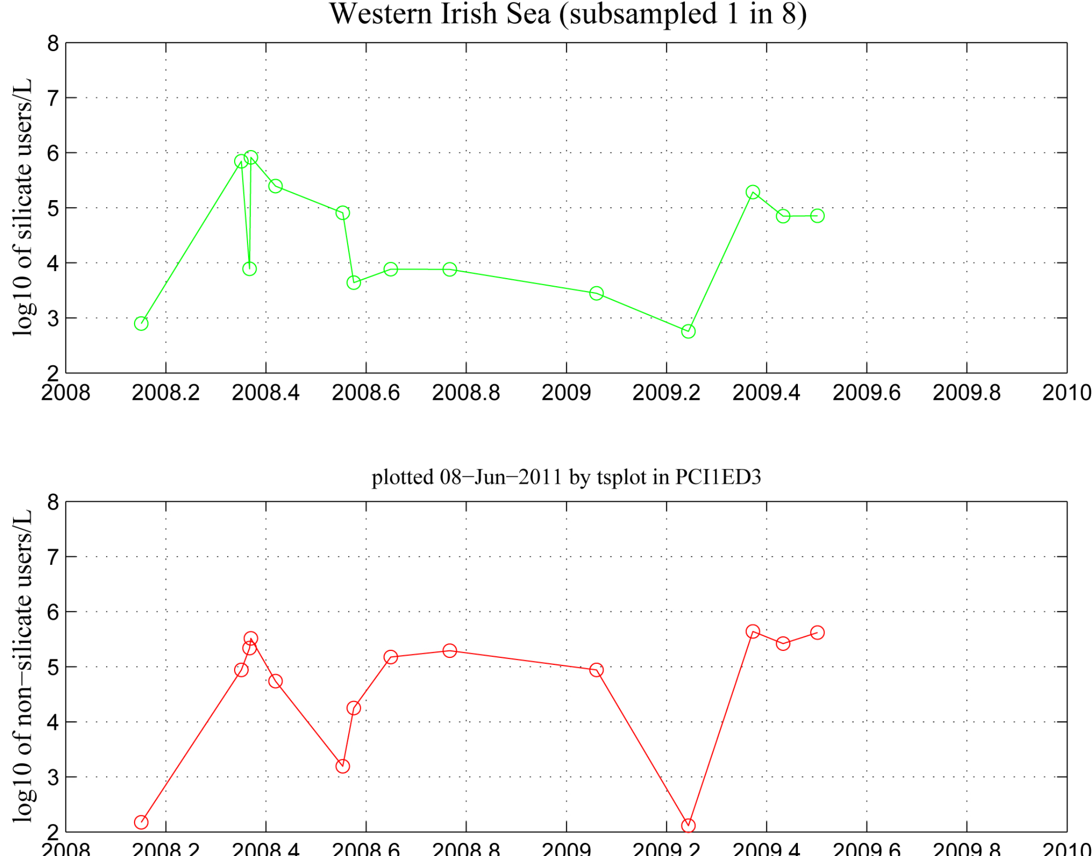
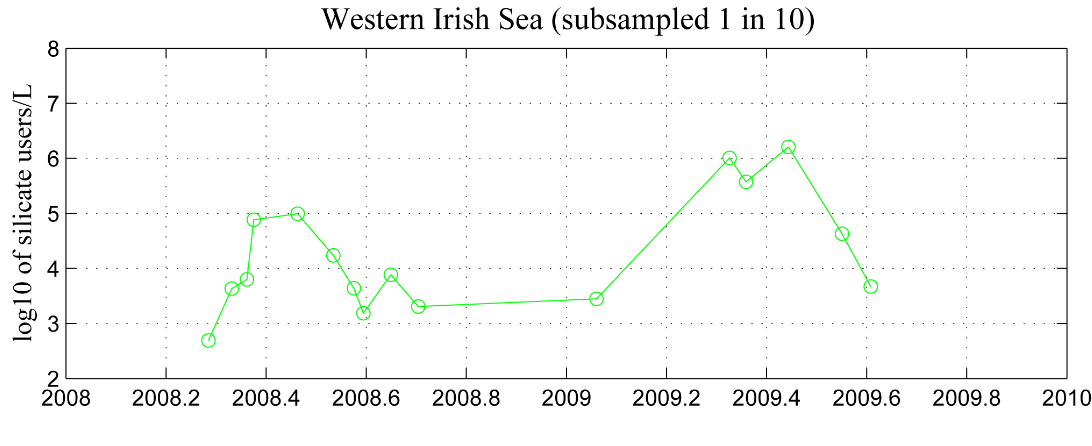


Figure 6.10: Time series of data analysed at station 38A considering considering one sample in eight randomly chosen. The top plot of the diagram displays the silicate users over the sampling period and the bottom plot showed the non-silicate users over the sampling period. Both lifeforms on a $\log_{10}$ scale.



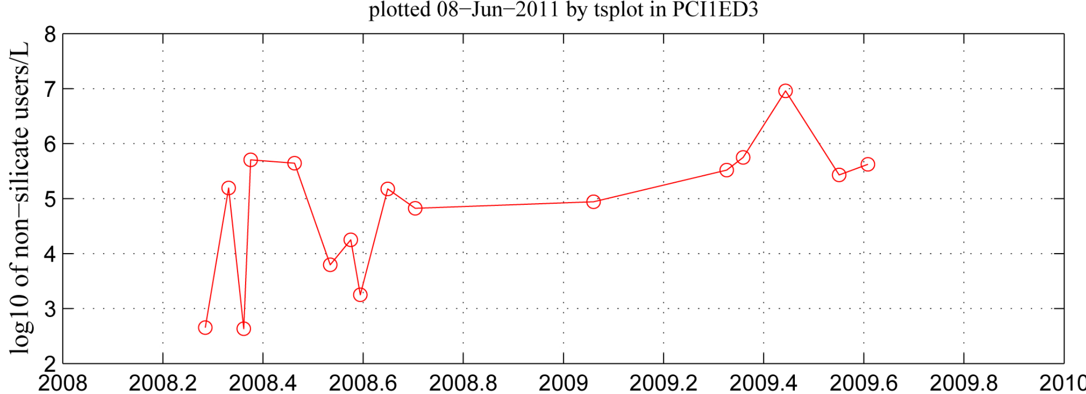


Figure 6.11: Time series of data analysed at station 38A considering considering one sample in ten randomly chosen. The top plot of the diagram displays the silicate users over the sampling period and the bottom plot showed the non-silicate users over the sampling period. Both lifeforms on a $\log_{10}$ scale.

From the results it was obvious that 1 in 10, 1 in 8, and 1 in 6 sub samples picked at random did not reflect the seasonal succession of the silicate and non-silicate users that was found when all data were plotted. Even the pattern that resulted from 1 in 4 sub samplings did not appear to represent the pattern of the actual resolution in a satisfying way. Especially, the silicate users were not displayed correctly toward the end of the plot where the illustration did not pick up the late summer and autumn peaks that were apparent when all data were included. The 1 in 2 sub sampling option was found to be representing the pattern of the two lifeforms in an acceptable way and so was the monthly average. The monthly means were calculated by an average of six samples per month and ought to be representative for the real pattern. The plot of the 1 in 2 sub sampling option was created by an average of three samples per month. For the robustness and reliability of reference envelopes the ideal dataset would incorporate daily samples that were monthly averaged and plotted into state space. This way the great variability evident in daily samples would be kept low and an extensive reference envelope would be avoided while the seasonal succession would be captured and reliability would be provided. However, from an operational point of view this is not practical because daily sample analysis

would exceed time and financial budgets. A dataset of two years for a reference condition does not provide enough information about the annual variability in microplankton community structure and lacks the required robustness despite a high (daily or weekly) sampling frequency. A dataset containing five years worth of data is more desirable as it results in a more robust reference condition and has a higher confidence level in detecting unusual inter annual events. However, five years of sampling are half a decade and a change in microplankton community structure could be directed to a climatic cause rather than a change in nutrients. It also has to be kept in mind, that Plankton indices are tools to examine change in time. Therefore, a dataset containing four years of monthly data is suggested as the ideal time period. This period allows inter annual variability and provides the certain confidence in detecting unusual events while it gives enough data points to create a reference envelope and minimises a change due to climate influence. A minimum dataset of three years for the reference condition with a frequency of at least monthly samples is a good compromise to the four year dataset.

To create a dataset for a time period over three years was not possible within this project, therefore it was decided to collect and analyse daily to weekly samples for the development of the MCI to provide a detailed and large dataset for experimenting, investigating and evaluating ideal options for a robust reference envelope. I suggest that two samples per month over three years (n = 72) are the minimum number that a dataset should incorporate for the development of a reference envelope and the actual data, the monthly average or the 1 in 2 sub sampling option should be used given that climate and pressures are thought to be fairly constant over this period. For the comparison site fewer data points are necessary. I suggest that a fourteen day frequency or at least monthly sampling should be maintained and the minimum time period should be one year in order to be able to detect a shift or change. With all datasets it is important that the sampling is throughout the year as seasonal variation is seen as an essential part of the structure of the microplankton community.

A required dataset over a 3 year period for the reference condition was not archived during this study as fieldwork was only account for over a 2 year period. The dataset established for this period at station 38A incorporating 135 analysed samples was considered a compromise providing a dataset large enough to create a reference envelope and also carry out different experiments and comparisons needed for the development and investigation of the MCI tool. With 34 analysed samples, the West Gabbard was considered a reliable comparison site. To consider the West Gabbard samples for a reference envelope however, the available dataset was not found to be sufficient. Regarding the suggestion made above it would need 72 samples for a three year period or at least 44 samples for a period about 22 months. A less favourable option was to use monthly data which required 22 data points and so the 34 samples

were used to create a reference envelope choosing this option. This was done to find out whether the MCI was able to detect differences in lifeform combinations between sites with similar hydrodynamics and different nutrient loadings. The reason why I used the two year period also for the comparison site was that I was interested in the difference of microplankton community structure at two different site (spatial comparison) over the same time period.

For Liverpool Bay a total of seventeen samples were analysed which was not considered to be sufficient for either the reference or the comparison site. Although a monthly sampling frequency was performed for the Cefas plankton recorder, some samples were found to contain too much sediment (even after dilution) and were rejected. The Liverpool Bay dataset was found to be too small. Comparing the nutritional modes applying the intersection of datasets resulted in two data points for the heterotrophs/autotrophs and mixotrophs/heterotrophs combinations and no conclusion could be drawn. Applying the union option, resulted in 17 new data and a comparison of the nutritional modes between the study sites was possible. The issue of small datasets and union options is discussed in the following section.

6.3.5 Intersection and Union

The key element of the second function in the MCI script as described in the MCI subsection of chapter 5 is the intersection or union of data. The intersection creates a point (Y_1, Y_2) when a number for both lifeforms is apparent on the same day. The union option ought to deliver more data points for the state space plot that can be desirable sometimes when the dataset provided is rather small. It takes a data pair anytime it can find either Y_1 (lifeform 1) or Y_2 (lifeform 2) on the same day. If it can only find one of Y_1 or Y_2, it creates the pair with NaN (not a number) in place of the missing value. With another function of the model, NaN is replaced by a 'no-see' value, which is set individually for each sampling site. The 'no-see' value exists because it is believed that although a lifeform was not found in an analysed sample by chance, it was still present in the water column in low abundance. A suitable number for the 'no-see' value is assumed to be half the minimum of one cell observed in a sample. Thus when 50ml are examined and one cell was counted 20 cells L^{-1} were present so the suitable number for the 'no-see' value in this case would be 10 cells L^{-1}. In the case of biomass as a measure of abundance, a suitable value would be obtained by multiplying this "no-see" value by the smallest biomass found during sample analysis. Assuming that the smallest biomass was 0.00029 mg C m^{-3} this value is multiplied with the "no-see" value of 10 and the minimum value for biomass would 0.0029 mg C m^{-3}.

The diagram in Figure 6.12 gives an example of the issue of small datasets and

illustrates the differences in using the intersection and union. Plotting the data from Liverpool Bay into the reference state space using the intersection of the data sets resulted in only two data points and so no MCI could be obtained. Using the union of the dataset resulted in 17 points and an MCI = 0.12 with $p < 0.00$ indicating a significant difference. The main data points lay along the bottom (south) of the plot area suggesting that most of them were present only at the 'no-see' value (minimum number). When the union option was applied to both datasets, additional eleven data points for the reference condition (West Gabbard) extended the envelope and the MCI value resulted in 0.82 with a shift not statistically significant ($p = 0.24$). I have no answer for an optimal solution and further investigation needs to be considered. As mentioned before such small data sets showed that reliable analyses were not possible resulting in values with no significant meaning. However, the fact that only two data points were available for the comparison dataset confirms that a minimum amount of heterotrophic microplankton was present at the Liverpool Bay station. When the union option was applied the main part of the data points were found in the bottom part of the diagram and the significant difference to the data from the WGabb station was confirmed.

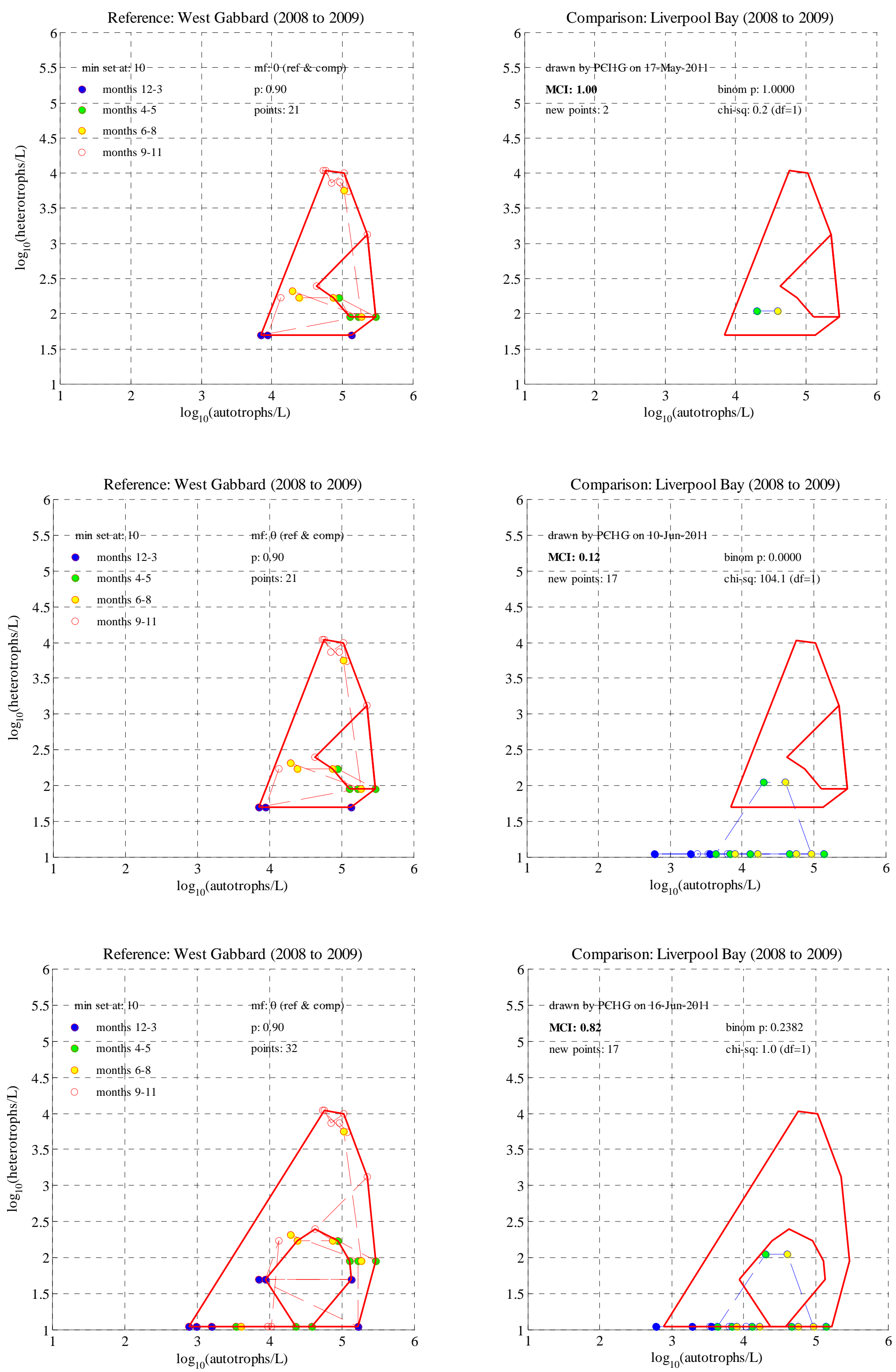


Figure 6.12: The application of the intersection and union option to the datasets of the West Gabbard station as the reference site and the Liverpool Bay station as the comparison site. The comparison comprises of the autotrophic and heterotrophic microplankton with a 90% data consideration. The top diagram shows the intersection of both datasets. The diagram in the middle refers to the intersection applied

to the West Gabbard and union of the Liverpool Bay. The bottom diagram shows the union option applied to both datasets. All three options resulted in different MCI values with differences in their statistical significance.

6.3.6 Flat Bottom

In their publication of the PCI in 2008 Tett *et al.* faced the issue of a "flat bottom" and vertical left site in the PCI plots reflecting an insufficient dynamic range in the data, caused by minimum cell abundance mainly in winter. This issue was avoided in the MCI by following Tett's suggestion to settle larger sampling volumes (100ml) during the winter period.

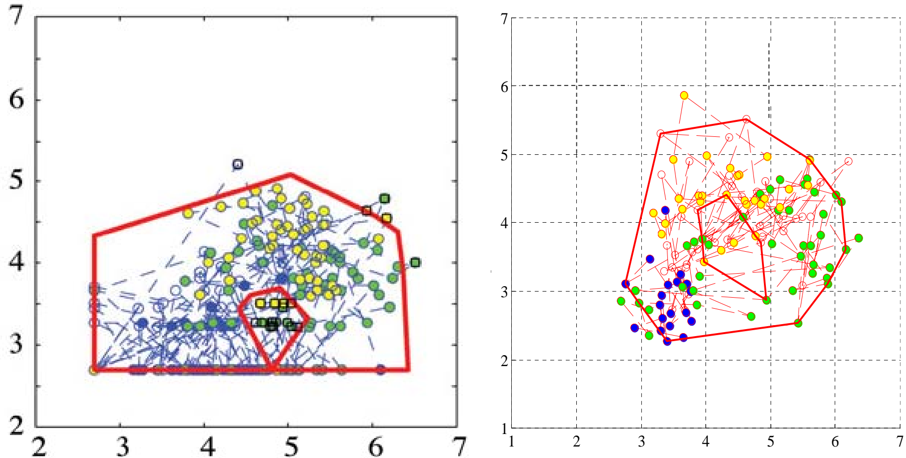


Figure 6.13: An illustration of the difference between the "flat bottom" issued in the PCI publication by Tett *et al.* (2008) on the left and an MCI plot avoiding the "flat bottom" by settling larger sampling volumes during winter (blue data points).

6.3.7 Conclusion of Methods

The 10% outlier exclusion, the investigation of the minimum dataset, the intersection and union options, and the prevention of the "flat bottom" plots added to the improvement of the methods used in the PCI as published by Tett *et al.* (2008). The next step was to extend these methods to a wider set of lifeforms, i.e. to extend the PCI to an MCI.

6.4 Results

6.4.1 Comparison of the PCI and MCI

To demonstrate that the MCI extends the PCI and gives more detail about the nutritional mode of microplankton and therefore more insight into its structure by incorporating heterotrophic and mixotrophic non-silicate users, autotrophic silicate users were plotted against autotrophic non-silicate users (the state space that the PCI originally described), heterotrophic non-silicate users, and mixotrophic non-silicate users (Figure 6.14). Data from the development site 38A in the western Irish Sea as reference state space anf the test site WGabb as comparison state space were used for this demonstration. In all three diagrams 135 data points were plotted into a state space on a $\log_{10}$ scale and categorised into winter (months 12-3, blue), spring (months 4-5, green), summer (months 6-8, yellow), and autumn (months 9-11, no colour) and 21 to 30 comparison points were available.

