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## **DOCTOR OF PHILOSOPHY**

**Seasonal variation of dissolved organic carbon (DOC) and nitrogen (DON) in relation to primary production and yellow substrates (g440). in the Menai Strait, North Wales**

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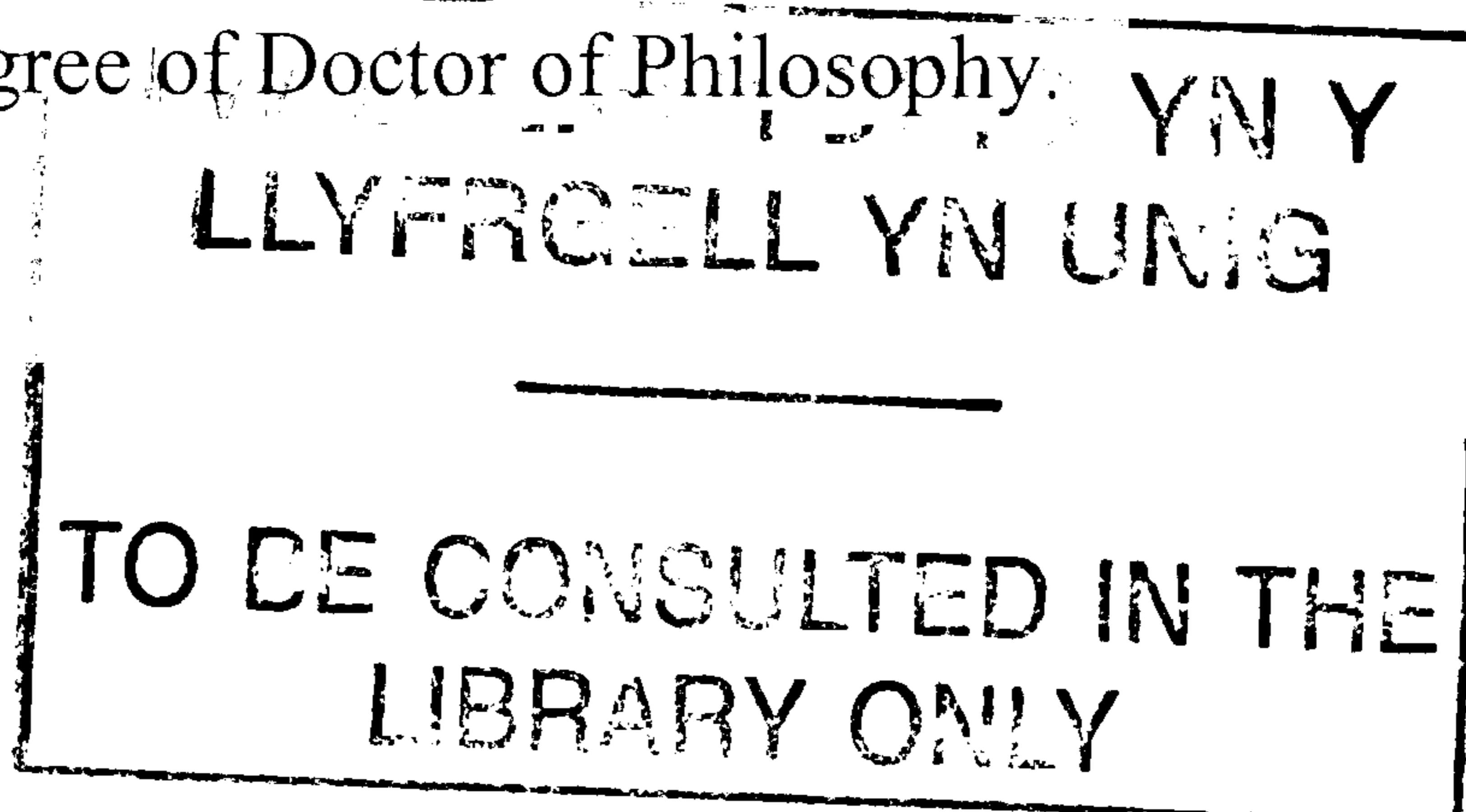
**Seasonal Variation of Dissolved Organic Carbon (DOC) and Nitrogen (DON) in Relation to Primary Production and Yellow Substances ( $g_{440}$ ) in the Menai Strait, North Wales**

By

A.R.N. Al-Azri

A thesis in partial fulfillment of the requirements of the University of Wales

for the degree of Doctor of Philosophy.



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University of Wales, Bangor

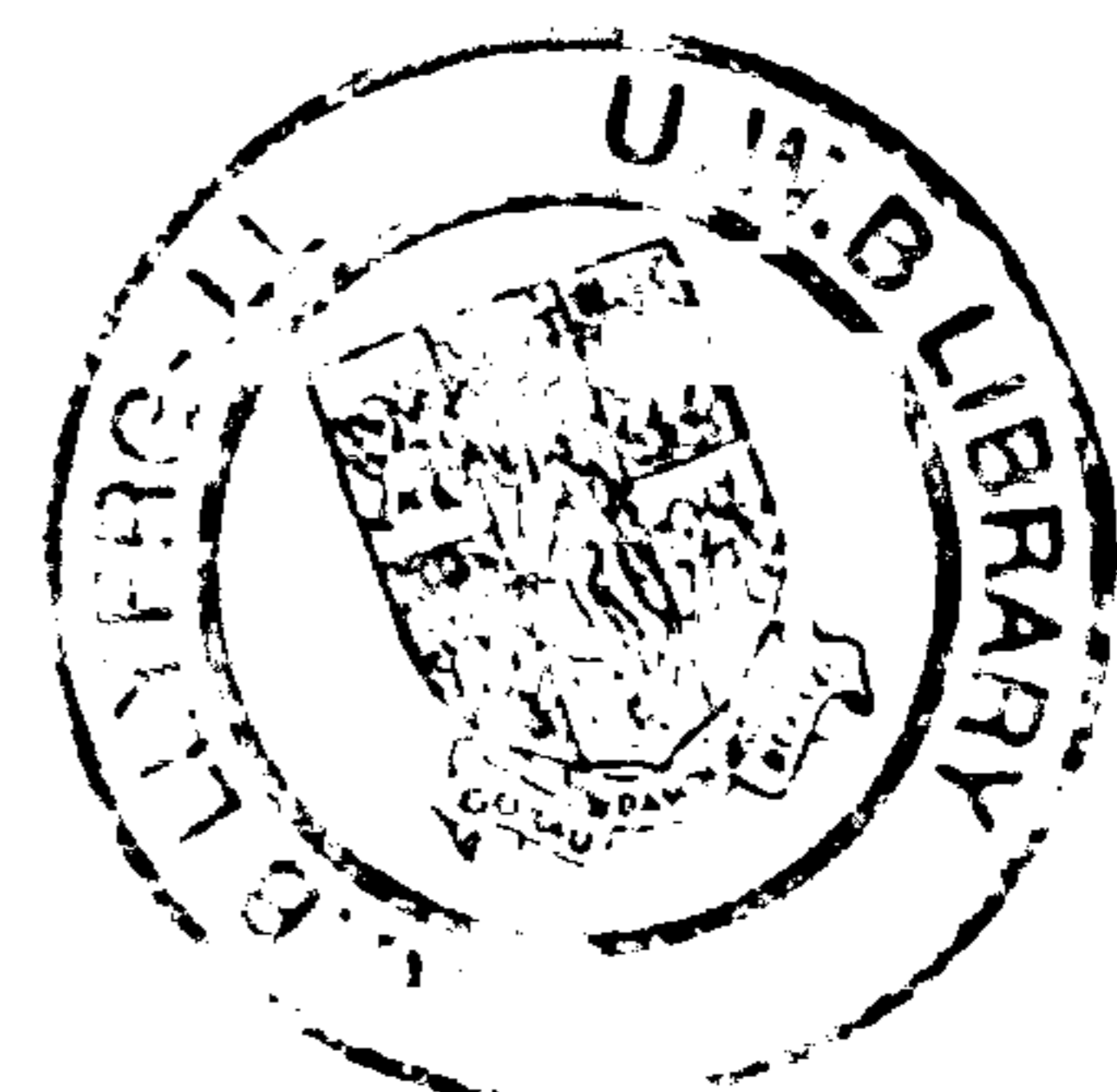
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**Abstract:**

During the year 1998-1999 samples of seawater were collected fortnightly except during the spring bloom in 1999 when samples were collected on a daily basis from the Menai Strait North Wales, U.K. Seasonal cycles of dissolved organic carbon (DOC), nitrogen (DON), yellow substance (YS), particulate organic carbon (POC), nitrogen (PON), and primary productivity were followed. Furthermore Chlorophyll *a* concentrations and counts of bacterial abundance and phytoplankton species were made along with measurement of physical and chemical parameters.

The phytoplankton species succession followed more or less similar pattern to previous studies in the region but with minor differences such as the occurrence of *Leptocylindrus sp.* blooms during summer in both years. Significant correlations were found between DOC and POC as well as DON and PON. YS was significantly correlated with DOC but not with salinity showing less evidence of riverine and runoff contribution. There was proportionality between the intensities of chlorophyll *a* and the subsequent DOC peaks and the time lag between primary production and increase in bacterial abundance, showing that a major part of the DOM in the Menai Strait is produced through degradation of particulate organic matter.

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# CHAPTER I

## 1. INTRODUCTION

Dissolved organic matter (DOM) accounts for more than 90% of the organic matter in the sea surface (Williams & Druffel 1987; Druffel *et al.* 1992; Bada & Lee 1977). DOM in seawater is predominantly of *in situ* origin, however other sources such as from the atmosphere and river flow into coastal water may significantly contribute to the total DOM. DOM is produced through different methods, as will be discussed in detail later in this chapter.

In the marine environment, dissolved organic carbon (DOC) represents one of the largest reservoirs of exchangeable organic matter at the earth's surface (Williams & Druffel 1987; Druffel *et al.* 1992; Bada & Lee 1977). Further, this fraction is influenced by both biological and physicochemical processes depending on time and place (Lancelot 1983). However little is known of the specific mechanisms leading to the coupling of the processes of production and consumption which are considered as the main processes in the carbon cycle (Williams & Druffel 1987).

It is therefore the aim of this research to investigate the dynamics of the DOM in the coastal waters in relation to primary production and heterotrophic uptake of DOM. This can be achieved by studying phytoplankton dynamics and observation of other biological and physicochemical parameters.

## 1.2. BIOGEOCHEMICAL PROCESSES

### 1.2.1 The production of dissolved organic matter (DOM)

The term organic matter refers to all organic compounds which are composed mainly of elements such as carbon, nitrogen, oxygen, phosphorus and sulphur. In the marine environment the primary producers are considered as the main source of particulate organic matter. These organisms transform inorganic matter into particulate organic matter (POM), using light energy converted to chemical energy. In addition to carbon dioxide and water, inorganic nutrients such as nitrogen, phosphorus and sulphur are required. Nitrogen is assimilated as nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), and ammonium ( $\text{NH}_4^+$ ), while phosphorus is assimilated as phosphate ( $\text{PO}_4^{3-}$ ) and sulphur as sulphate ( $\text{SO}_4^{2-}$ ).

Most organic matter falls into one of the following general classes biomolecules, hydrocarbons, carbohydrates, lipids, fatty acids, amino acids, or nucleic acids (Libes 1992). Due to the complex nature of the individual chemical compounds in organic matter, its concentration is often determined according to the defined fraction (POM and DOM, %C, C/N), total concentrations and absorbance spectra.

### 1.2.2. Characterisation of Organic Matter

Oceanic dissolved organic matter (DOM) represents one of the largest exchangeable reservoirs of organic carbon at the earth's surface ( $\sim 1.7 \times 10^{18}$  g C) (Druffel *et al.* 1992). The interest in studying the sources and fate of DOM appeared after a realisation of the role of bacteria as the primary consumers of DOM (Cherrier *et al.* 1996). Despite the difficulties in distinguishing between the DOM and particulate organic matter (POM), in most studies DOM is defined as the organic material passing through a  $0.45\mu\text{m}$  filter and the POM as the material retained on the filter (Sharp 1973). However, it has been suggested that even with this size of filter, most bacteria, all viruses and small-suspended particles may pass through it (Druffel *et al.* 1992). About 5-30 % of marine primary production is directly released as DOM

(Baines & Pace 1991) and the dissolved materials produced by phytoplankton consists of all types of biochemical products such as carbohydrates (mono, di, oligo and polysaccharides) nitrogenous compounds (amino acids, proteins, and polypeptides), lipids (fatty acids) and organic acids (glycolate, vitamins) (Biddanda & Benner 1997). They can be classified into two groups according to molecular weight: high and low molecular weight (HMW and LMW respectively).

### **1.2.2.1 Fractionation of DOM**

In the sea phytoplankton along with other types of algae are the main producers of organic matter from inorganic compounds. In addition, natural phytoplankton populations are known for extracellular release of dissolved organic matter (DOM) (Kirchman 2000). The liberation of such products could be quantitatively important in the way that it is used by heterotrophic bacteria, or it could be qualitatively important since among the substances liberated some could act as inhibitors of growth of some species (Al-Hassan 1975). DOM can be classified into two major categories, either by size as explained in the previous paragraph or by molecular weight. The classification of DOM by molecular weight consists of two categories, the low molecular weight (LMW) and the high molecular weight (HMW) or colloidal. The LMW consists of compounds < 1000 Daltons (Da) while the HMW compounds have a molecular weight > 1000 Da. (Jensen 1983). Some studies reported that 20-30% of the DOM is HMW (Benner *et al.* 1992) and 65-80% is LMW (Amon & Benner 1994), resulting in two types of pools: a carbon rich HMW fraction and a nitrogen rich LMW fraction (Biddanda & Benner 1997). It is believed that LMW material is rapidly remineralized, while HMW compounds are refractory (Amon & Benner 1994). However a recent study (Amon & Benner 1996) revealed that the HMW DOC pool is larger than the reactive pool of LMW DOC. Consequently, DOM seems to decrease with decreasing size which means the HMW DOM tends to react faster than the LMW DOM.

The compositional differences between different molecular size fractions produced by phytoplankton have an impact on the rates at which organic matter can be broken



down and returned to DOM pools (Amon & Benner 1994), i.e the HMW DOM supports high rates of bacteria respiration, while the LMW DOM supports higher bacterial growth (Gardner *et al.* 1996). This has been reported by Amon & Benner (1994), in an experiment where rates of carbon consumption were examined by measuring changes in dissolved oxygen. DOC concentrations in HMW incubations decreased rapidly being mineralized to carbon dioxide by bacteria, on the other hand low consumption was observed in LMW DOM incubation indicating that small fraction of LMW DOM was used by bacteria. This has a big impact on the microbial loop, as differences in the compositions of HMW and LMW DOM seem to be reflected in microbial growth and respiration and contributed to the variations of the dissolved organic carbon (DOC) in the surface water of oceans.

#### **1.2.2.2. Sources of DOM**

DOM in seawater is predominantly of *in situ* origin, however there is also contribution from external sources such as fresh water input from river and atmosphere (Wafar 1984). The production of DOM is through degradation of dead and decaying organic matter, microbial secretions, algal exudates, and of terrigenous origin.

##### **1.2.2.2.1. Microbial secretions:**

Bacteria and microalgae play an important role in the marine food web in the mineralisation of DOM and inorganic compounds and the cycling of carbon and nitrogen. The contribution of bacteria as a source to the cycling of DOC is through the secretion of the mucous material known as exopolymers secretions (EPS) (Decho 1990). EPS are of high molecular weight and are used by bacteria as a protective shield from different aspects in the water column. In the marine environment they occur in two forms: dispersed HMW secretions and particulate slime components. In addition, they are considered as a reliable source of food for other microbial communities. Bacteria cannot utilise their own EPS, but they can utilise the EPS produced by other bacteria (Tago & Aida 1977; Bell & Mitchell 1972).

#### **1.2.2.2. Algal Exudation:**

During phytoplankton blooms excess organic material is produced and released as exudates (Bjornsen 1988; Larsson & Hagstrom 1982). These exudates are often dominated by low molecular weight (LMW) compounds characterised by high concentration of nitrogen and consequent low C/N ratios (Mague *et al.* 1980; Biddanda & Benner 1997). There is a strong debate concerning the rate of phytoplankton excretion, some studies (Sharp 1973; Larsson & Hagstrom 1982) have reported that healthy phytoplankton contribute 0 - 10% of primary production as extracellular releases, while Fogg (1976) argued against this view and suggested that the figure could be as high as 5 - 50% and on some occasions as much as 90%. Sharp (1973) considered that previous work overestimated the algal excretion and that the true value is about 25%. Despite these different views, it is known that the algal excretion is related to the type of species and varies according to the physiological state and growth-rate of phytoplankton (Baines & Pace 1991). In addition it has been shown that phytoplankton excretion of DOM is a continuous process (Lancelot 1983), but others (Malinsky-Rushansky & Legrand 1996; Zlotnik & Dubinsky 1989) suggest that changes in environmental conditions such as nutrient depletion (Larsson & Hagstrom 1982; Lancelot 1983; Kiorbe *et al.* 1990), high irradiance (Thomas 1971), and viral infection (Gobler *et al.* 1997) could influence the excretion rates.

#### **1.2.2.3. Terrigenous dissolved organic matter:**

It is known that terrigenous dissolved organic matter is continuously discharged by rivers into ocean (Opsahl & Benner (1997), but its distribution and role in the global carbon cycle is poorly defined. Terrigenous DOM can be detected by recognising the lignin-a biopolymer found only in terrestrial vegetation. The lignin could also provide taxonomic information about the source of the terrigenous organic matter (Rochelle-Newall & Fisher 1999). It is thought that terrigenous DOM could be a significant source of nutrients in the coastal ocean, which accounts for ~25 % of global ocean productivity (Opsahl & Benner 1997).

Opsahl & Benner (1997) reported that the terrigenous DOM concentration is 2.6

times higher in the Atlantic than the Pacific, as a result of the 3.6 times greater riverine water discharge to the Atlantic ocean. However, the terrigenous DOM comprises a very small portion (0.7-2.4 %) of the total DOM in the ocean and has an oceanic residence time of (21-132 years) which again is shorter than that of the marine DOM. The regeneration of nutrients during rapid cycling of terrigenous DOM could contribute to high rates of primary production in the coastal ocean.

#### **1.2.2.2.4. Sloppy feeding:**

The major methods of DOM release by zooplankton includes: sloppy feeding, excretion, egestion, and release from faecal pellets (Kirchman 2000). In an environment where the availability of food is high, especially during phytoplankton blooms, DOM can be introduced as by-products from the incomplete ingestion or digestion of food by zooplankton (Jumars *et al.* 1989). The cell size of the dominant phytoplankton is important in determining the amount of DOM released. Lampert (1978) found that the release of DOC from small cells when swallowed was low compared to large cells. The contribution from both the leakage during ingestion and subsequent diffusion could reach as much as 30% of ingested carbon (Lampert, 1978). Furthermore, it has been suggested that the DOC released by zooplankton can represent roughly 10-20% of ingestion (Strom *et al.* 1997). Another method of DOC release from zooplankton is from faecal pellets. Faecal pellets are released by zooplankton and DOC is released by diffusion, mechanical breakage and enzymatic hydrolysis (Jumars *et al.* 1989; Strom *et al.* 1997). Among the above mentioned methods, egestion and rapid dissolution of solutes from pellets are considered to be the dominant control of DOM which is released by zooplankton (Jumars *et al.* 1989).

### **1.2.3 Degradation of organic matter**

#### **1.2.3.1. The classical food chain**

In many marine environments, the rate of organic matter available from primary production is higher than the riverine input of terrestrial particulate organic matter (Carlson *et al.* 1998). Therefore the major source of marine organic matter originates

from *in situ* primary production. The utilisation and transfer of dissolved organic matter from primary producers to higher trophic levels has been investigated for a long time based on the concept of a marine food chain or so called “classical food chain”. This has been established for a long time and it involves the energy transfer from organic matter from primary producers to higher trophic levels i.e. phytoplankton-zooplankton-small fish-large fish to mammals. This food chain concept may vary from place to place depending on number of food levels from primary producers to mammals and the effect of the environmental conditions such as nutrient, light and temperature among others (Ryther & Menzel 1965, Amon & Benner 1996).

#### **1.2.3.2. The microbial loop**

Degradation of organic matter and returning of inorganic nutrients to the environment has been regarded as the only role of bacteria in the marine environment. Not until researchers recognised the role of bacteria and other microorganisms in the marine ecosystems. This role was defined as the ‘microbial loop’ (Azam et al. 1983). This was achieved with the development of epifluorescence techniques to estimate bacterial abundance (Porter & Feig 1980) and techniques to estimate bacterial production (Fuhrman & Azam 1980).

The phrase “microbial loop” was used to describe the bacteria-flagellate-microzooplankton pathway (Azam *et al.* 1983). These organisms are responsible for break down of organic matter and the regeneration of nutrients (Joint & Pomeroy 1987) and are known to be efficient consumers of organic matter as will be discussed in the next section.

In the microbial loop, the dissolved organic matter (DOM) released by phytoplankton and other organisms, is utilised by bacteria and the resulting biomass is in part consumed by heterotrophic microflagellates. These flagellates are in turn preyed upon by microzooplankton, thus energy released by phytoplankton is returned to main food web via a microbial loop of bacteria -flagellates-microzooplankton.

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organisms, both autotrophic and heterotrophic. Scientists agree on the role of bacteria, flagellates and microzooplankton as remineralisers, responsible for converting organic matter to inorganic and recycling nutrients to primary producers. However, there is no consensus of opinion on the form in which nutrients are assimilated by these organisms. Many studies (Jumars *et al.* 1989; Currie 1990; Bratback *et al.* 1994; Kirchman 1994) have reported that bacteria can assimilate both inorganic and organic nitrogen and phosphorus and can compete with phytoplankton for these inorganic nutrients. The proportion of inorganic nutrient assimilation can vary depending on the concurrent availability of dissolved organic nutrients. It has been shown that bacteria can assimilate 60 % of their total phosphorus as phosphate and 30% of their nitrogen as ammonium (Kirchman 1994). On the other hand, other studies (Wheeler & Kirchman 1986; Hoch & Kirchman 1995) have shown that the assimilation rate of ammonia could be as high as 75%. Bacteria were thought to be unimportant utilisers of nitrate (Wheeler & Kirchman 1986). However it has been reported that they are capable of assimilating nitrate (Kirchman *et al.* 1991) in the presence of sufficient supplies of dissolved organic compounds such as sugars and carbohydrates (Parson *et al.* 1981; Kirchman *et al.* 1991). Despite the fact that bacteria can assimilate the two major forms of nitrogen, dissolved free amino acids are the most preferred form followed by ammonia and nitrate (Kirchman *et al.* 1991). Having reviewed the role of bacteria in the cycling of organic matter and the competition between bacteria and phytoplankton for growth limiting nutrients, it is worthwhile summarising the factors controlling bacterial growth.

#### **1.2.3.2 2. Factors controlling bacterial growth**

The control and regulation of bacterial utilisation of both organic and inorganic matter are still to be fully understood. However, bacterial growth and abundance clearly are controlled by temperature, availability of the dissolved organic matter, predation (grazing), phytoplankton exudates and viral mortality (Riemann *et al.* 1984; Cole *et al.* 1988; Shiah & Ducklow 1994). These factors have been examined singly rather than considered together or under different environmental conditions. For example temperature has often been demonstrated to be one of the major

factor controlling the seasonal production and growth of bacteria in low water temperature such as in the temperate regions (White 1991). While in tropical regions, the supply of dissolved organic matter, grazer, predation, UV radiation and viral mortality are considered the major factors controlling the production and growth of bacteria (Shiah & Ducklow 1994; Hoch & Kirchman 1995; Kirchman 2000).

The interaction between DOM and bacteria plays an important role in the marine environment (Rainer & Benner 1996). The utilization of DOM by bacteria depends on the molecular size of DOM produced. For example, dissolved organic matter produced by phytoplankton and other algae consists of biochemical compounds such as polysaccharides, amino acids, glycollate and organic acids (Eberlein *et al.* 1985). These different compounds seem to affect different microbial activities. Low molecular weight organic matter increase bacterial growth, while high molecular weight organic matter increases bacterial respiration (Gardner *et al.* 1996). However several studies (Joint & Pomeroy 1987; Chin-Leo & Benner 1992; Thingstad & Rassoulzadegan 1995) reported that in some seasons dissolved inorganic compounds such as  $\text{PO}_4^{3-}$  and sometimes  $\text{NH}_4^+$  rather than DOC control bacterial activity. Billen (1984) argued that bacteria assimilated or regenerated ammonium depending on the relationship between C:N composition of the organic substrate and the bacterial biomass. This argument was supported by Goldman *et al.* (1992) who suggested that based on the low C:N ratio of bacterial biomass, nitrogen not carbon limits bacterial growth. In addition Kirchman (1990) suggested that organic nitrogen is most stimulatory to bacterial growth in marine systems. Recently Rivkin (1997) reported that the pattern of utilisation by bacteria of specific organic carbon and nitrogen compounds could change spatially and temporarily in response to changes in phytoplankton exudation. In addition carbon seemed to be the major limiting factor but they did not rule out the possibility of nitrogen as a limiting factor for bacterial growth. It should be noted that the influence of individual or a combination of more than one factor might well differ in different habitats or in different seasons (Shiah & Ducklow 1994).