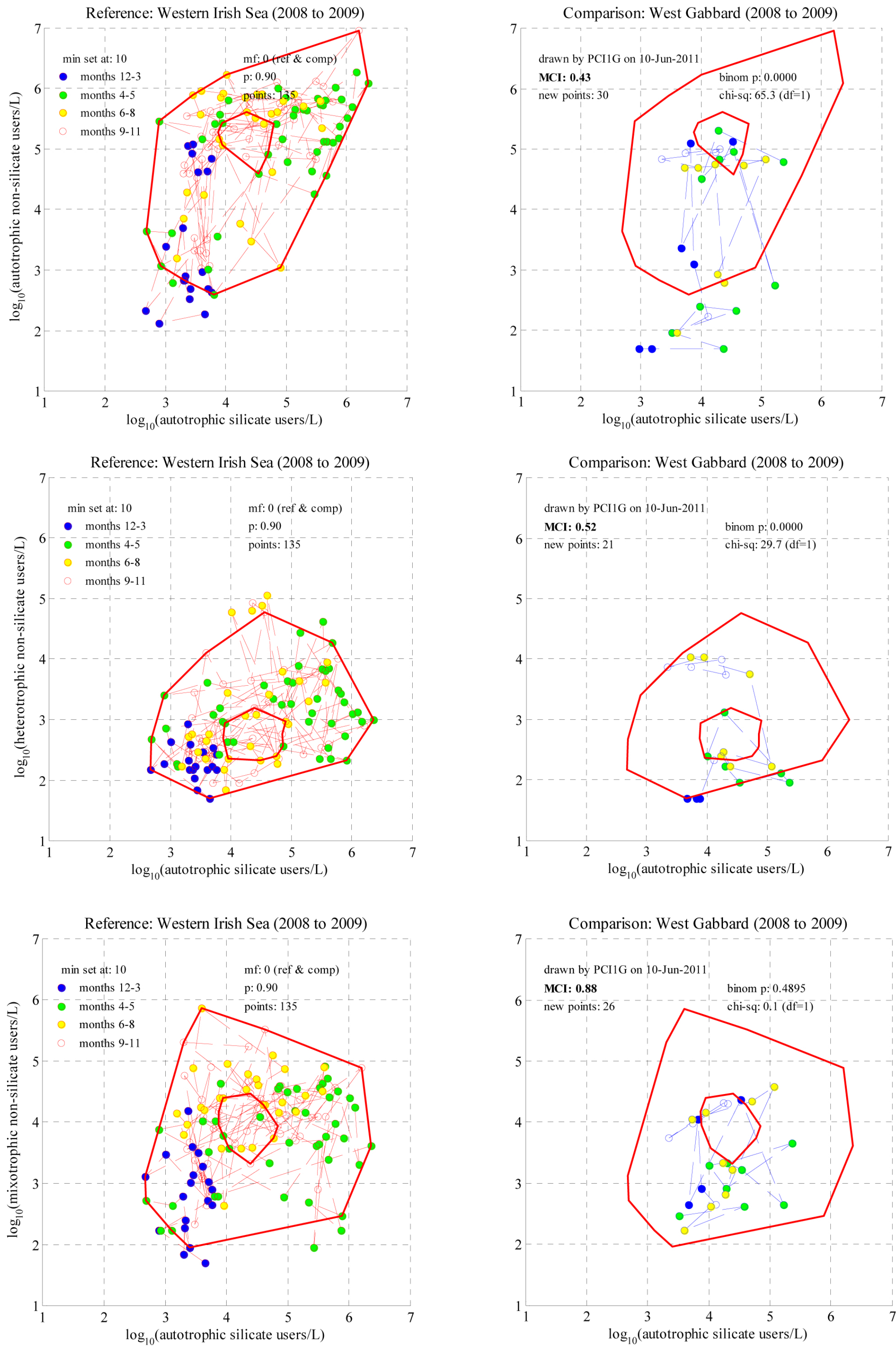


Figure 6.14: A comparison between the PCI and the MCI. The top row shows the diagrams of the results using the MCI program to calculate a PCI value (using only autotrophs for the data). The middle and the bottom row show the diagrams of the results of using the MCI program to calculate an MCI value using data for heterotrophs and autotrophs and data for mixotrophs and autotrophs and silicate users.

The shapes of the envelopes in all plots were different from each other which was expected as different lifeforms described different state spaces. The data of autotrophic silicate and non-silicate users were widely spread and highest numbers were reached in spring (months 4-5) and also in autumn (months 9-11). While it seemed that both lifeforms were equally represented during spring and summer, more autotrophic non-silicate users were present during autumn (month 9-11) and partly during the winter months (12-3). In the comparison of autotrophic silicate and heterotrophic non-silicate users, the data were more compressed and autotrophic silicate users were present in higher numbers. Spring was dominated by autotrophic silicate users while the summer and autumn showed a few high data for heterotrophic non silicate users and low numbers for all microplankton in general. The winter months were represented by low numbers of both lifeforms. Comparing autotrophic silicate users with mixotrophic non-silicate users the data points were widely spread. Autotrophic silicate users dominated the spring period although mixotrophic non-silicate users were also present in high numbers. In the summer and autumn months the presence of both lifeforms seemed equally high while more mixotrophic non-silicate users were present in the winter period. A 90%ile was used to exclude outliers in all three plots. Looking at the spatial comparison with the West Gabbard station all three MCI values were different from each other. The state space described by autotrophic silicate users and autotrophic non-silicate users at the West Gabbard comparison site was significantly different from the reference condition and the MCI value was 0.43. The difference of autotrophic silicate users and heterotrophic non-silicate users was also significant ($p = 0.00$; $MCI = 0.52$), while the comparison of autotrophic silicate users and mixotrophic non-silicate users was not significant ($p = 0.5$) and the MCI = 0.88 cannot be considered. The combination of two lifeform categories (silicate users/non-silicate users and nutritional mode) in this case was done on purpose to show that only in the non-silicate user lifeforms different nutritional modes existed (i.e. heterotrophic and mixtrophic dinofagellates and micro-flagellates, ciliates). For all following MCI plots lifeform combinations were not performed.

6.4.2 Cell Abundance and Carbon Biomass

Some pre-plots were performed to compare cell abundance (cells L^{-1}) and carbon biomass (μg C L^{-1}). In chapter 4 most of the results were presented in carbon biomass (i.e. seasonal succession, silicate and non-silicate users, small and large sized microplankton plots) as the variability of numbers in cell abundance was sometimes too high and not practical to plot. The main reason for using carbon biomass instead of cell abundance here was that of a better comparison with the results in chapter 4. The carbon biomass was determined in chapter 3 using the formulas given in 3.8, 3.9, 3.11 and 3.11, 3.12. For the sake of completeness an MCI cell abundance

diagram was compared to an MCI carbon biomass diagram in Figure 6.15. For all other following diagrams carbon biomass was used.

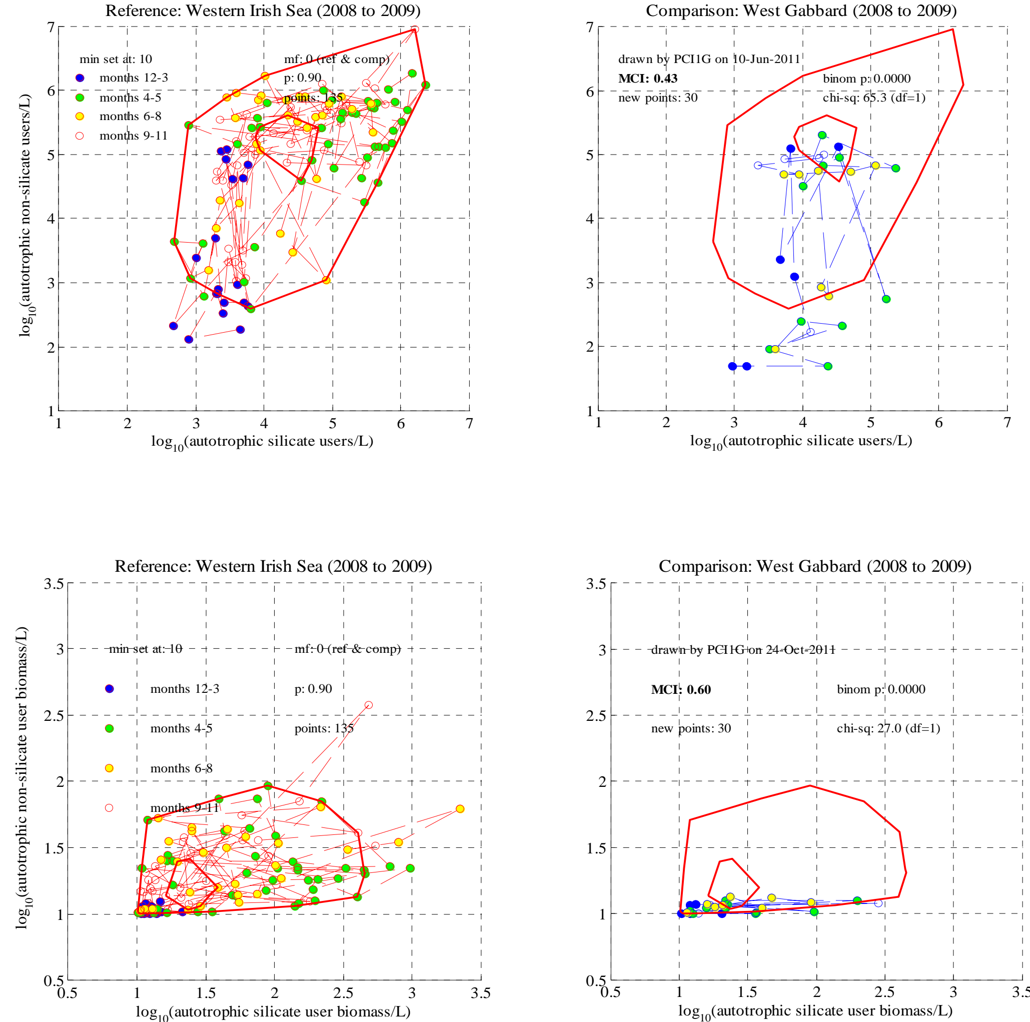


Figure 6.15: Illustration of a cell abundance diagram (top) compared to a carbon biomass diagram (bottom) for autotrophic silicate and autotrophic non-silicate users. The cell abundance diagram decribes a wide envelope with high numbers up to 7 on the $\log_{10}$ scale while the envelope described by carbon biomass is more compressed and reaches up to 3.5 on the $\log_{10}$ scale. Both comparisons with data from the West Gabbard show a significant MCI value although the values indicate that 17% more of the new data points fall out of the reference condition when considering cell abundance. The carbon biomass comparison shows more agreement between the sites (60%).

The diagrams in Figure 6.15 show that the envelope described by the cell abundance of autotrophic silicate users and autotrophic non-silicate users was wider and reached higher numbers compared to the carbon biomass envelope that seemed more compressed and was plottd on half the $\log_{10}$ scale of that of the abundance plot. The MCI values were both significantly different from 1, but 60% of the new points

of the carbon biomass comparison fell inside the reference envelope while more than half of the new point (57%) in the cell abundance plot fell outside the reference envelope although the envelope was bigger. The chi-square values give the propability of confidence that the community structure has changed. In the cell abundance comparison the chi-square value was 65.3 while it was 27.0 in the carbon biomass comparison giving a higher propability of confidence that a change in cell abundance between the autotrophic silicate users and autotrophic non-silicate users in the Western Irish Sea and the West Gabbard has taken place.

6.4.3 The MCI applied

The comparisons of the lifeforms defining state space plots decided upon in chapter 5 are presented in this subsection. First the results between station 38A and the West Gabbard station are given and then the comparisons of station 38A and the station in Liverpool Bay followed. The results of the comparison between the two test sites West Gabbard and Liverpool Bay are presented last.

6.4.4 Comparison of Stations 38A and West Gabbard

Silicate and non-silicate users

The hypothesis for the comparison of silicate and non-silicate users between station 38A and the West Gabbard station was that the MCI value significantly differs from 1 and the shift in the two lifeforms is toward south east due to increased mixing at the West Gabbard site which is expected to result in a lower biomass of non-silicate users, but also due to an increase in nitrogen and phosphate, which was expected to lead to an increase in biomass in general.

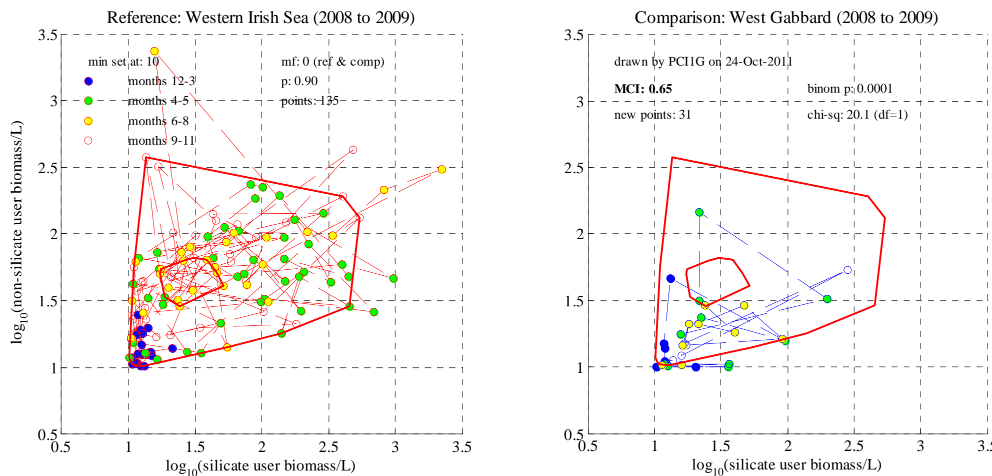


Figure 6.16: Spatial comparison of the carbon biomass of silicate users and non-

silicate users between station 38A and the West Gabbard station with 90% data consideration for the reference envelope.

A hundred and thirty five data points were used to create the reference envelope for silicate and non-silicate users and 31 data points from the WGabb station were plotted into the envelope for comparison (Figure 6.16). The value of MCI = 0.65 was significant (p = 0.00) and most of the WGabb data points were located in the south western part of the reference envelope. While there was a distinct seasonal pattern at station 38A (low numbers in winter, high numbers in spring and summer), the distinction was not so apparent at the WGabb station. Spring points varied from low silicate and non-silicate users numbers (1.1; 1.0) to high numbers (2.3; 1.5). The pattern indicated that there were not as many non-silicate users as there were at station 38A. The biomass of total silicate users was also less than at the reference site. The results confirmed half of the a priori hypothesis that was a move toward south. The move toward east was not observed and the pattern indicated a shift toward west rather than east.

Nutrition

For the nutrition comparison three combinations are presented (Figure 6.17). The top diagram shows the comparison of autotrophs and heterotrophs, the second diagram displays the autotrophs and mixotrophs comparison and the bottom diagram shows the comparison of mixotrophs and heterotrophs. The hypotheses for all combinations (autotrophs/heterotrophs; autotrophs/mixotrophs; mixtrophs/heterotrophs) was that the MCI value is significantly different from 1 and the shift is toward south west. It was expected that a lower biomass of non-silicate users were present at the WGabb station due to the mixed hydrodynamics in that region and consequently a reduction of all three nutritional modes despite the increased nutrients. The decrease in heterotrophs and mixotrophs was expected to be more pronounced than the decrease in autotrophs.

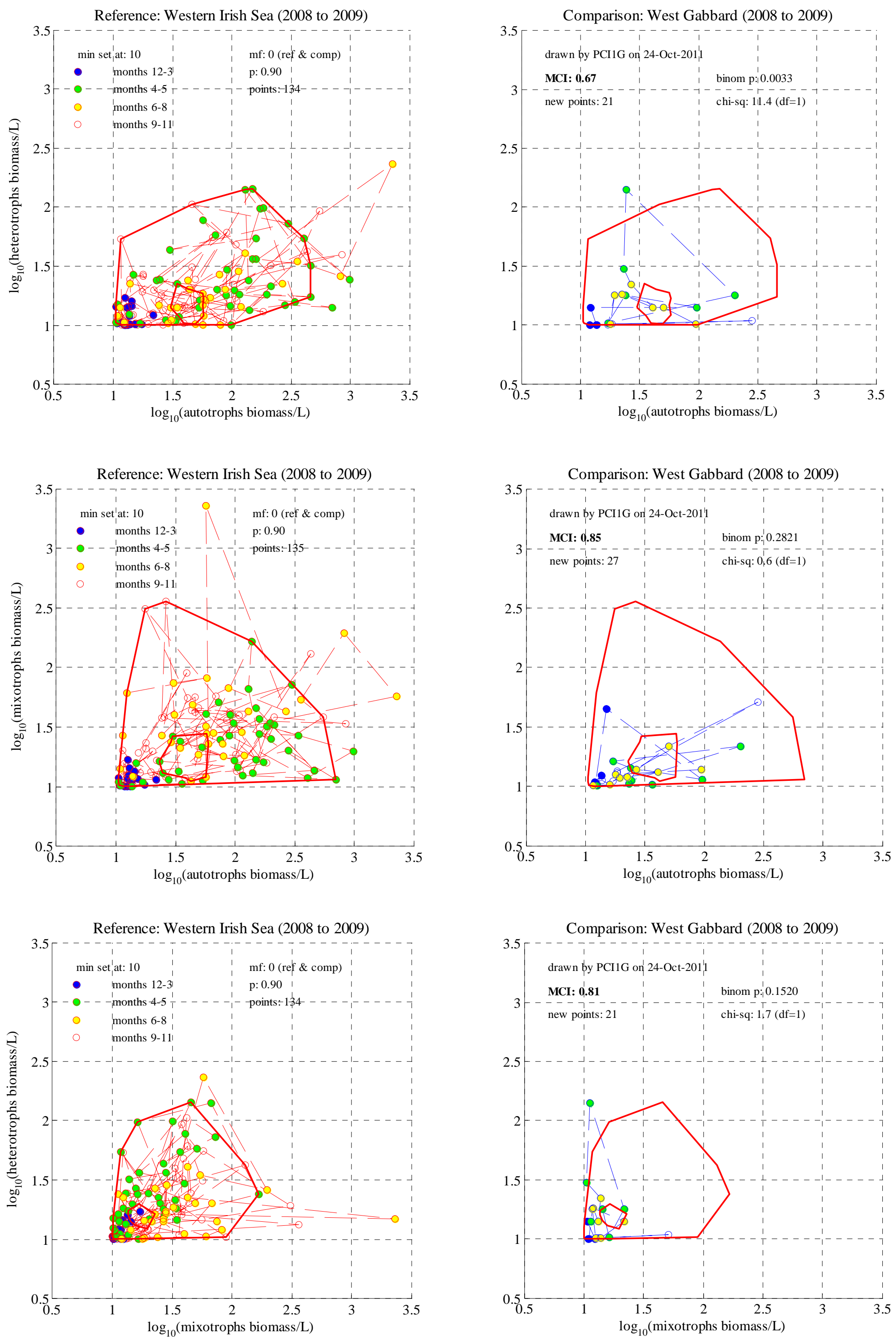


Figure 6.17: Spatial comparisons of the carbon biomass of autotrophic and heterotrophic, autotrophic and mixotrophic, and mixotrophic and heterotrophic microplankton between station 38A and the West Gabbard station with 90% data consideration for the reference envelope.

For the autotrophs/heterotrophs reference condition, 134 data points were available to create the envelope. Autotrophs were present in high carbon biomass in spring.

The highest number of autotrophs was observed in summer and this was also ture for the heterotrophs (Figure 6.17). Twenty-one data points from the West Gabbard were plotted into the state space and 67% of the new points (MCI = 0.67) fell into the reference envelope. The p value of 0.00 suggested that the difference between autotrophs and heterotrophs at reference and comparison site was significant. Generally, the new points were located in the south western part of the reference envelope indicating that there was lower biomass of heterotrophs and autotrophs. The predicted shift toward decreased carbon biomass and the a priori hypothesis was true.

For the reference condition of autotrophs and mixotrophs 135 data points were available to create the envelope. The winter data were concentrated in the south western corner of the diagram while the points for spring and especially summer showed a wide range. The data distribution indicated that mixotrophic biomass was nearly the same to the autotrophic biomass. For the comparison twenty-seven of the West Gabbard data were plotted into the reference envelope and the MCI resulted in 0.85 with p = 0.28 indicating that the difference was not significant. The comparison data points were mainly located in the south western part of the reference condition with high single data points for spring, autumn and winter indicating increased carbon biomass for the occassions. The observed pattern toward south west was not as expected and the MCI value was not significantly different from 1 and so the hypothesis was not supported.

The reference envelope of the mixotrophs and heterotrophs combination was create with 134 data points that were widely spread. The colour coded data points indicate that heterotrophs represented a higher carbon biomass than mixotrophs in the spring and mixotrophs contributed more to the summer carbon biomass. The twenty-one new points from the West Gabbard station that were plotted into the reference envelope showed a very similar pattern to the one displayed in the autotrophs and hetertrophs comparison but more compressed. The MCI resulted in 0.81 and the change was not significant (p = 0.15). The expected shift toward south west due to the mixed water column at the comparison site could not be confirmed and so the hypothesis was not true.

Size

For the size comparison a distinction between small ($<40\mu$M) and large ($\geq 40\mu$M) microplankton was made. For the comparison of the test site West Gabbard with the reference site 38A the hypothesis was that the MCI value is significantly different from 1 and the shift in the lifeforms is toward north west. It was expected to have lower biomass of small sized microplankton in the West Gabbard region due to mixing and higher biomass of large sized microplankton due to mixing and increased nitrogen and phosphate.

The reference envelope from station 38A was created with 135 data points (Figure 6.18). Both lifeforms contributed similarly to the carbon biomass during spring and summer while the biomass of the large sized microplankton was higher in autumn and winter. Highest numbers for small sized microplankters were recorded for a sample in summer (also apparent in the autotrophs comparison) while the highest number for large sized microplankton was also apparent in the summer period. Plotting the comparison data points (31) from the West Gabbard station into the reference envelope resulted in an MCI value = 0.65 with p = 0.00 indicating that the difference was significant. Most data points from the West Gabbard were located in the north western part of the reference envelope illustrating that there were not as many small microplankton compared to station 38A. Highest carbon biomass in the large sized lifeform was reached in spring while it was highest in autumn for the small sized lifeform. The shift was toward north west as predicted and the hypothesis was therefore true.

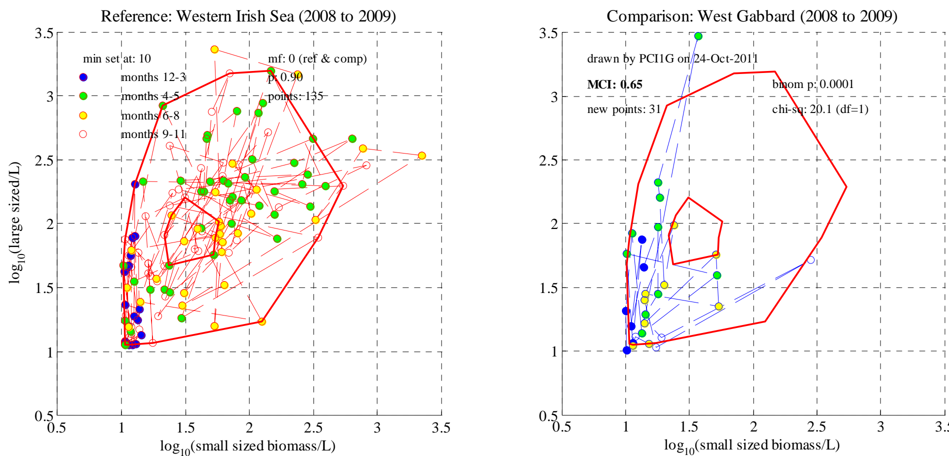


Figure 6.18: Spatial comparison of the carbon biomass of small sized and large sized microplankton between station 38A and the West Gabbard station with 90% data consideration for the reference envelope.

6.4.5 Comparison of Stations 38A and Liverpool Bay

Diatoms and Dinoflagellates

For the Liverpool Bay station comparison of diatoms and dinoflagellates, fifteen data points were plotted into the reference envelope of 135 data points (Figure 6.19). The hypothesis for the comparison of diatoms and dinoflagellates between the reference site 38A and the comparison site Liverpool Bay was that the MCI value is significantly different from 1 and the shift in the lifeforms is toward south east. The a priori reason for that was that due to mixing at the LBay station lower dinoflagellate biomass was expected (shift south) and due to increased nitrogen and

phosphate a shift toward higher diatom biomass (east) was expected. MCI value = 0.47 was significant (p = 0.00) and all data points were located in the southern part of the reference envelope. At the comparison site generally diatoms presented a higher carbon biomass than dinoflagellates although there were two occassions, one in summer, when dinoflagellates contributed similarly high amounts to the carbon biomass. Highest values for diatoms were recorded for summer. A shift toward increased diatoms was not apparent. The hypothesis was true for the shift toward south, but not toward east.

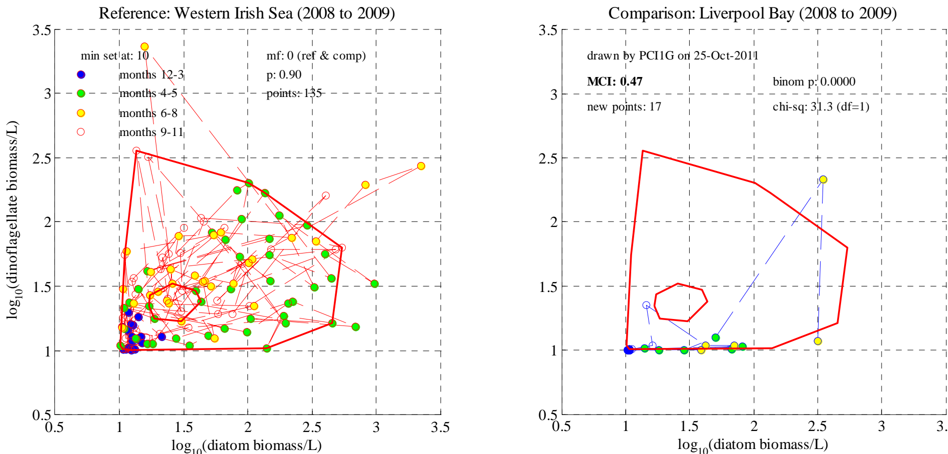


Figure 6.19: Spatial comparison of the carbon biomass of diatoms and dinoflagellates between station 38A and the Liverpool Bay station with 90% data consideration for the reference envelope.

Nutrition

The comparison of station 38A with the Liverpool Bay station in the different nutritions is illustrated in Figure 6.20. The hypotheses for all three combinations (autotrophs/heterotrophs; autotrophs/mixotrophs; mixtrophs/heterotrophs) was that the MCI value is significantly different from 1 and the shift is toward south west. It was expected that fewer dinoflagellates were present at the LBay station due to the mixed hydrodynamics in that region and consequently a reduction of all three nutritional modes, while the decrease in heterotrophs and mixotrophs was expected to be more pronounced than the decrease in autotrophs. As discussed in the method section only two observations that included heterotrophs were available using the intersection of the two datasets and no conclusion could be drawn. Therefore, the union of both datasets was considered for the heterotrophs comparison. Plotting the Liverpool Bay data into the western Irish Sea reference envelope resulted in an MCI value of 0.00 for autotrophs/ heterotrophs and mixotrophs/heterotrophs indicating a complete shift. Thirteen data points were available using the union and the shift was statistically significant. Apart from one data point indicating high carbon biomass contribution by heterotrophs in summer, the other twelve points

were located in the southern part of the autotrophs/heterotrophs lifeform diagram and south western part in the case of the mixotrophic/heterotrophic lifeform diagram indicating minimum carbon biomass values. The comparison of autotrophs and mixotrophs consisted of eleven new data points of which 45% fell into the reference envelope and the difference was significant ($p = 0.00$). The new data points were all located in the southern part along the x-axis of the reference condition. For this comparison the hypothesis was true considering the shift toward the south, but not toward the west.

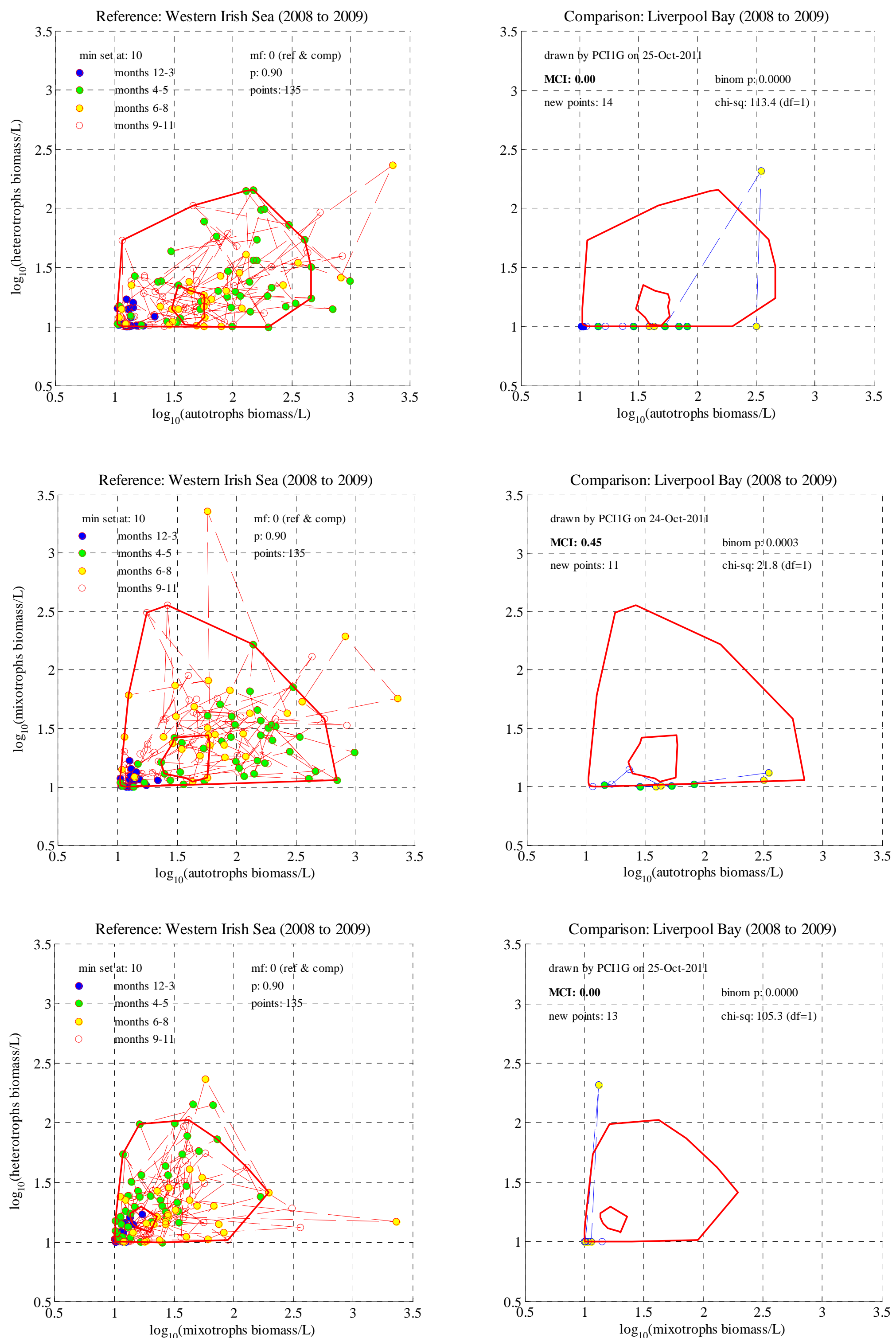


Figure 6.20: Spatial comparisons of the carbon biomass of autotrophic and heterotrophic, autotrophic and mixotrophic, and mixotrophic and heterotrophic microplankton between station 38A and the Liverpool Bay station with 90% data consideration for the reference envelope. For the comparisons with heterotrophs the union rather than the intersection of the datasets was considered.

Size

The size comparison with LBay station is given in Figure 6.21. The hypothesis for this lifeform combination was that the MCI value is significantly different from 1 and the shift is toward north west. The mixed hydrodynamics in the Liverpool Bay region were hypothesised to provide unfavourable conditions for small sized microplankton and therefore a decrease was expected (shift west). Due to increased nitrogen and phosphate at LBay station an increase in the large sized (diatoms) component was expected (shift north). Fourteen new data points were plotted into the state space and the MCI value resulted in 0.57 with p = 0.00 indicating a significantly shift. The diagram showed that the data points from Liverpool Bay were located in western part of the reference envelope indicating a similar pattern to that from the WGabb station and suggesting that compared to station 38A less small microplankton was present. Most of the data falling inside the envelope were pointing toward higher numbers of large sized microplankton indicating that this lifeform was more abundant at Liverpool Bay than it was at station 38A. Therefore the hypothesis was true.

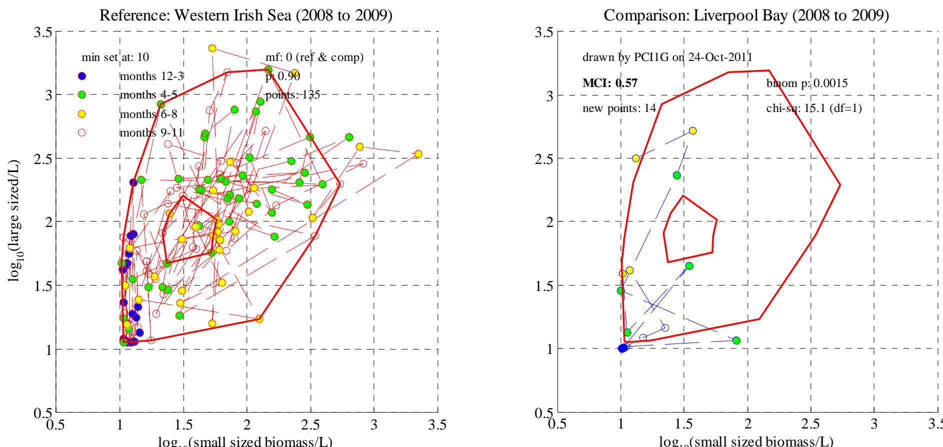


Figure 6.21: Spatial comparison of the carbon biomass of small sized and large sized microplankton between station 38A and the Liverpool Bay station with 90% data consideration for the reference envelope.

6.4.6 Comparison of West Gabbard and Liverpool Bay

Diatoms and Dinoflagellates

The comparison of diatoms and dinoflagellates between the two study sites West Gabbard and Liverpool Bay is given in Figure 6.22. The hypothesis for this lifeform combination was that the MCI value is significantly different from 1 and the shift is toward the east, because it was expected that an increase in nutrients at Liverpool Bay led to an increase in diatom biomass.

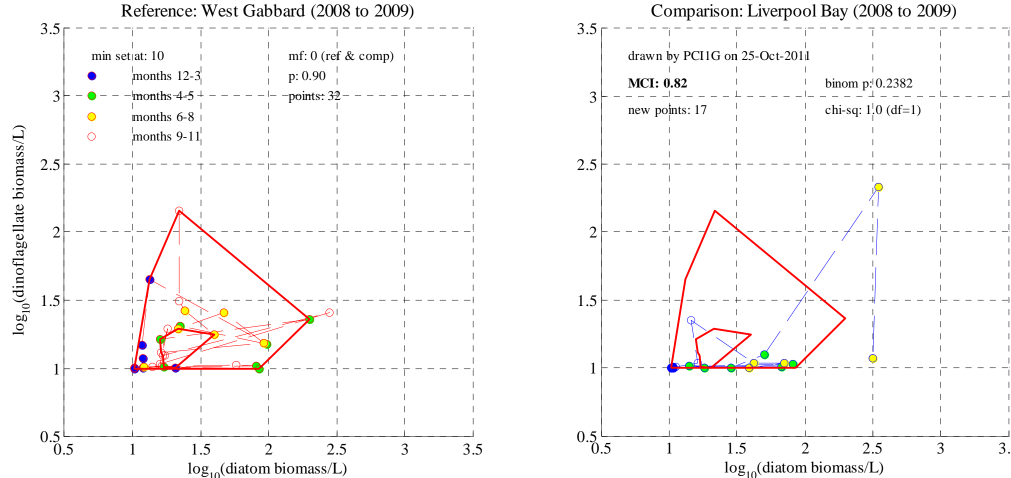


Figure 6.22: Spatial comparison of diatoms and dinoflagellates between the West Gabbard and Liverpool Bay stations with 90% data consideration.

Thirty data points were used to create the reference envelope. The data characterisation was described in the comparison with station 38A. A clear distinction of the seasons was not apparent. Fifteen new data points from the Liverpool Bay site were plotted into the reference envelope resulting in an MCI value = 0.82, that was not significant (p = 0.24). The shift of the diatoms and dinoflagellates lifeforms was toward the south east of the reference condition indicating that diatom biomass was increased at the LBay station and also that dinoflagellate biomass was decreased. The hypothesis was not found to be true as the shift was not significantly different from one.

Nutrition

For the comparison of the autotrophs and heterotrophs and the comparison of the autotrophs and mixotrophs between the West Gabbard as the reference site and Liverpool Bay as the comparison site the hypothesis was that the MCI value is significantly different from 1 and the shift is toward east. It was expected that more autotrophs were present at the comparison site due to increased nutrients. For the comparison with the heterotrophs only one data point was available using the intersection and so the union of both datasets was generated in those cases.

Thirty-one data points were used to create the reference envelope for the autotroph and heterotroph comparison and fourteen new data were plotted into that reference envelope. The MCI value resulted in 0.79 and the shift was not statistically significant (p = 0.16). Therefore the hypothesis for this comparison was not true.

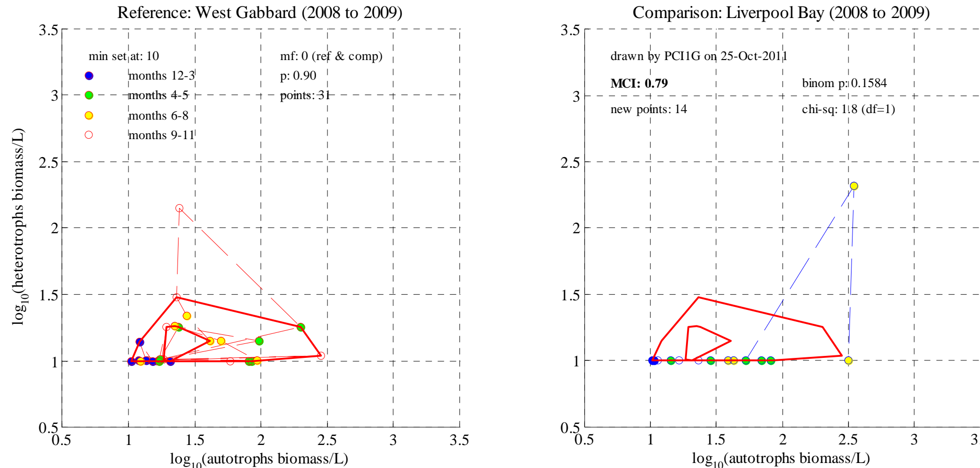


Figure 6.23: Spatial comparisons of the carbon biomass of autotrophic and heterotrophic microplankton between the West Gabbard and the Liverpool Bay stations with 90% data consideration for the reference envelope and using the union option for both datasets.

The reference envelope for the comparison of the autotrophs and mixotrophs between the West Gabbard as the reference site and Liverpool Bay as the comparison was created with twenty-seven data points (Figure 6.24). Eleven new data points from Liverpool Bay were plotted into the state space and 36% fell out of the reference envelope. p = 0.00 indicated a significant difference and the data located along the southern part of the diagram were shifted toward east. The hypothesis was true in its first part that the MCI value is significantly different from 1 and also that the shift was toward east, but the shift was also toward south and not so much pronounced toward east which was not expected.

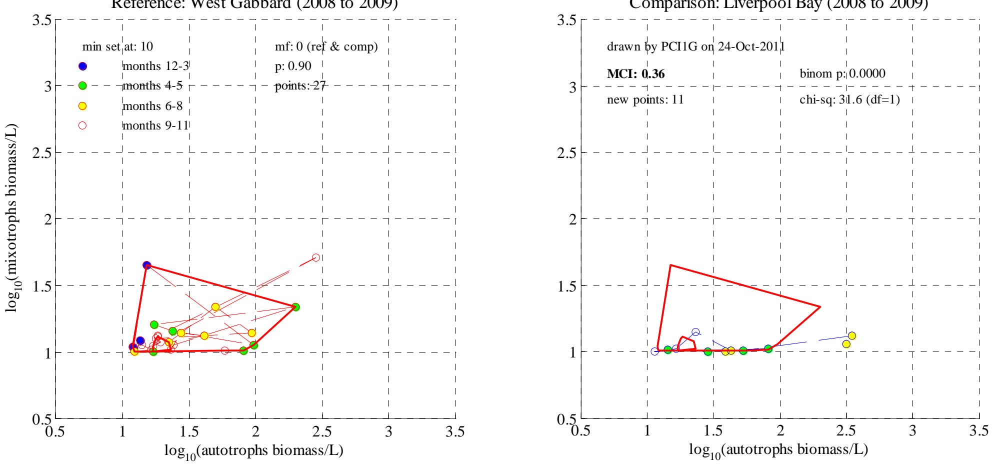


Figure 6.24: Spatial comparisons of the carbon biomass of autotrophic and mixotrophic microplankton between the West Gabbard and the Liverpool Bay stations with 90% data consideration for the reference envelope.

For the comparison of mixotrophs and heterotrophs between the WGabb station and the LBay station the hypothesis was that the MCI value does not differ from 1 and no shift is apparent. This was hypothesised because due to the mixed water column that was assumed to be similarly strong at both sites elevated dinoflagellate (i.e. heterotrophs and mixotrophs) growth was not expected. Applying the union to both datasets resulted in 27 data points for the reference envelope and thirteen data points for the comparison (Figure 6.25). The MCI value was 0.54 and the shift toward north west was found to be statistically significant (p = 0.00) proving the hypothesis wrong.

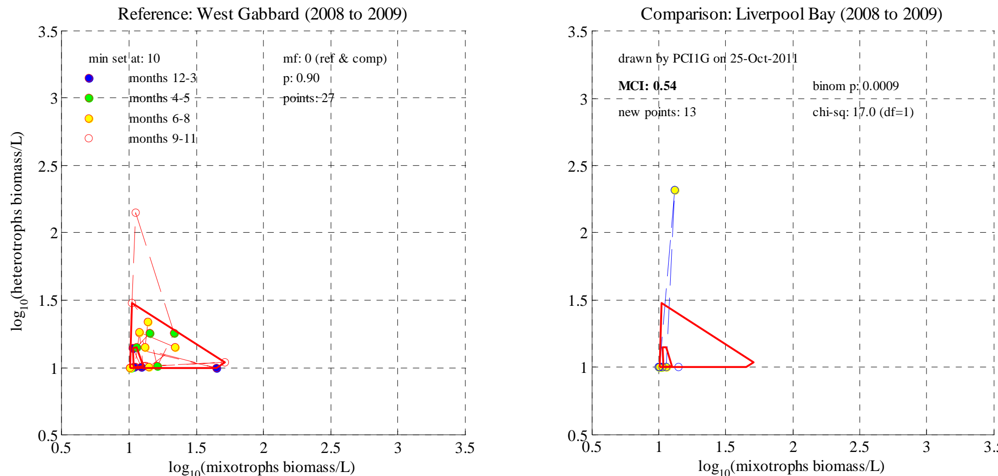


Figure 6.25: Spatial comparisons of the carbon biomass of mixotrophic and heterotrophic microplankton between the West Gabbard and the Liverpool Bay stations with 90% data consideration for the reference envelope and using the union option for both datasets.

Size

The comparison of the small and large sized microplankton between the two test sites West Gabbard and Liverpool Bay is illustrated in Figure 6.26. The hypothesis for this lifeform combination was that the MCI value is significantly different from 1 and the shift is toward north because it was expected to find increased carbon biomass of large sized microplankton at the Liverpool Bay station due to increased nitrogen and phosphate. Thirty-one data points were available to create the reference condition for the West Gabbard station. Twenty-one% of the fourteen new data points available from the Liverpool Bay station fell into the reference envelope and p = 0.00 indicated that this shift was significant. The data were widely spread but the tendency of most of the data outside the envelope was toward north west and the hypothesis was only half true.