## **1.2.4. Definitions and dynamics of the DOM**

The major components of the DOM can be characterised as dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and gelbstoff or yellow substance. These fractions are examined in order to understand the seasonal changes of DOM as whole.

### **1.2.4.1. Dissolved Organic Carbon (DOC)**

DOC in seawater plays a significant role as a major reservoir of reactive organic carbon. Druffel *et al.* (1992) suggested that the concentration of DOC in seawater is more than that of POC by 10 to 100 times in surface waters and by 100 to 1000 times at depths. Newly produced DOC has been shown to accumulate during or after phytoplankton blooms (Carlson *et al.* 1994). However, there is a combination of factors which influence the production and accumulation of DOC. For example, nutrient depletion, the coupling between phytoplankton primary production and bacteria biomass and the role of other DOC production mechanisms such as grazing interactions and viral lysis which could produce a more refractory DOC (Carlson *et al.* 1998). The mean age of DOC through  $^{14}\text{C}$  measurements was estimated to be around 4000 to 6000 years (Williams & Druffel 1987). DOC turnover rates may vary from place to place and about 40-50% of the DOC is biologically degradable (Kirchman *et al.* 1991). However, it has been suggested that the transformation of carbon from biological community could play a big role as a source of refractory DOC in the oceans. Nevertheless, the low transfer and growth efficiencies associated with interaction of microbes and grazer make it difficult to accumulate as refractory DOC (Brophy & Carlson 1989).

#### **1.2.4.1.1 Oceanic Distribution**

DOC is produced by biological processes in the upper ocean and mixed downward and diluted by physical processes (Figure 2). In general the upper ocean distribution of DOC reflects the balance between these processes. During the productive season



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Several studies ( Copin-Montegut & Avril, 1993; Carlson *et al.* 1994; Pakulski & Benner, 1994; Zweifel *et al.* 1995;) have reported a gradient in DOC between surface waters and the deep aphotic zone.

**Table 1. DOC gradient between surface and deep waters (values are mean)**

| Area                  | DOC surface<br>( $\mu\text{M C}$ ) | DOC deeper<br>water<br>( $\mu\text{M C}$ ) | Depth<br>(m) | References                 |
|-----------------------|------------------------------------|--|--------------|----------------------------|
| Southern Ocean        | 50                                 | 44   | 3000         | Ogawa <i>et al.</i> 1999   |
| Mediterranean Sea     | 73.5                               | 53.5                                       | 2000         | Doval <i>et al.</i> 1999   |
| North Atlantic        | 71                                 | 50   | 5000         | Zweifel <i>et al.</i> 1995 |
| Sargasso Sea          | 90                                 | 55   | 250          | Carlson <i>et al.</i> 1994 |
| English channel       | 72.5                               | 72   | 70           | Banoob & Williams 1973     |
| Gulf of Mexico        | 130                                | 45   | 1600         | Thomas <i>et al.</i> 1997  |
| North-central Pacific | 72                                 | 34   | 6000         | Druffel <i>et al.</i> 1992 |

All of these results show high concentrations of DOC in the surface waters, which is believed to be triggered by primary production and sometimes the input from rivers in coastal regions. The degradation of POC to form DOC and the continuous oxidation of DOC by respiration are considered the major processes in the transport of DOC between coastal and open oceans (Kepkay *et al.* 1997a). In addition, the offshore DOC transport is regulated by a combination of physical and biological processes, such as the coagulation of colloids into larger aggregates, the vertical mixing of DOC and aggregates between surface and deep water, and the respiratory degradation of colloidal fractions of DOC and aggregates of larger colloids (Kepkay *et al.* 1997b). The cycling of DOC in deep oceans is considered to be controlled by the major processes such as the recycling of DOC within the surface and deep ocean and the deep-water mass circulation.

#### 1.2.4.2 Dissolved Organic Nitrogen (DON)

The nitrogen cycle is very important in the marine system due to its role as a limiting factor for primary productivity. Dissolved inorganic nitrogen (DIN) species ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and urea) are assimilated by phytoplankton. This is due to the fact that phytoplankton are generally not nitrogen fixers. They tend to use the DIN species because they require less energy to be converted into an organic form.

In coastal areas and estuarine environments, an average of 25 to 41% of the dissolved inorganic nitrogen taken by phytoplankton is released as dissolved organic nitrogen (DON) (Bronk *et al.* 1994). The DON pool is thought to contain a large number of compounds that are unknown due to the difficulties involved in methodologies (Bronk *et al.* 1994). The typical concentrations in oceanic and coastal waters range between 3-7 and 3-20 $\mu\text{M}$  respectively (Kirchman 2000). Release rates for DON in oceanic systems range from 4 to 26 nanogram-atoms of nitrogen per litre per hour (Bronk & Glibert 1991). During the phytoplankton blooms, more DON is produced, which is degraded by bacteria which are in turn consumed by zooplankton and protozoa. Subsequently, the DIN is returned into the water column through the remineralization process, thus providing the inorganic N species required by phytoplankton to continue the cycle. However, there is a net loss of N in the cycle by sinking of detrital material known as particulate organic nitrogen (PON). It has been reported that uptake of  $\text{NO}_3^-$  into the surface waters by phytoplankton is equal to the upward flux of  $\text{NO}_3^-$  and that this is balanced by the flux of particulate nitrogen (PN) out of the sea (Bronk & Glibert 1991). Bronk *et al.* (1994) reported that the total DON turnover times in oceanic, coastal, and estuarine sites were 10, 18, and 4 days respectively, which indicates that although a fraction of the DON pool is likely to be refractory, another fraction appears to be turning over rapidly. For example urea and free amino acids are considered as the major labile component of DON (Fuhram, 1990), whereas others, such as humic acids, are more stable (Gardner & Stephens, 1978).

### 1.2.4.2.1. Oceanic Distribution

Oceanic distributions of DON show high concentrations near the surface but decreasing with depth, indicating the role of organisms in the euphotic zone in producing DON, followed by bacterial degradation in deep waters (Jackson & Williams, 1985). DON have been examined in various ocean environment (Williams, 1995; Ogawa et al., 1999; Doval *et al.* 1999).

**Table 2. Typical DON concentrations of different types of water. Most of the concentration shown in the table were reported in studies conducted in coastal regions (values are mean).**

| Regions                            | DON concentrations<br>( $\mu\text{M}$ ) | References               |
|------------------------------------|---|--------------------------|
| Strait of Georgia                  | 8.5                                     | Williams 1995            |
| Coastal Pacific                    | 3                                       | Williams 1995            |
| English Channel                    | 5.4                                     | Banoob & Williams 1973   |
| Southern Ocean (Australian sector) | 6.5                                     | Ogawa <i>et al.</i> 1999 |
| North-western Mediterranean        | 4.7                                     | Doval <i>et al.</i> 1999 |

Specific DON compounds, such as urea, also exhibit the same gradient. For example it has been reported that the concentration of urea in surface waters ranges from 0 to 0.5  $\mu\text{M}$ , while below 100m it is undetectable (Butler *et al.*, 1979). DON is produced mainly during phytoplankton metabolism in surface waters and is removed by bacterial activities at depth. This is the opposite behaviour to nitrate which is assimilated in surface waters and regenerated at depth. Consequently an inverse relationship between nitrate and DON has been seen (Jackson & Williams, 1985).

### 1.2.4.3. Seasonal Cycle of DOC and DON

It has been reported that the general pattern of seasonal variation of DOC and DON is characterised by lower concentrations during winter and a slight increase in spring and summer followed by highest concentration during late summer and autumn (Banoob&Williams, 1973). These authors reported the changes in the organic forms of carbon, nitrogen and phosphorus in seawater in the English Channel. High concentrations of DOC and DON increased slightly during April-June and the highest values were recorded in September. Morris & Foster (1971) also reported a similar trend to that of Banoob & Williams (1973). Other studies (Al-Hasan *et al.* 1975;Wafar 1984;Williams 1995) reported seasonal variation of DOC and DON characterised by low concentration during winter followed by an accumulation during spring reaching the maximum during summer. The seasonality of DOC and DON most of the time are linked to phytoplankton blooms. However other factors may dominate depending on the surrounding environment and whether it is in the coastal or oceanic regions. For example the oceanic DOC is mainly produced by phytoplankton primary production *in situ* (Duce & Duursma, 1977). By contrast DOC in estuarine and coastal areas has been reported to be mainly dominated by riverine input as primary production is low because of light limitation due to high turbidity (Cadee 1984) or due high primary production from phytoplankton blooms (Carlson *et al.*, 1994; Williams 1995). The fate of riverine DOC in estuaries and coastal areas would be affected by removal and addition processes which involve various mechanisms such as precipitation and adsorption (Hunter 1980). On the other hand the accumulation of DOC and DON in coastal areas is explained as a result of excretion by phytoplankton, dead and decaying of organic material and losses into soluble form during zooplankton grazing and excretion (Wafar 1984). Each of the above mentioned pattern of accumulation influence the seasonality of DOC and DON. In addition the seasonal variation of DON and DOC show different patterns and the changes in the two parameters are not closely correlated (Williams 1995). It is therefore difficult to draw a general conclusion on the seasonality of DOC and DON which is applicable to all environments.

#### 1.2.4.4. Yellow Substances

Yellow substances are also known as aquatic humus. They are composed of humic and fulvic acids (Carder & Steward 1989). The highest concentrations occur in the regions of high production such as areas influenced with land drainage (e.g. coastal regions). Typical profiles of yellow substance (absorption at 440nm) in different type of waters (Kirk 1976; Bricaud et al. 1981; Maritorea & Guillochea, 1996; Kratzer et al., 2000) are shown in Table 3.

**Table 3 Typical concentration of yellow substances as represented by the absorption coefficient at 440nm ( $g_{440}$ ). The values are mean from surface waters.**

| Regions                          | Yellow substance ( $m^{-1}$ ) | References                  |
|----------------------------------|-------------------------------|-----------------------------|
| Menai Strait                     | 0.15                          | Kratzer et al. 2000         |
| South-Eastern Australia          | 0.045                         | Kirk, 1976                  |
| Pacific Ocean (French Polynesia) | 0.06                          | Maritorea&Guillochea 1996   |
| Chesapeake Bay                   | 3.15                          | Rochelle-Newall&Fisher 2002 |
| Baltic Sea                       | 0.018                         | Karabashev 1977             |

In oceanic regions, which are unaffected by land drainage or freshwater runoff, the concentration of yellow substances could be related to primary production as a by-product of algal cell degradation (Harvey *et al.* 1983). Bricaud *et al.* (1981) reported that in certain upwelling areas the concentration of yellow substances does not follow that of chlorophyll *a* (i.e high concentrations of yellow substances might be observed where the chlorophyll concentration is low) indicating a long life for yellow substance compared to the algae that produce them.

Besides pigments and other nonliving materials, yellow substances are considered to be the major factor responsible for the changing colour in aquatic environment (Bricaud *et al.* 1981). As a result, measurement of yellow substances is used to study

the optical properties of seawater. However, measurement of yellow substances could also provide vital information on the past primary productivity and the origin of DOC in a particular environment (Carder & Steward 1989).

### **1.2.5. Aims of the research**

Several authors have documented the observation of DOC, DON and yellow substance. Most of their studies have been focusing on production and consumption of individual fractions or two fractions. In addition most of the studies reported on yellow substances are related to optics. However according to my knowledge, there are no studies in the coastal area investigating the combination of DOC, DON and yellow substance and their interaction with biological and physico-chemical parameters

It is therefore the aim of the present work to: (i) study the seasonal variations of DOM (DOC, DON and yellow substances) in relation to primary production and bacterial abundance; (ii) understand the role of seasonal variation of phytoplankton abundance and species succession on the seasonal variations of DOC, DON and yellow substance; (iii) study the influence of the seasonal variation of DOC in relation to yellow substance and vice versa. Thus, in order to achieve the aims of this project, the following investigations were conducted:

1. To determine the seasonal variation of phytoplankton and bacteria abundance in association with changes in nutrients, temperature, light and salinity.
2. To investigate the seasonal variation of particulate organic carbon (POC), nitrogen (PON), DOC, DON and yellow substance, in relation to phytoplankton and bacteria abundance.
3. To assess the seasonal variation of DOC, DON and yellow substance in relation to primary production and respiration.

4.To carry on experiments investigating the relationship between DOC and yellow substance under different physical and chemical parameters, and attempt to combine the field and laboratory studies in order to understand their dynamics.



# CHAPTER II

## 2.THE MENAI STRAIT

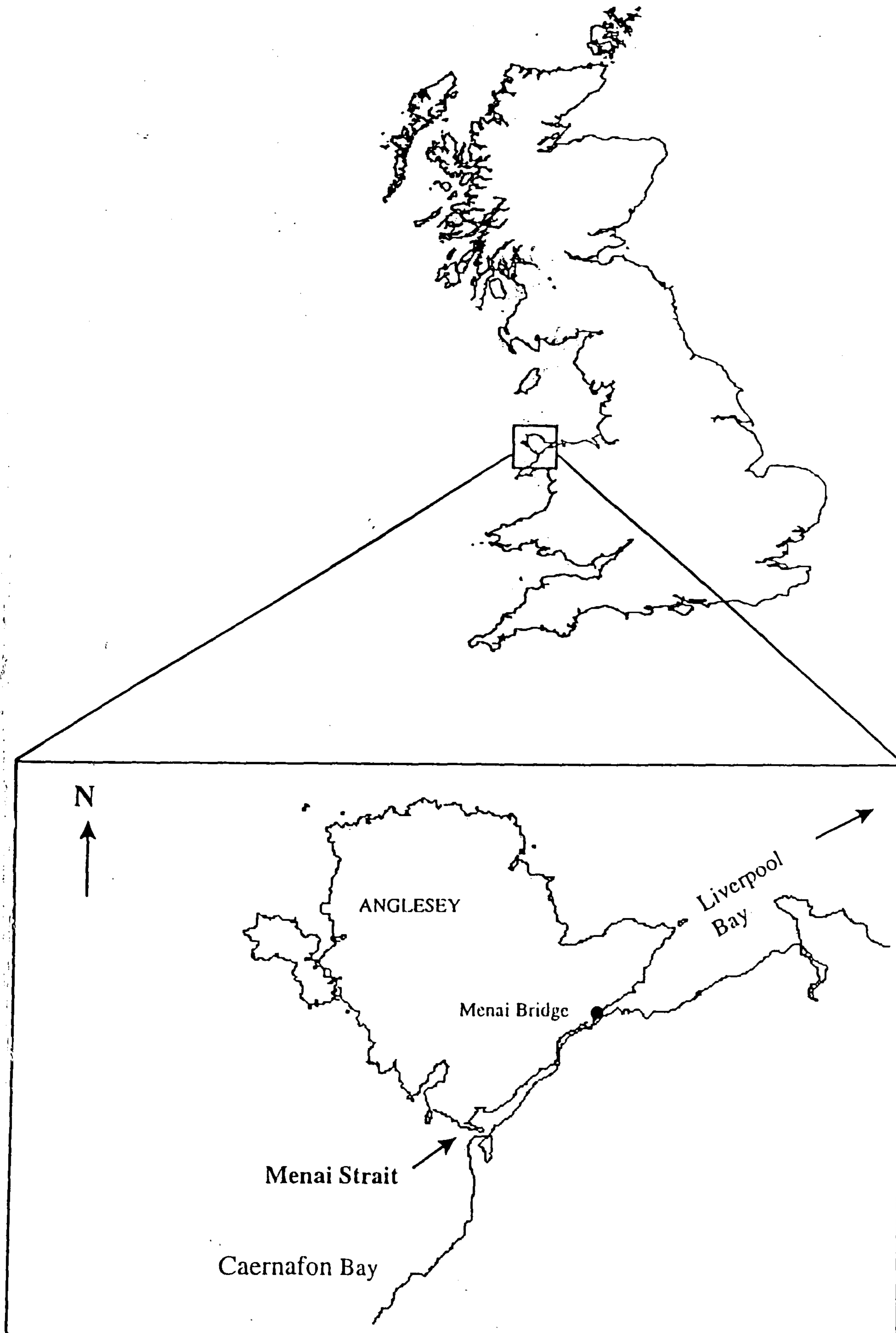


Figure 2.1. Map showing study area (The Menai Strait).

## 2.1. Study area

The Menai Strait is a shallow turbulent stretch of water separating the Island of Anglesey from the mainland of North Wales. The Strait is over 20km in length and has a mean width of 800m narrowing to about 300m at either end of the Swellies (Harvey 1968).

The mean spring and neap tidal ranges at Menai Bridge are 6.6m and 3.4m respectively. Maximum velocities within the Strait during the north-easterly and south-westerly tidal flow are approximately  $80\text{cm s}^{-1}$  and  $120\text{cm s}^{-1}$  respectively (Harvey 1968). It is indicated that the south-westerly flowing tidal current is typically stronger than that flowing to the north-east and that there is a possibility of a small residual flow of water from Liverpool Bay into Caernafon Bay through the Menai Strait (Harvey 1968).

The bed of the Menai Strait consists mainly of solid rocks and stones, especially in the mid channel region where the high energy of the tidal currents prevents significant sedimentation. The water flow in the Menai Strait is affected by weather conditions. A significant correlation between changes in the water level along the channel with a decrease in the atmospheric pressure has been reported (Harvey 1968). Strong north-westerly winds can increase the flow whereas the south-westerly winds can reduce or even reverse the residual flow from its usual direction. There are several freshwater streams that flow into the Strait but the major ones are the Ogwen and the Seiont which enter at the north-east and south-west ends respectively (Al-Hasan *et al.* 1975). The discharge of these streams is small compared with either the tidal prism or with the residual flow of seawater through the Menai Strait.

With the knowledge of the circulation pattern in the eastern part of the Irish sea and the origin of the water passing through the Menai Strait, it could be anticipated that the influence of run off and river drainage from the north-west coast of England and Wales on the concentration of nutrients would be detectable in the Menai Strait water (Ewins & Spencer 1967). Consequently, the plankton sampled from Menai

Strait mostly originates from the adjacent coastal water of Liverpool Bay (Blight *et al.* 1995).

## 2.2. Temperature

The water temperature in the Menai Strait has been monitored for a long period by a number of authors (Al-Hasan *et al.* 1975; Lennox 1979; Blight *et al.* 1995; Rodrigues 1998). These studies showed a seasonal trend characterized by low temperature values (6.2 to 8°C) during winter and highest (12 to 17°C) during summer (Al-Hasan *et al.* 1975). Short-term period variations have been reported (Harvey 1972). The seasonal variation has been explained in term of changing weather conditions. During summer, temperatures remain high as a result of shallow water and exposure of sand and mud to the sun during low tide. In winter, the Menai Strait is more exposed to cold weather and the input of cold water from rivers which lowers water temperature. Short-term period fluctuations have been explained as a result of input of fresh water of different temperature from the surrounding environment (Al-Hasan *et al.* 1975) and the daily heating cycle (Harvey 1972). The Menai Strait tidal oscillation and shallowness create turbulent water and prevent thermal stratification.

## 2.3. Salinity

The seasonal variation of salinity in Menai strait water has been determined by a number of authors (Ewins & Spencer 1967; Jones & Spencer 1970; Al-Hasan *et al.* 1975). The results of these studies showed that the salinity values remain within the range of 32 to 36 psu but can occasionally drop below these values during abnormal hydrographic conditions like the high input of fresh water during the rain seasons. In general, salinity values increase during summer; in autumn and winter it is lower but variable as result of changes in rainfall and river discharge (Al-Hasan *et al.* 1975).

## 2.4. Secchi reading

The transparency of Menai Strait water is characterized by a seasonal trend: low transparency during winter which increases towards the summer months (Jones & Spencer 1970). The seasonal variation is influenced by physical and biological parameters with the latter having more effect. In Menai Strait water the particulate suspended matter appears to be allochthonous with no differences between the load carried at the surface water and bottom water (Buchan *et al.* 1967). Unlike the organic particles, the inorganic particles are characterized by minimum loads during summer and maximum during winter. This variation is more influenced by biological rather than physical effects, since rainfall, fresh water, run-off and salinity showed insignificant effect on the suspended inorganic fraction in the long term (Buchan *et al.* 1967). The main biological processes influencing the variations of the suspended inorganic fraction are reduced erosion and dispersion of film particles by film-forming micro-organisms which bind the surface of the particles. The other process is removal of suspended particles by filter-feeding organisms (Buchan *et al.* 1967). These processes predominate during summer as a result of increasing micro-organism abundance. In addition the weather conditions contribute to the variation of the secchi reading depending when the reading was taken. For example, during winter, cloud cover could give a low secchi reading. In addition the time during which the measurement was taken could also give different reading i.e. early morning measurement may differ to measurement taken during mid-day.

## 2.5. Nutrients

Nutrients (nitrate, nitrite, ammonia, phosphate and silicate) in Menai Strait water also have been studied by a number of authors (Ewins & Spencer 1967; Jones & Spencer 1970; Al-Hasan *et al.* 1975; Blight *et al.* 1995; Rodrigues 1998). These nutrients show similarity in annual trends and sometimes in magnitudes except for ammonia. There are several factors influencing the seasonal variations of these nutrients. Among these factors, the input of land run-off, sewage discharges, rivers and the interaction of the biological uptake and regeneration. Generally nutrient seasonal trends are characterized by high concentrations during winter and low

concentrations during spring and summer, due to low and high biological activities respectively. The tidal cycle from and into Liverpool Bay and the Irish Sea could also influence the variation in nutrient concentrations (Ewins & Spencer 1967; Al-Hasan *et al.* 1975).

### **2.5.1. Nitrate:**

The seasonal trend of nitrate in the Menai Strait is characterized by high concentrations during winter and low during spring and summer. The concentrations may vary slightly in magnitude from year to year but generally fall within the range of 0.05 and 23 $\mu$ M as reported from previous studies (Ewins & Spencer 1967; Lennox 1979; Blight *et al.* 1995; Rodrigues 1998). Nitrate generally increases during winter reaching a maximum concentration (14 $\mu$ M) as reported in studies of Blight *et al.* (1995) and Rodrigues (1998) in March. The continuous accumulation of nitrate during winter months could be due to land drainage, run-off and the slow processes of regeneration and remineralization due to low biological activities (Al-Hasan *et al.* 1975; Blight *et al.* 1995; Rodrigues 1998). The decline in concentration usually starts in May with concentration generally falling below 1 $\mu$ M. The decrease in nitrate concentration during spring and summer is mainly associated with the development of the spring phytoplankton bloom. The initial decrease during May could be the result of the movement of high-salinity low-nitrate water into the Menai Strait (Ewins & Spencer 1967) followed by the increase of biological activities.

### **2.5.2. Nitrite:**

Nitrite concentration shows an irregular trend with low concentration (less than 0.010 to 0.80 $\mu$ M (Rodrigues 1998). Maximum concentration occurs during autumn and lowest during summer. These changes could be related to phytoplankton as they may release nitrite in low light and the slight increase during summer could be attributed to bacterial activity with increasing temperature (Alice 1986).

### **2.5.3. Ammonia:**

The seasonal variation of ammonia was reported by Rodrigues (1998). There is no clear seasonal pattern as for that of nitrate; instead short-term accumulation and exhaustion of ammonia is observed during the study period. In general high

concentration ( $0.70\mu\text{M}$ ) was recorded in autumn and the lowest below detection limit ( $0.030\mu\text{M}$ ) in summer. A maximum of about  $3\mu\text{M}$  was reported by the same author who did not rule out the possibility that this could have been due to contamination.

#### **2.5.4. Silicate:**

The seasonal pattern of silicate in the Menai Strait is more or less similar to that of nitrate. Silicate concentration starts to increase in autumn and unlike nitrate which continues to increase throughout winter, it reaches a maximum concentration (about  $14\mu\text{M}$ ) by November (Ewins & Spencer 1967; Lennox 1979). However Al-Hasan *et al.* (1975) reported a maximum concentration of  $15.6\mu\text{M}$  in January. During spring silicate tends to be removed initially due to species succession during the spring phytoplankton bloom which starts with the dominance of diatoms (Ewins & Spencer 1967; Lennox 1979; Blight *et al.* 1995). In March and April, silicate reaches its lowest concentration ( $0.37\mu\text{M}$ ), while in summer months a low concentration ( $0.87\mu\text{M}$ ) is maintained until September when it starts to increase again. An inverse relationship between salinity and silicate concentration is found supporting the hypothesis that accumulation of silicate during autumn is thought to be a function of increased contribution from land drainage rather than mineralisation (Ewins & Spencer 1967). However Hunt & Foster (1985) suggested that on some occasions the riverine water might dilute the seawater silicate instead of increasing it.