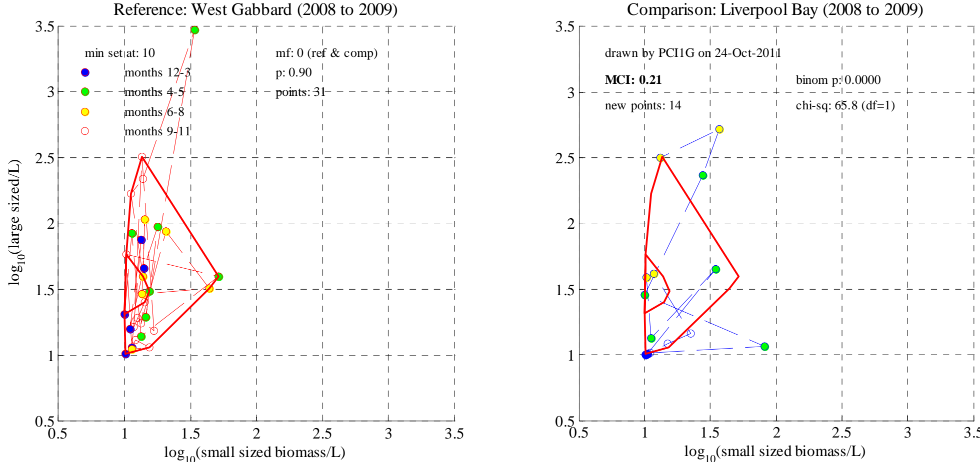


Figure 6.26: Spatial comparison of the carbon biomass of small sized and large sized microplankton between the West Gabbard and the Liverpool Bay station with 90% data consideration for the reference envelope.

6.5 Summary

6.5.1 Stations 38A and West Gabbard

The hypothesis that the MCI value significantly differs from 1 and the shift of silicate and non-silicate users is toward south east between station 38A and the West Gabbard station was found to be true in terms of the significant shit toward south, but not toward east. The hypotheses for all three nutrition combinations (autotrophs/heterotrophs; autotrophs/mixotrophs; mixtrophs/heterotrophs) between station 38A and the West Gabbard station was that the MCI value is significantly different from 1 and the shift is toward south west. This was true for the autotrophs and heterotrophs combination. For the autotrophs and mixotrophs this was not true although most of the points were located as hypothesised, the shift was not significant. For the mixotrophs and heterotrophs this was similar, the main part of the points were located as hypothesised, but the shift was not significant. The hypothesis that the MCI value is significantly different from 1 and the shift in small and large sized microplankton between station 38A and the West Gabbard station is toward north west was found to be true.

6.5.2 Stations 38A and Liverpool Bay

The hypothesis for the comparison of diatoms and dinoflagellates between the reference site 38A and test site Liverpool Bay was that the MCI value is significantly different from 1 and the shift in the lifeforms is toward south east. This was found to be true as the shift was significantly different from 1 toward south east.

The hypothesis for all three nutrition combinations (autotrophs/heterotrophs; autotrophs/mixotrophs; mixtrophs/heterotrophs) between station 38A and the Liverpool Bay station was that the MCI value is significantly different from 1 and the shift is toward south west. This was not true for any of the lifeforms. The shift of autotrophs and mixotrophs was toward south but not west. For the autotrophs and heterotrophs and the mixotrophs and heterotrophs one high carbon biomass peak pointed toward north, but the remaining data points were concentrated in the southern/south western part of the reference envelope. Therefore, the hypothesis could not found to be true.

The hypothesis that the MCI value is significantly different from 1 and the shift in the small and large sized microplankton combination between station 38A and the LBay station is toward north west was found to be true.

6.5.3 Stations West Gabbard and Liverpool Bay

The hypothesis for diatoms and dinoflagellates between the West Gabbard station and the Liverpool Bay station was that the MCI value is significantly different from 1 and the shift is toward east. The hypothesis was not true as the shift was toward south east and was not significant.

The hypothesis for the autotrophs and heterotrophs combination between the WGabb and LBay station was that the MCI value is significantly different from 1 and the shift is toward east. When the union option was applied to both datasets, the detected shift was not statistically significant although it was toward east. The hypothesis was not true.

The hypothesis for the autotrophs and mixotrophs between WGabb and LBay was that the MCI value is significantly different from 1 and the shift is toward east. This was found to be half true. Although the shift was significant most of the data were located in the south eastern part of the reference envelope.

The hypothesis for the mixotrophs and heterotrophs combination between WGabb and LBay was that there is no shift between the two sites. The hypothesis was not true, because the MCI value differed significantly from 1.

The hypothesis that the MCI value is significantly different from 1 and the shift in small and large sized lifeforms is toward north between WGabb and LBay was found to be half true because the shift was also toward west.

6.6 Discussion

Microplankton as primary and secondary producers play the key role in the production of organic matter and transferring the energy of this matter to other components of the marine food web. Thus these micro-organisms are critical to the health of marine ecosystems. Changes in microplankton structure may influence this health and could therefore provide important information about the status of the pelagic part of the marine ecosystem. For this reason a microplankton community index (MCI) was developed during this study. It is based on the concept of the phytoplankton community index (PCI) and it was aimed to extend the PCI to a more complete indicator of change by incorporating the heterotrophic and mixotrophic components of microplankton. The distinction between autotrophs, heterotrophs and mixotrophs can be a difficult task, it requires special equipment and time, both often not given in monitoring programmes of microplankton analyses. For these reasons the distinction of microplankton nutrition is often not integrated in the routine to establish long-term data bases. However, it is important that nutritional modes

of the microplankton are distinguished because they are of vital importance as they bear directly on trophic interactions as discussed in chapter 2 and can provided information on eutrophication in relation to biodiversity and food webs. With the directives requirement of an ecological approach in assessing ecosystem structure and functioning it is inevitable that this important component is included in a tool that describes microplankton community structure.

6.6.1 PCI and MCI comparison

The microplankton community index incorporates the heterotrophic and mixotrophic part of the microplankton in order to provide more insight into microplankton structure than the published and tested PCI (Tett *et al.*, 2008) already does. The results of the comparison of the PCI and the MCI were expected to differ (Figure 6.14), because different lifeform plots lead to different state spaces. In this comparison, the results showed that at station 38A more autotrophic non-silicate users were apparent than at the West Gabbard station. The heterotrophs were also more abundant at station 38A than they were at the comparison site. These differences provide more insight into microplankton structure than the examination of only the autotrophic part of microplankton and can indicate the level of primary and secondary production giving useful information in relation to trophic levels and in the context of eutrophication and human pressure on the marine pelagic food web that the autotrophic component of microplankton alone could not provide. However, the two sites differed in hydrodynamics and therefore the causes of the differences between reference and comparison microplankton here need to be interpreted with care. If hydrodynamics were the only difference between the two sites, a shift toward south would have been expected as diatoms (autotrophs) typically dominate mixed and intermittently stratifying regions. If nutrient differences were the only difference between the two sites, a shift toward north east (i.e. an increase in biomass of all microplankton) would have been expected due to a nutrient increase. As mentioned in the results section, the combination of two lifeform categories (silicate users/non-silicate users and nutritional mode) in this case was done on purpose to show that only in the non-silicate user lifeforms different nutritional modes existed (i.e. heterotrophic and mixtrophic dinofagellates and micro-flagellates, ciliates). For the following MCI plots this combination approach was not applied because it was aimed for one distinct lifeform set (two state variables) that was able to define a state space that could provide information on the structure of microplankton community and its potential change due to eutrophication. For further development of the MCI it was important that each state variable that was added to the state space in order to provide more information on microplankton community structure was independent of the existing set on a statistical basis (degree of freedom), as

discussed in chapter 5 (subsection 5.4.1). This step in the development of the MCI was also a reason for keeping lifeform combinations simple.

6.6.2 Station 38A and the West Gabbard station

As discussed in chapter 4, the western Irish Sea was considered to be in a near pristine state, with low maximum nutrient concentrations (Gowen & Stewart, 2005; Gowen *et al.* 2008, and the results from this study) and high chlorophyll and carbon biomass during spring with growth limitation during summer (Gowen & Bloomfield 1996; Trimmer *et al.* 1999; Gowen *et al.* 2008). Data from the region around the AFBI mooring site described a seasonally stratified water body with little or no freshwater influence (Gowen *et al.* 1995; Horsburgh *et al.* 2000). In contrast, the West Gabbard was moderately nutrient enriched with low chlorophyll and carbon biomass probably due to light limitation (Tett *et al.*, 1993), a region of freshwater influence (Tett *et al.*, 2007) and high tidal energy perhaps with intermittent stratification because of freshwater effect (Sanders *et al.* 2001; Mills *et al.* 2003). As discussed in chapter 4, the two stations were expected to differ in their microplankton composition because of differing hydrodynamic conditions and also because of their different nutrient loadings. The general shifts were assumed to be toward south, because of the mixed water column at the West Gabbard station, less non-silicate users were expected and east, because higher nutrients at the southern North Sea site were expected to result in higher biomass. The shift toward south east in silicate and non-silicate users between station 38A and WGabb was not found to be true. In contrast, a shift toward south west was found to be statistically significant (MCI= 0.65, $p<0.05$). The results from chapter 4 showed that the West Gabbard station had a lower average carbon biomass than station 38A and the dominant lifeform was diatoms which are autotrophic silicate users.

Considering the nutritional lifeform combinations, the shift between autotrophs and heterotrophs was the only statistical significant shift recorded. For the autotrophs and mixotrophs and the mixotrophs and heterotrophs the shift was in the hypothesised direction but was not significant ($p > 0.05$). This suggested that the community structure for the autotrophic and mixotrophic microplankton at WGabb was similar to the one at 38A. The findings were unexpected. However, as reported in chapter 4 both stations had a similar amount of *Prorocentrum* species and small *Gymnodinimum* species that were distinguished as mixotrophic species. The shift in small and large sized category was expected to be toward north west, because the results from chapter 4 showed that the diatoms mainly identified at the West Gabbard station were large sized diatoms such as *Guinardia flaccida* and *Navicula* sp. . The MCI result was significant and the shift confirmed this expectation. Thus it has been demonstrated that the MCI tool could detect the expected differences

and shifts in microplankton community structure between station 38A and WGabb related to the differences of prevailing hydrodynamics.

6.6.3 Station 38A and the Liverpool Bay station

As reported in chapter 4, Liverpool Bay was considered an intermittently stratifying (Gowen & Bloomfield, 1996) region of freshwater influence (Tett *et al.*, 2007) with high nutrient enrichment (Gowen *et al.*, 2002; Gowen & Stewart, 2005; Mills *et al.* 2005) and high chlorophyll and carbon biomass (Allen *et al.* 1998; Mills *et al.* 2005; Gowen *et al.* 2008). It was subject to nutrient enrichment as it has demonstrably suffered the first two stages of eutrophication (nutrient enrichment and enhanced biomass). However, Gowen *et al.* (2008) concluded that there was no eutrophication in the strict sense of the UWWTD definition of eutrophication as the region had not appeared to have suffered "undesirable disturbance".

The comparison of station 38A and Liverpool Bay data showed similar results to the comparison of station 38A with the West Gabbard. Diatoms were found to be the dominant lifeform and dinoflagellates did not contribute a big amount to the total carbon biomass in Liverpool Bay. This was consistent with the expectation that diatoms have a higher biomass input in mixed conditions while non-silicate users such as dinoflagellates and micro-flagellates were found in stratified conditions as discussed in chapter 4. The shift of the diatom and dinoflagellate state space was expected to be toward south due to low biomass of dinoflagellates and east due to the high biomass of diatoms at this station as it was observed in chapter 4. This expectation was confirmed by the MCI results. From the results in chapter 4 one high data point for dinoflagellates was expected in the summer months that reflected the occurrence of the large dinoflagellate *Noctiluca scintillans* on day 189 (8th July 2009). The high peak was apparent in the MCI plot.

The expected shift for the nutritional modes of the microplankton at station Liverpool Bay could not fully be investigated. The hypothesis for the autotrophs and mixotrophs was true and the expected shift toward south west was confirmed by the tool. However, the other two combinations (autotrophs and heterotrophs and mixotrophs and heterotrophs) provided only two new data points for the comparison site using the intersection option. The union option brought different results. The MCI value was not significantly different from 1 ($p<0.05$) for the autotrophs/heterotrophs state space, but for the mixotrophs/heterotrophs state space (MCI = 0.54; p = 0.00). However, a lot of points (12 data points) represented the "no-see" value, indicating that heterotrophs and mixotrophs were present in very low abundance. From the results in chapter 4 it was apparent that the presence of non-silicate users was generally low and most of them were exclusively autotrophic.

Therefore, low numbers were expected for these nutritional comparisons. A big part of the microplankton samples collected at the Liverpool Bay station were impossible to analyse due to too much sediment in the sample, which led to only five samples in 2009 for Liverpool Bay. The appearance of high suspended sediment could be an indication of strong mixing with expectation of either low total microplankton or a low proportion of dinoflagellates. Confirmation of the assumption could not be made and the results raised issues on minimum datasets that were discussed earlier on.

The expected shift toward the west in the comparison of small and large sized microplankton between station 38A and the Liverpool Bay station was found to be statistically significant. The shift was expected because as reported in chapter 4, carbon biomass in Liverpool Bay was dominated by large diatoms including *Coscinodiscus* spp., *Rhizosolenia* spp. and *Guinardia* spp.. One data point in spring (green) indicated high carbon biomass for small sized microplankton and could be related to maximum numbers of the flagellate *Phaeocystis* spp. that was observed on day 146 26th May 2008. The MCI tool was able to detected the differences between small and large sized microplankton as well as the differences between the diatoms and dinoflagellates and confirmed the interpretation of the results in chapter 4. Like the results from the comparison of station 38A and the West Gabbard, the differences between station 38A and Liverpool Bay seem more explicable by the differences in hydrodynamics than those by nutrients.

6.6.4 Reference Conditions

A key element of the MCI application is the comparison to a reference condition. According to the MSFD these reference conditions should be representing GES, which means that a condition has to be found in which the status of microplankton is deemed to represent good environmental status. It is a challenging task to find and define reference conditions like that as it needs "expert opinion" on the consideration of GES in microplankton. One option of identifying a "normal domain" (i.e. condition that reflects good environmental status) could be to use multi factorial analysis of time series of a microplankton community index and the environmental conditions and pressures together with ecosystem - model derived simulations (Paul Tett pers. comm.). But to reach this stage a lot of work and financial support is required and the time frame given by the MSFD to achieve GES in 2020 and the budget available for all member states might not allow this investigation. An alternative approach is a "trend" approach that is more immediately useful because it does not require further research. This "trend" approach is looking at no trend in a microplankton community index and where large datasets exist "no persistent change from the normal domain". In problem areas a persistent change away from the normal do-

main would be apparent and in this case a trend back toward the normal domain correlated with a decrease in pressure such as nutrients would be desirable. The difficulty to identify change due to nutrients is the influence of long term changes such as climate change. With assessing the ecosystem health it might be possible to deal with those changes, but that would exceed the structure methodologies focused on in this study as ecosystem health is described not only by its structure but also by its function and resilience. When assessing a GES for microplankton in a spatial comparison, it is important that the hydrodynamical characteristics of the sites are similar because as demonstrated in the comparisons between station 38A and the two test sites, hydrodynamics can have a bigger influence on the microplankton community structure than pressures such as nutrient enrichment. For this study I chose the West Gabbard station as a reference condition to the Liverpool Bay station because I knew that the hydrodynamics were alike and the West Gabbard station was half as nutrient enriched as the Liverpool Bay station. However, the West Gabbard station was not proven to be representing GES as required by the MSFD. The reference condition here was aimed to represent a better or healthier status than the comparison site judged by the nutrient concentrations. In my opinion this was the case. However, the main objective in this study was to aim for providing a part of a tool for management, able to show whether a condition is changing, regardless of the direction toward good or bad. Plotting values of the abundance of several lifeforms belonging to the microplankton into state-space, enables to track changes in the condition of the microplankton community by means of comparing state-spaces of new points with a reference envelope. A confirmation as to whether the West Gabbard station indeed represents GES merits further investigation and temporal analysis including the trend approach described.

6.6.5 The West Gabbard and the Liverpool Bay Stations

The results of the lifeform comparisons between the development site and the two test sites showed that the MCI was indeed able to detect and indicate shifts in microplankton community structure that were hypothesised and expected from the results in chapter 4. With the confidence that the MCI operated and functioned correctly and could detect change, it was then applied to the two test sites that were hydrodynamically similar but differed in their nutrient loadings. In some cases, change in the microplankton community (from diatoms to flagellates) would be observed as a consequence of change in N:Si ratios caused by anthropogenic nutrient enrichment (Cadeée & Hegemann 1986; Riegman, 1992). Liverpool Bay, as mentioned before, was subject to the first two stages of eutrophication (Gowen *et al.* 2008). However, the anthropogenic nutrient enrichment in this Bay (Gowen *et al.* 1999, 2000) did not cause an obvious increase in non-silicate users and hence no ev-

idence of a shift from the dominant diatoms to another lifeform was observed (Tett *et al.* 2007). In order to assess whether the status of Liverpool Bay has remained the same, the MCI was applied to compare it to a less nutrient enriched, hydrodynamically similar site (West Gabbard). The overall hypothesis for this comparison was that the MCI values significantly differ from 1 and that a shift is always toward higher biomass due to the higher nutrient levels observed in Liverpool Bay.

Results from the first PCI application published by Tett *et al.* (2007) comparing the less-enriched outer and more-enriched inner waters (Liverpool Bay) of the eastern Irish Sea showed no significant difference in plankton community structure and confirmed the finding by Gowen *et al.* (2008) that Liverpool Bay has not suffered "undesirable disturbance" in the context of eutrophication. The results of this study showed that the diatom/dinoflagellate state space in Liverpool Bay did not differ significantly from one and therefore from the reference condition. The MCI values for the autotrophs/mixotrophs and the mixotrophs/heterotrophs conditions were significantly different from one and so was the MCI value for the small/large sized lifeform condition between the West Gabbard and Liverpool Bay. The shifts were toward the hypothesized direction that was expected due to increased nutrient input at the comparison site (Liverpool Bay) but were not toward increased biomass of diatoms and dinoflagellates or a shift toward more flagellates, nor was there an increase in the small sized fraction of microplankton - all shifts that would indicate an alteration of the N:Si ratio from 1:1, elevated chlorophyll and biomass, different trophic levels (less export of energy caused by moving toward smaller organisms) and eventually eutrophication. Two of the hypothesis were rejected because the shift was found not statistically significant. The one hypothesis where no shift was hypothesized had to be rejected because the shift between the mixotrophs/heterotrophs condition at WGabb and LBay was found significant. Two hypothesis were supported. But even in these two cases the shift toward increased carbon biomass in autotrophs in the autotrophs/mixotrophs comparison and the increase in large sized microplankton in the small/large sized comparison due to increased nitrate and phosphate was minimal. Considering the nutrient and chlorophyll concentrations of Liverpool Bay data along with the prevailing hydrodynamics, it was assumed that low numbers of dinoflagellates were found similar to those in the West Gabbard and due to higher nutrient loadings in Liverpool Bay, it was assumed that more diatoms would cause a shift toward east. The reason why the MCI did not reflect the high biomass at Liverpool Bay that was reported in chapter 4 and by Gowen *et al.* (2008) for previous years was a bit surprising and an explanation for this could not be found. If the MCI results were reliable, it would indicate that the third stage of eutrophication was still not reached at Liverpool Bay in 2008 and 2009. However, only a small dataset of microplankton samples was available for Liverpool Bay in 2008 and 2009 and this was assumed to have had a big influence on the results of the MCI values.

In 2009, only five samples between mid spring and late summer were available and hardly captured the seasonal cycle of the microplankton. The indication that less dinoflagellates were apparent at Liverpool Bay was probably due to the incomplete sample analyses and led to the assumption that more dinoflagellates were apparent at the West Gabbard station which could not be confirmed. The lower diatom numbers recorded for Liverpool Bay could possibly be explained by a possible TOxN limitation indicated during early summer in 2009. The silicate limitation (Sanders *et al.* (2001) and findings in this study) suggested for the WGabb site could have had a small influence on the higher abundance of dinoflagellates. Both reasons, the incomplete data set for LBay and the possible silicate limitation at WGabb, were also likely to explain the higher numbers of mixotrophs at WGabb. For the higher numbers of autotrophs and small sized microplankton at WGabb an additional reason was found. At Liverpool Bay micro-flagellates were not counted but recorded with a roughly estimated number when they occurred in high abundances. The different examination methods by the analysts, the silicate limitation at the West Gabbard site and the possible TOxN limitation at the Liverpool Bay site could explained the detected shifts by the MCI in the comparison between those two sites.

6.6.6 Interpretation of the Results

The differences between the two test study sites could be interpreted in several ways:

1. The West Gabbard reference condition did not represent good environmental status and the small shifts indicated that the microplankton community structure at this site is in similar condition to the Liverpool Bay site. This was doubtful, because Liverpool Bay was subject to eutrophication in the near past (2007 as reported by Gowen *et al.* 2008) and although the values of nutrient, chlorophyll and biomass recorded for this study were lower than in former years they were still higher than the values recorded for the WGabb station.

2. The prevailing hydrodynamics were not as similar as it was assumed when the sites were chosen and therefore the microplankton communities were different due to difference in hydrodynamics. This could be possible, because many data at both study sites were not available and for the West Gabbard sites not much information was provided by publications as it is a relatively new mooring site and it has not been fully investigated. The light conditions of the two site were assumed to be different which ought to have an effect on the microplankton community structure. This could not be investigated during this study, but it merits further analysis. Another point that could indicate more turbid conditions in LBay were the many samples excluded from analysis as too much sediment prevented reliable counts. More such

samples were dismissed at Liverpool Bay than at the West Gabbard. However, two hypothesis made about the five lifeform comparisons were true and indicated a significant shift, the others indicated that there was no significant difference between the lifeform state spaces of WGabb and LBay.

3. The dataset of the comparison site was not large and detailed enough to delivered reliable results. This interpretation seemed the most likely. The incomplete data implied the possibility of missing the highest levels of nutrients and microplankton chlorophyll preventing any conclusion on the relationship of increased nutrient enrichment and changes in microplankton community structure.

6.6.7 Conclusions of the Results

In the attempt to gather all information about the two dimensional comparison sets between the West Gabbard and the Liverpool Bay stations, an overall MCI value was calculated. This was done by adding all single MCI values for each lifeform comparison providing an average MCI value. Where the MCI value was not significant (in the case of the autotrophs and heterotrophs comparison), it was assumed that there was no shift and an MCI value of 1 was added. For the nutrition category the three combinations (autotrophs/heterotrophs, autotrophs/mixotrophs, mixotrophs/heterotrophs) were added and divided by three before the value was added to the taxonomy and size values. The resulting overall MCI value for the comparison of the two test sites was MCIall = 0.61 indicating that 61% of the data points from Liverpool Bay fell inside the reference envelope of the West Gabbard data. What does this difference mean? It could be the result of a "normal" inter annual variation without indicating a persistent shift in regime. Nutrient effects could be possible as some of the shifts were in the direction expected in the context of eutrophication. To interpret this MCIall value and fit it in the context of assessing GES, the trend approach mentioned in subsection 6.5.4 could be applied. The idea was to plot this MCIall value over time and examine whether or not a trend was apparent and if this trend could be related to a trend in anthropogenic pressure such as nutrient enrichment over the same time period. If this was the case then deviation from GES could be confirmed. This approach was a result from a workshop of experts held in Belfast on 2nd and 3rd June 2011 in which I participated.

6.7 Further Work

The next step would be to examine if the shifts detected between Liverpool Bay and West Gabbard followed a trend related to time. With sufficient data a comparison could be made for individual years and the overall MCI value could be calculated for

each of those years and displayed in a time series plot. For example, if a decreasing trend for MCIall was apparent over a period for e.g. four years and this trend was related to a decreasing trend in nutrients this could indicate a shift away from eutrophication for Liverpool Bay.

One state variable space that maybe should be considered in the future, because it is explicitly mentioned and dealt with in the MSFD are harmful algal blooms. They were not in the focus of my study and also not considered, because it is difficult to direct them to lifeforms. As reviewed in chapter 2 there is no function or “deep taxonomy” that unites them. For example *Alexandrium minutum* has strains that produce toxins and some that do not (Touzet *et al.* 2007). However, as HABs are important and explicitely recognised by the MSFD in D5 of annex 2, one idea could be to define a state variable space with potential toxin producing dinoflagellates and *Pseudonitzschia* spp. the only toxin producing diatom that we know of. Because any toxin producing microplankton is ‘undesirable’ when it occurs beyond the natural abundance and frequency, any change from the south western part of the state space (low numbers of both lifeforms) and thus an increase in either lifeform could be regarded as ‘undesirable’. This idea also evolved during a Defra workshop held in Belfast 2-3 June 2010 on defining lifeforms for plankton indicators in which I participated.

6.8 Conclusion

The purpose of this study was to investigate and examine issues such as minimum datasets, weaknesses in the MCI approach, and to lay out a path for solutions and provide suggestions in order to improve, change and apply the microplankton community index. Most of the results demonstrated here supported the purpose to turn the MCI tool into a robust and reliable indicator of change. It was important to highlight where the approach merits further work and investigation in the future and where results were already satisfying.

The study showed that the MCI is fully functional and can detect differences in microplankton community structure. The results also showed that the MCI extended the PCI to a more complete indicator of change by incorporating the nutritional modes of the microplankton. By restricting the data consideration to the 90%ile it is believed that the MCI provides a reference envelope more sensitive to change than using a greater %ile. The minimum dataset investigation and the application of the intersection and union of data showed that small datasets cause problems. For reliable results it is of vital importance that datasets are established by consistent sampling throughout the year and consist of at least monthly sample points over a period of 3 years for the reference condition. For the comparison condition at least

monthly samples are required over a twelve months period. The “flat bottom” issue is avoided by settling a larger sample volume, especially in the winter when growth is minimal. Furthmore, the results showed how important it is to follow the same identification and enumeration procedure to arrive at reliable MCI values.

It could not be investigated if there was a relationship between the decrease of nutrients in Liverpool Bay and the shift in microplankton community structure. This could be a future aim embedded in further investigation of small datasets.

Conclusively, this study laid a path for the conceptual ideas in detecting change in microplankton community structure and proved that the MCI is operational and can be applied to other monitoring sites in UK waters. The following list enumerates the key points that are required to deliver reliable MCI values:

1. reference dataset needs to comprise fortnight or at least monthly samples over three years
2. comparison dataset needs to comprise fortnight or at least monthly samples over one year
3. datasets have to be established incorporating the same sample analysis procedures
4. similar hydrodynamics for spatial comparisons
5. reference site should be in GES

Chapter 7

General Discussion

7.1 Synthesis

Changes in microplankton composition have been used as evidence of undesirable disturbance in the context of eutrophication. However, in trying to place undesirable disturbance uniquely in the context of eutrophication, two difficulties remain (Tett *et al.*, 2007). First, some disturbances of marine ecosystems occur naturally and second, undesirable disturbance might be caused by a mixture of pressures of which nutrient enrichment is only one. Traditionally, bulk indicators such as chlorophyll and winter nutrient concentrations were used to assess the status of marine ecosystems in relation to anthropogenic nutrient enrichment. However, these univariate indicators do not provide adequate insight into ecosystem structure and functioning that is required by the Marine Strategy Framework Directive (MSFD 2008/56/EC). Microplankton is the base of the marine pelagic food web and their productivity and seasonal and inter-annual variability are key elements of the structure and functioning of the pelagic ecosystem. Changes in microplankton can influence the supply of organic matter and hence energy to higher trophic levels. However, it is also evident that top down changes such as the removal of top fish predators can cascade through the food chain and influence lower trophic levels and possibly even the microplankton (so called fishing down the food chain) (Frank *et al.* 2005). The close coupling between the microplankton and environmental change (Hays *et al.*, 2005) therefore makes it a sensitive indicator of environmental disturbance. This is especially true with respect to anthropogenic nutrient enrichment of coastal waters. The pivotal role of microplankton in the pelagic ecosystem and its sensitivity to environmental change, argues for the inclusion of the state of the microplankton community structure to be an integral part of any assessment of ecosystem structure and function. However, detecting change in microplankton community structure due to anthropogenic nutrient enrichment is not easy. Some of the changes attributed

to enrichment (e.g. an increase in the occurrence of harmful algal bloom species, for example *Prorocentrum minimum* (Heil *et al.* 2005)) are similar to those that are reportedly due to climate variation (Edwards, 2009). Furthermore, it is necessary to distinguish natural short-term (days to weeks), seasonal and inter-annual variability from change that might be due to anthropogenic pressure.

7.2 Aim and Objectives

The overall aim of this study was to develop an indicator to detect changes in microplankton community structure related to anthropogenic nutrient enrichment. The first objective was to extend an existing phytoplankton community index (PCI) to a more complete indicator by encompassing other important lifeforms (micro-flagellates and heterotrophic protozoa) that contribute to microplankton structure and to distinguish between the different nutritional modes of the microplankters. The second objective was to apply and test this microplankton community index (MCI) and evaluate its performance as a tool for providing insight into changes in microplankton community structure in the context of anthropogenic nutrient enrichment.

To achieve the first objective of this study, high frequency sampling was carried out over a two year period (Feb 2008- Dec 2009) in the western Irish Sea at the AFBI mooring station 38A to characterise the main environmental properties of the region and to establish a microplankton dataset. To achieve the second objective, data and samples were used from two test sites (West Gabbard in the Outer Thames Estuary, Southern North Sea and Liverpool Bay in the Eastern Irish Sea both operated by Cefas). Both sites have similar hydrodynamic conditions but differed in their nutrient loadings.

7.3 Observational Results

7.3.1 Testing Hypotheses

To test the hypotheses that different nutrient loadings result in different microplankton community structure, several sub-hypothesis were expressed (chapter 4). Hypotheses concerning the hydrodynamic characteristics of the three sampling sites were established and tested first. These were that there was only a low level of nutrient enrichment at station 38A and the microplankton biomass season was closely coupled to the seasonal pattern of stratification; station LBay was highly nutrient enriched, intermittently stratified and located in a ROFI with restricted coupling

of the microplankton biomass season to the stratification pattern; station WGabb was moderately nutrient enriched, intermittently stratified, influenced by freshwater with restricted coupling of the microplankton biomass season to the intermittent pattern of stratification.

Station 38A as the development site for the MCI was shown to be seasonally stratified and with low nutrient enrichment. Average winter concentrations of the two year sampling period were 6.5 μM TOxN, 7.36 μM SiO_2 and 0.71 μM ${PO_4}^{-3}$ and maximum winter levels reached 8.52 μM TOxN, 8.68 μM SiO_2 and 0.91 μM ${PO_4}^{3-}$. A stratification period of approximately six to seven months allowed a microplankton growing season of four to five months with TOxN (lowest concentrations 0.01μM) as the limiting nutrient during summer and light as the limiting factor to microplankton growth in autumn. Silicate was assumed as the limiting factor (minimum concentration 0.37μM) of the spring bloom which was dominated by diatoms in respect of carbon biomass. The succession from diatoms in the spring to heterotrophic microplankton and autotrophic dinoflagellates associated with stronger stratification and a well illuminated euphotic zone and the occurrence of another, weaker (autumn) bloom dominated by diatoms is a classical pattern (Chang *et al.*, 2003) for off shore waters in temperate shelf seas. Three succession stages were identified following Margalef's stages of succession (1963, 1967).

Stations WGabb and LBay were both intermittently stratified and similar in their hydrodynamic characteristics. Nutrient concentrations at both sites were associated with more variability than at sampling site 38A due to the strong tidal mixing throughout the year and freshwater influence. The average winter nutrient concentrations at the Liverpool Bay site were nearly double the average concentrations at the West Gabbard site in the Outer Thames estuary. The results from this study support the results from earlier studies that Liverpool Bay is nutrient enriched (Jones & Folkard, 1971; Foster, 1984; Gillooly *et al.*, 1992, Gowen *et al.* 2000; Gowen *et al.* 2002). However, in 2008 and 2009 lower average winter nutrient levels (TOxN 18.2 μM, Si 8.6 μM, phosphate 0.9 μM) were observed than in earlier years for example by Gowen *et al.* 2002 who recorded average winter levels of 30.3 μM DIN, 13.6 μM Si and 1.6 μM DIP. Minimum summer nutrient concentrations (0.1μM TOxN) in 2008 and reduced growth in early summer could have suggested nutrient limitation during this period.

At the WGabb station average winter nutrient levels were (11.63 μM TOxN, 5.82 μM SiO_2) indicative of a moderate level of enrichment and compared well with concentrations recorded by Sander *et al.* (2001) (12 μM nitrate, > 6.0 μM silicate) for the Outer Thames region. Sanders *et al.* (2001) suggested that during summer diatom growth was silicate limited and dinoflagellate growth was light limited. During this study, the minimum silicate concentration was 0.40μM for a short pe-

riod during summer and there were generally low values in carbon biomass and cell abundance. These observations support the conclusions of Sanders *et al.* (2001). A pronounced shift in the dominance of lifeforms (from diatoms to dinoflagellates or micro-flagellates) was not obvious at either station and diatoms remained the dominant lifeform throughout the year. These observations were consistent with findings by Gowen *et al.* (2000) for Liverpool Bay and with findings by Mills *et al.* (2005) for the southern North Sea. However, detailed assessment is precluded by the limited data set available for the LBay station.

Considering the results of chapter 4, the hypotheses about the samplings sites could all be confirmed.

7.3.2 Methodological Issues

Incomplete Datasets

A consistent problem case that became apparent during data analysis was the incomplete datasets for some key environmental data from both test sites and also the small dataset of microplankton data provided by Cefas for the Liverpool Bay site. This limited interpretation of the results and some confirmation of physical and chemical seasonal patterns could not be given. The data are suggestive of nitrogen limitation during the early summer period in both sampling years at Liverpool Bay, but because data for mid and late summer were missing, this suggestion could not be confirmed. Although data published by Gowen *et al.* 2008 suggested that there was no nutrient limitation in 2003 and 2004 there is no certainty that this remained true for 2008 and 2009. With the small microplankton datasets it was also difficult to confirm the presence or absence of a succession from diatoms to dinoflagellates with the data available for the intermittently stratified sites during the sampling period in 2008 and 2009. This particular problem however, could be partly directed to the different counting methods that were applied by different analysts. To minimise this problem, inter-calibrations were performed. These led to better agreement in the second sampling year (2009) at station WGabb. At the Liverpool Bay station this problem remained as analysts other than those involved in the inter-calibration scheme analysed the microplankton samples that were provided. The low microplankton sample number provided by Cefas for Liverpool Bay in 2009 arose because many samples were rejected as too much sediment and other detrital matter prevented accurate analysis. One of the reason why micro-flagellates represented so little of the overall biomass and cell abundance at Liverpool Bay and the West Gabbard (in the first year) was also down to counting procedures as not much attention was given to these small microplankters by the analysts. A great presence of flagellates (dinoflagellates and micro-flagellates) in these mixed regions

was not expected believing Margalef's (1963, 1978) succession theory and published confirmations on this matter (e.g. Smayda, 1980; Reynolds & Smayda, 1996). However, it is important to include the micro-flagellates in future counts as they also provide insight into ecosystem structure, especially in the context of nutrient enrichment, energy flow, and eutrophication (see *Phaeocystis* spp. in Ryther & Officer, 1981).

Sampling Method and Fixatives

The distinction of the nutritional mode of microplankton collected by the remote access sampler (RAS) proved to be more difficult than first thought. The autofluorescence of microplankton was masked by the preservative lugol's iodine that was used and in experiments the bleaching method using saturated sodium thiosulphate did not bring a satisfying result. Formaldehyde proved to be a better preservative for this purpose but with the samples being left in the sampler attached to the mooring for a six to eight week period, there was a problem of fading autofluorescence. An experiment of a time series with fresh and up to four months old samples resulted in a "cut off" point of 6 weeks. Autofluorescence in any sample older than six week could not reliably be distinguished without light exposure of a specific camera attached to the microscope which was not available for the microplankton analysis during this study. To resolve the problem of fading autofluorescence in long-term samples, fresh samples from every survey were additionally analysed in the second year and a fluorescence library (look up table) was established from the results from this part of the study and the literature. Experiments were also performed with a DAPI-stain method, but were not included in the nutrition mode analysis due to inadequate results. It was difficult to find a method for the distinction of the nutritional mode that could be integrated into routinely counting programmes as most of the moethods are time consuming, require additional and appropriate equipment and samples cannot be stored longer than six weeks. However, there is a need to distinguish between the nutritional modes of microplankton as it provides detailed information in assessing ecosystem health that the primary producers (autotrophs) alone cannot provide. Approved and quality assured look up tables appropriate for UK waters created by literature reviews and randomly chosen live or fresh samples might be the key in identifying the nutritional mode of each species and may allow this distinction in routinely sample programmes. The micro-flagellate counts presented a similar issue concerning the additional time it takes to count samples and the difficulty in identifying the organisms because they are so small. Flow cytometry provides one solution, but requires unpreserved samples for reliable analysis and these cannot be provided when a remote access sampler is the methodology of choice. Another disadvantage of the RAS method is the fixed depth of the sampler. This means that deep chlorophyll maxima or thin layer blooms such as those of *Dinophysis* sp. (Gentien *et al.*, 2005) could be missed and a high variability in cell

numbers could occur if such layers move up and down the water column relative to the sampler. Using a sampler fixed at one depth could also under-sample dinoflagellates because of their vertical migration pattern in stratified waters. Advection might introduce additional variability in the data and lead to difficulties in interpreting changes in species succession. An example during this study was delivered by the occasional appearance of large *Noctiluca scintillans* cells at the Liverpool Bay site which had an impact on the overall microplankton biomass and indicated a succession from diatoms to dinoflagellates in the summer 2009. However, being heterotrophic, *Noctiluca scintillans* is only indirectly related to elevated biomass in response to nutrient enrichment.

Despite these limitations, the advantage of using a RAS method is that a comprehensive and detailed temporal picture of seasonal microplankton succession and variability in microplankton can be obtained. The data from station 38A showed how variable the microplankton can be even on a daily basis and highlight why it is necessary to conduct high frequency sampling in order to obtain detailed information about microplankton biomass and succession and the factors influencing it. Another benefit of using the remote access sampler is that the biological data (i.e. microplankton data) can be supported and explained by the chemical (i.e. nutrients) and physical (i.e. temperature, salinity, turbidity, transparency, oxygen) data as instruments can all be attached to the same mooring site and used to obtain data at the same or even a higher recording frequency.

7.4 The Microplankton Community Index

7.4.1 Lifeforms

In this study, lifeforms were chosen to provide insight into the process of eutrophication, the biogeochemistry and energy flow of the microplankton and possible linkages to higher trophic levels. Silicate and non-silicate users or diatoms and dinoflagellates were proxies for biogeochemistry and therefore possible changes in microplankton biodiversity linked to nutrient enrichment. The nutritional mode of microplankton, autotrophs, heterotrophs, and mixotrophs, were proxies for the energy flow through the system and provided information about food web structure and functioning, and consequently also provided information on eutrophication. The third pair of lifeforms used to characterise the microplankton component of the pelagic ecosystem were small and large microplankters. These also provided a proxy for energy flow. This conceptual idea would enable the MCI to be used as part of the assessment of three of the eleven MSFD descriptors that relate to biodiversity (QD1), food web (QD4), and eutrophication (QD5) in respect of the planktonic part. There is

also the possibility of using the MCI to develop a holistic indicator for these three MSPD descriptors as suggested in 6.6.7.

Many other lifeform categories were tested and it is possible to describe ecosystem state using the state variable space approach with pairs of lifeforms other than the ones chosen here. However, it is important to recognise that many long term datasets do not distinguish between the nutritional modes of microplankton or quantify micro-flagellates on a routine basis. The use of look up tables in which species are assigned a nutritional mode, may overcome some of these limitations but any proposals for monitoring programmes should take into account what information particular lifeforms provide on ecosystem health and balance this against the practicalities and costs of obtaining the necessary data on a routine basis.

7.4.2 Hypotheses and Results

In chapter 6 it was hypothesised that differences in microplankton community structure between 38A and the two enriched sites would be detected by the MCI. It was further hypothesised that these differences were assumed to be due to the different hydrodynamic conditions and nutrient loadings. This hypothesis was confirmed. The differences that were derived from the observational results in chapter 4 were also detected by the MCI. The results from this study further show that using the MCI made it possible to extend the PCI and provide a more complete assessment of change in microplankton community structure by incorporating the different nutritional modes of microplankters. With confidence that the MCI could be used to reliably show change, it was applied to the nutrient enriched sites to test the main hypothesis that different nutrient loadings result in different microplankton community structure. The results presented in the MCI chapter (chapter 6) showed that the two sites were significantly different in their microplankton community structure compared to the West Gabbard station, the autotrophs/mixotrophs state space plots showed that autotrophic biomass at the LBay station was elevated. The MCI value of the small/large sized microplankton comparison showed a difference between the communities at the two sites with a higher biomass of the large sized lifeform at LBay. Comparisons with the heterotophic lifeform were difficult, because only one data point was available. Although, a higher carbon biomass at Liverpool Bay was generally observed, it could not be related to the nutrient and chlorophyll concentrations at the Liverpool Bay site recorded during this study due to the low number of data available.

7.4.3 The MCI and GES

The Marine Strategy Framework Directive (MSFD 2008/56/EC) requires each member state to ensure that good environmental status (GES) for all coastal and shelf sea regions within 200 nautical miles from the shore is achieved by 2020. One approach to define GES for monitored sites would be to set a threshold for the MCI value. Thus, a value equal to or greater than 0.7 with $p < 0.05$, could be set for the MCI to represent GES. However, this raises the issue of what microplankton community structure is like when it is at good environmental status. Furthermore, such an approach assumes that the reference site lifeform state space plot against which comparisons are made represents GES. Based on our understanding of microplankton ecology, there is an expectation of observing certain assemblages of species and their seasonal succession (e.g. diatoms to dinoflagellates) coupled to the hydrodynamic conditions of a particular water body when this water body is substantially free from anthropogenic pressure such as nutrient enrichment. In terms of GES, it is possible to argue that we know what GES is not (absence of a spring bloom or seasonal succession overall dominance of one species, increase in harmful nuisance species and blooms). However, it is less clear what GES of the microplankton community should be.

7.4.4 Further Considerations

Data sets used for the MCI can be either spatial or temporal. In this study only the spatial comparisons were performed, because the datasets were not comprehensive enough to perform temporal comparisons. An alternative approach was proposed by Gowen *et al.* (2011). These workers suggested that time-series data could be used to calculate a trend in the index. Such a trend could then be compared with trends in anthropogenic pressure. An example is shown in Figure 7.1. As suggested by Gowen *et al.* 2011, the target for GES could be "no significant trend in the MCI correlated with a significant trend in pressure". However, there is again the problem of what the initial starting conditions represent and how to interpret any trend in the MCI. The MCI is designed as a tool for management. That is a method of detecting a change in microplankton, but it cannot be used to determine whether a shift is "good or bad". It is clear that further work is required to characterise microplankton reference conditions and the best approach to this is likely to combine current understanding of microplankton ecology with ecological modelling and expert judgement.