#### **2.5.5. Phosphate:**

The seasonal variation of phosphate in Menai Strait waters is characterized by high concentrations in October and November. The annual concentration varies between  $0.42\mu\text{M}$  and  $1.26\mu\text{M}$  (Ewins & Spencer 1967; Al-Hasan *et al.* 1975). Unlike nitrate, phosphate shows a sharp drop in December followed by fluctuations and an increase throughout winter until April reaching the minimum concentration at the end of May ( $0.43\mu\text{M}$ ). As with nitrate and silicate, the observed fluctuations in phosphate concentration in winter are associated with the input of high salinity waters of different origin. Phosphate exhibits a different pattern from other nutrients as it is not exhausted by phytoplankton blooms and it is maintained at high concentration ( $0.42$  to  $0.64\mu\text{M}$ ) during summer months (Al-Hasan *et al.* 1975). Unlike Ewins &

Spencer (1967) who suggested the insignificant effect of *Phaeocystis sp.* bloom on phosphate concentration, Jones (1962) found that the annual bloom of *Phaeocystis sp.* in the Menai Strait often resulted in the soluble reactive phosphate being reduced to a very low concentration for a short time, thus suggesting the uptake of phosphate by *Phaeocystis sp.* during summer.

## 2.6. Chlorophyll *a* and phytoplankton

### 2.6.1. Chlorophyll *a*:

The seasonal variation of chlorophyll *a* in the Menai Strait has been reported by a number of authors (Ewins & Spencer 1967; Al-Hasan *et al.* 1975; Lennox 1979; Newton 1986; Blight *et al.* 1995; Rodrigues 1998). It is characterized by a high concentration between April and June (maximum  $16 \mu\text{g}\cdot\text{dm}^{-3}$ ) which coincides with the phytoplankton bloom. During summer chlorophyll *a* decreases to low a level (ranging between 1 and  $4 \mu\text{g}\cdot\text{dm}^{-3}$ ). This is followed by a minor peak in autumn and decreasing to the lowest level (below  $1 \mu\text{g}\cdot\text{dm}^{-3}$ ) during winter months (Lennox 1979; Newton 1986; Blight *et al.* 1995; Rodrigues 1998).

### 2.6.2. Phytoplankton:

Phytoplankton studies in the Menai Strait have been made by Jones & Spencer (1970), Al-Hasan (1976), Lennox (1979), Newton (1986) and Blight *et al.* (1995). In general the annual cycle of phytoplankton growth exhibits a similar pattern from year to year, despite the fact that the start of the phytoplankton bloom and its magnitude may vary from year to year with the major bloom usually occurring between April and June.

Two peaks characterize the annual pattern: a major peak in spring and summer and a minor one in autumn (Blight *et al.* 1995). The major spring bloom is characterized by 3 peaks: the first peak is composed of mixed diatoms with *Asterionella glacialis*, *Dytilum brightwelli*, *Skeletonema costatum* and *Guinardia flaccida* (Jones & Spencer 1970; Al-Hasan *et al.* 1975; Blight *et al.* 1995). This is followed by a mixture of diatoms (*Rhizosolenia sp.* dominating) and flagellates (*Phaeocystis sp.*). The highest

peak of phytoplankton abundance is dominated by *Phaeocystis sp.* with cell numbers ranging between  $4.6 \times 10^5$  and  $3.4 \times 10^7$  cells.dm<sup>-3</sup> (Jones & Spencer 1970; Blight *et al.* 1995). The timing of the start of the flagellate dominance may vary from year to year but it always follows the end of the major diatom bloom. This suggests that the growth of *Phaeocystis sp.* is limited by the nutrient concentration. Flagellates may not be able to compete with diatom species until diatom growth is limited by silicate depletion; then flagellates start to dominate (Lennox 1979). After the major phytoplankton peak, the biomass remains low during summer. Although the phytoplankton is dominated by small flagellates during summer (due to the decline in the inorganic nutrient content) sometimes one species may dominate the species composition (Al-Hasan *et al.* 1975). A peak of coccoid cyanobacteria has been observed in late July and August (Blight *et al.* 1995). The low phytoplankton biomass during summer is followed by a minor peak which varies in magnitude from year to year and sometimes does not occur (Jones & Spencer 1970; Blight *et al.* 1995).

## 2.7. Bacteria and Primary production

### 2.7.1. Bacteria:

Blight *et al.* (1995) and Rodrigues (1998) recently studied the seasonal variation of bacterial abundance in Menai Strait waters. It is characterized by a low number ( $0.42 \times 10^6$  cells.cm<sup>-3</sup>) during winter months. This is followed by an increase in spring reaching the major peak ( $7.3 \times 10^6$  cells.cm<sup>-3</sup>) in June. A minor peak is observed in autumn followed by a decline towards lower numbers during winter months. Despite the variation in magnitude of peaks, both studies (Blight *et al.* 1995; Rodrigues 1998) reveal a similarity in seasonal trends.

The maximum bacterial numbers during summer are associated with the spring peak of primary production, with the time lag between the maxima of primary production and that of bacterial number ranging in length from a week to one month (Blight *et al.* 1995). It has been suggested that bacteria account for about 50% of the total respiration and ciliates for about 25%, with the rest (nano and picoflagellates) making a minor contribution in the total respiration. Three peaks in cell-specific



respiration are identified, concurrent with the three chlorophyll *a* peaks during the spring bloom. The phasing of the gross community production and respiration include positive net production relaxing into negative net production especially during *Phaeocystis sp.* bloom. This was explained as being due to the high rates of respiration during the break down of the *Phaeocystis sp.* bloom, which sometimes can reduce the oxygen saturation to 60% (Blight *et al.* 1995).

The phasing of the gross community production and respiration of the three types of blooms are characterized. No obvious respiration maxima are associated with mixed diatoms but distinct respiration maxima are associated with the development of *Rhizosolenia sp.* and *Phaeocystis* blooms. The flow of organic matter and time are considered to be the major reasons for these observations (Blight *et al.* 1995). It is suggested that during the mixed diatom bloom, the flow of dissolved organic matter from the diatoms to the bacteria is insufficient to sustain bacterial growth. As a result a low bacterial number is observed. During the *Rhizosolenia sp.* bloom, more dissolved organic matter is thought to be available for bacteria and thus the number of bacteria and ciliates increase. During *Phaeocystis* blooms, even more dissolved organic matter is available for bacteria and ciliates thereby increasing their abundance to the maxima (Blight *et al.* 1995).

### **2.7.2. Primary production:**

Primary production in the Menai Strait, was recently studied by Blight *et al.* (1995) and Rodrigues (1998) using oxygen measurements. The general pattern of primary production in the Menai Strait exhibited 2 peaks during spring and early summer (max.  $50\mu\text{M.O}_2.\text{d}^{-1}$  and  $47\mu\text{M.O}_2.\text{d}^{-1}$ ). Al-Hasan (1976) studied primary production in the Menai Strait using  $^{14}\text{C}$  during 1974 and 1975. The general patterns and scales of primary production were similar over the two years and were in broad agreement with Blight *et al.* (1995) and Rodrigues (1998). In contrast to Blight *et al.* (1995) and Rodrigues (1998), Al-Hasan (1976) study showed elevated rates of primary production throughout the summer and early autumn (max.  $75\text{mg C m}^{-3}\text{ h}^{-1}$ ).

## 2.8. Organic Matter

### 2.8.1. Particulate organic matter:

Although the Menai Strait receives particulate organic input from different sources such as land run-off and sewage, the major source appears to be the phytoplankton blooms. The particulate organic matter concentration in the Menai Strait has been reported by a number of authors (e.g. Blight *et al.* 1995; Rodrigues 1998 among others). It is characterized by high concentration during spring and autumn followed by low concentration during winter. Both the particulate organic carbon (POC) and nitrogen (PON) appear to be associated with the spring and autumn blooms. The annual concentration of POC and PON varies in magnitude from year to year. However, their concentrations vary generally between 17 and 160 $\mu$ M of carbon and 2 and 8 $\mu$ M of nitrogen respectively (Blight *et al.* 1995; Rodrigues 1998).

### 2.8.2. Dissolved organic matter:

#### 2.8.2.1. Dissolved organic carbon (DOC):

Despite the importance of dissolved organic matter in the ecology of the marine environment, few studies (Morris & Foster 1971; Al-Hasan *et al.* 1975) have investigated variations in its concentration, composition and its relation to the seasonal variation of phytoplankton in the Menai Strait. The seasonal variation of dissolved organic carbon (DOC) in the Menai Strait was studied from May 1968 to May 1970 (Foster & Morris 1971). Both years show similar annual variation patterns in which short-term variations are apparent. The annual trend was characterized by a minimum monthly mean concentration during winter months (83 $\mu$ M) followed by a slight increase during spring and summer reaching a monthly maximum (333 $\mu$ M) in October and November. This is followed by a sharp decline to winter levels. Highest concentrations of DOC (500 $\mu$ M) were recorded over a period of 7 days in October 1968. The maximum DOC concentrations were not concurrent with high rates of primary production. Despite this, Morris & Foster (1971) concluded that the increase in DOC in spring and summer comes from the *in situ* biological production rather than from a terrestrial origin. Thus the rate of supply of DOC by excretion from live and dissolution from dead and decaying organisms

continues in excess of bacterial and chemical oxidative regeneration while nutrients and sunlight are adequate for primary production. When phytoplankton production becomes limited by the amount of available light in autumn, DOC accumulates in the water column causing the maxima observed in October.

#### **2.8.2.2. Glycollate:**

Al-Hasan *et al.* (1975) also studied the seasonal variation of glycollate and phytoplankton extracellular products. The seasonal variation of glycollate in the Menai Strait varies between 0 and 60  $\mu\text{g}\cdot\text{dm}^{-3}$  with minimum values occurring in August, February and May. The maximum values are recorded early winter and summer. Statistically, a significant correlation between glycollate concentration, chlorophyll *a* and percentage extracellular releases were found. However there was no significant correlation with the numbers of any phytoplankton species. This suggests that if phytoplankton is the major source of glycollate then the production rate of several species must be considered. Thus the seasonal variation of glycollate must represent a balance between release and uptake mainly by bacteria. The low concentration of glycollate in August was explained as being due to a low rate of release and high rate of uptake by bacteria associated with relatively high temperature (Al-Hasan *et al.* 1975).

#### **2.8.2.3. Yellow substance (Gelbstoff):**

Seasonal variation of yellow substance in the Menai Strait was determined by Frost (1973). According to this study, the seasonal trend of yellow substance is characterized by lowest values in July followed by an increase reaching maximum values in early November and decreasing to low levels in late November and December (Frost 1973). A different pattern has been reported more recently by Kratzer *et al.* (2000) who measured highest values during the summer (maximum  $0.25\text{m}^{-1}$ ) followed by a decrease during autumn. This is followed by a slight increase in late November, declining to low values during winter months. The lowest value (about  $0.07\text{m}^{-1}$ ) is recorded in February. Yellow substance exhibits short-term variations which could be due to heterogeneity of the water caused by incomplete

mixing or input of water of different biological activity coming from different regions (Frost 1973).

Yellow substance levels follow closely that of dissolved organic carbon as determined by Foster & Morris (1971). This suggests that the high concentration of yellow substance in Menai Strait waters occurs a few weeks after the maximum concentration of dissolved organic carbon, thus supporting the hypothesis that the major source of yellow substance in coastal waters is from the exudates and decay products of the brown algae (Frost 1973). Although there is no direct link between phytoplankton concentration and yellow substance, the increase of yellow substance during summer is thought to be due to phytoplankton and bacterial activity (Kratzer *et al.* 2000). The high phytoplankton primary production during spring and summer and low DOC uptake by bacteria due to refractory nature of yellow substance, allow the maximum accumulation of yellow substance during summer (Kratzer *et al.* 2000). It has been suggested that the decrease in yellow substance could be due to the fact it is adsorbed mostly to smaller inorganic particles which themselves form strong bonds over the year and sink to the sediments. In autumn these particles are resuspended in the water column due to the effect of strong winds mixing water layers. However, during winter months land drainage and river run-off could influence the yellow substance variation (Frost 1973).

# CHAPTER III

## 3. METHODOLOGY

Physical, chemical and biological parameters were monitored from June 1998 to September 1999 inclusive in order to investigate seasonal changes and the processes that control the temporal distribution of dissolved organic matter. A number of methods used in this study will be reviewed in this chapter.

Seawater was collected at the surface (1m deep) from the middle of the Menai Strait away from St. Georges Pier (Figure.2.1) avoiding any contamination originating from boats docking at the Pier. Water samples were collected within two hours of high tide. A clean 25dm<sup>3</sup> polypropylene aspirator (covered with a thick black plastic bag to block light penetration) was used to collect and transport the sample to the laboratory. A weekly sampling protocol was followed except during spring 1999 (May-June) when sampling was undertaken on a daily basis.

### 3.1. PHYSICAL PARAMETERS

Water temperature was measured by dipping a digital thermometer (Digitron T208) directly into the surface. Salinity measurements were made using a Salinometer (Autosal 8400 A) and water transparency was determined using a Secchi disc.

### 3.2. CHEMICAL PARAMETERS

#### 3.2.1 Nutrients:

The concentration of inorganic nutrients in the Menai Strait was determined using the colorimetric techniques described by Parsons *et al* (1984) and Grasshoff (1983a). Seawater used for nutrient determination was collected using a Niskin water bottle and samples were filtered through pre-combusted (500°C for 3 hours) Whatman

GF/F filters (ca. 0.75 $\mu$ m pore size) 47mm diameter. All the containers used to collect and transfer nutrient samples were acid-washed (10% HCl v/v), rinsed thoroughly with distilled water and rinsed twice with sample water before transferring and collecting the water sample. Nutrient analysis was normally carried out immediately after sampling. However, a few samples were stored frozen and analysed later.

#### **3.2.1.1. Nitrate:**

The preparation and use of the cadmium coated reduction column were carried out in accordance with the techniques described by Parsons *et al* (1984) and Grasshoff (1983a). Cadmium filings (0.5 to 2mm in size) were washed in 500ml copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) until the blue colour had left the solution. A final rinse was made using dilute ammonium chloride and the cadmium was transferred to the reduction column. Nitrate determination involves the quantitative reduction (97-99%) of nitrate to nitrite by running the sample through a column containing copper-coated cadmium which has been activated with a high nitrate concentration spike (concentrated ammonium chloride). The length of time the sample is in contact with the cadmium and the efficiency of the column is very important (Grasshoff 1983a). The flow rate of the sample through the reduction column was adjusted so that 5  $\text{cm}^3$  was collected between 4 to 6 minutes and the efficiency of the column not less than 95%. Therefore, flow rate and efficiency of the column were checked regularly and adjusted when necessary. Nitrate concentrations were determined using a calibration curve ranging between 0.1 to 20 $\mu$ M (Menai Strait range). Standard nitrate solutions were prepared with synthetic seawater as described by Parsons *et al* (1984) due to the small salt effect.

#### **3.2.1.2. Nitrite:**

Determination of nitrite was carried out according to the techniques described by Parsons *et al* (1984). In this procedure nitrite reacts with an aromatic amine (sulphanilamide hydrochloride) to form a diazonium compound which couples with a second aromatic amine (n- (1-naphthyl) – ethylenediamine dihydrochloride) to form an azo dye. The extinction of the azo dye at 543nm is measured and is proportional

to the nitrite concentration over the range 0-10 $\mu$ M. Nitrite concentrations were determined using a calibration curve ranging between 0.1 to 2 $\mu$ M (Menai Strait range). Deionised water was used to prepare standard dilutions since there is no interference by salinity (Grasshoff 1983b).

### 3.2.1.3. Ammonia:

Despite the existence of a number of methods for measuring ammonia (Solorzano 1969; Matsunaga & Nashimura 1974; Garside *et al.* 1978; Parsons *et al.* 1984; Genfa & Dasguta 1989; Goyal *et al.* 1988; Gibb *et al.* 1995; Kerouel & Aminot 1997) extra care should be taken in order to choose the most appropriate method. Information such as the sensitivity of the method, types of chemical used and time required for ammonium determination is critical.

The indophenol blue technique which has been widely used (Solorzano 1969; Catalano 1987) but was not adopted as it involves the use of toxic phenol reagent which requires a fume cupboard for handling and must be disposed of with extra care as hazardous waste. Although the ammonium method described by Parsons *et al.* (1984) provides some advantages, such as greater sensitivity and avoidance of the use and handling of many toxic substances, it requires more time to do the analyses. Therefore the ammonium determination in this study was carried out using the fluorometric method as described by Kerouel & Aminot (1997). This method is very sensitive, involves simple procedures and uses a single working reagent which is a mixture of orthophthalaldehyde (OPA), sodium sulphite and sodium borate. The reagent is stable for months when stored in the dark. Samples and standards were prepared at the same time in the room with low ammonia contamination and all the standards and reagents were prepared with ammonia free water and corrected with blank while determining the final concentration. All glassware and polycarbonate bottles used in this method were soaked overnight in dilute (10%) HCl and rinsed thoroughly with distilled water followed by a final rinse with sample water prior to its use. The volume of the sample was reduced from the original volume (80cm<sup>3</sup>) to 20cm<sup>3</sup>, and that of the working reagent (20cm<sup>3</sup>) to 5cm<sup>3</sup>. Correction for background and matrix effect was followed in accordance with Kerouel & Aminot (1997).

### 3.2.1.4. Phosphate:

Determination of phosphate was carried out according to the technique described by Parsons *et al* (1984). In this procedure the sample reacts with the mixed reagent (ammonium molybdate, sulphuric acid, ascorbic acid and potassium antimonyl-tartrate) to form a complex which is reduced to form a blue solution. The extinction of this solution is measured at 885 nm. A calibration curve was established and phosphate standards ranged between 0 and 1.5 $\mu$ M (range of phosphate concentration in the Menai Strait). The phosphate standards were prepared with distilled water since there are no matrix effects (Parsons *et al* 1984).

## 3.3. BIOLOGICAL PARAMETERS

### 3.3.1. Chlorophyll *a*:

The concentration of chlorophyll *a* was determined fluorometrically using a Turner 10 Designs Fluorimeter following the technique recommended by Tett (1987). Subsamples (250 - 1000cm<sup>3</sup>) were filtered in duplicate through 47mm Whatman GF/F filters. These filters were either processed immediately, or after storage at -20°C and defrosting, placed in clean stoppered plastic centrifuge tubes into which 8 cm<sup>3</sup> of neutralised acetone (90% v/v dilution and sodium bicarbonate) was dispensed. Extraction was carried out in a refrigerator at 4°C for a minimum period of 18 hours but not exceeding 24 hours. The fluorimeter cuvette was washed twice with 90% acetone between samples to avoid cross-contamination. Acetone blanks and sample readings were made before and after acidification with 2N HCl. The chlorophyll concentrations (C) were calculated according to the following equations:

$$f_o = (f_o^* - f_o(b))/R$$

$$f_a = (f_a^* - f_o(b))/R$$

$$C = K_f (f_o - f_a) * E/V \text{ (}\mu\text{g.dm}^{-3}\text{)}$$

where  $f_o^*$  and  $f_a^*$  represent the fluorescence of the sample at 670 nm in instrument units before and after addition of 2N HCl respectively;  $f_o(b)$  and  $f_a(b)$  represent the recorded fluorescence of the blank before and after acidification respectively; R is



the range factor; E is the extract volume ( $8\text{cm}^3$ ); V is the volume of sample filtered ( $250\text{-}1000\text{cm}^3$ );  $K_f$  is the calibration factor (0.99) resulting from instrument calibration according to the calibration method of Tett (1987).

### 3.3.2. Total Bacteria Number:

Despite the availability of a wide range of methods for estimating bacteria abundance in the marine environment, many techniques prove inaccurate because of cross-contamination. The two most common techniques for estimating bacterial number using epifluorescence microscopy are: the use of the fluorochrome acridine orange (AO) (Hobbie *et al.* 1977) and the use of another fluorochrome 4'-6-diamino-2-phenylidole (DAPI) by Coleman (1980) and Porter & Feig (1980). The acridine orange binds to both DNA and RNA, and when exposed to blue light it fluoresces apple green. DAPI binds to DNA and fluoresces blue when excited with ultraviolet light.

There are advantages and disadvantages when using both methods (AO and DAPI) for the enumeration of total bacterial number. For instance, the DAPI technique allows a relatively long period between slide preparation and microscopic counts of bacteria since DAPI fluorescence fades less rapidly compared to acridine orange. Moreover, DAPI has the advantage that it does not fluoresce when bound to detrital matter (Sieracki *et al.* 1985). In contrast, acridine orange can bind to detritus, clays and colloids (Porter & Feig 1980) which could be counted as bacterial cells resulting in overestimated counts. For example, (Suzuki *et al.* 1993) reported that estimating bacterial abundance using DAPI staining underestimates the total counts as well as the size of the bacterial cells in comparison with acridine orange technique. In addition, they considered that DAPI could only stain specific parts of the cell (such as nucleic acid) whilst the acridine orange stains other parts of the cell providing a clear size of the cell.

However, in study by Zweifel & Hagstrom (1995), they diluted their samples with freshwater prior to DAPI staining in order to overcome the problem of poor binding

of DAPI to DNA at high salt concentrations (Wilson *et al.* 1990). They found that 2-32% of the total particles counted as bacteria were nucleiod containing cells (NUCC). The rest were cell residues of virus-lysed bacteria (ghosts) or remains of grazing protozoa.

Having in mind the advantages and disadvantages of the two techniques, and the nature of the Menai Strait water, which contains a heavy load of suspended matter, the DAPI method was preferred over the acridine orange technique.

### 3.3.2.1. Procedures:

The total number of bacteria was determined using the DNA-specific stain DAPI and epifluorescence UV microscopy (Leitz Ortoplan Microscope; x 1250 magnification). Subsamples of 20 cm<sup>3</sup> were preserved with 50% gluteraldehyde solution (0.5% final concentration) and stored in the refrigerator at 4°C prior to the preparation of slides. Preservation time prior to slide preparation ranged between 1 day to 2 weeks as suggested by Porter & Feig (1980). Subsamples of 1 to 2cm<sup>3</sup> were dispensed into a 15 cm<sup>3</sup> filter funnel. The subsample was stained with DAPI (Sigma, USA final concentration of 1.25µg.cm<sup>-3</sup>) for 10 minutes in the dark. The subsample was then filtered through 0.2µm black polycarbonate filters, 25mm (Poretics Inc.). The stained filter was mounted on a microscope slide with immersion oil (PAN SCAN) and a coverslip and immediately stored at -20°C until counted. The slides count was achieved within 16 weeks which fell within the period indicated (24 weeks) by (Porter & Feig 1980). Bacterial enumeration was made with a Leitz Ortoplan fluorescence microscope at x 1250 magnification using a Patterson Globe and circle NG1 Graticule. A minimum of 300 cells or 30 fields were counted per filter. The length and width of the Patterson Globe were 47.5µm and 21.4µm respectively and the diameter of the active area of the filter was 16mm. The active area of the filter was therefore 208.9 mm<sup>2</sup> and the area of the graticule grid was 1009.4µm<sup>2</sup>. The number of bacteria on the filter surface was calculated as follows:

$$Y = (X/1009.4) * (2.08672 * 10^8)$$

The number of bacteria (Z) in subsample (V) cm<sup>3</sup>

$$Z = Y/V \text{ cells.cm}^{-3}$$

where Y is the number of bacteria on the filter surface

X is the number of bacteria on the grid

V is the volume of subsample.

### 3.3.3. Oxygen Measurement (Respiration, gross and net community production)

The rate of primary production in the Menai Strait was estimated by measuring the changes in the concentration of dissolved oxygen in three sets of bottles (zero, dark and light) according to Gaarder & Gran (1927). Dissolved oxygen concentrations were determined using the Winkler titration method. The changes in dissolved oxygen concentration which are caused by production and respiration are expressed as primary production and calculated as follows:

$$\text{Gross Community Production (GCP)} = \text{Light}_{O_2} - \text{Dark}_{O_2}$$

$$\text{Net Community Production (NCP)} = \text{Light}_{O_2} - \text{Zero}_{O_2}$$

$$\text{Respiration (R)} = \text{Dark}_{O_2} - \text{Zero}_{O_2}$$

where Light<sub>O<sub>2</sub></sub> = the mean oxygen concentration in the light bottles.

Dark<sub>O<sub>2</sub></sub> = the mean oxygen concentration in the dark bottles.

Zero<sub>O<sub>2</sub></sub> = the mean oxygen concentration at the start of the incubations.

In order to achieve accurate results caution should be taken while preparing and analysing samples. For instance, the optimum standardisation of the thiosulphate, using calibrated bottles for volume, a thorough flushing of the burette to get rid of bubbles, and high precision and sequence in adding reagent (manganese sulphate and alkaline iodide). Moreover, the temperature at the start and end of incubation should not vary more than 3°C above the *in situ* temperature. Where samples were needed for future analyses, they were stored under water to avoid evaporation and this allows

a long storage period. The Winkler titration was performed using a PC-based system with a photometric endpoint detector (Williams & Jenkinson 1982).