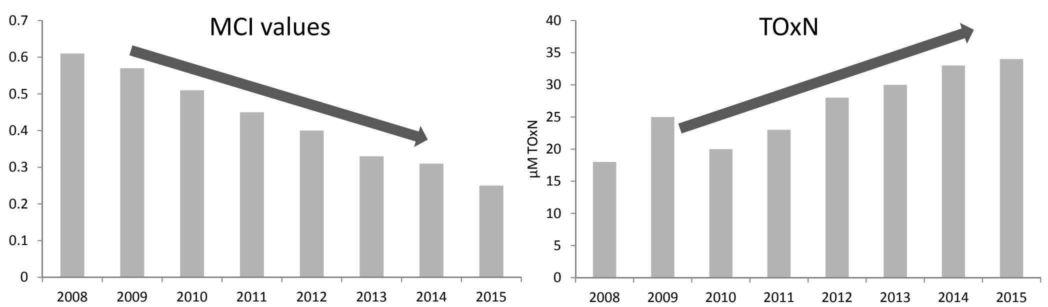


Figure 7.1: An illustration of an example approach for MCI values plotted in relation to time. This plot is compared to a plot of anthropogenic nutrient enrichment over the same time period. If a significant trend in the MCI can be related to an increasing trend in the anthropogenic pressure that is nutrients, then the microplankton are not in good environmental status (GES) (redrawn from Gowen *et al.* 2011).

7.4.5 Methodological Issues

Minimum datasets for the MCI

The MCI was used to show a statistically significant difference in the microplankton community structure between Liverpool Bay and the West Gabbard. The index appears to provide a robust method for detecting changes in the microplankton component of marine pelagic ecosystems. However, the robustness of an indicator is dependant in part on the datasets used. The results of this study showed that for reference conditions, a dataset should consist of a minimum of three years with monthly or better fortnightly samples throughout the year to provide confident in the results. For comparisons between water bodies the same frequency should be kept, but a one year dataset would be sufficient. It is important that the standard operating procedures are common and that it is clear which microplankton are included in the analysis and which are not. This is very important for the routine analysis of samples collected for the purposes of assessing the state of the microplankton for policy requirements. This is especially the case when there may be a requirement to combine data from different institutes to provide a regional seas assessment. Counting errors for individual analysts can be quantified, but careful inter-calibration is required to minimise taxonomic miss-identification. This is also of vital importance when choosing lifeforms as state variables to describe ecosystem state variable space.

7.4.6 Applicability of the MCI

The MCI should be broadly applicable, because the lifeforms used and the stressors (i.e. nutrients) that these organisms respond to are common to most coastal and offshore temperate shelf seas of the world. With further validation the index could be applicable for other marine ecosystems (e.g. permanently stratified offshore regions, estuaries, sea loughs) and possibly for freshwater bodies. The advantages of using the theory-based approach of lifeforms in a state variable space are of practical nature as state space plots are not as sensitive to the limitations in sampling regimes that statistics based on time-series graphs are. The lifeform approach can also be applied at any taxonomic level at which it is possible to distinguish kinds of organisms using morphology, life-history and biogeochemistry (Tett *et al.* 2003). The MCI has an advantage over other indicators of structure is that it takes into account the natural, short-term, seasonal and inter annual variability in the microplankton that is an essential part of microplankton ecology.

Harmful algal blooms (HABs) are explicitly mentioned in the MSFD in relation to eutrophication. However, harmful algae were not considered as lifeforms within this study mainly because they were not a key objective of this study and they cannot be categorised into one functional group (i.e. lifeform). As shown in chapter 2, toxin producing microplankton do not originated from one distinct lineage, and no “deep taxonomy” unites them. Some species incorporate toxin and non-toxin producing strains (e.g. *Alexandrium* sp.) and so it is difficult to distinguish between them. Nevertheless, there is need to include nuisance and toxin producing species when assessing microplankton community structure. One suggestion is to integrate HABs into the MCI scheme (Gowen *et al.* 2011). Thus, a state variable space could be defined by potential toxic dinoflagellates and *Pseudo-nitzschia* species as state variables with any shift in state regarded as undesirable.

7.5 Further Development of the MCI

Taking the results of this study into account the MCI should now be applied to and tested with other and larger datasets from around the UK. Spatial comparisons could be performed and it should be possible to make temporal comparisons that were not possible within the three years of this project. It would be interesting to see how the MCI would perform with datasets from Stonehaven, the CPR data for the North Sea, and Loch Creran to determine whether trends are apparent and can be related to trends in nutrient concentrations or other human pressures. For the MCI to be a useful tool in the assessment of marine pelagic ecosystems for the MSFD there is a need in make it operational. That is, to develop a method of data

synthesis and integration that takes data from multiple sources (and analysts) and converts the data into standardised sets of lifeforms and outputs the state space plots and values of the MCI.

7.6 Proposal for a Microplankton Reference Site

The MSFD explicitly requires that the ecosystem within assessment regions is in good environmental status (GES) and that GES is maintained. To do this, reference conditions need to be identified that are representative of GES. Thus, reference conditions of microplankton communities that are characteristic of each of the different hydrodynamic water types found in UK waters need to be identified.

The Irish Sea is partially landlocked and would appear to be potentially at risk of eutrophication (Gowen *et al.*, 2000) on similar basis to other partially landlocked basins such as the Baltic Sea where nutrient enrichment is a major concern (Lancelot *et al.*, 2001). However, time series analysis showed that phosphate declined over the last decade and nitrogen was stable (Gowen *et al.* 2008). The western Irish Sea was slightly nutrient enriched in the late 1990s (Gowen & Stewart 2005; Gowen *et al.* 2008) compared to near ocean (Atlantic) nutrient levels. The results of this study support these earlier studies that there is a low level of enrichment in the seasonally stratified region in the western Irish Sea.

The microplankton species assemblages and succession of species were closely coupled to the seasonal dynamics in this area and comply with Margalef's model of succession of spring diatoms –> summer dinoflagellates –> autumn diatoms. The nutrient uptake by microplankton at station 38A in the western Irish Sea in 2008 and 2009 indicated that silicate was the limiting factor during spring and nitrogen was the limiting factor during summer, which was consistent with published work in this area (Gibson *et al.* 1997, Gowen *et al.* 2008). On the basis of current understanding of microplankton biogeography, seasonal dynamics and succession theory, although slightly nutrient enriched, the microplankton at station 38A represents a community structure that might be expected to be seen in low nutrient, seasonally stratifying conditions. Therefore, I propose that the microplankton at this station is representative of GES and that for the purposes of the MSFD could be used as reference conditions for seasonal stratifying temperate shelf sea regions.

7.7 Conclusion

Coastal eutrophication is considered a severe problem worldwide and can lead to severe economic and ecological consequences by affecting coastal marine resources

and coastal marine ecosystems and eventually human health (with shellfish poisoning and respiratory illness) (Nixon, 1995). It is apparent that traditional assessment of eutrophication that relied on bulk indicators of ecosystem state and elements of floristic composition of the phytoplankton do not provide adequate insight into the effects of anthropogenic nutrient enrichment on ecosystem health: structure and function resistance, and resilience. However, new environmental policies such as the Marine Strategy Framework Directive (MSFD) require a holistic approach to the assessment of environmental status that is expressed in such properties as structure and function. Microplankton forms the base of marine food webs and its sensitivity to change in nutrient supply makes changes in the microplankton community structure of potential use as an indicator of pelagic ecosystem health in the context of eutrophication.

The aim of this study to develop and test an indicator of change in microplankton community structure in the context of eutrophication was achieved. The microplankton community index (MCI) is based on the theoretic approach of an existing phytoplankton community index (PCI) but incorporates the heterotrophic, mixotrophic, micro-flagellate and ciliate lifeform components of the microplankton as lifeforms. The index provides a means of detecting a change in microplankton community structure in response to environmental forcing such as anthropogenic nutrient enrichment. Lifeforms were chosen to provide information on the process of eutrophication.

The MCI was developed using a two-year microplankton dataset from a low nutrient enriched region of the western Irish Sea. By including the microplankton lifeforms listed above the MCI extended the PCI approach and can be used to provide a more complete assessment of change in microplankton community structure.

Results from this study show that the seasonal dynamics and community structure of the microplankton at this study site are consistent with current understanding of microplankton ecology in seasonally stratifying shelf seas. Station 38A in the western Irish Sea is therefore proposed as a reference site for the microplankton in seasonal stratifying regions in temperate shelf seas. Three pairs of lifeforms, were used for the MCI and applied to datasets from enriched sites in Liverpool Bay ($\approx 30\mu$M winter DIN) and the Outer Thames estuary ($\approx 15\mu$M winter DIN). Use of the MCI revealed significant differences in the microplankton community structure. Autotrophic biomass was elevated in Liverpool Bay and there was a higher biomass of the large sized lifeform. However, incomplete datasets meant that the overall aim of this study (to related a change in microplankton community structure to increased anthropogenic nutrient enrichment) was not achieved.

7.8 Future Work

Further work is required to further develop the MCI. In particular, the datasets should consist of a minimum of one year for comparison sites and three years for reference sites with monthly or better fortnightly samples throughout the year. The standard operation procedures should be commonly known to all analysts. Inter-calibrations to minimise counting errors for individual analysts should be held on a regular basis.

It has been shown that the MCI can be used to detect changes in microplankton community structure but it does not provide information on whether the change is "good or bad". Evidence for a shift away from GES requires that baseline conditions for microplankton community structure in all of the hydrographical regions found in UK waters need to be established. One means of doing this would be to combine current understanding of microplankton dynamics and modelling with expert opinion. In the short term, correlating trends in the MCI with trends in pressure might provide a means of determining whether GES has been met. The conceptual idea of lifeform pairs being proxies for MSFD descriptors (biodiversity, food web and eutrophication) could form the basis for developing a single holistic indicator of changes in the microplankton component of the pelagic ecosystem.

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Appendix A

Listing 1

A.1 Species Lists

Table A.1: Species list of all microplankton that occurred in the samples from station 38A in the western Irish Sea in 2008 and 2009.

Alexandrium spp.	*Euglenida*	*Proboscia alata*
Amphidinium	*Gonyaulax spp.*	*Prorocentrum micans*
Asterionella glacialis	*Guinardia delicatula*	*Prorocentrum rostratum*
Cerataulina pelagica	*Guinardia flaccida*	*Prorocentrum spp.*
Ceratium furca	*Guinardia striata*	*Prorocentrum triestinum*
Ceratium fusus	*Gymnodinium* large (>75um)	*Protoperidinium bipes*
Ceratium horridum	*Gymnodinium* medium (35-75um)	*Protoperidinium brevipes*
Ceratium lineatum	*Gymnodinium* small (10-35um)	*Protoperidinium crassipes*
Ceratium longipes	*Gyrodinium* large (>75um)	*Protoperidinium spp.*
Ceratium tripos	*Gyrodinium* medium (35-75um)	*Prymnesiophycea (Phaeocystis)*
Chaetoceros curvisetus	*Gyrodinium* small (10-35um)	*Pseudonitzschia delicatissima* group
Chaetoceros danicus	*Heterocapsa triquetra*	*Pseudonitzschia seriata* group
Chaetoceros debilis	heterotrophic flagellates	*Pyrocystis lunula*
Chaetoceros decipiens	*Karenia mikimotoi*	*Rhizosolenia imbricata/styliformis*
Chaetoceros diadema	*Laboea*	*Rhizosolenia pungens*
Chaetoceros didymus	*Laudaria annulata*	*Rhizosolenia setigera*
Chaetoceros eibenei	*Leegardiella*	*Rhodomonas spp.*
Chaetoceros large (>40)	*Leptocylindrus danicus*	*Scenedesmus spp.*
Chaetoceros medium (21-40um)	*Leptocylindrus minimus*	*Scrippsiella*
Chaetoceros small (10-20um)	*Licmorphora spp.*	*Skeletonema*
Corethron criophilum	*Lingulodinium*	small armoured dinos (10-35μm)
Coscinodiscus spp	*Lohmaniella strobilidium*	small centric diatoms (10-25μm)
Cryptophyceae	*Melosira nummuloides*	small naked dinos (10-35μm)
Cylindrotheca closterium	*Mesodinium myrionecta*	*Stephanopyxis turris*
Dictyocha fibula	*Meuniera membranacea*	*Strombidium*
Dictyocha speculum	*Navicula* large (150-300μm)	*Thalassionema nitzschioides*
Dididium	*Navicula* medium (50-150μm)	*Thalassiosira angulata*
Dinophysis acuminata	*Navicula* small (50μm)	*Thalassiosira anguste-lineata*
Dinophysis acuta	*Nitzschia*	*Thalassiosira nordenskioeldii*
Dinophysis fortii	*Odontella sinensis*	*Thalassiosira rotula*
Dinophysis norvegica	other microplankters	Tintinnids
Diploneis	*Paralia sulcata*	*Torodinium*
Ditylum brightwellii	*Pleurosigma*	undetermined ciliates
Eucampia zodiacus	*Prasinophyceae*	undetermined flagellates

Table A.2: Species list of all microplankton that occurred in the samples from the West Gabbard station in the Outer Thames estuary, southern North Sea in 2008 and 2009.

Actinastrium	*Dinophysis dens*	*Paralia sulcata*
Actinoptychus	*Dinophysis norvegica*	*Phaeocystis globosa*
Akashiwo sanguinea	*Dinophysis rotundata*	*Plagiogrammopsis*
Alexandrium	*Dissodinium pseudolunula*	*Pleurosigma*
Amphidinium	*Ditylum brightwelli*	*Podosira stelligera*
Amphidinium carterae	*Eucampia zodiacus*	*Polykrikos*
Ankistrodesmus	*Euglena*	*Prorocentrum*
Armoured dino indet	*Fragilaria*	*Prorocentrum balticum*
Asterionella	*Gonyaulax*	*Prorocentrum cordatum*
Asterionella formosa	*Guinardia*	*Prorocentrum gracile*
Asterionellopsis glacialis	*Guinardia delicatula*	*Prorocentrum micans*
Asterionellopsis kariana	*Guinardia flaccida*	*Prorocentrum triestinum*
Asteromphalus	*Guinardia striata*	*Protoperidinium*
Bacillaria	*Gymnodinium*	*Protoperidinium bipes*
Bacteriastrum	*Gyrodinium*	*Protoperidinium brevipes*
Bellerochea	*Gyrodinium spirale*	*Protoperidinium crassipes*
Cerataulina	*Heliotheca tamesis*	*Protoperidinium depressum*
Ceratium	*Heterocapsa niei*	*Protoperidinium leonis*
Ceratium furca	*Heterocapsa triquetra*	*Protoperidinium oceanicum*
Ceratium fusus	*Karenia mikimotoi*	*Protoperidinium steinii*
Ceratium horridium	*Lauderia*	*Pseudoguinardia recta*
Ceratium lineatum	*Leptocylindrus*	*Pseudo-nitzschia*
Ceratium minutum	*Licmophora*	*Pyrophacus horologium*
Ceratium tripos	*Licmophora (cluster)*	*Raphoneis amphiceros*
Chaetoceros	*Melosira*	*Rhizoselenia*
Chatonella	*Meuniera*	*Rhizoselenia imbricata*
Corethron	*Monad flagellate*	*Rhizoselenia setigera*
Coscinodiscus	*Naked dino indet*	*Rhizoselenia styliformis*
Cyclotella	*Naviculaceae*	*Scenedesmus*
Cylindrotheca	*Navicula vanhoeffenii*	*Scrippsiella*
Dactlyliosolen	*Nitzschia*	*Skeletonema*
Dictyocha speculum	*Noctiluca scintillans*	*Stephanopyxis turris*
Dinophysis	*Odontella*	*Thalassionema nitzschoides*
Dinophysis acuminata	*Odontella mobilensis*	*Thalassiosira*
Dinophysis acuta	*Odontella sinensis*	*Triceratium*

Table A.3: Species list of all microplankton that occurred in the samples from the Liverpool Bay station in Liverpool Bay, eastern Irish Sea in 2008 and 2009.

Actinastrium	Dinophysis acuta	Proboscia alata
Actinastrium colony	Dinophysis dens	Prorocentrum
Actinoptychus	Dinophysis norvegica	Prorocentrum cordatum
Akashiwo sanguinea	Dinophysis rotundata	Prorocentrum gracile
Alexandrium	Ditylum brightwelli	Prorocentrum micans
Amphidinium	Eucampia zodiacus	Prorocentrum triestinum
Amylax	Euglena	Odontella mobiliensis
Ankistrodesmus	Fragilaria	Odontella sinensis
Armoured dino indet	Gonyaulax	Paralia sulcata
Asterionella	Guinardia	Phaeocystis globosa
Azadinium spinosum	Guinardia delicatula	Plagiogrammopsis
Bacillaria	Guinardia flaccida	Pleurosigma
Bacteriastrum	Guinardia striata	Protoperidinium
Cerataulina	Gymnodinium	Protoperidinium bipes
Ceratium	Gyrodinium	Protoperidinium brevipes
Ceratium furca	Gyrodinium spirale	Protoperidinium crassipes
Ceratium fusus	Heliotheca tamesis	Protoperidinium depressum
Ceratium horridium	Heterocapsa triquetra	Protoperidinium steinii
Ceratium lineatum	Karenia mikimotoi	Pseudoguinardia recta
Ceratium minutum	Lauderia	Pseudo-nitzschia
Ceratium tripos	Leptocylindrus	Pyrophacus horologium
Chaetoceros	Leptocylindrus danicus	Raphoneis amphiceros
Chaetoceros socialis	Leptocylindrus minimus	Rhizoselenia
Chatonella	Licmophora	Rhizoselenia imbricata
Corethron	Licmophora (cluster)	Rhizoselenia setigera
Coscinodiscus	Melosira	Rhizoselenia styliformis
Cyclotella	Meuniera	Scenedesumus
Cylindrotheca	Monad flagellate	Scrippsiella
Dactlyliosolen	Naked dino indet	Skeletonema
Dictyocha fibula	Naviculaceae	Stephanopyxis turris
Dictyocha speculum	Nitzschia	Thalassionema nitzschoides
Dinophysis	Noctiluca scintillans	Thalassiosira
Dinophysis acuminata	Odontella	Triceratium

A.2 Dinoflagellate Library

Table A.4: Library for identified dinoflagellate species and groups and their nutritional mode.

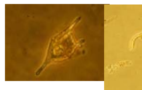
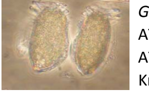
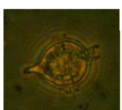
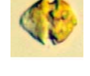
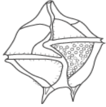
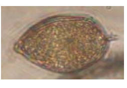
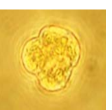
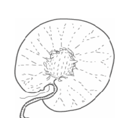
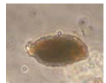
Dinoflagellate Library				
nutritional mode	*Alexandrium species*		*Ceratium species*	
my findings	AT (consistenly)		AT (consistenly)	
public literature	AT		MT	
source:	http://www.liv.ac.uk/hab		http://www.sahfos.ac.uk/pil	
nutritional mode	*Dinophysis species*		*Gonyaulax spp.*	
my findings	MT (consistenly)		AT summer, autumn	
public literature	MT		AT	
source:	Kraberg *et al.* 2010		Kraberg *et al.* 2010	
nutritional mode	*Heterocapsa triquetra*		*Lingulodinium*	
my findings	AT summer, autumn		AT summer	
public literature	MT		oligate AT	
source:	Kraberg *et al.* 2010		http://www.liv.ac.uk/hab	
nutritional mode	*Prorocentrum species*		*Protoperdidinium species*	
my findings	AT (consistenly)		HT (consistenly)	
public literature	MT		HT	
source:	Kraberg *et al.* 2010		Kraberg *et al.* 2010	
nutritional mode	*Pyrocystis*		*Scrippsiella*	
my findings	HT (summer)		AT summer, autumn	
public literature	parasitic		AT	
source:	Kraberg *et al.* 2010		Kraberg *et al.* 2010	

Table A.5: Dinoflagellate library continued.

nutritional mode	*Amphidinium*		*Gymnodinium small (<35um)*	
my findings	AT spring,summer		HT winter,spring; AT summer, autumn	
public literature	AT		species dependent AT,HT, MT	
source:	Kraberg *et al.* 2010		Kraberg *et al.* 2010	
nutritional mode	*Gymnodinium medium (25-75um)*		*Gymnodinium large (>75um)*	
my findings	AT winter, summer; HT spring, autumn		AT winter, summer; HT spring, autumn	
public literature	species dependent AT,HT, MT		species dependent AT,HT, MT	
source:	Kraberg *et al.* 2010		Kraberg *et al.* 2010	
nutritional mode	*Gyrodinium small (<35um)*		*Gyrodinium medium (35-75um)*	
my findings	HT (winter, spring); MT summer, autumn		HT (winter, spring); MT summer, autumn	
public literature			HT	
source:			Kraberg *et al.* 2010	
nutritional mode	*Gyrodinium large (>75um)*		*Karenia mikimotoi*	
my findings	HT (winter, spring); MT summer, autumn		AT (consistenly)	
public literature	HT		AT	
source:	Kraberg *et al.* 2010		Kraberg *et al.* 2010	
nutritional mode	*Noctilluca*		*Torodinium*	
my findings	HT autumn		AT summer	
public literature	bioluminescent, HT		HT with chloroplasts	
source:	http://www.liv.ac.uk/hab		Kraberg *et al.* 2010	

legend: AT = photoautotrophic HT = heterotrophic MT = mixotrophic

A.3 Standard Operation Procedures

A.3.1 Standard Operation Procedure to prepare samples for microplankton analysis

Introduction

This procedure describes the settling of preserved marine water samples prior to the identification and enumeration of microplankton.

Scope

A sub-sample of preserved seawater is allowed to settle for sufficient time to allow the phytoplankton species to sink to the bottom of the sedimentation chamber. The sedimentation chamber (Utermöhl chamber) is placed on the stage of an inverted light microscope and the sample is examined for the presence of microplankton species. The volume settled is 50 mL. It is important that samples are settled on a level surface. Microplankton species are identified using expert knowledge and specified literature and cell numbers are recorded. Different counting methods can be employed depending on the density of different species present in the sedimentation chamber. If cells are present in low densities the entire bottom of the chamber is scanned and cells are counted. This is the preferred method of cell enumeration. If cells are present at high densities then a central strip or a number of fields of view (FOV) can be counted. Final cell concentrations are expressed as cells per litre. The sample details (collection date, depth, analysis date) are written on a sample analysis form (counting sheet) when a sub-sample is set up.

Safety Precautions

Before performing this procedure read the COSHH & risk assessments for acidified Lugol's iodine and for working in offices and laboratories.

Equipment/Apparatus

- 50 mL settling chamber
- Glass cover slip
- Tissues
- Counting sheet

- Pencil
- Permanent marker
- Acidified Lugol's iodine
- Rubber gloves
- Fume cupboard

Procedure

The base of the settling chamber is labelled with the date of when the sample was taken (use permanent marker for this) and the date and time it has been set up. These details are also recorded on the counting sheet.

The sample bottle is gently inverted 20 times to homogeneously mix the water containing the microplankton cells.

In the fume cupboard a sub-sample is immediately poured into a 50 ml settling chamber until it begins to overflow.

A glass cover slip is slid onto the top of the chamber, in such a way as to cut off the excess liquid and ensure no air bubbles are trapped.

The outside and bottom of the chamber are carefully dried with a tissue and the settling chamber is place level on the bench in a fume cupboard.

The sample is left to settle for a minimum of 20 hours.

Identification and enumeration of cells is then conducted in accordance with the standard operating procedure for 'Microscope Analysis of Microplankton samples'.

Epi-fluorescence

The above procedure is followed using the formalin preserved samples except that these samples are kept away from direct light and placed in a darkened corner of the fume cupboard to sustain auto-fluorescence.

Chamber Calibration

The volume of each sedimentation chamber used in the procedure must be verified annually by weighing. Chambers are rejected for use if they fall outside weighing $\pm$ 10 % of their stated volume.

A.3.2 Standard Operation Procedure for Microscopic analysis of microplankton samples

Introduction

In this study the primary purpose for monitoring microplankton in Irish Sea waters was to develop and test a Microplankton Community Index (MCI). It was hoped that this MCI can be used to identify changes in microplankton compositions in the Irish Sea and the North Sea to provide a tool for the detection of undesirable disturbance. This procedure describes the identification and enumeration of microplankton in marine water samples.

Scope

Preserved water samples are set-up ready for analysis as described by the SOP "to prepare samples for microplankton analysis" (see A.3.1). Cells are counted and identified in accordance with the analyst's best knowledge and an approved species list.

To save time and to achieve reasonable accuracy in counting, the sample is first examined superficially as provisional assessment of abundant species and their size on the chamber bottom with a magnification x10. The area to be counted and the magnification used depends on the size of the organisms and the cell number present. If microplankton cells are present in low densities (less than 4 cells per field of view) the entire bottom of the chamber is counted. This is the preferred method of cell enumeration. If cells are present at high densities (more than 4 cells per field of view) then either the central strip or a number of fields of view (FOV) can be counted. Final cell concentrations are expressed as cells per litre.

Occasionally some of the water sample may leak from the sedimentation chamber if there is an incomplete seal, or the glass is invisibly broken. Should leakage occur, this sub-sample is disposed down a sink and a second sub-sample, from the original sample, is re-settled following the procedures described in the SOP A.3.1. The cause of the leak is investigated and if possible rectified. If the chamber is beyond immediate repair then another settling chamber is selected for use.

Some samples may contain excessive amount of detritus making species identification and enumeration problematic, as there is a risk of cells being hidden by debris. Should this occur, the sample is rejected, and another sample is settle with a smaller volume. If cells are still being hidden by debris the sample is rejected as accurate analysis is not possible. A note is written on the batch sheet 'unable to analyse' and a photographic record of the sample's base plate is taken.

Safety Precautions

Before start performing this procedure read the COSHH and risk assessments concerning this procedure.

Reference and associated documents

Corporate Health and Safety Laboratory Protocol according to the AFBI standard

- COSHH regulations
- About Chemical Safety
- Warning labels and Safety Data Sheets
- About Handling Hazardous Materials

Material Safety Data Sheets: acidified Lugol's Iodine Solution

Use the provided up to date and relevant reference documents in the plankton laboratory listed at the end of the SOP. Each document is uniquely identified by an alpha/numeric and colour coded label attached to the spine of the document. This list is reviewed annually to ensure that the literature available to the microplankton analysts is both relevant and the most up-to-date.

Equipment/Apparatus

- High power inverted microscopes with mechanical x/y stage, objectives for x10, x20, and x40 magnification
- Sedimentation chamber (Utermöhl chamber) to hold a preserved water sample (50 mL)
- Perspex stage holder for chambers
- Level bench
- Microplankton counting sheet
- Pencil
- Reference books and taxonomic keys
- Nikon Diaphot inverted micrometer
- Clicker counter
- Lens tissue

– detergent
– millipore filtered water

Procedure

Carefully remove the 50 mL plastic cylinder from a sample that has been settled for at least 20 hours by sliding it gently off the chamber while placing glass cover slip over the chamber bottom. It is important to ensure that no air bubbles are trapped.

The sedimentation chamber containing a preserved water sample, which has been left to settle for a minimum of 20 hours, is carefully mounted into a Perspex stage holder on a Nikon Diaphot inverted microscope.

The microscope is focused and the light level adjusted to ensure maximum resolution for resolving morphological features of the microplankton cells (the shutter should be fully opened). This will aid cell identification to the lowest taxonomic level.

The level of magnification, the volume of sample settled and analysed and the sample date, are recorded on the counting sheet. Details about the station, depth, etc. of collected samples need to be recorded on the counting sheet when the samples are collected from different sites. Details of the counting procedure are recorded on the counting sheet. Since the counting procedure depends on the abundance of the individual species, different counting procedures may be used for the same sample.

A quick scan of the sample is performed in a magnification of x10 to determine the method of analysis to be employed (whole chamber, central strip, field of view (FOV)) which is also recorded in the counting sheet after a decision is made. Once a decision about the counting procedure was made and counting has started the procedure cannot be switched. There are four methods that can be employed.

The first method is employed when any of the microplankton that have been identified are present in low concentrations (less than approximately 4 cells per FOV). In this case the whole base of the chamber is scanned for the presence of these cells at a magnification of x20. This allows a minimum detection level of 20 cells per litre if a 50 ml chamber were used. However, species identification may require higher magnification (possibly up to x40).

The second method is used when cells of a particular species/genus are too numerous to ensure accurate counting over the whole base of the chamber (greater than approximately 4 cells per FOV). In this case a central strip is counted until a minimum of 100 cells is reached. If that is not the case the chamber is turned 90 ° and another central strip is counted.

The third method is employed when there are more than approximately 50 cells in one FOV (e.g. in a spring bloom). Then 20 FOVs are counted along the central strip.

The fourth method is applied when there are more than approximately 10 cells in one FOV or for species smaller than 20 μm (micro-flagellates or small diatoms or small dinoflagellates). FOVs in a magnification of x40 are used in this case until a minimum of 200 cells are counted. It is possible that all techniques may be used in counting the same sample if different species/genus are present in different cell concentrations.

If concentrations of algae or sediment are too great, then a smaller volume of sample should be settled into a 25 ml or 10 ml sedimentation chamber. Again, cells should be counted from the whole base of the chamber at a magnification of x20 whenever possible.

Counting procedure

Whole base plate

Starting at the top outer edge of the base plate, the microscope stage is moved so that the field of view (FOV) tracks from left to right. When the right edge of the chamber is reached a reference point, (e.g. cell or a piece of detritus) is identified and the stage moved so that the FOV moves vertically downwards one field of view and cells in this transect are identified and counted moving the microscope stage from right to left. This procedure is repeated until the whole base plate has been viewed. If necessary a clicker counter may be used if cells are present in high numbers.

Central Strip

The central strip can be found where the edge of the chamber is parallel to the edge of the field of view. The stage is moved so that the field of view tracks across the centre line of the base plate.

Fields of View

Fields of view (FOV) are counted along the central strip by counting every second or third FOV. If the required number of total cells or each species is not reached counting the central strip, the chamber is turned 90 degree and the procedure is repeated.

Enumeration

The total number of cells of each species or genera counted is recorded on the counting sheet. Empty cells are only included if they are part of a chain like the diatom *Paralia sulcata* or when it is clearly visible that the cell content is right next to the frustle. Broken cells should only be counted if they have contents and

represent more than half of the cell.

Calculations

Cells per Litre for the different methods were calculated as follows:

For counts in the whole chamber:

$$\text{total number of cells} \cdot \left(\frac{1000}{\text{volume settled (ml)}}\right) \tag{A.1}$$

For counts in the central strip:

$$\left(\frac{\text{total number of cells}}{\text{area of central strip}}\right) \cdot \text{Microscope field factor} \cdot \left(\frac{1000}{\text{volume settled (ml)}}\right) \tag{A.2}$$

$$\left(\frac{\text{total number of cells}}{\text{area of central strip}}\right) \cdot \text{Microscope field factor} \cdot \left(\frac{1000}{\text{volume settled (ml)}}\right)$$

For counts in fields of view (FOV):

$$\left(\frac{\text{total number of cells}}{\text{Number of FOV}}\right) \cdot \text{Microscope field factor} \cdot \left(\frac{1000}{\text{volume settled (ml)}}\right) \tag{A.3}$$

Sample Disposal

On completion of analysis, the settled sub-sample is discarded down the laboratory sink with plenty of water. The remainder of the sample is returned to the store room.

The chamber is rinsed with fresh water then left to soak in detergent solution over night. On the next day the chambers are washed, and rinsed first with fresh water then with Millipore water, and put to dry in the drying area.

Records

The counting sheets and other records (station name and number, depth of samples collected, settled volume, concentration of preservative used) are kept in a lever arch file. Before beginning another count the data from the completed count are entered into an excel spread sheet. The samples are stored for one year before disposal and the counting sheets are archived.

Microscope Calibration

The microscope is serviced annually by a certified engineer. Should a problem occur between services the laboratory will request an engineer immediately and the microscope will not be used until corrected. The microscope eyepieces, lenses and filters are wiped with lens tissue before use.

A.3.3 Standard Operation Procedure for analysis of heterotrophic and autotrophic micro-flagellates using epifluorescence microscopy

Introduction

This procedure describes the identification and enumeration of heterotrophic and phototrophic (micro-) flagellates in water samples collected as part of a study to development a Microplankton Community Index.

Scope

Preserved water samples are set-up ready for analysis as described by the SOP A.3.1. All cells are counted and identified in accordance to the analyst's best knowledge and an approved species list.

Safety Precautions

Before starting this procedure read the COSHH and risk assessments concerning this field.

Reference and associated documents

Corporate Health and Safety Laboratory Protocol:

- COSHH regulations
- About Chemical Safety
- Warning labels and Safety Data Sheets
- About Handling Hazardous Materials

Material Safety Data Sheets: Formaldehyde

Literature References: N/A

Equipment/Apparatus

- Work space in a dark room
- High power inverted microscopes with mechanical x/y stage, objectives for x10, x20, and x40 magnification
- Auto-fluorescence filter BP-450-490nm,
- Super high pressure mercury lamp, model HB-10101AF
- Sedimentation chamber (Utermöhl chamber) to hold a preserved water sample
- Perspex stage holder for chambers
- Level bench
- Microplankton counting sheet for fluorescence analysis
- Pencil
- Clicker counter
- Lens tissue
- detergent
- millipore filtered water

Procedure

Gently slide a glass cover slip across the top of the base plate by displacing the 50 mL plastic cylinder (sedimentation tube). Ensure the glass cover slip covers the chamber of the base plate and there are on air bubbles trapped. Then carefully carry the sedimentation chamber to the microscope for analysis.

The sedimentation chamber containing a preserved water sample, that has been left to settle for a minimum of 20 hours, is carefully mounted into the Perspex stage holder on the Nikon Diaphot inverted microscope equipped with epi-fluorescence attachments.

The microscope is focused and the light level adjusted to ensure maximum resolution for resolving morphological features of the microplankton cells (the shutter should be fully opened). This will enable cell identification to the lowest taxonomic level.

The mercury lamp for fluorescence should be switched on 30 minutes before analysis ensuring enough time for the lamp to heat up for performance.

The UV filter has to be inserted and the shutter to the UV-light source should be closed until the light is needed.

The level of magnification, and details relating to the sample (volume of sample settled, the sample date, and sample depth) and the applied counting methods (i.e. FOVs, central strip, whole chamber) are recorded on the counting sheet.

Heterotrophic and autotrophic micro-flagellates are discriminating on the basis of their colour emissions under fluorescent light. There are three categories:

1. 'red' for strong photoautotrophic activity and photoautotrophic organisms
2. 'orange' for weak photoautotrophic activity and therefore autotrophic organisms and potentially mixotrophic organisms
3. 'blue-green' for heterotrophic organisms

Total flagellate cell counts are achieved by switching from fluorescent light to transmitting light. All micro-flagellate cells visible in the transmitting light are counted. The sum of micro-flagellate cells is gained by adding all cells that are visibly emitting auto-fluorescence in the fluorescent light. The sum of cells is subtracted from the total cell counts to identify the discrepancy of fading auto-fluorescence in the sample.

The area to be counted and the magnification used depends on the number of cells present. However, because the cells of interest are less than 20μm all counts are performed at a magnification of x40. A minimum of 200 cells are counted. If there are approximately 4 cells in one FOV, a central strips is counted. If one central strip does not provide enough cells, then the sample is turned 90° and another central strip is counted. This is the preferred method of cell enumeration for microflagellates. If cells are present at > 4 cells in one FOV, then cells are counted in 10 fields of view. Final cell concentrations are expressed as cells per litre.

Total Cell Calculation per Litre

Cells per Litre for the different methods were calculated as follows:

For counts in fields of view (FOV):

$$\left(\frac{\text{total number of cells}}{\text{Number of FOV}}\right) \cdot \text{Microscope field factor} \cdot \left(\frac{1000}{\text{volume settled (ml)}}\right) \qquad \text{(A.4)}$$

For counts in the central strip:

$$\left(\frac{\text{total number of cells}}{\text{area of central strip}}\right) \cdot \text{Microscope field factor} \cdot \left(\frac{1000}{\text{volume settled (ml)}}\right) \qquad \text{(A.5)}$$

Records

The counting sheets and other records are kept in a file and are regularly entered into an excel spread sheet. The samples are stored for another full year before disposal and the counting sheets are archived.

A.4 Fluorescence Counting Sheet

Counting sheet for fluorescence counts

Flagellates Fluorescence counting sheet		volume: 50ml	formalin [3.6%]
Date:	date counted:	comment:	
red (photoautotrophic)			
orange (mixotrophic)			
green (heterotrophic)			
Total			
difference (total-sum)			

Figure A.1: Counting sheet for sample analysis with epi-fluorescence. The samples were all pre-fixed with formaldehyde. Information about sampling date, analysis date, settled volume and time, and any other information was recorded at the top of each counting sheet.

The mixotrophic category changed during counting as I realised after some counts that it was not mixotrophic micro-flagellates appearing orange but autotrophic micro-flagellates that responded in a weak signal.

A.5 RAS-500 manual

The RAS-500 (McLane Research Laboratories Inc., East Falmouth, Massachusetts) was a time-series sampler that could collect up to 48 individual 500 mL water samples in a wide temperature (0 - 50°) and depth range (up to 5500 meters). Pre and post sample acid cleaning cycles removed bio-fouling of the inlet tubing and other contaminants to keep the samples pure. The RAS-500 user interface controlled a multi-port valve and displacement pump, directed the acid wash, cleaning cycles and fluid volume, and programmed the sampling schedule. During deployment, the system logged data such as electrical parameters, sampling timing (e.g. 13:00:00 GMT), volume rate (e.g. 75 mL per minute) and volume flow (e.g. 400 mL per bag). These data were off loaded after the RAS-500 was recovered to check the

performance of the sampler. All components like the controller housing, pump assembly, multi-port valve, and sample containers were mounted inside a protective stainless steel frame. The sampler was about 128 cm high, 73 wide and 73 cm long.

A pump drew seawater into the sample containers at a fixed rate of 75 mL/min. A multi-port valve directed the seawater to the sample bags and could be programmed to flush old water from the tubes and valve before each sample was collected to help prevent sample contamination and reduce accumulated bio-fouling. Each bag was connected in series between the intake head (top half of valve) and the exhaust head (lower half of valve). The pump drew water out of the sample container in which the collapsed sample bag was mounted. This pumping created a pressure gradient that drove the flow of ambient seawater through the intake and into the sample bag. After each sample was taken, the multi-port valve returns to the Home Port (0), sealing the sample in the bag. The whole system was operated by an alkaline battery pack, that had to be checked every other survey and was replaced when the voltage of 31.5 volt dropped to less than 28.0 volt. A backup battery sustained the controller memory in the event of a main battery failure. Before the battery was replaced all data were downloaded from the RAS-500. Removing the main battery erases all the deployment data stored. The programme to set-up the sampler was called ‘ras.exe’. The main menu showed several options. The previous deployment settings pump flow, battery voltage, date and time were always checked first. Once these were found to be fine the option ‘new deployment settings’ was chosen. Within this, the choice of ‘enter each event individually’ was chosen for this sampler set-up and the new deployment programme was entered. Before the system was deployed it was important to check that the battery was connected, the end cap was closed, the programme was entered correctly, the COM cable was disconnected (first from the sampler then from the computer!) and the dummy plug was attached. (An example sheet for a sampling schedule for a particular programme can be found in subsection A.5.1). For the consistent nutrient data a very similar smaller model (RAS-100) was used.

A.5.1 Example of Sampler Programme

Bottle no.	Date (us date)	Hour (GMT)	fixative	Volume [mL] + fixative [mL]
1	09/22/2009	12:00:00	Lugol's	400 +2
2	09/22/2009	13:00:00	formalin	400 +10
3	09/25/2009	12:00:00	Lugol's	400 +2
4	09/28/2009	12:00:00	Lugol's	400 +2
5	09/28/2009	13:00:00	Formalin	400 +10
6	10/01/2009	12:00:00	Lugol's	400 +2
7	10/04/2009	12:00:00	Lugol's	400 +2
8	10/04/2009	13:00:00	Formalin	400 +10
9	10/07/2009	12:00:00	Lugol's	400 +2
10	10/10/2009	12:00:00	Lugol's	400 +2
11	10/10/2009	13:00:00	Formalin	400 +10
12	10/13/2009	12:00:00	Lugol's	400 +2
13	10/16/2009	12:00:00	Lugol's	400 +2
14	10/16/2009	13:00:00	Formalin	400 +10
15	10/20/2009	12:00:00	Lugol's	400 +2
16	10/24/2009	12:00:00	Lugol's	400 +2
17	10/24/2009	13:00:00	Formalin	400 +10
18	10/28/2009	12:00:00	Lugol's	400 +2
19	11/01/2009	12:00:00	Lugol's	400 +2
20	11/01/2009	13:00:00	Formalin	400 +10
21	11/05/2009	12:00:00	Lugol's	400 +2
22	11/09/2009	12:00:00	Lugol's	400 +2
23	11/09/2009	13:00:00	Formalin	400 +10
24	11/14/2009	12:00:00	Lugol's	400 +2
25	11/18/2009	12:00:00	Lugol's	400 +2
26	11/18/2009	13:00:00	Formalin	400 +10
27	11/22/2009	12:00:00	Lugol's	400 +2
28	11/26/2009	12:00:00	Lugol's	400 +2
29	11/26/2009	13:00:00	Formalin	400 +10
30	11/30/2009	12:00:00	Lugol's	400 +2
31	12/03/2009	12:00:00	Lugol's	400 +2
32	12/03/2009	13:00:00	Formalin	400 +10
33	12/06/2009	12:00:00	Lugol's	400 +2
34	12/06/2009	13:00:00	Formalin	400 +10
35	12/10/2009	12:00:00	Lugol's	400 +2
36	12/10/2009	13:00:00	Formalin	400 +10

The recommended final concentration of 1% formaldehyde could not be used as there was a risk that the combined volume of sample and preservation would lead to burst the sample bag. The maximum volume of a single bag was 500 mL and it was noticed that the volume pumped into the bags varied (250-500 mL). A burst bag could have resulted in destruction of the instrument as the electronic pump system is highly sensitive to moisture or liquid.

A.6 Quality Assurance

Cefas species list of inter-calibration

Table A.6: Species list of the inter-calibration with Mr Thomas McGowan from Cefas for Quality Assurance in microplankton counts from Cefas's West Gabbard mooring station in the Outer Thames, southern North Sea.