### 3.3.3.1. Procedure:

A hand bilge pump was used to pump water into a clean 25dm<sup>3</sup> polypropylene aspirator (covered with a thick black plastic bag to block light penetration). The pumping process was done gently in order not to introduce bubbles into the water samples. Borosilicate bottles (50 - 150cm<sup>3</sup>) were rinsed and a set of 3 bottles (4-6 replicates each) were over-filled with three times the bottle's volume and stoppered carefully so that no air bubbles were enclosed. Temperature of the zero bottles was determined using a digital thermometer (Digitron Instrumentation, UK). Subsequently manganese sulphate (1cm<sup>3</sup>) was added to each zero bottle water sample using a multipipette (BCL 8000 with a 15cm<sup>3</sup> syringe) followed by alkaline iodide solution (1cm<sup>3</sup>). In the case of small bottles (50cm<sup>3</sup>) the volumes of reagents were reduced to 0.5cm<sup>3</sup>. The bottles were immediately stoppered and shaken until the water samples were homogeneously mixed. The light and zero bottles were then exposed to light in one incubator whilst the dark bottles were kept in another incubator in the dark. The water samples were kept within 3°C above *in situ* temperature by flushing water through the incubators.

After 24 hours the light and dark bottles were then treated as described for the zero bottles and were left until the precipitate settled. Before titration, 1cm<sup>3</sup> of sulphuric acid was added to each bottle and a magnetic stirring bar was carefully placed in the bottom of the bottle. The light beam was adjusted (0% and 100%) by placing a bottle filled with distilled water in the light beam path. Then the sample was placed in the light beam of the photometric detector and the computer was initiated to start titration. The 100% light beam was adjusted again at the end of the first titration and the sample was titrated until the end point is reached. The values the titrated samples were automatically calculated and printed out.

### **3.3.4. Particulate Organic Carbon and Nitrogen (POC and PON):**

#### **3.3.4.1. Sample preparation:**

A known volume of water sample (150 - 500cm<sup>3</sup>) was filtered onto pre-combusted (500°C for 4 hours) 25mm diameter GF/F filters under gentle vacuum. The filters were then placed on petri slides and immediately frozen until prepared for analysis. The filters were removed from the freezer and placed with the petri slides uncovered on a plate in a Perspex box with fuming concentrated hydrochloric acid for 24 hours to remove any carbonate. Subsequently they were dried at 50°C overnight, wrapped in pre-combusted aluminium discs (Elemental Microanalyses, Ltd), scrunched into small balls and then stored in a desiccator until analysed. To avoid any cross-contamination, gloves and clean forceps were used while wrapping the filters.

#### **3.3.4.2. Sample Analysis:**

The concentrations of particulate organic carbon (POC) and nitrogen (PON) were determined using a Roboprep C/N Elemental Analyser. The C/N analyser is based on the combustion technique where filters with samples are combusted in the furnace (1020°C) and oxygen is introduced to form CO<sub>2</sub> and N oxides. Complete oxidation to N<sub>2</sub>O, CO<sub>2</sub> and H<sub>2</sub>O is reached by passing the oxidised samples through chromium trioxide. A stream of helium (He) carries the produced gases through a reduction furnace (600°C) where N oxides are reduced to dinitrogen gas. Subsequently the gases are carried further through a water trap, through a packed G.C column at 105°C (where these gases are separated) and finally on to T.C.D. detector. The CO<sub>2</sub> and N<sub>2</sub> peaks are integrated and sent to the computer.

To determine the final concentration of POC and PON, calibration curves were established before and after sample analysis. Known weights of acetanilide (C<sub>6</sub>H<sub>5</sub>NHCOCH<sub>3</sub>) were prepared depending on the range of POC and PON concentration in Menai Strait water. The known weights were carefully wrapped in

pre-combusted aluminium foil, scrunched into small balls and kept in a desiccator with silica gel until analysed.

### **3.3.5. Phytoplankton Counts:**

Accurate determination of total number of phytoplankton depends on several factors such as the type of fixative used, concentration techniques of samples prior to counts, amount of detrital material in the samples and the period of preservation.

The most widely used fixatives for phytoplankton are formaldehyde and potassium iodide plus iodine (Lugol's solution) (Thronsen 1978). Both fixatives have advantages and disadvantages depending on the content of samples and storage time. Formaldehyde preserves the shape of coccolithophores, diatoms and thecate dinoflagellates in good condition, whilst Lugol's solution dissolves coccoliths and silica after long storage. Lugol's solution preserves a large number of flagellates and other phytoplankton community species (Thronsen 1978). However, care should be taken when identifying and counting cells since Lugol's solution might stain many organic and inorganic particles in the sample. In addition a large amount of detritus in the samples collected from a very dynamic environment such as the Menai Strait could result in underestimating cell abundance using the inverted microscope technique.

#### **3.3.5.1. Procedures:**

Clean glass bottles (50 -100cm<sup>3</sup>) with Lugol's solution (4-8 drops) were used for phytoplankton samples. After sampling, subsamples (50-100cm<sup>3</sup>) were drained from the aspirator (covered with plastic bag) using silicon tubing into the bottles, which were shaken to mix the fixative and the samples. The bottles were then stored in the refrigerator. Prior to counting and identification, the sample bottles were gently inverted a few times to suspend settled cells. The content were transferred into graduated cylinders (100cm<sup>3</sup>) and left for 24 hours to settle. Samples were then siphoned to 20cm<sup>3</sup> without disturbing the settled cells at the bottom of the cylinder.

Subsequently, the 20cm<sup>3</sup> samples were gently shaken and poured into settling chambers (25cm<sup>3</sup>) and left for 24 hours to settle. Transects were made in the counting chambers using the inverted microscope at 100x magnification bearing in mind that this is insufficient resolution to accurately count nanoplankton or picoplankton. The total cell numbers were estimated using the following equations:

$$T = A * 13.77$$

$$Z = (T * 1000) / 20$$

where T is the number of cells in 20cm<sup>3</sup>

A is the average number of cells counted

13.77 is the calculated constant using x100 magnification

Z is the total number of cells in a litre.

### 3.3.6. Dissolved Organic Carbon (DOC):

The DOM is measured as the total carbon, because of its structural complexity. The carbon is separated from the dissolved inorganic carbon (DIC) by acidification and oxidized to CO<sub>2</sub>, DOC can then be measured using different methods. Despite the availability of these, the important consideration is the efficiency of each method in completely oxidising the DOM to an equivalent amount of CO<sub>2</sub>. The most common techniques are the Wet-Chemical Oxidation (WCO) and the High-Temperature (600-900 °C) Oxidation (HTO).

High temperature oxidation was reported to provide more accurate measurement than the WCO (Benner *et al.* 1992). Despite its efficiency in oxidising the organic matter, some HTO instruments have problem of poor reproducibility and accuracy, which could be the result of contamination of samples during or after sampling, storage of samples and incomplete sparging of inorganic carbon (Qian & Mopper 1996).

An Automated High-Performance High-Temperature Combustion Total Organic Carbon Analyzer (Qian & Mopper 1996) was used in this study because the entire

injection process is closed to the atmosphere hence eliminating contamination during injections, and providing good reproducibility and accuracy. The instrument's main operation principles are described in Sugimura & Suzuki (1988).

#### **3.3.6.1. Procedures:**

Immediately after sampling, water samples were filtered through pre-combusted (500°C for 4 hours) GF/F Whatman filters (47 mm diameter; see appendix 3 for filter efficiency). The first 200cm<sup>3</sup> aliquots of the filtrate were used to rinse the container and discarded, and a 1dm<sup>3</sup> sample was filtered. Approximately 3cm<sup>3</sup> of filtrate was transferred to pre-combusted (500°C for 4 hours) glass vials. These were sealed and stored frozen at -20°C until analyzed.

Prior to analysis the instrument was flushed with UV-irradiated MilliQ-water and calibrated using potassium hydrogen phthalate (Sigma Product) as standard. The samples were left at room temperature to thaw and a number of blanks (UV-Irradiated MilliQ-water) were prepared according to the number of standards and samples to be analysed. One drop of 85% phosphoric acid was added to each vial using a pre-combusted Pasteur pipette. Consequently each vial was stoppered with a piece of pre-combusted aluminium foil and placed in racks which were then placed in an autosampler. The instrument was activated using a PC program which controls the injection and analysis of samples. A calibration curve was prepared for each run using potassium hydrogen phthalate (Sigma Product) as standard. The regression line equation was used to calculate the final concentration of dissolved organic carbon.

#### **3.3.7. Dissolved Organic Nitrogen (DON):**

The DON is a major intermediate for nitrogen cycling in aquatic systems where nitrogen availability limits photosynthesis (Benner *et al.* 1992). DON measurements



involve oxidation of samples to various nitrogen oxides which are determined by spectrophotometric means (Koroleff 1983).

In this study, the wet oxidation technique was used as described by (Koroleff 1983). The final concentration of DON is determined by calculating the difference between nitrogen oxides before and after oxidation.

#### **3.3.7.1. Procedures:**

Water samples were filtered through pre-combusted GF/F Whatman filters. Approximately 500cm<sup>3</sup> of sample were transferred into acid-washed polyethylene bottles and stored frozen at -20°C. Before analysis, samples were left at room temperature to thaw.

Aliquots of 60cm<sup>3</sup> were transferred to clean Teflon bottles and 9cm<sup>3</sup> of digestion reagent (25g potassium peroxodisulphate, 7.5g sodium hydroxide and 15g boric acid dissolved in 500cm<sup>3</sup> of UV-Irradiated MilliQ-water) were added. The bottles were closed tightly with screw caps and autoclaved for 2 hours at 120°C and allowed to cool for 2 hours. Subsequently, the nitrite, nitrate and ammonium concentrations were measured as described previously. The final concentration of DON was calculated as the difference between dissolved inorganic nitrogen (DIN = nitrate + nitrite + ammonium) before and after digestion. To test the efficiency of the method, the calibration was performed in accordance with the technique described by (Koroleff 1983).

#### **3.3.8 Yellow Substance (Light Absorption at 440 nm)**

The dissolved and colloidal organic compounds present in natural waters are collectively called yellow substances or Gelbstoff (Bricaud *et al.* 1981). Understanding the origin and role in the ocean of this substance has become of great interest. It has been reported that yellow substance is among the major causes of ocean colour variations (Bricaud *et al.* 1981;Kalle 1996). The seasonal variation of

yellow substance was determined using the method described by Bricaud *et al.* (1981).

### 3.3.8.1. Procedures

Water samples were collected in acid-washed polycarbonate bottles and filtered through a pre-combusted GF/F filters (47mm) using a glass filter holder. Aliquots of 100 cm<sup>3</sup> were transferred to a clean (acid-washed and rinsed with sample water) dark bottles and left to reach room temperature. A 10cm cuvette was rinsed twice with sample water and the final filtrate was transferred to the cuvette and measured against distilled water as a blank.

The absorbance was measured using a UV-1601 Shimadzu spectrophotometer.

The optical density (O.D) at 440nm and 750nm was measured and the absorption coefficient at 440 nm ( $g_{440}$ ) was calculated from the following equation:

$$g_{440} = \ln(10) * (O.D._{440} - O.D._{750}) / L \quad m^{-1}$$

where  $\ln(10) = 2.3$ , and L is the path length of the cuvette, in this case 0.1m.

# CHAPTER IV

## 4. RESULTS

### 4.1. Introduction

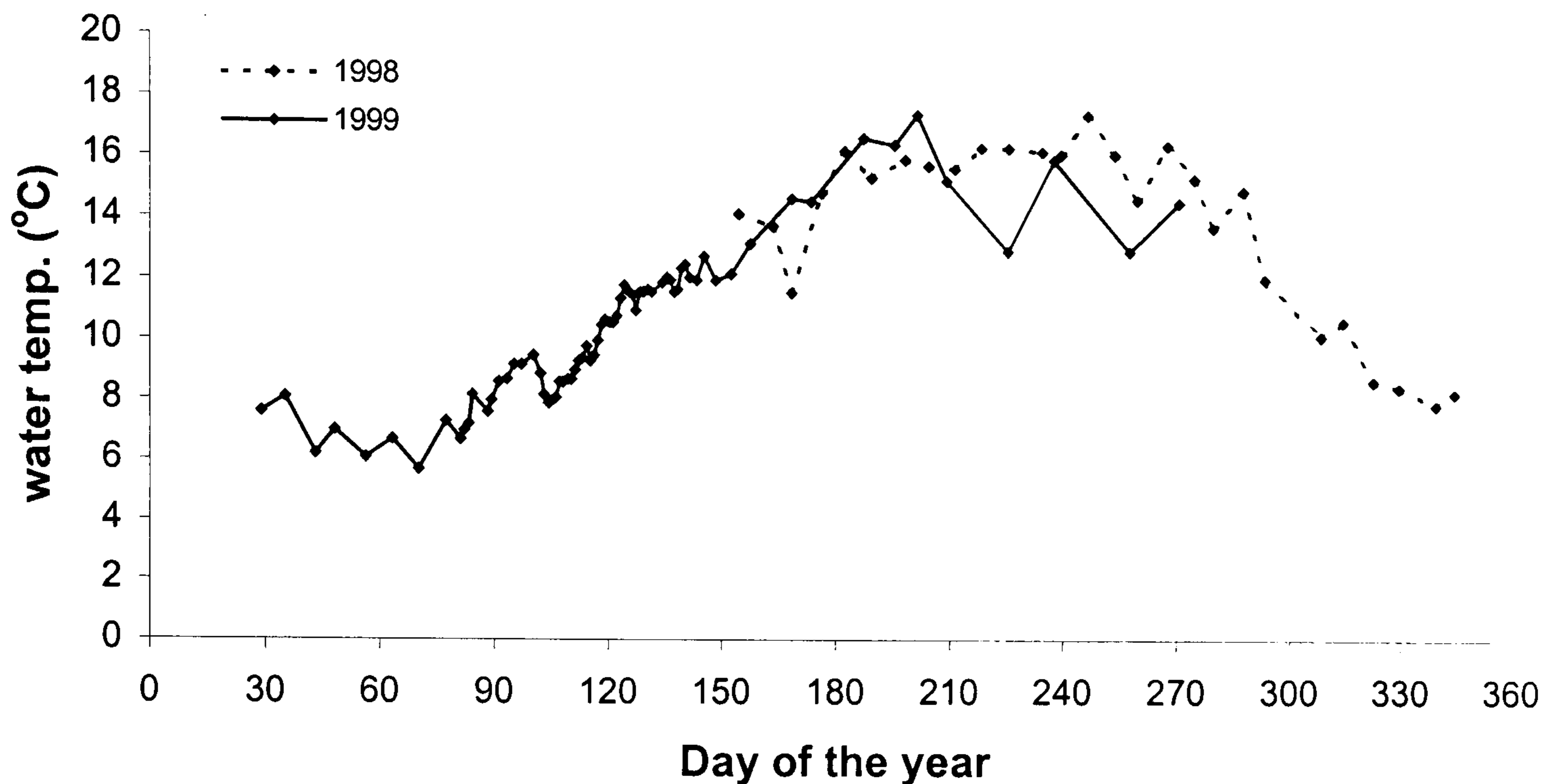
The seasonal cycle of phytoplankton and primary production in the Menai Strait in this study was characterised into four different phases: spring (March to May), summer (June to August), autumn (September and October) and winter (November to February). Measurement started in June 1998 and was continued on a weekly basis until April 1999. For the following seven weeks (from 22/03/99 to 28/05/99) sampling frequency was increased to daily basis to monitor changes over the bloom period in detail. The final period of sampling was reduced to weekly in June and July and twice a month in August and September 1999.

### 4.2. Physical parameters

#### 4.2.1. Temperature

The seasonal variation of water temperature in Menai Strait (Figure 4.1) followed a similar pattern as reported in previous studies (Al-Hasan 1976, Blight 1996, Rodrigues 1998). The highest water temperature was recorded during summer followed by a gradual decrease in autumn, reaching the lowest values during winter.

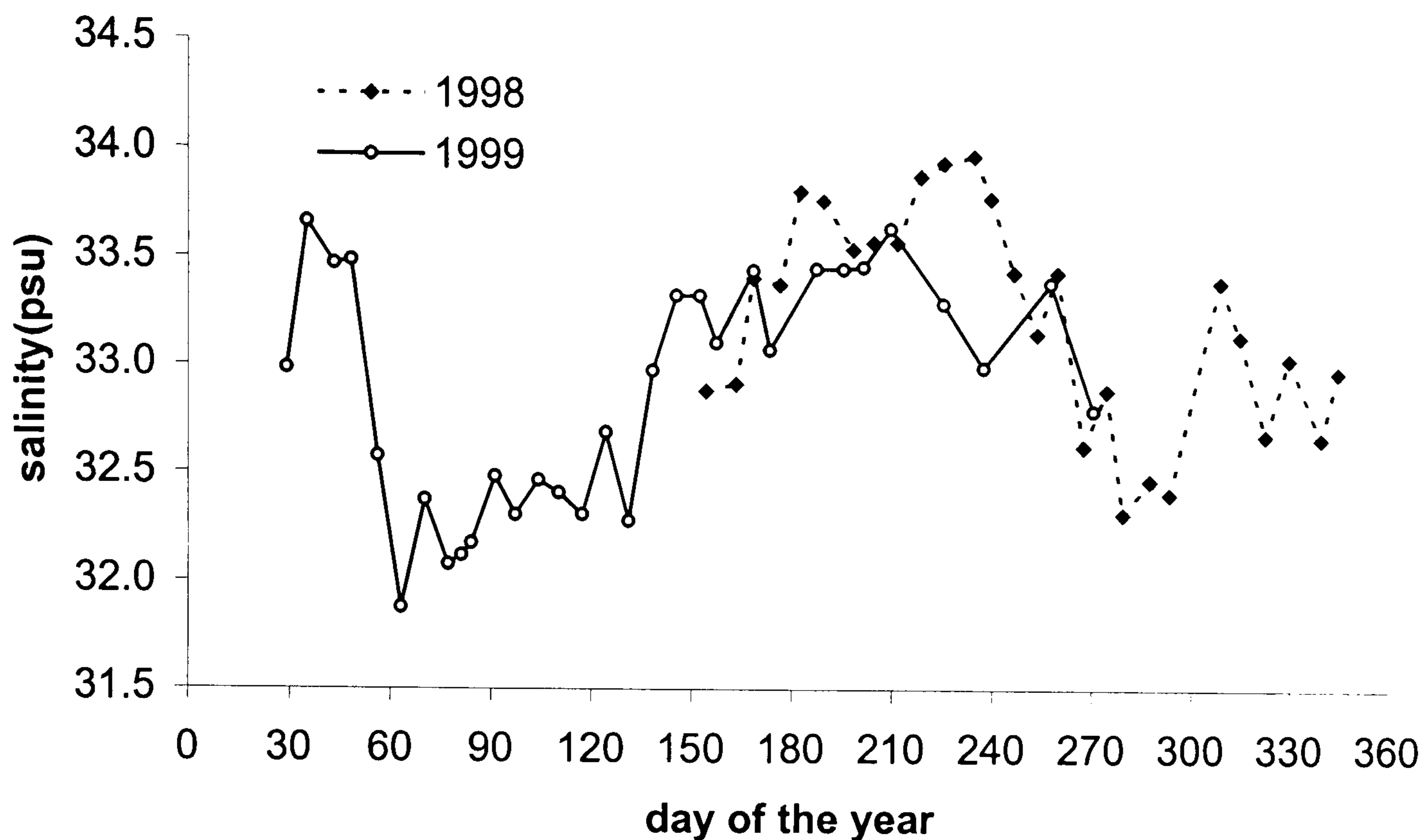
During summer 1998, the water temperature ranged between 11.6°C and 17.5°C. The lowest temperature of 11.6°C was recorded on day 168 when a drop in temperature of about 2°C was recorded. The highest temperature of 17.5°C was measured on day 246. The water temperature started to decrease in late October 1998 with the cooling air temperature, reaching the lowest value (8.3°C) on day 344. In 1999, temperature continued to drop reaching the lowest value (5.7°C) on day 70. As expected it started to increase with the warmer weather in spring reaching the highest temperature (10.7°C) on day 120. It remained high during summer 1999 reaching 17.5°C on day 201. Similar patterns were observed in both years with slight differences in values.



**Figure 4. 1. Variation in Menai Strait water temperature during 1998 and 1999.**

#### **4.2.2. Salinity**

As shown in Figure 4.2, the seasonal variation of salinity in the Menai Strait in 1998 and 1999 showed temporal fluctuations that were small in magnitude. Salinity minimum and maximum values were 31.9 (day 63) and 34 (day 234). A seasonal pattern was observed, low values during winter and high during summer. At the beginning of 1999, unusual high values (33; 33.7; 33.5) were recorded on days 29, 35, and 43.



**Figure 4.2. Salinity variation in Menai Strait during 1998 and 1999.**

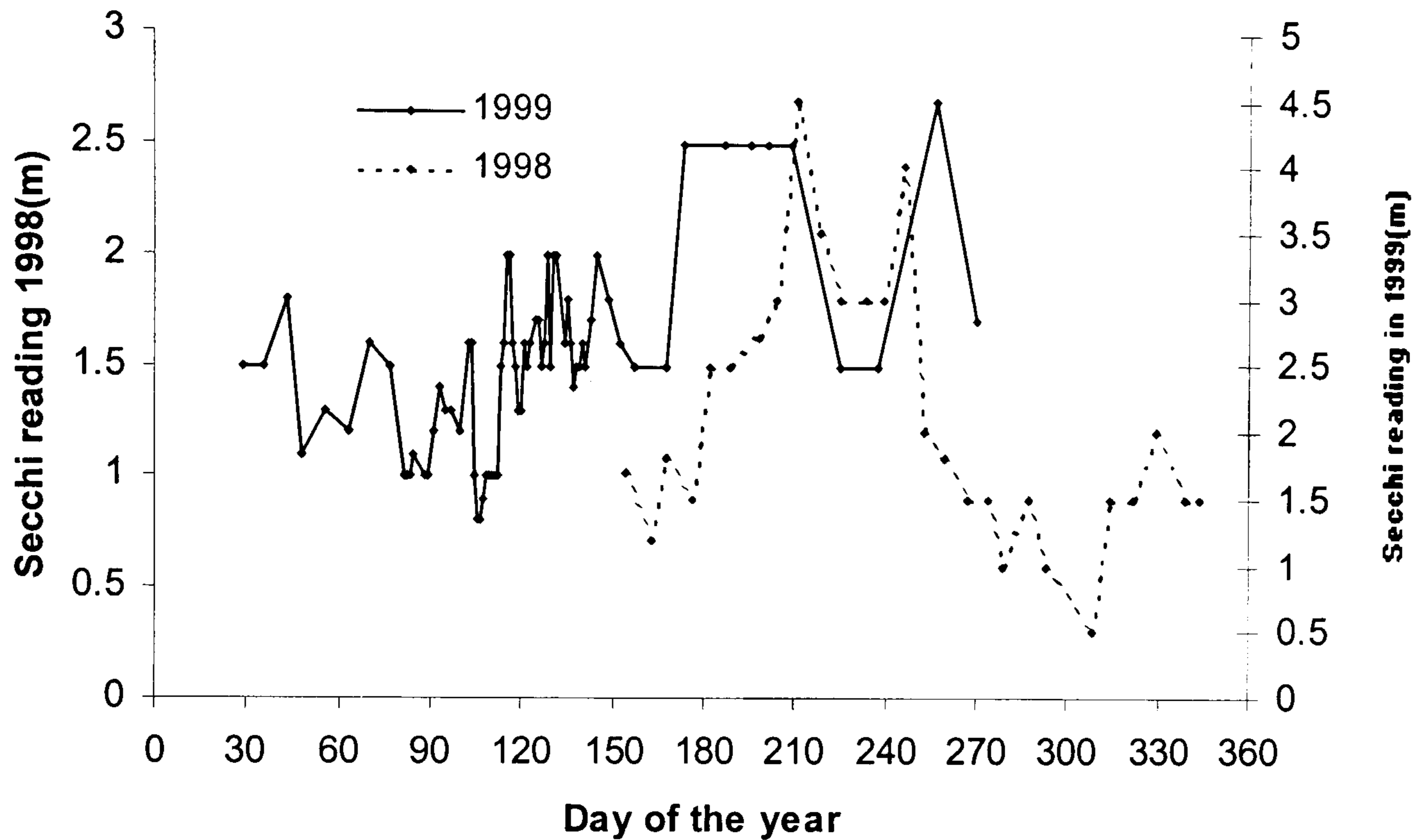
### 4.2.3. Secchi Disc Depth

In this study secchi disc is reported as water transparency. The seasonal variation of water transparency in 1998 and 1999 (Figure 4.3a) followed the usual trend with the lowest transparency in winter and highest in summer (Lumb 1990). The mean for 1998 (7 months) and 1999 (9 months) were 2 m and 1.7 m. The annual mean in this study falls within the range of the historical data of the Menai Strait (Figure 4.3b).