Month:														
Mooring Depl No.:			W G 58		W G 59		W G 60		W G 60		W G 62		W G 62	
Bag Number:			3		3		6		18		3		12	
Sample Date:			10/01/09	10/01/09	18/01/09	18/01/09	22/04/09	22/04/09	24/05/09	24/05/09	19/07/09	19/07/09	12/08/09	12/08/09
Analyst>			TM	CS	TM	CS	TM	CS	TM	CS	TM	CS	TM	CS
Analysis Date:			19/11/09	19/11/09	19/11/09	19/11/09	23/11/09	23/11/09	23/11/09	23/11/09	23/11/09	23/11/09	24/11/09	24/11/09
Sample size:			25ml	25ml	25ml	25ml	25ml	25ml	25ml	25ml	25ml	25ml	25ml	25ml
Genus/Assembly	**Species/Grou**	**(um)**												
Actinoptychus	sp													
Akashiwo	sanguinea													
Alexandrium	species													
Alexandrium	species	<20												
Alexandrium	species	20-50											40	
Alexandrium	species	>50												
Amphidinium	species													
Amphidinium	cartarae													
Amylax	triacantha													
Asterionella	formosa													
Asterionellopsis	glacialis					520	200	500	160	3520	120	80	920	
Asterionellopsis	kariana			80	160		160	220						
Asteromphalus	species													
Atheya	Attheya													
Bacillaria	paxillifera			440	80									
Bacteriastrum	species													
Bellerochea	species													
Cerataulina	pelagica								400	1760	80	400		240
Ceratium	species													
Ceratium	species	<20												
Ceratium	species	20-50												
Ceratium	species	>50												
Ceratium	tripos													
Ceratium	furca													

Table A.7: Species list of the inter-calibration continued

			TM	CS	TM	CS	TM	CS	TM	CS	TM	CS	TM	CS
Genus/Assembly	**Species/Group**	**(um)**	10/01/09	10/01/09	18/01/09	18/01/09	22/04/09	22/04/09	24/05/09	24/05/09	19/07/09	19/07/09	12/08/09	12/08/09
Ceratium	fusus													
Ceratium	macroceros													
Ceratium	horridum													
Ceratium	lineatum													
Ceratium	platycorne													
Ceratium	compressum													
Chaetoceros	sp											280	40	640
Chaetoceros	Phaeroceros		40						680	1260	120	40	2360	2640
Chaetoceros	Hyalochaetae			40	80	200	120	160	840	1056		160	2160	6880
Coccolithophorid indet														
Cochlodinium	species													
Corethron	hystrix					40								
Coscinodiscus	species													
Coscinodiscus	species	<20		40										
Coscinodiscus	species	20-50												
Coscinodiscus	species	>50				120		120						80
Cyanobacteria indet														
Cylindrotheca	gracilis													
Cylindrotheca/Nitzschia	closterium/longissima		120	240	680	960	720	1800	120	880	19080	12160	155000*	82800
Dactyliosolen	fragilissimus								220					
Desmid indet														
Detonula	confervacaea													
Detonula	pumila													
Dictyocha	fibula										40			
Dinophysis	species													
Dinophysis	species	<20												
Dinophysis	species	20-50												
Dinophysis	species	>50												
Dinophysis	acuminata													
Dinophysis	acuta													
Dinophysis	caudata													
Dinophysis	dens													

Table A.8: Species list of the inter-calibration continued

			TM	CS	TM	CS	TM	CS	TM	CS	TM	CS	TM	CS
Genus/Assembly	Species/Group	(um)	10/01/09	10/01/09	18/01/09	18/01/09	22/04/09	22/04/09	24/05/09	24/05/09	19/07/09	19/07/09	12/08/09	12/08/09
Dinophysis	fortii													
Dinophysis	hastata													
Dinophysis	nasutum													
Dinophysis	norvegica													40
Dinophysis	odiosa													
Dinophysis	ovum													
Dinophysis	pulchella													
Dinophysis	punctata													
Dinophysis	sacculus													
Dinophysis	skagii													
Dinophysis	tripos													
Diplopsalis	group													
Dissodinium	species													
Ditylum	brightwellii				40	40	40	1320		440	40	40	280	640
Eucampia	cornuta													
Eucampia	groenlandica													
Eucampia	zodiacus					80								
Euglenophyceae indet			40								80			
Fragilaria	species		1040	920	2560	11040	2280		680	700				
Fragilariopsis	species						13520	9000	6840	9420		1840		
Gonyaulax	species													
Gonyaulax	species	<20												
Gonyaulax	species	20-50						80						
Gonyaulax	species	>50												
Gonyaulax	spinifera													
Gonyaulax	verior													
Guinardia	cylindrus													
Guinardia	delicatula							240	3880	7040	240	120	200	320
Guinardia	flaccida								640	2640	40	160	160	270
Guinardia	striata								9080	16720	5920	9040	240	1120
Gymnodinium	species													
Gymnodinium	species	<20												40 (large)

Table A.9: Species list of the inter-calibration continued

			TM	CS	TM	CS	TM	CS	TM	CS	TM	CS	TM	CS
Genus/Assembly	Species/Gr	(um)	10/01/09	10/01/09	18/01/09	18/01/09	22/04/09	22/04/09	24/05/09	24/05/09	19/07/09	19/07/09	12/08/09	12/08/09
Gymnodinium	species	20-50		200 (med)		9000 (med)		160 (med)		440 (med)		320 (med)		
Gymnodinium	species	>50		9000 (small)		10800(small)		400 (small)		3600 (small)				12600 (small)
Gyrodinium	species													
Gyrodinium	species	<20						240 (small)						
Gyrodinium	species	20-50				40 (med)		40 (med)				160 (med)		40 (med)
Gyrodinium	species	>50												40 (large)
Gyrosigma/Pleurosigma	species		40	40	40	40	40	200	40					
Helicotheca	tamesis						80	80						
Hemiaulus	species													
Heterocapsa	species												120	900
Heterocapsa	minima													
Indet. araphiated diatom	species													
Indet. araphiated diatom	species	<20												
Indet. araphiated diatom	species	20-50				80	80	80			120	160		
Indet. araphiated diatom	species	>50												
Indet. armoured dinos	species													
Indet. armoured dinos	species	<20	40	80			40	40	80		40	40	120	120
Indet. armoured dinos	species	20-50	120		80	120	160	240	160	80	200	1000	440	720
Indet. armoured dinos	species	>50			40	40	40	40			40	40		
Indet. centric	species													
Indet. centric	species	<20	80		40			80			80			
Indet. centric	species	20-50	160	720	200	160		320	40	2640	80			900
Indet. centric	species	>50			120	id as coscino	80	id as coscino						
Indet. chain diatom	ribbons								80					
Indet. naked dinos	species													
Indet. naked dinos	species	<20							40					
Indet. naked dinos	species	20-50												720
Indet. naked dinos	species	>50		360										
Indet. raphiated pennate	species													
Indet. raphiated pennate	species	<20								5400				
Indet. raphiated pennate	species	20-50	80							40	40	160	40	80
Indet. raphiated pennate	species	>50	160		120			240					240	560

Table A.10: Species list of the inter-calibration continued

			TM	CS	TM	CS	TM	CS	TM	CS	TM	CS	TM	CS
Genus/Assembly	**Species/Group**	**(um)**	10/01/09	10/01/09	18/01/09	18/01/09	22/04/09	22/04/09	24/05/09	24/05/09	19/07/09	19/07/09	12/08/09	12/08/09
Karenia	mikimotoi													
Katodinium	species													
Lauderia	species													
Lauderia	annulata											240		
Lennoxia	species													
Leptocylindrus	danicus									8800		240	720	2400
Leptocylindrus	mediterraneus													
Leptocylindrus	minimus				600	240	200							1360
Lingulodinium	polyedrum											40		
Lithodesmium	undulatum													
Mediopyxis	helysia													
Melosira	species													
Meuniera	membranacea										40	80	120	
Microflagellates	species		2022000*	55800	3258000*	127800	291000*	59400		109800	200	14160	80	68400
Myrionecta	rubra													
Neocalyptrella	robusta													
Noctiluca	scintillans													
Noctiluca indet														
Octactis	octonaria													
Odontella	species												120	120
Oxyrrhis	marina													
Oxytoxum	species													
Paralia	sulcata		2640	5520	840	3640	280		600	880	440	440		670
Pediastrum indet														
Phaeocystis	species													
Phalachroma	rotundata													
Planktoniella	sol													
Podosira	stelligera													
Polykrikos	species													
Proboscia	alata													
Pronoctiluca	species													
Prorocentrum	species													

Table A.11: Species list of the inter-calibration continued

			TM	CS	TM	CS	TM	CS	TM	CS	TM	CS	TM	CS
Genus/Assembly	**Species/Group**	**(um)**	10/01/09	10/01/09	18/01/09	18/01/09	22/04/09	22/04/09	24/05/09	24/05/09	19/07/09	19/07/09	12/08/09	12/08/09
Prorocentrum	species	<20												
Prorocentrum	species	20-50						240	40	40		80		
Prorocentrum	species	>50												
Prorocentrum	lima													
Prorocentrum	micans				40	40			40	80	600	600	640	600
Prorocentrum	triestinum												40	80
Prorocentrum	minimum/balticum								80	40	80	80		
Protoceratium	reticulatum													
Protoperidinium	species													
Protoperidinium	species	<20												
Protoperidinium	species	20-50												
Protoperidinium	species	>50						80		80		40		40
Pseudo-Nitzschia	species	(<5)			120	160	160	240				280	80	120
Pseudo-Nitzschia	species	(>5)	160	120	160								1320	960
Pseudo-nitzschia sp	species													
Pseudosolenia	calcar-avis													
Rhizosolenia	species	0-10											40	
Rhizosolenia	species	10-20					160		80		160		160	
Rhizosolenia	pungens													
Rhizosolenia	setigera							120				80		160
Rhizosolenia	styliformis							120		160		480		460
Rhizosolenia	hebetata													
Rhizosolenia	imbricata													
Rhizosolenia	stolterfothii													
Rhizosolenia	species													
Scrippsiella	species							240		120				
Silicoflagellates indet														80
Sinophysis	species													
Skeletonema	species			80	280	160	5580	8720						640
Spatulodinium	pseudonoctiluca													
Stephanopyxis	species													
Striatella	unipunctata													

Table A.12: Species list of the inter-calibration continued

			TM	CS	TM	CS	TM	CS	TM	CS	TM	CS	TM	CS
Genus/Assembly	**Species/Group**	**(um)**	10/01/09	10/01/09	18/01/09	18/01/09	22/04/09	22/04/09	24/05/09	24/05/09	19/07/09	19/07/09	12/08/09	12/08/09
Thalassionema	species													
Thalassionema	nitzschioides						80						800	960
Thalassiosira	species	10-50		40	80	160	2160	2340						
Thalassiosira	species	>50												
Thalassiosira	species	<10							200	280				
Thalassiosira sp	species			160										
Triceratium	alternans													
Others	others													
Other diatoms														
Other dinoflagellates														
Ciliates				160		120		480		880		320		120

Appendix B

Listing 2

B.1 The MCI Script

The MCI script, currently named `MCItaxPTa.m`, calls, successively, 3 main functions:

ENV - current version `ENV3Fpt2.m`, which loads a database and extracts data selected by species or lifeform, and other constraints;

WAVE - current version `WAVE2eb.m`, which loads the output files from ENV (data1 with species or lifeform (Y1) and data2 with species or lifeform (Y2)) and merges two files into one by intersection or union (data Y1+Y2);

PCI - current version `PCI1G.m`, which loads the output files from WAVE (wavedata1 Y1+Y2 and wavedata2 Y1+Y2) and creates the reference and comparison conditions, calculates the PCI/MCI value, performs statistically analysis and displays the output in a diagram

`cfxCSb.m` is the run control file/script, which is read by all three main functions in order to obtain values of parameters used during the run.

The crucial code of the script `MCItaxPTa.m` is shown in table B.1.

Table B.1: Part of MCItax

```
cfxCSb
for iter = 1:4,
    dataenv{iter} = ENV3Fpt2(cp(iter));
end;
for i = 1:2
    iter1 = i*2 - 1; iter2 = i*2;
    datawave{i} = WAVE2eb(cp(iter1), cp(iter2), ...
        dataenv{iter1}, dataenv{iter2});
end;
datapci = PCI1G(cp, datawave{1}, datawave{2});
```

B.2 Data Files

An MCI plot made by the function PCI requires two sets of 3 (or more) columns of data, the columns being: year.decyear, abundance or biomass of lifeform 1, abundance or biomass of lifeform 2 (and so on, if more columns). The first set provides a reference condition, the second set a comparison condition. They are provided to PCI in the form of either one or two 2-dimensional matrices, called `datawave{1}` and `datawave{2}` in table B.1. PCI must be instructed, through the control values passed in the structure `cp` (see section B.3), whether to draw both sets from a single matrix or whether to use both (and in which order). This is done by the control values `ndf` to state whether the second data set exists and by `compf` that instructs which data set to take for the comparison condition. The order is given by the order of the control variables `d1des` and `d2des` that identify the first and second dataset sites.

The WAVE function gets 4 files from the ENV function each file containing a 4-column matrix with column 1 = year, column 2 = day, column 3 = abundance, and column 4 = biomass of the selected lifeform. The `getruncontrols2` function and some control values instruct the WAVE function how to create the `datawave{1}` and `datawave{2}` matrices. `ninfile` instructs `getruncontrols2` how many data files to load at a time (one or two) from the passed on files `dataenv{iter1}` and `dataenv{iter2}`. The `getruncotrols2` function then uses the control variables `yname` to assign column 2 and 3 to the chosen lifeforms and control variable `ycol` instructs the function which column to use from the `dataenv{iter}` files which is either abundance labelled as cells/L by `col3name` or biomass labelled as mg C biomass/m3 by`col4name`. The key operation of this function is to merge a pair of 2-D matrices `dataenv` passed on from ENV into an individual `datawave` matrix. WAVE either takes the intersection of data `crflg = 2` or the union `crflg = 3` (switch statement) of those files and provides two 3-column matrices where the columns are

column 1= year.dec., column 2= abundance or biomass of lf1 (Y1) and column 3= abundance or biomass of lf2 (Y2) that are then passed on to the `PCI` function. The function ENV is run 4 times to make 4 `dataenv` matrices that are combined pairwise by WAVE. Each run of ENV is controlled by an appropriate control structure, such as `cp(3)`.

Control variables, that are needed to load the required information from a database into the script and functions are shown and explained in table B.2. For the ENV function and it's child functions these control variables are: `loaddt`, `uname`, `category`, `code`, `name`, `startyear`, `stopyear`, `startstat`, `stopstat`, `mindepth`, `maxdepth`, `envsw`, `zerocount`, `minmul`. Control variables that are used and needed by the WAVE function and it's child functions are: `dPF`, `dNF`, `dss`', `ninfile`, `yname`, `ycol`, `col3name`, `col4name`, `trans`, `z3`, `z4`, `crflag`, `defaf`. The control variables that are required by the PCI function and it's child functions are: `d1des`, `d2des`, `c1dat`, `c1sv1`, `c1sv2`, `dsv1`, `dsv2`, `mf`, `reff`, `refs`, `refe`, `p`, `COL`, `pt`.

B.2.1 Summary

Basically, function ENV loads the database into a big matrix of 14 columns (simply imports the database). Then `extractlifeform.m` picks the required information using `multfac.txt` and `volf.txt` and some control variables to put this information into a 4-column matrix. This it does 4 time as, later on in the `PCI` function, one pair of lifeforms or state variable (Y1+2) is required to describe the reference state space and another pair of the same lifeforms (Y1+2) is required to describe the comparison state space. Those matrices containing the year, the day, abundance and biomass of one chosen lifeform (e.g.Y1). Then those 4 files are loaded by the WAVE function, first the two datasets for datafile 1 which will be representing the reference condition and then two datasets for datafile 2 representing the comparison condition. `getruncontrols2.m` extracts the 2 lifeforms from the 4 column matrices passed on from ENV and merges them together into one file putting them into column 2 and 3. It extracts either the abundance or biomass from the passed on env files for each lifeform. It also converts the year and day into decimal year and puts it in the first column. The function repeats this twice, once for the reference condition and once for the comparison condition. The WAVE function and its child functions also log10 transform the data while creating two 3-column matrices. Eventually 2 datafiles are passed on to the PCI function. The PCI function takes those two 3-column matrices and creates a reference condition with outlier exclusion and the convex hull function in one diagram. The comparison data points are plotted into a new 2-dimensional system that holds the reference envelope. `PCIplot.m` visualizes the output in a diagram, where it displays the reference condition with state variable

1 and 2 and the drawn envelope and the comparison condition with state variable 1 and 2 and the reference envelope. The MCI value is calculated by `PCIcalc.m` that calculates how many data points of the comparison data fall inside the reference envelope.

B.3 Control Variables

In Table B.1, the statement `cfxCSb` includes lines of Matlab code, stored in a separate script-file, that give values to fields in a multiple control structure called `cp` in this subsidiary script and in the main script. The fields of `cp(1)` are used in the first call of ENV (which outputs `dataenv{1}`), `cp(2)` in the second call (which outputs `dataenv{2}`), and so on. When it comes to running WAVES, the relevant `cp` structures are passed along with the `dataenv` (e.g. `cp(1)` and `cp(2)` with `dataenv{1}` and `dataenv{2}`). When it comes to running PCI, the whole `cp` is passed (including all 4 structures) but `cp(1)` and `cp(2)` are used to control `datawave{1}` and `cp(3)` and `cp(4)` are used to control `datawave{2}`.

The control script contains a structure of cp(1), cp(2), cp(3), and cp(4). All structures contain control variables needed by the ENV, WAVE, and PCI functions and their child functions. Each cp structure provides control variables to help extract the lifeform and station information needed to create the reference and comparison state spaces. Some control variables are the same, e.g. `.mul`,`.colname`, `.mf`, `.p`, etc. and can be passed to the next cp structure by simply writing cp(2)= cp(1).

Table B.4 lists the fields of these structures and describes their purpose and function. Values might be passed to child functions such as `extractlifeform`, `getruncontrols2` within the main functions.

Table B.2: Fields of the control structure `cp`

Field name	What does it contain and use of its value	ENV	WAVE	PCI
.volf	(quoted string) maps species to lifeforms, e.g. `'volftax.txt'`	+	-	-
.mul	(quoted string) holds multiplication factor code, e.g. `'CordulaMultfac.txt`	+	-	-
.dfn	(quoted string) holds database as plain txt file, e.g. `'AllphytoplanktonCordulaOct10.txt'`	+	-	-
.uname	(quoted string) initials of the user	+	+	-
.loaddt	(coded scalar) loads raw of existing data	+	-	-
.category	(switch scalar) determines lf type loads it from `.volf` e.g. `'LFtype1=size'`	+	-	-
.code	(real scalar) determines lf code within category e.g. `'code 1 = silicate users'`	+	-	-
.name	(quoted string) defines name for lf Y1 e.g. `'silicate users'`	+	-	+
.startyear	(real scalar) first year of reference dataset	+	-	+
.stopyear	(real scalar) end year of reference dataset	+	-	+
.startstat	(real scalar) first station of reference dataset	+	-	-
.stopstat	(real scalar) end station of reference dataset	+	-	-
.defpf	(coded scalar) requires output type e.g. `'0 = no output'`	+	-	+
.envsw	(real scalar) defines %ile of envelope around extracted lifeform data, e.g. 90	+	-	-
.dPF	(coded scalar) requires output type of extracted data, e.g. time series plots of selected lfs	+	-	-
.zerocount	(real scalar) sets number for 'no-see' value for zero sample counts, e.g.`'zerocount=0'`	+	-	-
.randmeth	(coded scalar) undertakes subsampling if required	-	+	-
.dNF	(coded scalar) gives default number of pairs of	-	+	-
	terms set on 3, which means 2 pairs are used	-	+	-

Table B.3: Fields of the control structure `cp` continued

Field name	What does it contain and use of its value	ENV	WAVE	PCI
.c1sv1	(integer scalar)determines column to find state variable (sv)1 in	-	-	+
.c1dat	(integer scalar) determines column where to find year of datafile 1	-	-	+
.c1des	(quoted string) name of site of datafile 1 e.g. `‘Western Irish Sea’`	-	-	+
.dsv1	(quoted string) name of state variable one e.g. `‘silicate users’`	-	+	+
.c1sv2	(integer) determines column in which to find sv2	-	+	+
.dsv2	(quoted string)name of sv2, e.g. `‘non-silicate users’`	-	+	+
.c2des	(quoted string) name of site of datafile 2 e.g. `‘Liverpool Bay’`	-	-	+
.z3	(real scalar) minimum/‘no-see’ value for column 3 e.g. 295	-	+	+
.z4	(real scalar) minimum/‘no-see’ value for column 4 e.g. 1e-5	-	+	+
.mf	(switch(controls whether data are being reduced or all data are used	-	+	-
.ninfile	(case) tells function how many data files to load	-	+	-
.ndf	states if second dataset exists	-	+	+
.compf	(switch) instructs which dataset to take for the comparison condition	-	+	+
	e.g. `‘pdf’`			
.crflag	(switch) merges datafile 1 and 2	-	+	-
	if set on 2 = intersection; 3 = union	-	+	-
.trans	(switch) transforms data into log10 (x+z)	-	+	-
	e.g. if 1=transformation; if 0=no transformation	-	+	-
.yname	(quoted string) name of sv 1, e.g. `‘silicate users’`	-	+	-
.ycol	(switch) instructs which column of extracted data to use, e.g. `‘3=cell abundance’`	-	+	-

Table B.4: Fields of the control structure `cp` continued

Field name	**What does it contain and use of its value**	**ENV**	**WAVE**	**PCI**
.colname	(quoted string) label for units in column 3 or 4 e.g. `‘col3name = cells/L’`	+	+	+
.defaf	(coded)ends `‘WAVE’` function	-	+	-
.z1	(scalar) minimum value for abundance in sample (replaces zeros) (redundant)	-	+	+
.reff	(coded scalar)holds information on how many reference years are used (redundant)	-	-	+
.refs	(scalar) start year of reference condition	-	-	+
.refe	(scalar) end year of reference condition	-	-	+
.p	(scalar) holds the %ile of outlier exclusion	-	-	+
.COL	(coded)determines the colour in the diagram	-	-	+
.pt	(coded)determines format of saved diagram,	-	-	+