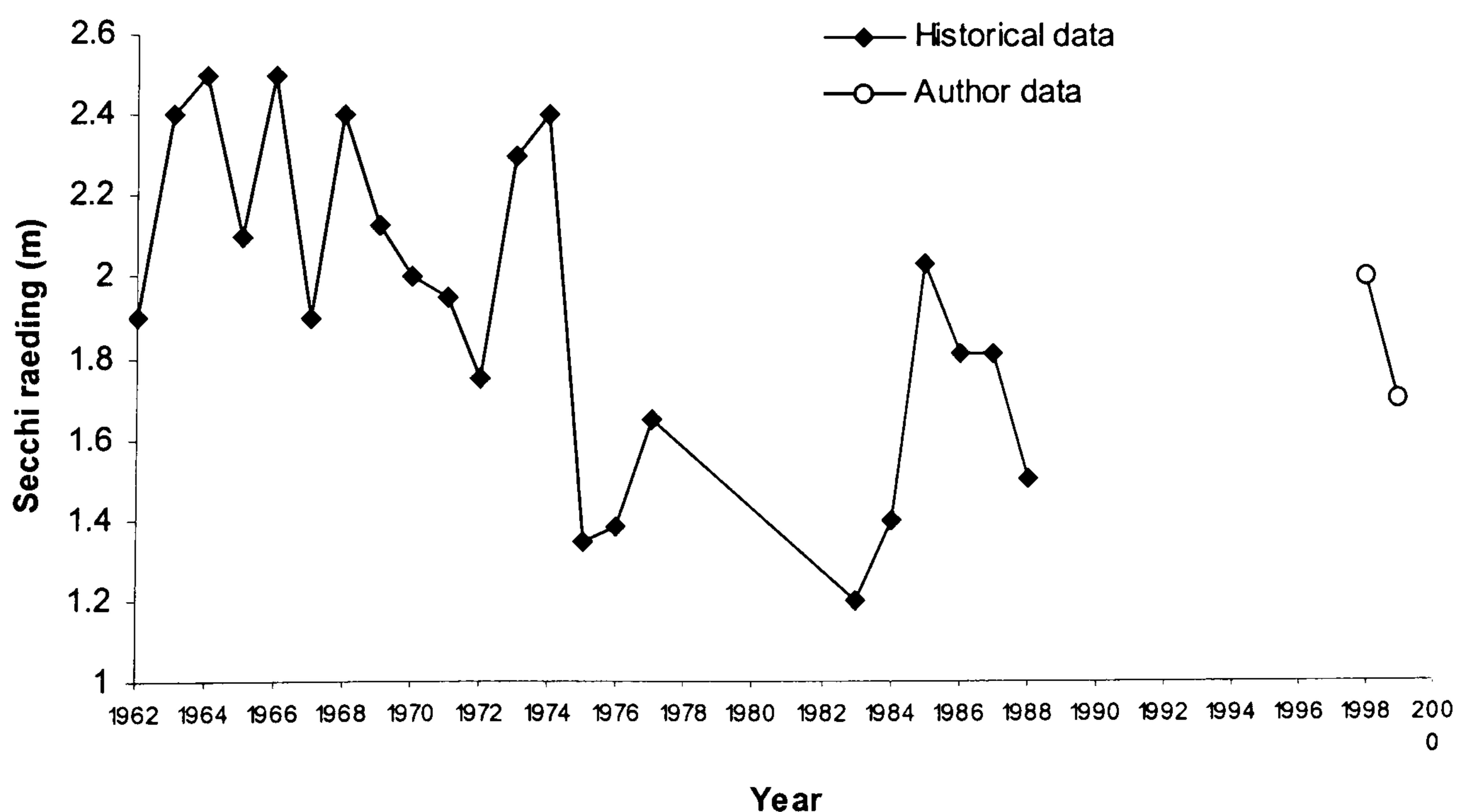
During 1998, the transparency of the water gradually increased from 1.2 m on (day 163) to the highest of 4.5 m on (day 211). Subsequently transparency decreased slightly to 3.5 m, and then remained constant for the rest of the summer. In autumn, it started to decrease with values ranging between 1 to 2 m. The lowest water transparency of 0.5 m was recorded on day 308.

During 1999, the water transparency continued to drop reaching its lowest (0.8 m) in early April (day 105 and 106). This was followed by more variable readings although gradually increasing to 1.6m. A drop occurred during the period of high

chlorophyll (day 148 to 168). There was an increase in transparency which was maintained until day 209. This was followed with values ranging between 1.5 to 2.7 m towards autumn.



**Figure 4. 3a. Seasonal variation of secchi reading in Menai Strait water during 1998 and 1999.**



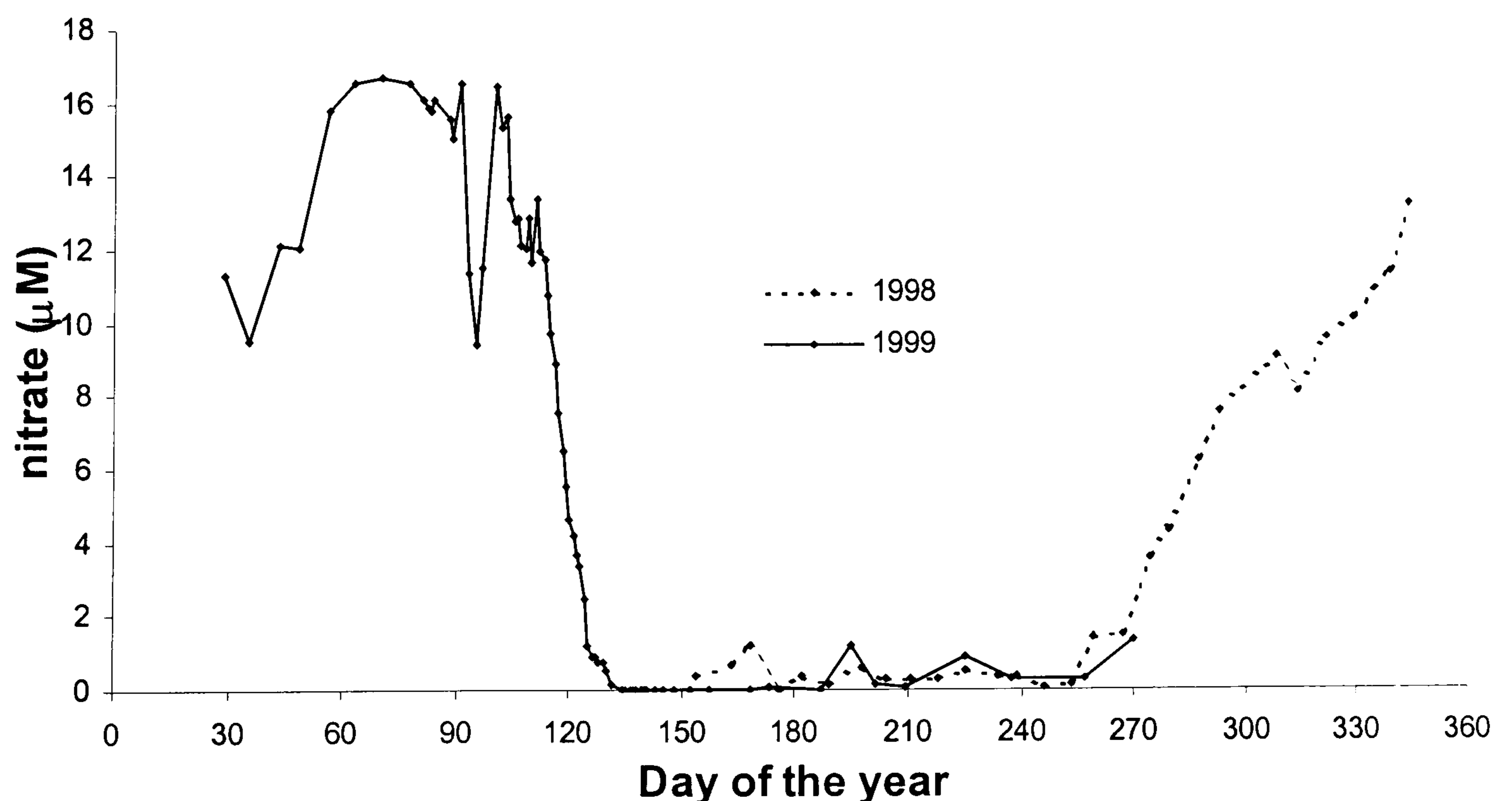
**Figure 4. 3b. Compilation of Annual mean secchi reading in the Menai Strait reported from 1968 to 1999. (From Menai Strait data base and author's data).**

### 4.3. Inorganic nutrients

The seasonal variation of nutrients in the Menai Strait followed a strong seasonal trend that is found in most nutrient studies in the Menai Strait. This trend is represented by a high concentration of nutrients from winter to spring. A gradual decline then occurs until summer when nutrients are below detection. Concentrations gradually increase again in autumn (Al-Hasan *et al.* 1975; Blight *et al.* 1995; Rodrigues 1998).

#### 4.3.1. Nitrate:

The seasonal variation in nitrate concentration is shown in figure (4.4a). It followed a similar pattern of the area. During summer (day 152 to 239), nitrate concentration remained low (below  $1\mu\text{M}$ ). This equilibrium was maintained until the end of September when nitrate regeneration began to dominate and continued to throughout winter reaching the highest measured level in 1998 ( $13.3\mu\text{M}$ ) on day 344.



**Figure 4.4a. Seasonal variation of nitrate in the Menai Strait during 1998 and 1999.**

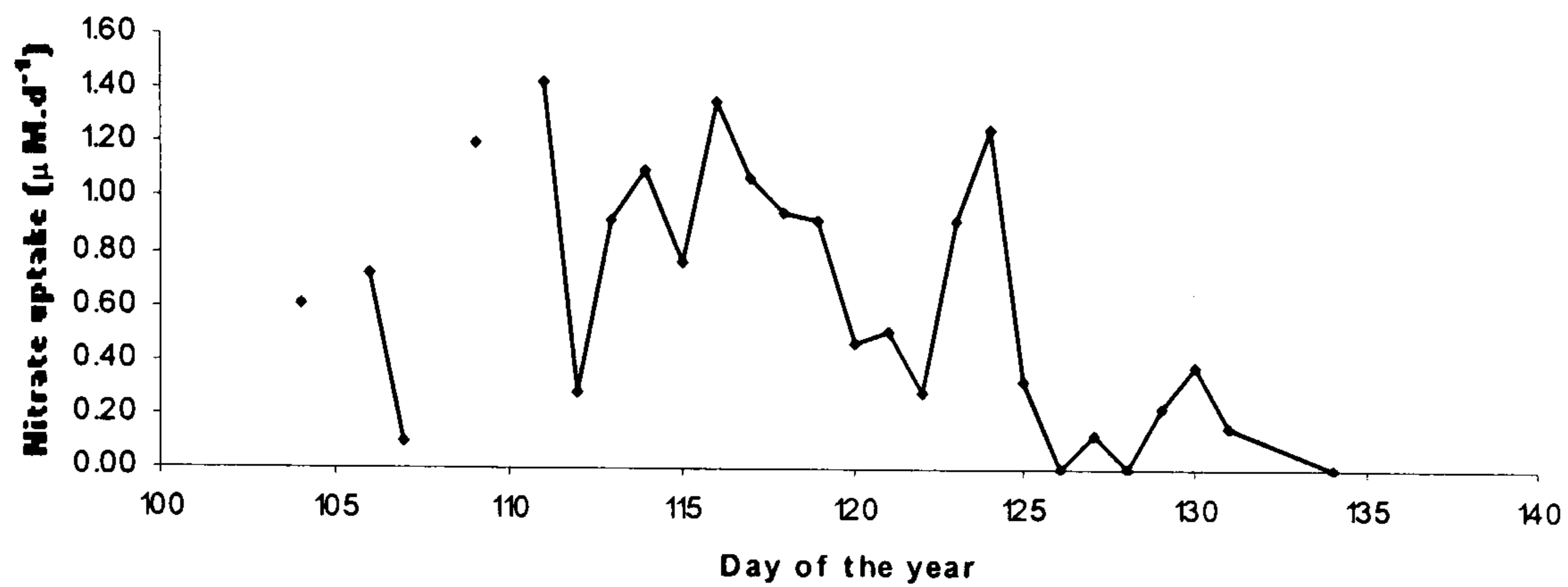
In January to March 1999, nitrate concentrations remained high ranging from 15.1 to 16.7  $\mu\text{M}$  from day 56 to day 91. An initial decrease in nitrate occurred on days 93 (11.4  $\mu\text{M}$ ), day 95 (9.4  $\mu\text{M}$ ) and day 97 (11.5  $\mu\text{M}$ ). Nitrate began to decrease again from day 104 until concentrations were below detection on day 134. From May to September nitrate remained low and constant with concentrations mostly below detection.

Daily net uptake rates for nitrate were calculated from differences in nitrate concentration measured during the intense sampling period (day 104 to 134). This method has been found to provide reliable estimate of new production (Boyd *et al.* 1995).

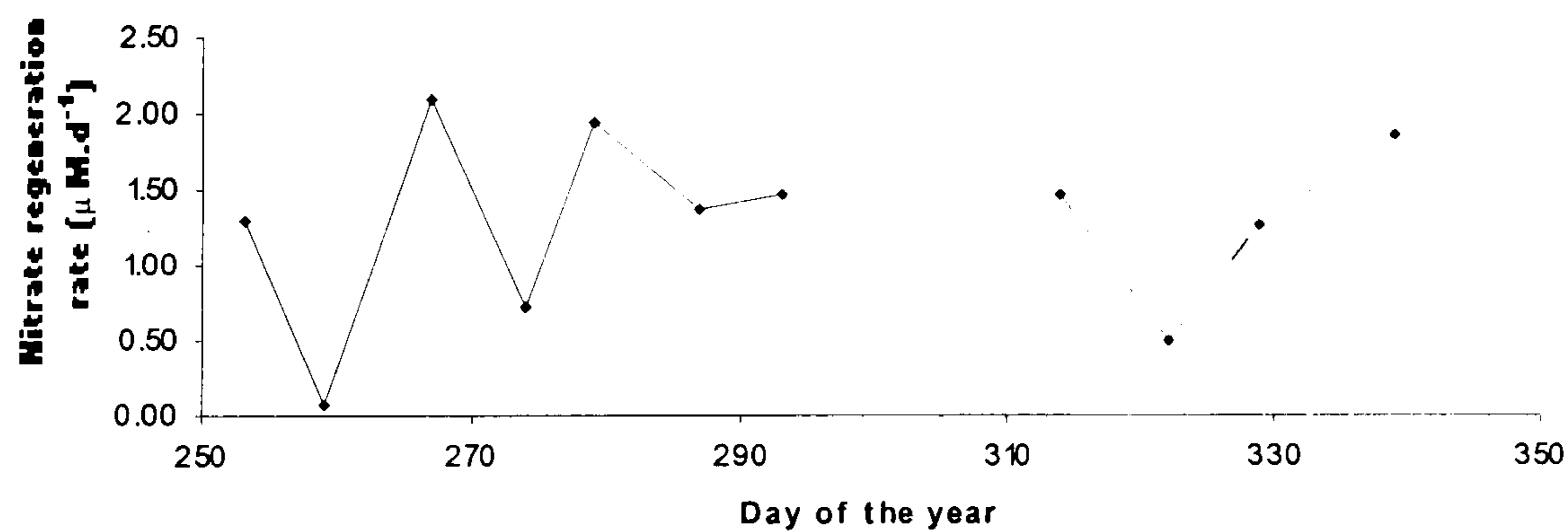
The net uptake for nitrate by the whole community showed variations during the study period (Figure 4.4b). Low net uptake rates occurred in the beginning of spring (April). This was followed by a rapid increase leading to maximum values ( $1.4\mu\text{M}\cdot\text{d}^{-1}$ ) during April and May. The period of high uptake rates coincided with a large increase in chlorophyll and resulting in the sharp decline of ambient nitrate concentration.

Net nitrate regeneration rates were calculated from weekly measurements when nitrate concentrations increased in the autumn and winter. Nitrate regeneration (Figure 4.4c) showed fluctuation with rates ranging from  $0.01\mu\text{M}\cdot\text{d}^{-1}$  (day 259) to  $0.3\mu\text{M}\cdot\text{d}^{-1}$  (day 267). The average value over this period was  $0.18\mu\text{M}\cdot\text{d}^{-1}$ . This variation could be associated with periods of low nitrate uptake especially during the increase in phytoplankton abundance during autumn. High regeneration rates occurred during late autumn and winter coinciding with the decline in phytoplankton abundance.





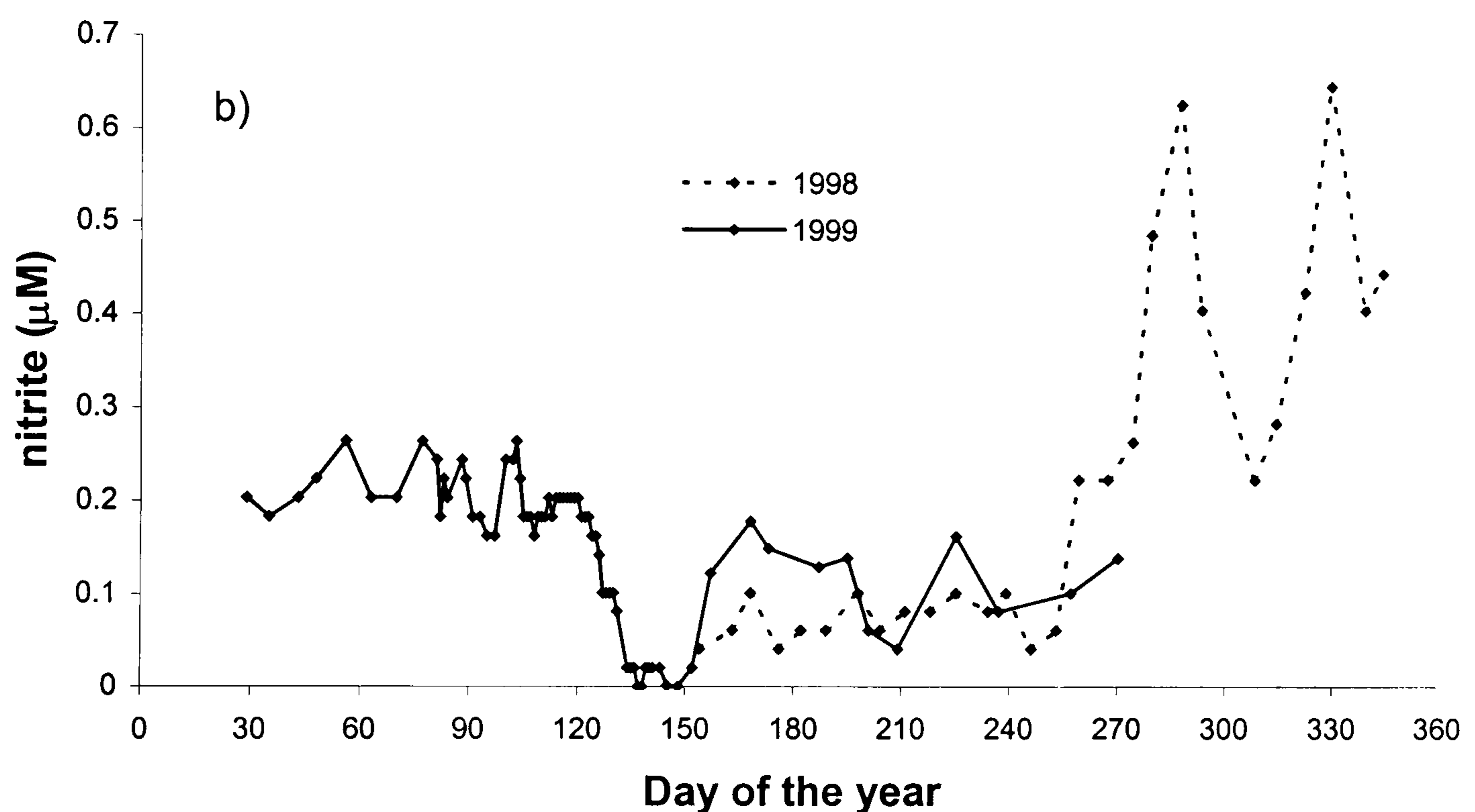
**Figure 4. 4b.** Net nitrate uptake rate in the Menai Strait in 1999 derived from the daily differences during the period of nitrate disappearance in spring.



**Figure 4. 4c.** Net nitrate regeneration rate as estimated from weekly increases in 1998 in the Menai Strait.

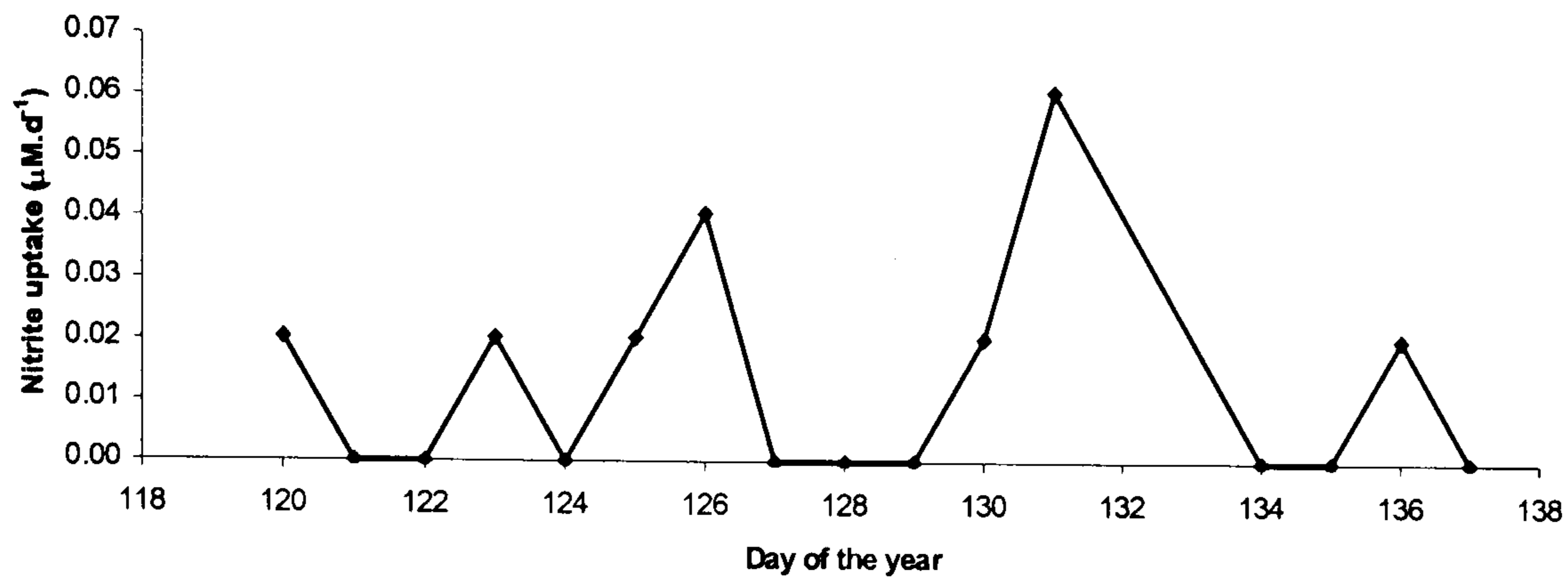
### 4.3.2 Nitrite:

Nitrite concentrations were low during summer 1998 with values generally  $\leq 0.1\mu\text{M}$  (Figure 4.5a). The rapid increase occurred in autumn (day 259) reaching the highest concentration ( $0.6\mu\text{M}$ ; day 329). Concentrations remained fairly stable (close to  $0.2\mu\text{M}$ ) from January to April. A rapid decrease during May was followed by a period of very low concentration (below detection) for about two weeks. Regeneration started rapidly in mid June (day 157) and concentration remained fairly constant for the rest of summer.

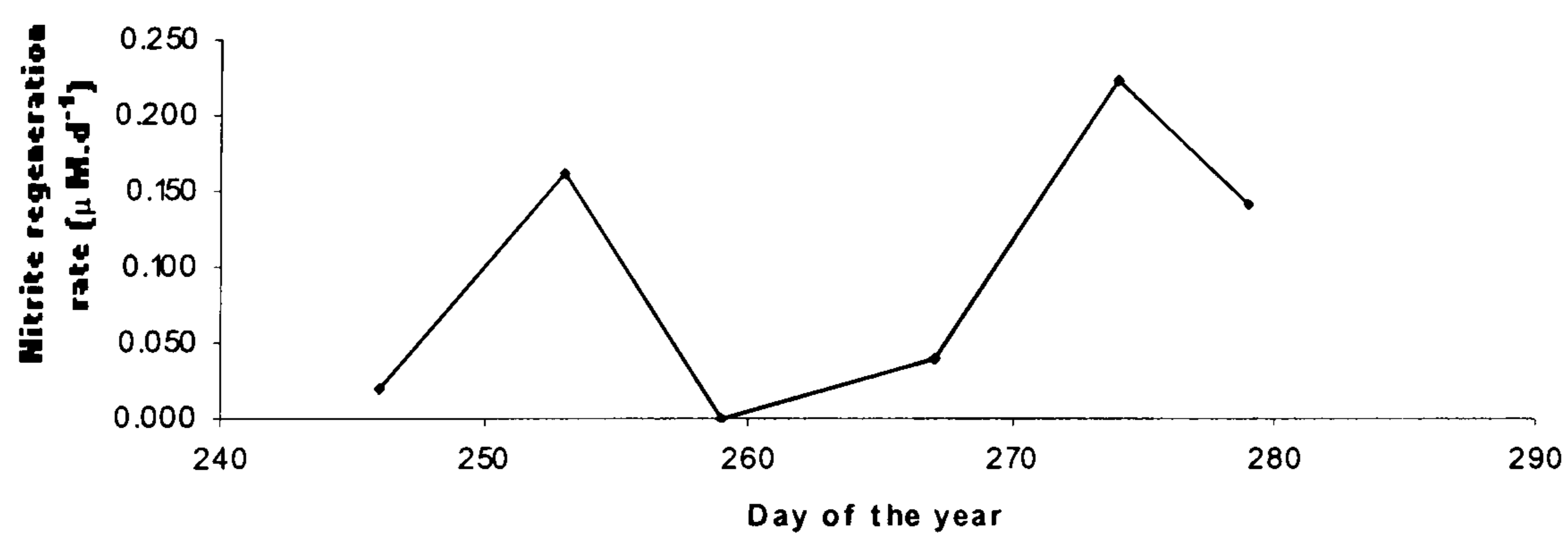


**Figure 4. 5a Seasonal variation of nitrite in the Menai Strait during 1998 and 1999.**

Net uptake of ambient nitrite (Figure 4.5b) ranged from  $\leq 0.02\mu\text{M}\cdot\text{d}^{-1}$  to maximum of  $0.061\mu\text{M}\cdot\text{d}^{-1}$  in May (day 131). Despite the high chlorophyll concentration, nitrite net uptake maximum followed the decline of nitrate net uptake. Unlike nitrate, net regeneration of nitrite was rapid (18 days) compared to nitrate. Regeneration rates ranged from  $0.02$  to  $0.22\mu\text{M}\cdot\text{d}^{-1}$  (Figure 4.5c) and increased following the decline of phytoplankton abundance. The decrease which occurred from day 260 coincided with the increase in chlorophyll *a*.



**Figure 4. 5b. Net nitrite uptake rate in the Menai Strait in 1999 derived from the daily differences during the period of nitrite disappearance in spring**

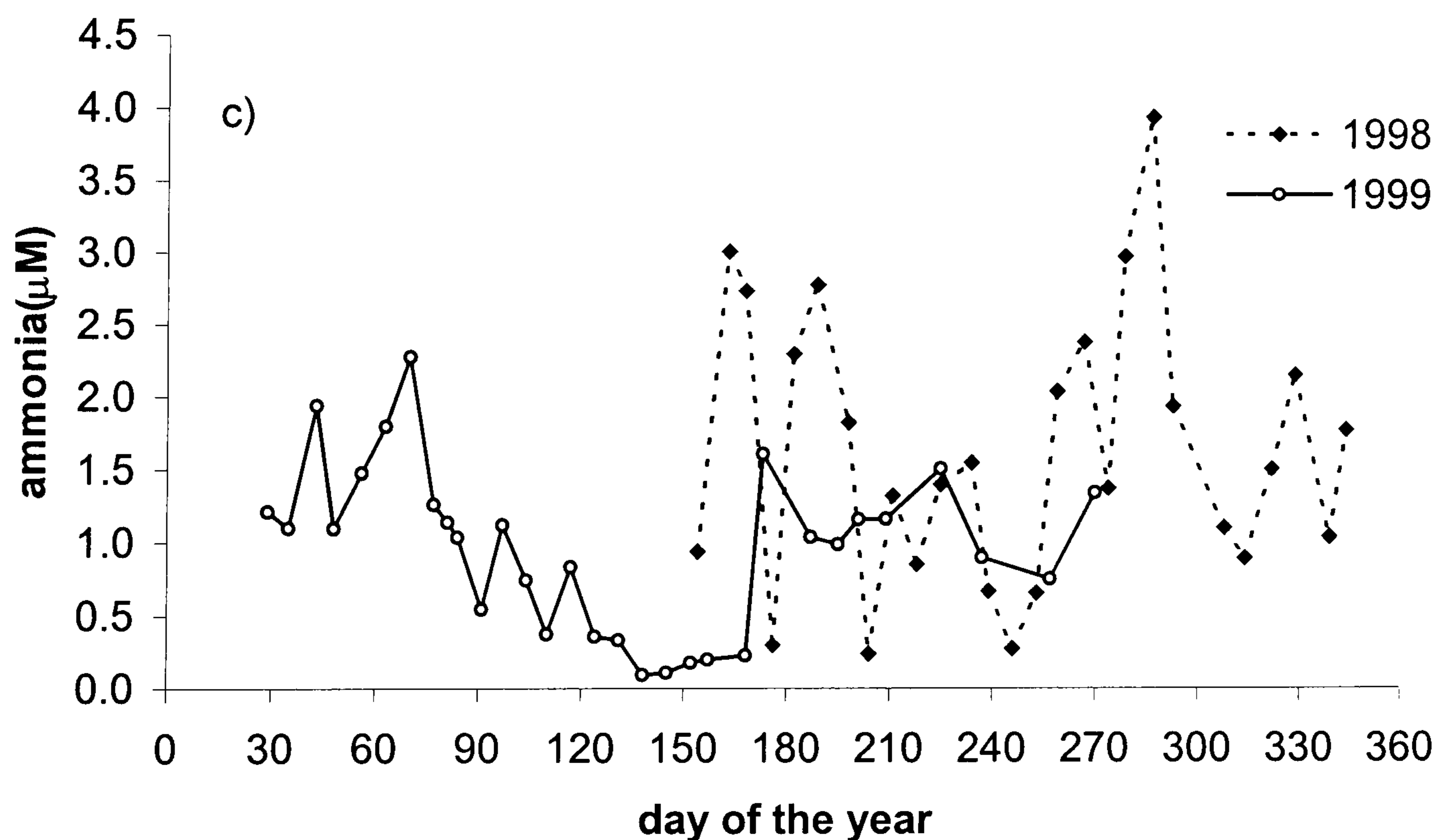


**Figure 4. 5c. Net nitrite regeneration rate in the Menai Strait as estimated from weekly increases in 1998.**

### 4.3.3. Ammonia:

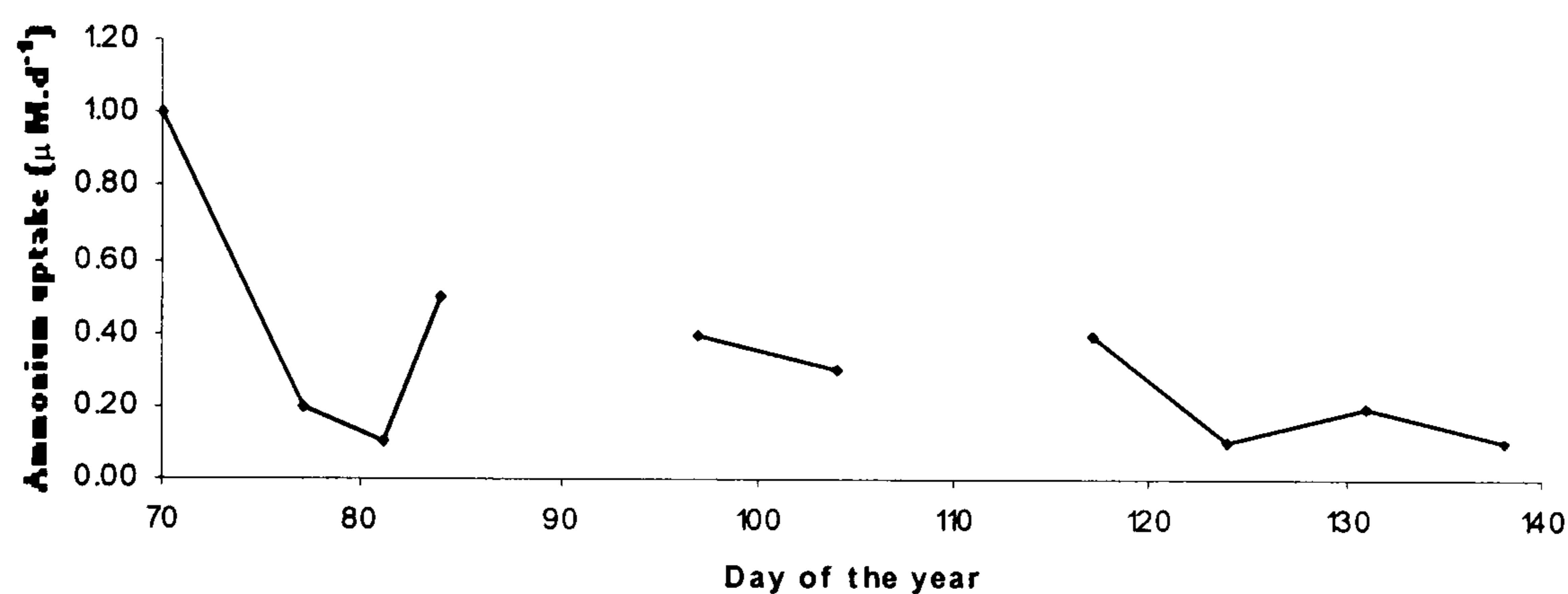
Ammonia concentrations (Figure 4.6a) fluctuated over very short intervals and the seasonal patterns in 1998 and 1999 were not clear, although there appeared to be periods of low concentration during summer months.

During June to August 1998, the concentration of ammonia was highly variable and ranged between  $0.2\mu\text{M}$  to  $4.0\mu\text{M}$ . The mean value between day 170 and 60 (1998&1999 data combined) was  $1.5\mu\text{M}$ . In 1999, concentration increased from  $1.2\mu\text{M}$  in January (day 29) to  $2.3\mu\text{M}$  (day 70) in March. The clearest trend was a sharp decrease starting in March and reaching the lowest concentration  $\leq 0.5\mu\text{M}$  on day 138. Ammonia regeneration occurred rapidly over a period of about 3 weeks in summer and concentration ranged between  $0.1$  to  $1.5\mu\text{M}$ .

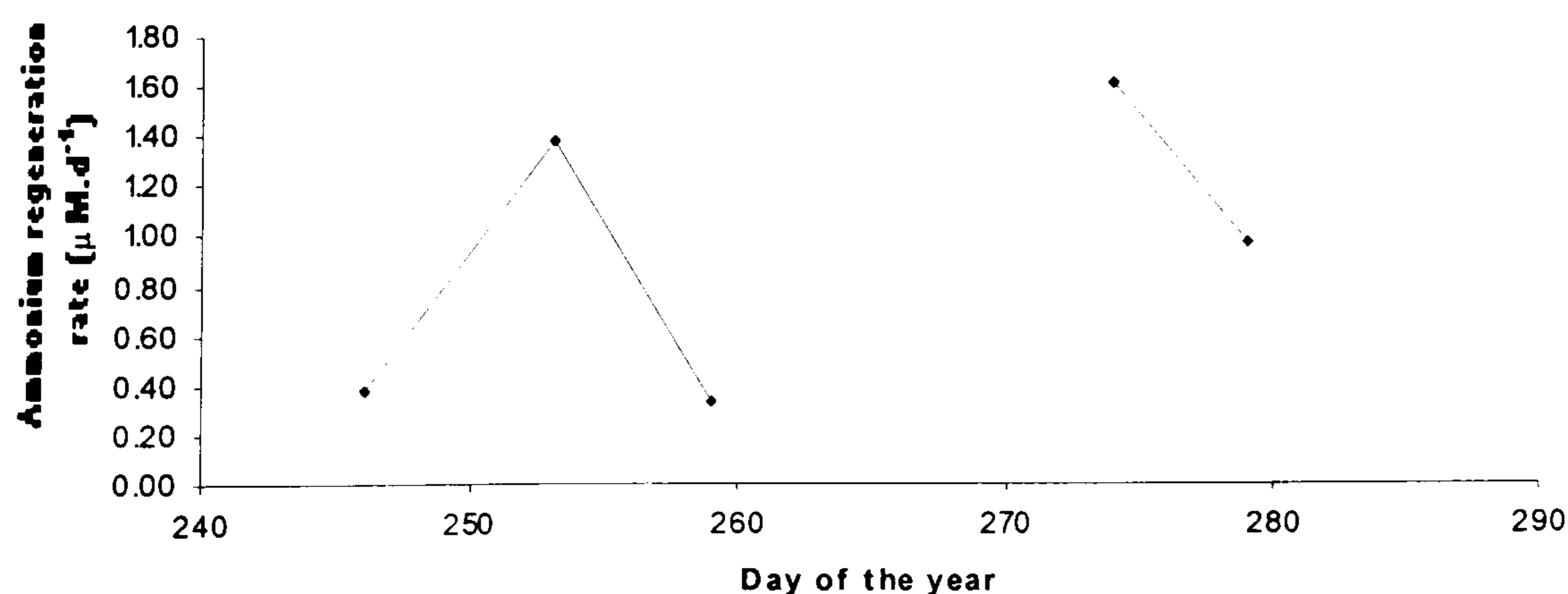


**Figure 4. 6a. Seasonal variation of ammonium in the Menai Strait during 1998 and 1999**

The net uptake rate of ammonium (Figure 4.6b) was high ( $1\mu\text{M}\cdot\text{d}^{-1}$ ) in March, and declined towards May reaching the lowest rates ( $0.1\mu\text{M}\cdot\text{d}^{-1}$ ). At the beginning of spring, ammonium was assimilated in preference to nitrate. Ammonium data were scattered, as a result the net regeneration rates calculated didn't show a clear pattern (Figure 4.6c). However the rate varied between  $0.3$  to  $1.6\mu\text{M}\cdot\text{d}^{-1}$ .



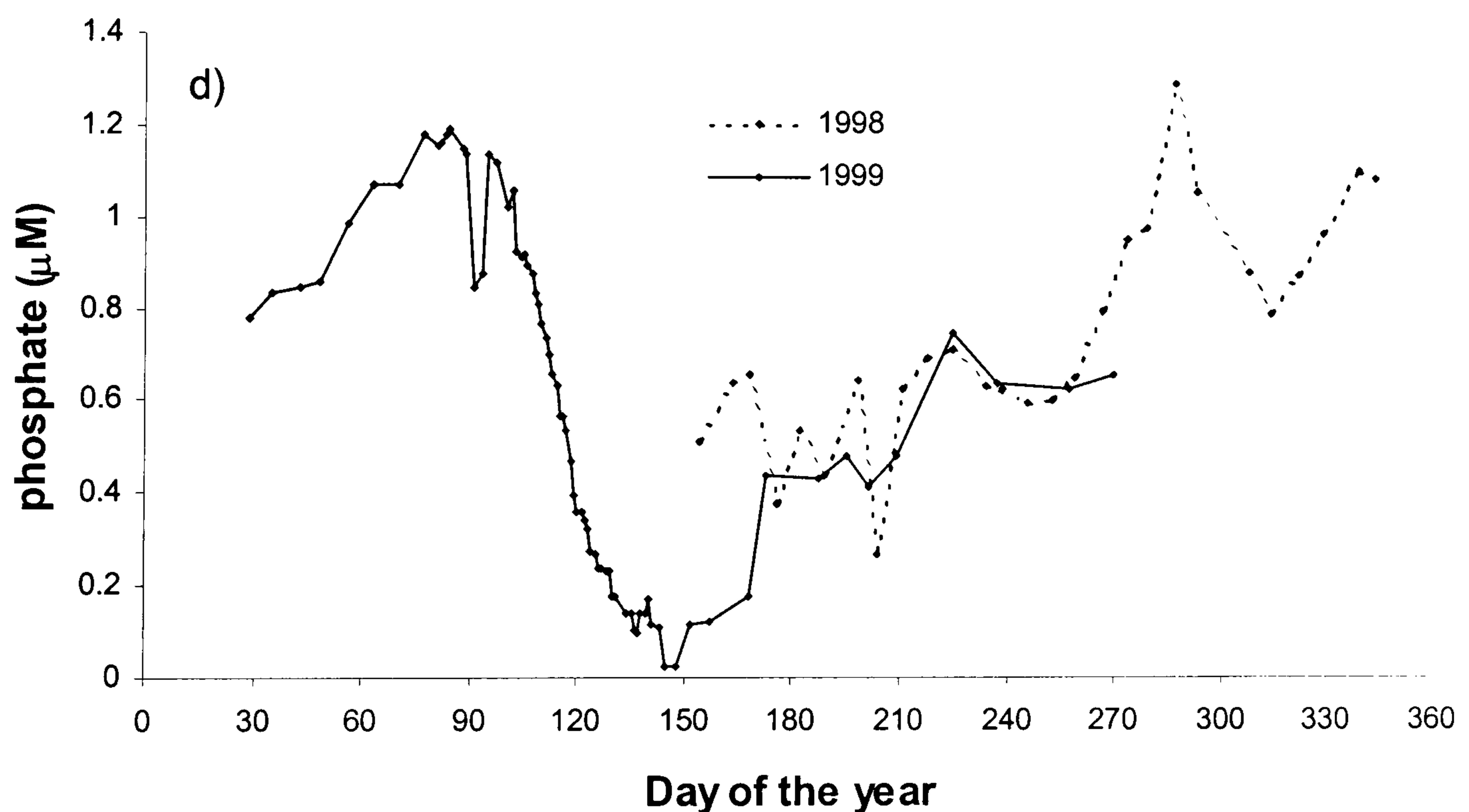
**Figure 4. 6b. Net ammonium uptake rate in the Menai Strait derived from the weekly differences during the period of ammonium disappearance in spring 1999.**



**Figure 4. 6c. Net ammonium regeneration rate in the Menai Strait as estimated from weekly increases in 1998.**

#### 4.3.4. Phosphate:

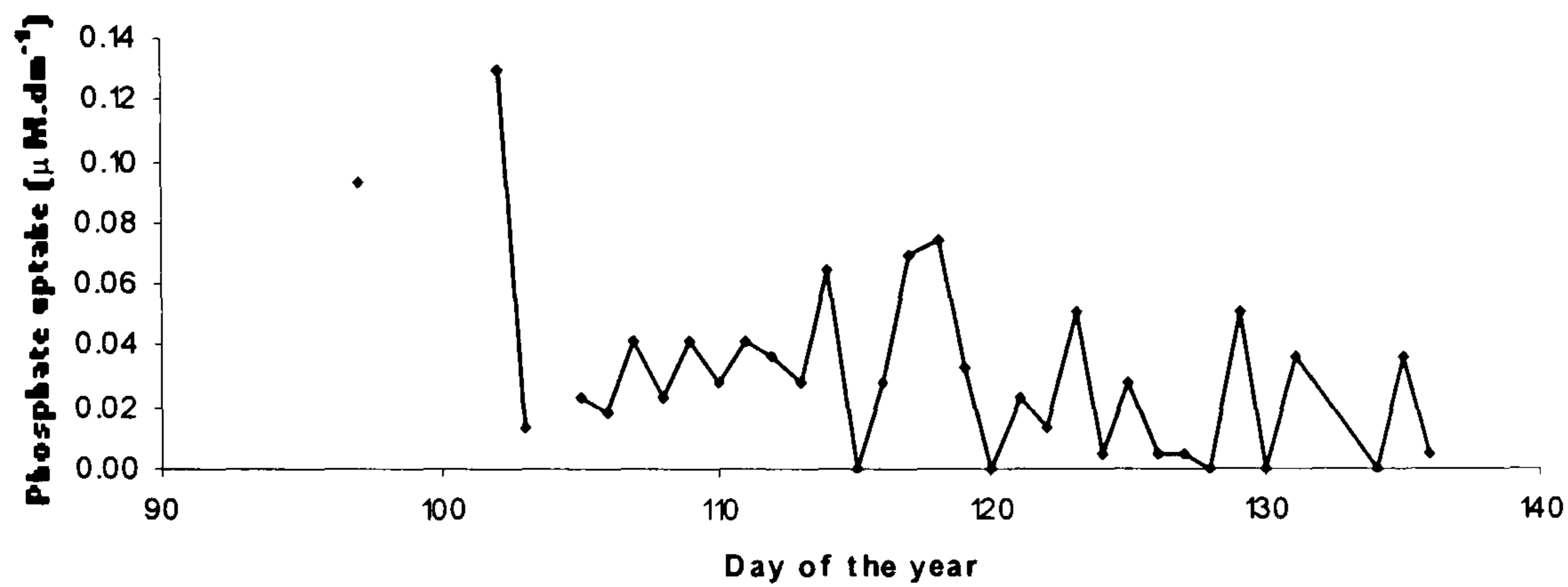
The seasonal variation of phosphate (Figure 4.7a) was characterised by relative constant concentrations during summer months of 1998, mean value  $0.60\mu\text{M}$ . The concentrations started to increase from day 267 and remained high for the rest of the year. When sampling continued during 1999, phosphate concentrations were found to be lower than in the previous months. Subsequently phosphate concentration gradually increased from  $0.8$  to  $1.2\mu\text{M}$  (day 88) and then remained close to  $1\mu\text{M}$  until day 103. Concentration started to decrease reaching the lowest value (below detection) on day 145. The low concentration remained for about 23 days. This was followed by regeneration with concentration rising from  $0.2$  to  $0.7\mu\text{M}$  between day 152 and 225. Relatively high concentrations were maintained during summer months, especially from the end of June to August ( $0.4$  to  $0.7\mu\text{M}$ ) and were similar to 1998 values.



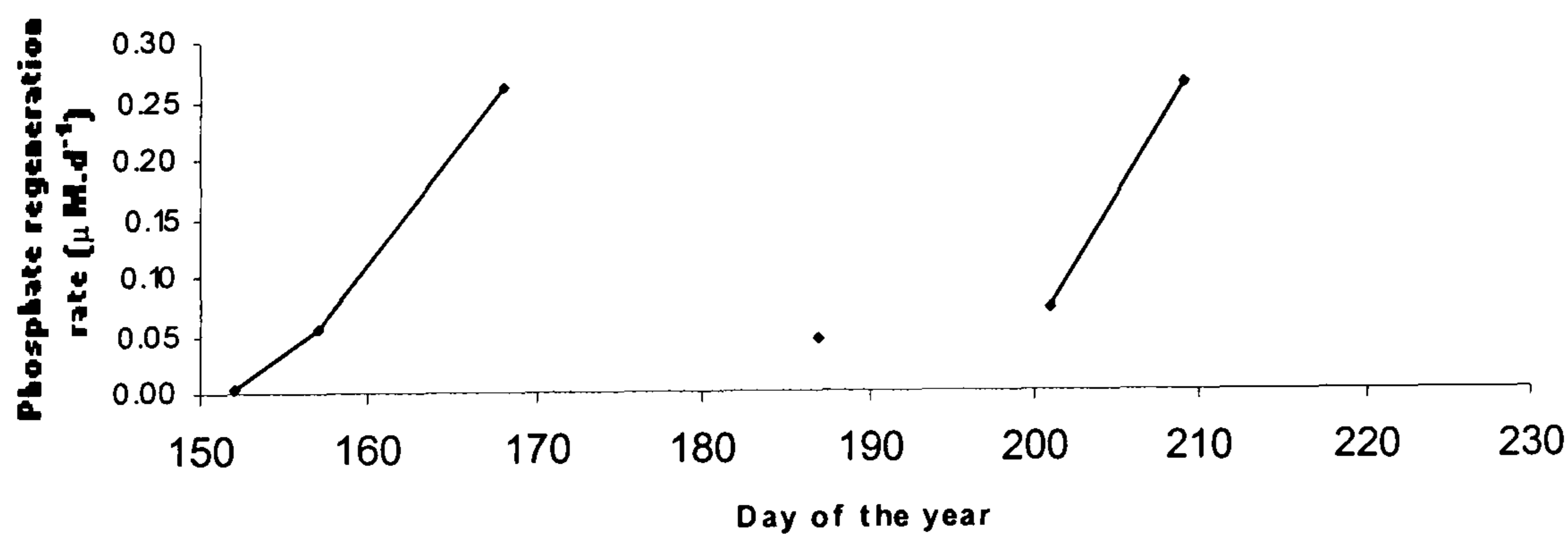
**Figure 4. 7a. Seasonal variation of phosphate in the Menai Strait during 1998 and 1999**

Phosphate uptake rates (Figure 4.7b) ranged between the maximum of  $0.1\mu\text{M.d}^{-1}$  to rates close to zero. In general a similar pattern was followed as that of net nitrate uptake rate. High net uptake coincided with the increase in chlorophyll *a* concentration. Phosphate regeneration rates (Figure 4.7c) were also calculated from weekly data. The regeneration rate ranged between  $0.01$  to  $0.26\mu\text{M.d}^{-1}$ . The decrease

in regeneration rate coincided with the decrease in chlorophyll. Overall, phosphate uptake occurred over the same period as nitrate coinciding with the phytoplankton abundance. Phosphate net regeneration occurred much more rapidly than nitrate after 2 days rather than 100 days.



**Figure 4. 7b. Net phosphate uptake rate in the Menai Strait in 1999 derived from the daily differences during the period of phosphate disappearance in spring.**

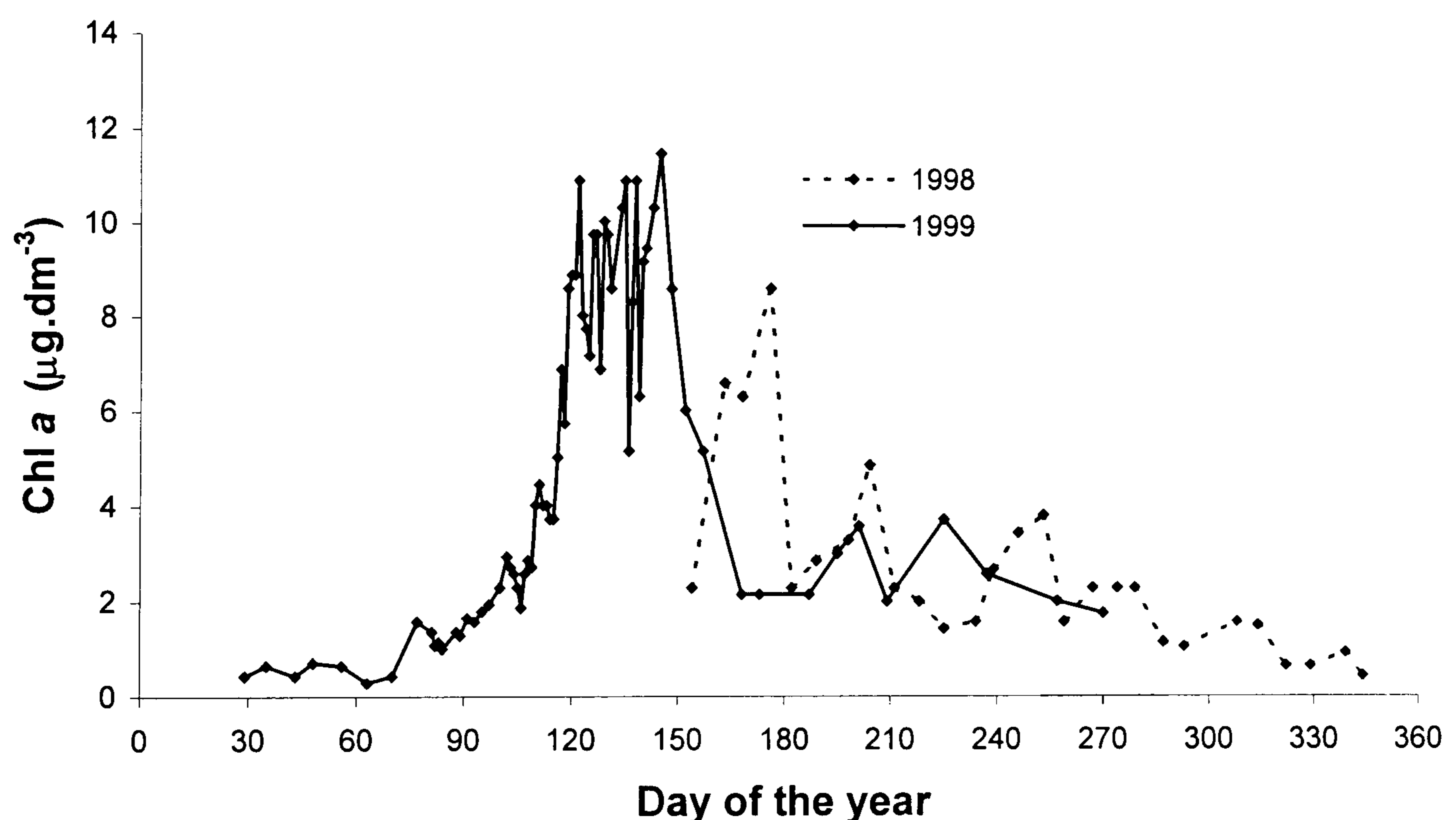


**Figure 4. 7c. Net phosphate regeneration rate in the Menai Strait as estimated from weekly increases in 1998.**

## 4.4. Biological Parameters

### 4.4.1. Chlorophyll *a*

The seasonal variation of chlorophyll *a* in 1998 and 1999 are shown in Figure (4.8). In 1998, chlorophyll *a* was characterised by high concentration (ranging between 6.3 to 8.7 $\mu\text{g}\cdot\text{dm}^{-3}$ ) in June (days 163, 168, 176). Concentration remained low for the whole summer (values <4.0 $\mu\text{g}\cdot\text{dm}^{-3}$ ) except on day 204 when the concentration was 4.9 $\mu\text{g}\cdot\text{dm}^{-3}$ . The winter period was associated with low chlorophyll concentration (<1.0 $\mu\text{g}\cdot\text{dm}^{-3}$ ) despite the high nutrient concentrations.



**Figure 4.8. Seasonal variation of chlorophyll *a* in the Menai strait in 1998 and 1999.**

From January (day 29) to March (day 70) in 1999, chlorophyll *a* concentration remained below 1.0 $\mu\text{g}\cdot\text{dm}^{-3}$ . Chlorophyll *a* concentration increased relatively slowly over March and early April before increasing rapidly from late April to May. Days 129 to 148 were characterised by high but variable concentrations (ranging from 5.2



to  $11.5\mu\text{g.dm}^{-3}$ ). Chlorophyll *a* decreased from late May to June, then concentration remained low until September (concentration ranged from 2.0 to  $3.6\mu\text{g.dm}^{-3}$ ).

#### 4.4.2. Phytoplankton Abundance

The seasonal variation of phytoplankton abundance in 1998 and 1999 (Figure 4.9a) followed that of chlorophyll. During 1998, phytoplankton abundance ranged between  $6.0 \times 10^4$  to  $8.2 \times 10^5$  cells.dm<sup>-3</sup> (days 198 and 168 respectively). The maximum abundance occurred during the third week of June (Figure 4.9b) (day 168, one week before the chlorophyll *a* maximum) and was associated with the growth of the diatoms and *Phaeocystis* (*Eucampia sp.*, *Phaeocystis sp.*, and *Rhizosolenia sp.*), but dominated by the later ( $>4.0 \times 10^5$  cells.dm<sup>-3</sup>). The second highest abundance ( $7.9 \times 10^5$  cells.dm<sup>-3</sup>) was dominated by *Leptocylindrus sp.* ( $>7.0 \times 10^5$  cells.dm<sup>-3</sup>) on day 176. During this period all nutrients were at their lowest concentration except phosphate and ammonia. A slight increase occurred in phytoplankton abundance in autumn ( $5.6 \times 10^5$  cells.dm<sup>-3</sup>) and this comprised of a combination of small diatoms, dominated by *Leptocylindrus sp.* ( $>4.0 \times 10^5$  cells.dm<sup>-3</sup>). During autumn and winter, phytoplankton abundance decreased reaching the lowest abundance ( $1.4 \times 10^4$  cells.dm<sup>-3</sup>) on day 344.

Low abundance persisted until March and small diatoms dominated during this period. From the beginning of April (day 91) to end of May (day 145), phytoplankton abundance increased gradually as nutrients started to decrease. Nutrients reached their lowest concentration at the time of highest phytoplankton abundance and chlorophyll concentration ( $7.7 \times 10^6$  cells.dm<sup>-3</sup> and  $11.5\mu\text{g.dm}^{-3}$  respectively) on day 145. A mixture of diatoms, and dinoflagellates persisted during summer. Dinoflagellates were dominated by the genus *Peridinium sp.* ( $2.1 \times 10^4$  cells.dm<sup>-3</sup>) in July (day 187), while among diatoms, *Leptocylindrus sp.* dominated ( $7.6 \times 10^6$  cells.dm<sup>-3</sup>) on day 152 and 187. A slight increase in phytoplankton abundance occurred in autumn (dominated by *Rhizosolenia sp.*).

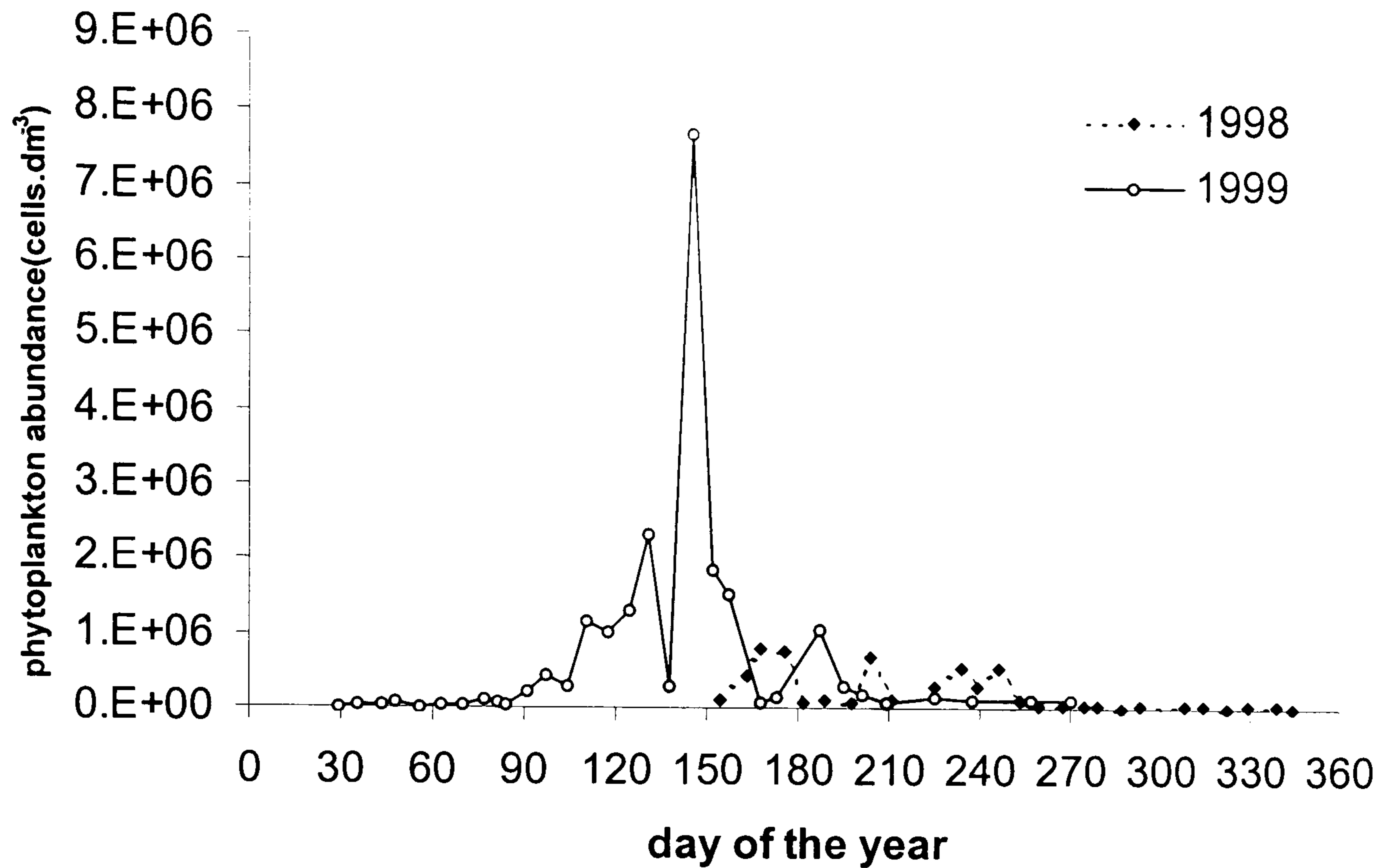


Figure 4.9a. Seasonal variation of phytoplankton abundance in the Menai strait water during 1998 and 1999.

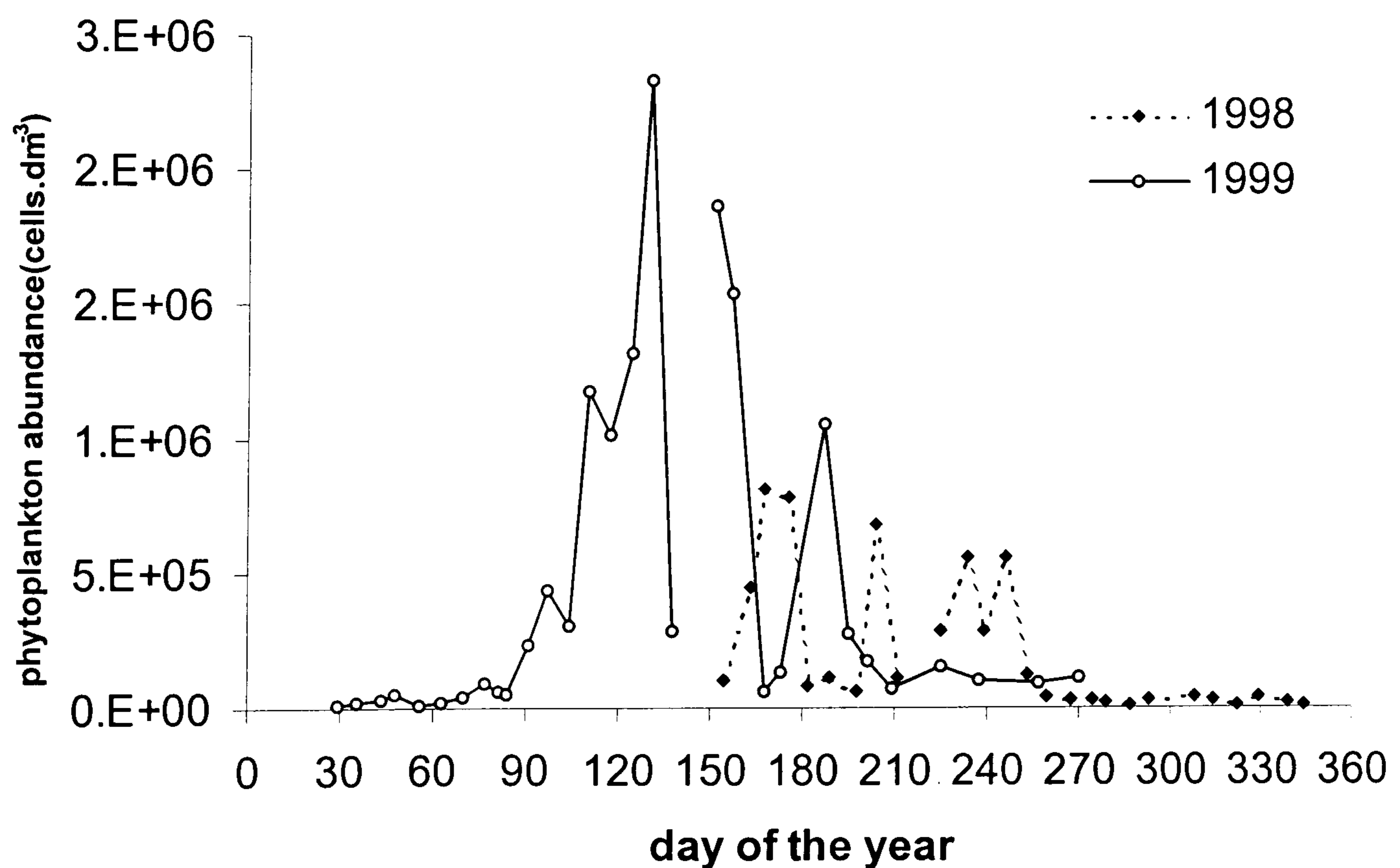
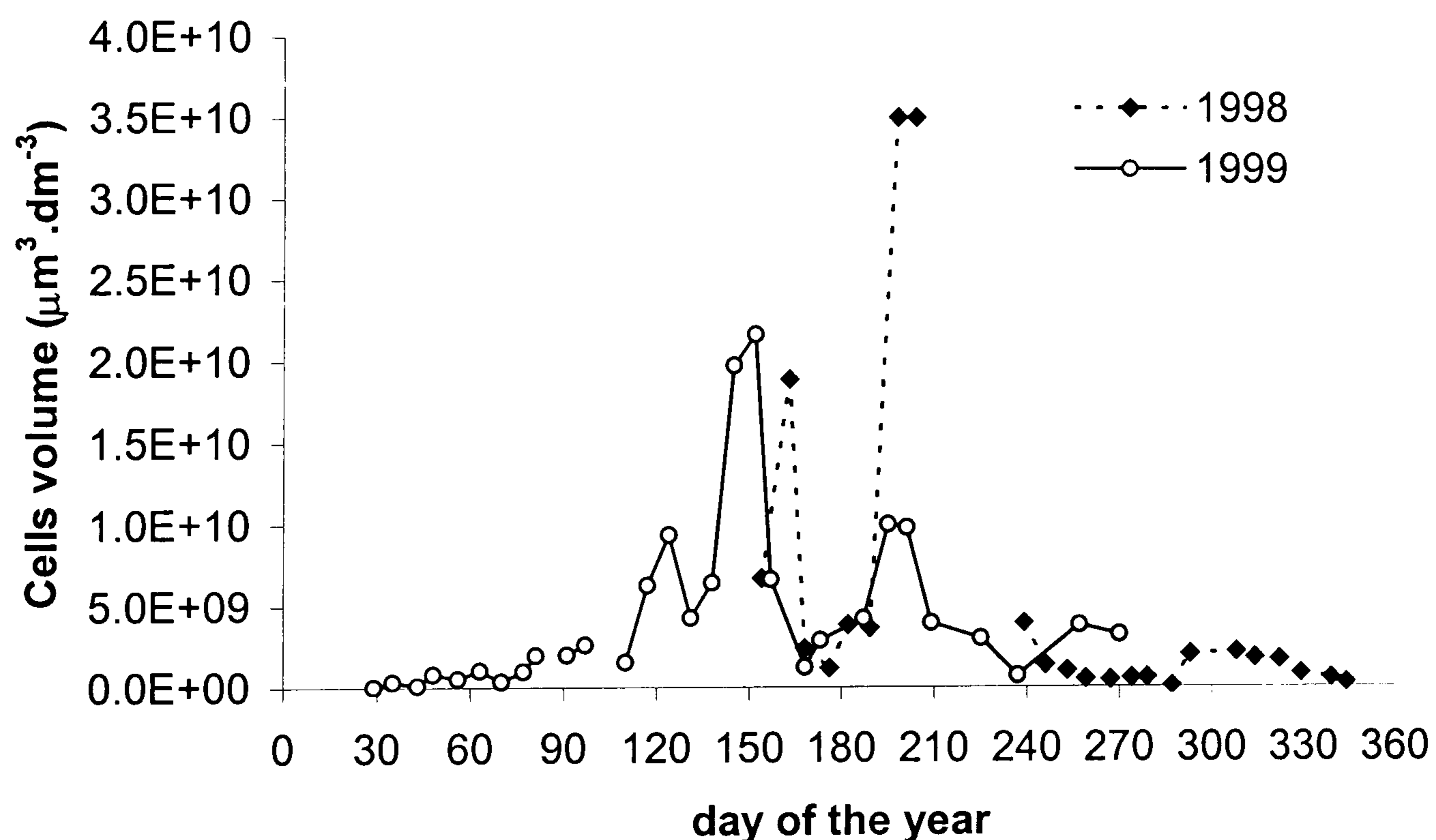


Figure 4.9b. Seasonal variation of phytoplankton abundance in the Menai Strait during 1998 and 1999. The major peak on day 145 was omitted to show the variation of other species occurring in low number.

The dynamics of the development of the standing phytoplankton population have been compared in terms of cell volume (Figure 4.9c). The volume for individual species used in this study is from (Newton 1986). The total volume of phytoplankton was calculated by multiplying the cell volume of individual species to the cell numbers (see Appendix 4).

The seasonal pattern of phytoplankton total volume was characterised by two peaks in summer 1998. A minor peak ( $1.9 \times 10^{10} \mu\text{m}^3.\text{dm}^{-3}$ ) occurred in June (day 168; mainly dominated by *Rhizosolenia*; *Leptocylindrus* and *Eucampia sp.*) followed by the major peak ( $3.5 \times 10^{10} \mu\text{m}^3.\text{dm}^{-3}$ ) in July (day 204 and 211; mainly dominated by *Rhizosolenia sp.*). Minimum values occurred during autumn and winter (ranging from  $2.1 \times 10^7$  to  $2.2 \times 10^9 \mu\text{m}^3.\text{dm}^{-3}$ ). The maximum value during spring ( $2.2 \times 10^{10} \mu\text{m}^3.\text{dm}^{-3}$ ) occurred on day 152 (dominated by *Rhizosolenia sp.*).



**Figure 4.9c. Seasonal variation of phytoplankton cell volume in the Menai Strait water during 1998 and 1999.**

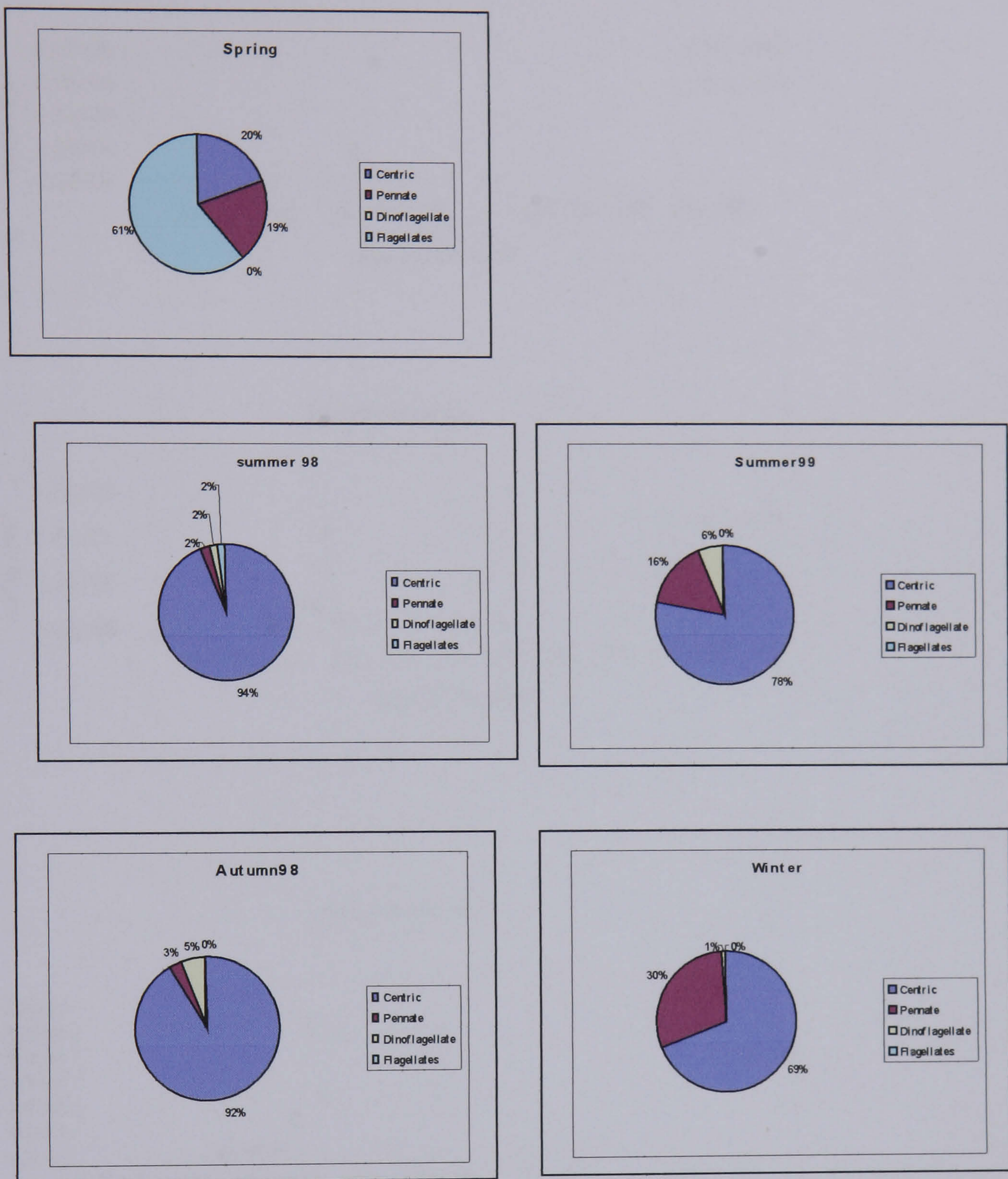
### 4.4.3. Species composition and distribution

Figure (4.10) shows the percentage of the total cell count attributed to the different taxonomic classes found during the study period. Bacillariophyceae were the most numerous class more than 94% during summer with centric diatoms dominating over pennate in all seasons. This class was dominant in all seasons except in spring when the Haptophyceae were more abundant. The Haptophyceae were found in late spring and dominated the percentage of the total cell count ( $\leq 61\%$ ). While Dinophyceae were found in small numbers ( $\leq 6\%$ ) throughout the study.

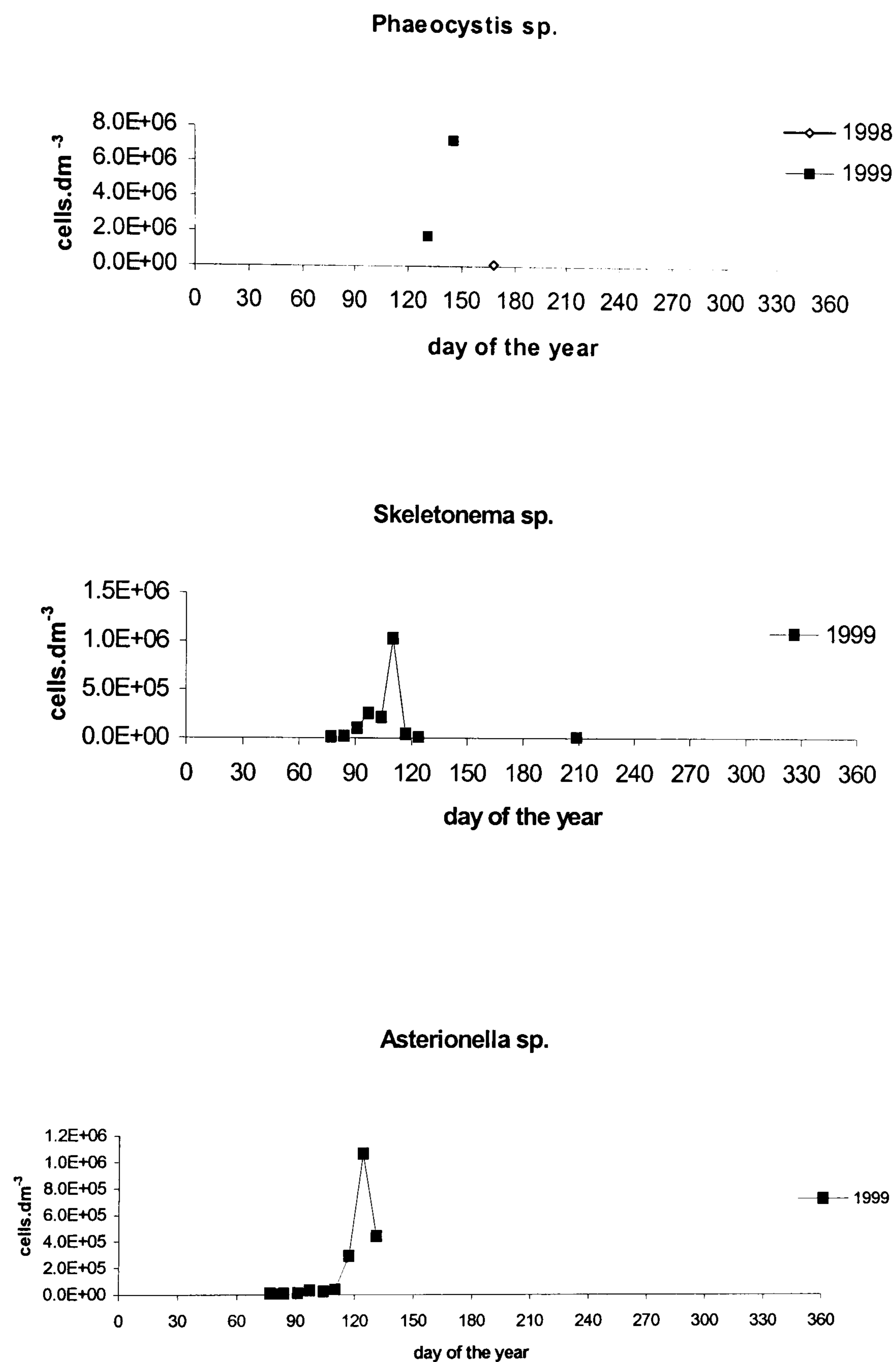
The phytoplankton species composition and succession could be classified into five groups according to their abundance and time of appearance: species with high abundance (up to  $10^6$  cells.dm<sup>-3</sup>), medium abundance (up to  $10^5$  cells.dm<sup>-3</sup>), low abundance (up to  $10^4$  cells.dm<sup>-3</sup>), and very low abundance (up to  $10^3$  cells.dm<sup>-3</sup>). These results will be presented separately as follows:

#### 4.3.3.1. High abundance

During this study three of the species (*Asterionella sp.*, *Skeletonema sp.*, and *Phaeocystis sp.*) were found in large numbers during a restricted period (March-June 1999) (Figure 4.11a). *Asterionella sp.*, occurred from March (day 84) to May (day 131) in 1999 with maximum cells number of  $1.07 \times 10^6$  cells.dm<sup>-3</sup>. *Skeletonema sp.* showed continuous appearance from March reaching the maximum cells number ( $1.03 \times 10^6$  cells.dm<sup>-3</sup>) in April (day 110). Despite few records of the *Phaeocystis sp.* it is worth mentioning that this species comprised the major component of the phytoplankton major peak. Maximum cells number ( $7.3 \times 10^6$  cells.dm<sup>-3</sup>) occurred in May 1999 (day 145). This species persisted for one month and disappeared during late summer.



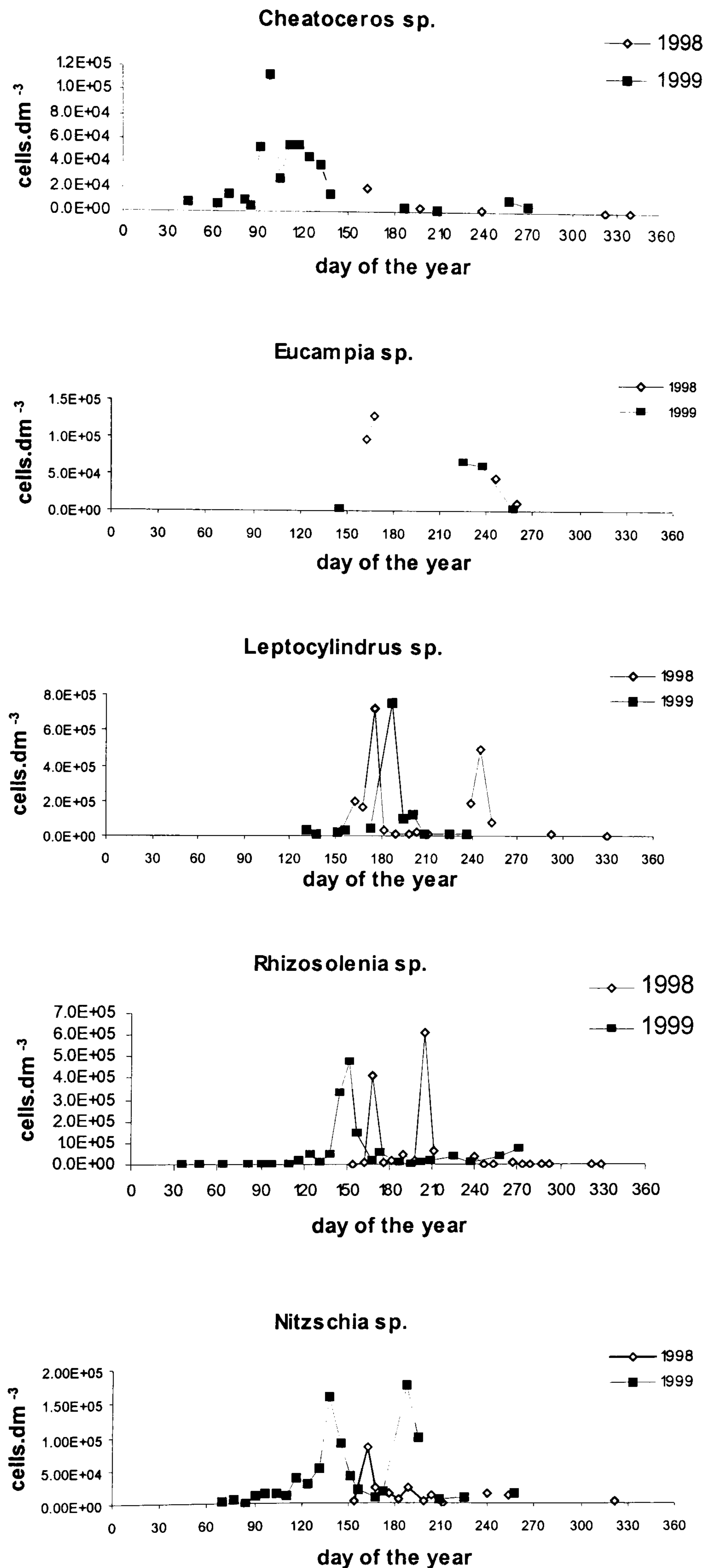
**Figure 4.10. Seasonal variation of phytoplankton taxonomical classes in the Menai Strait during 1998 and 1999.**



**Figure 4.11a. Phytoplankton species abundance in the Menai Strait during 1998&1999. These species were classified as high abundance.**

#### 4.3.3.2. Medium abundance

Species such as *Chaetoceros sp.*, *Eucampia sp.*, *Leptocylindrus sp.*, *Rhizosolenia sp.*, and *Nitzschia sp.* were the next more abundant (Figure 4.11b) (up to  $10^5$  cells.dm<sup>-3</sup>). *Chaetoceros sp.* were found throughout the study period, with highest abundance during spring. Maximum cell number ( $1.1 \times 10^5$  cells.dm<sup>-3</sup>) occurred in April (day 97). *Eucampia sp.* were restricted to summer period in both years. Maximum cell number in 1998 ( $1.2 \times 10^5$  cells.dm<sup>-3</sup>) occurred in June (day 168), whereas the highest cells number in 1999 ( $6.5 \times 10^4$  cells.dm<sup>-3</sup>) occurred in August (day 225). *Leptocylindrus sp.* occurred mostly in spring and summer. Maximum cell number in 1998 and 1999 occurred during summer ( $7.2 \times 10^5$  cells.dm<sup>-3</sup>) in June (day 176) and in July ( $7.6 \times 10^5$  cells.dm<sup>-3</sup>) day 187 respectively. *Rhizosolenia sp.* were found throughout the study with highest cell abundance occurring during summer ( $6.14 \times 10^5$  cells.dm<sup>-3</sup> in July 1998 (day 204), and ( $4.7 \times 10^5$  cells.dm<sup>-3</sup>) in June 1999 (day 152). *Nitzschia sp.* were abundant, especially during spring and summer. Maximum cell number in 1998 ( $8.3 \times 10^4$  cells.dm<sup>-3</sup>) occurred in June (day 163). In 1999 this species showed higher abundance with cell number increasing from  $1.6 \times 10^5$  cells.dm<sup>-3</sup> in May (day 138) to maximum cell number ( $1.8 \times 10^5$  cells.dm<sup>-3</sup>) in July (day 187).



**Figure 4.11b. Annual variation in phytoplankton species abundance in the Menai Strait during 1998&1999. These species were classified as medium abundance.**

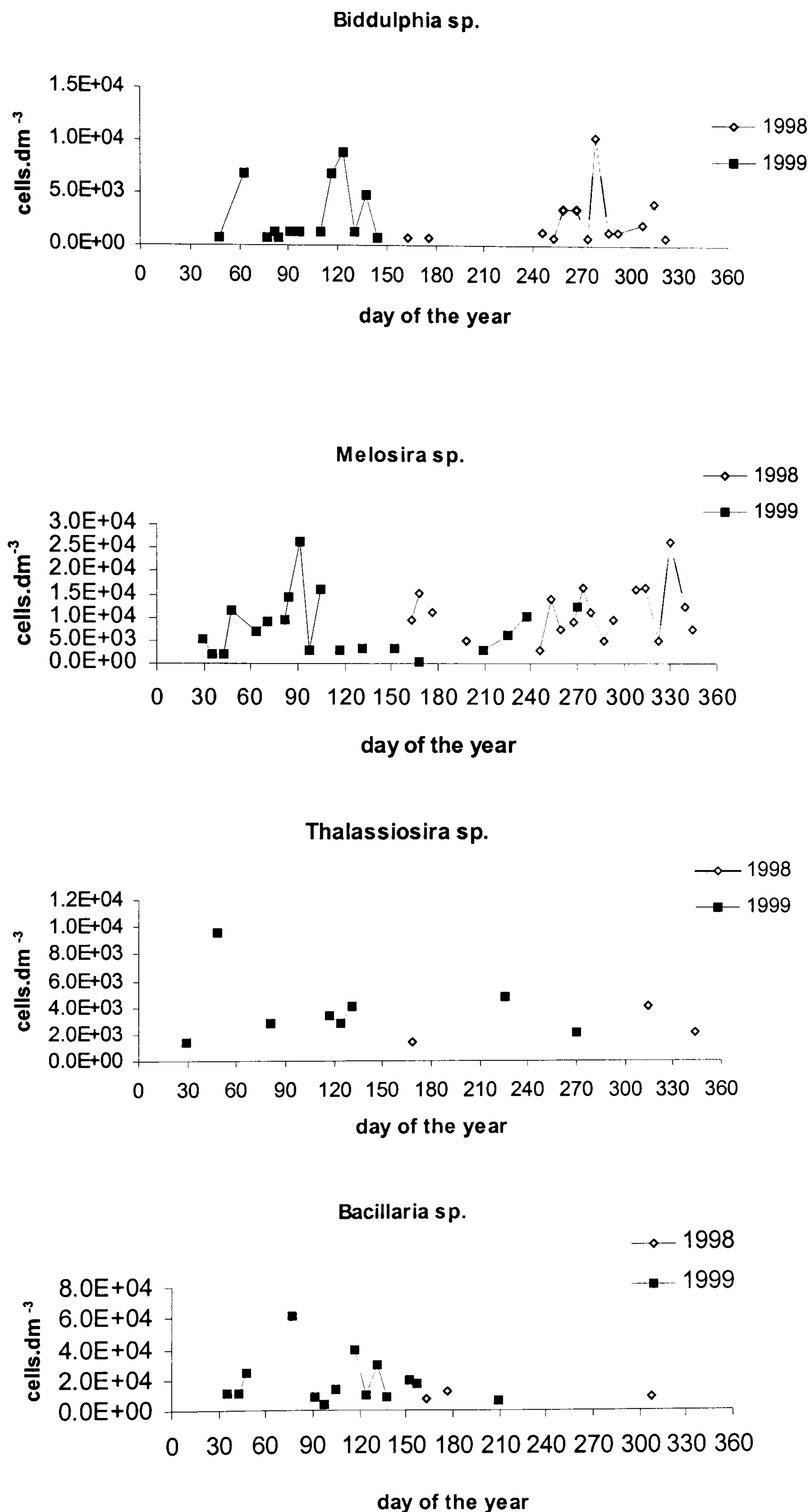


### 4.3.3.3. Low abundance

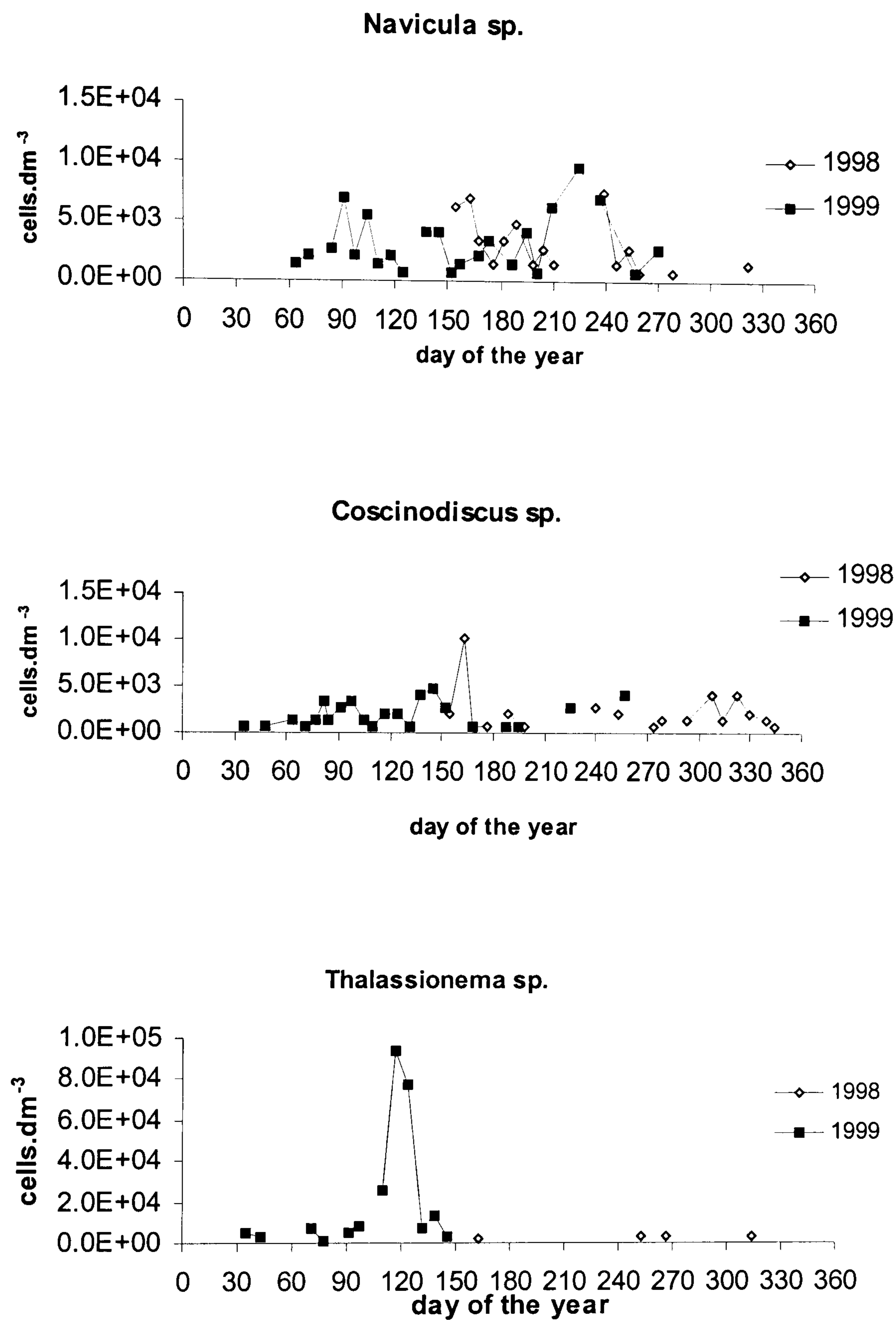
The phytoplankton species characterised as low abundance (up to  $10^4$  cells.dm<sup>-3</sup>) (Figure 4.11c) could be classified into a) species abundant throughout the study period (Figure 4.11c1) and b) those which are restricted to a particular period (Figure 4.11c2).

#### 4.3.3.3a. Low abundance throughout the study

The species found throughout the study were: *Biddulphia* sp., *Melosira* sp., *Thalassiosira* sp., *Bacillaria* sp., *Navicula* sp., *Coscinodiscus* sp., and *Thalassionema* sp. (Figure 4.11c1). *Biddulphia* sp. occurred in autumn 1998 with a maximum cell number ( $1.03 \times 10^4$  cells.dm<sup>-3</sup>) day 279. It disappeared during December and January and appeared again in early spring. Maximum cell number ( $8.9 \times 10^3$  cells.dm<sup>-3</sup>) in 1999 occurred in May, day (124). *Melosira* sp. occurred throughout the year with maximum cell number in autumn 1998 ( $2.6 \times 10^4$  cells.dm<sup>-3</sup>) in November (day 329) and spring 1999 ( $2.6 \times 10^5$  cells.dm<sup>-3</sup>) in April (day 91). *Thalassiosira* sp were mostly abundant in late autumn and winter. Maximum cell number in 1998 ( $4.1 \times 10^3$  cells.dm<sup>-3</sup>) occurred in November (day 314) and in 1999 ( $9.6 \times 10^3$  cells.dm<sup>-3</sup>) occurred in February (day 48). *Bacillaria* sp. occurred in June (days 163 and 176) and November (day 308) in 1998. The maximum cell number ( $1.1 \times 10^4$  cells.dm<sup>-3</sup>) occurred in June (day 176). In 1999 this species occurred from February (day 35) and increased gradually reaching the maximum cell number ( $6.2 \times 10^4$  cells.dm<sup>-3</sup>) in March (day 77) followed by a decline towards summer. *Navicula* sp. occurred in spring, summer and autumn. Maximum cells number in 1998 ( $6.9 \times 10^3$  cells.dm<sup>-3</sup>) occurred in June (day 163). In 1999 maximum cells number ( $9.6 \times 10^3$  cells.dm<sup>-3</sup>) occurred in August (day 225). *Coscinodiscus* sp. were found throughout the study. Maximum abundance in 1998 ( $1.03 \times 10^4$  cells.dm<sup>-3</sup>) occurred in June (day 163) whereas in 1999 ( $4.8 \times 10^3$  cells.dm<sup>-3</sup>) occurred in May (day 145). *Thalassionema* sp. were found in spring and autumn. Maximum cell number in 1998 ( $3.4 \times 10^3$  cells.dm<sup>-3</sup>) occurred in September (day 267). In 1999 (day 117) this species was more abundant with maximum cell number ( $9.4 \times 10^4$  cells.dm<sup>-3</sup>, day 110)



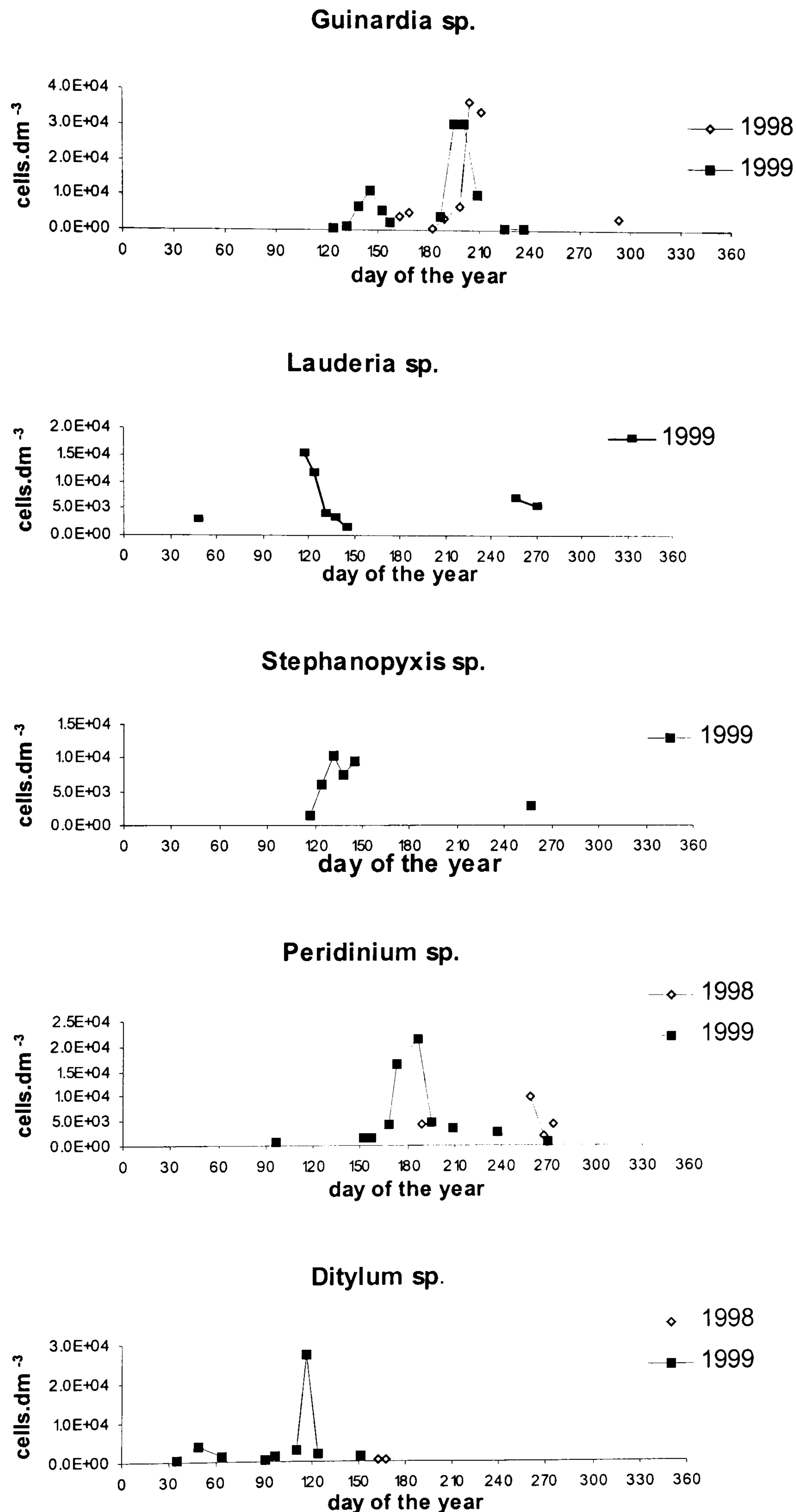
**Figure 4.11c1. Annual variation of phytoplankton species abundance in the Menai Strait during 1998&1999. These species were low in abundance but occurred throughout the study.**



**Figure 4.11c1(continued). Annual variation of phytoplankton species abundance in the Menai Strait during 1998&1999. These species were characterised with low abundance but occurred throughout the study.**

#### 4.3.3.3b. Temporally restricted low abundance

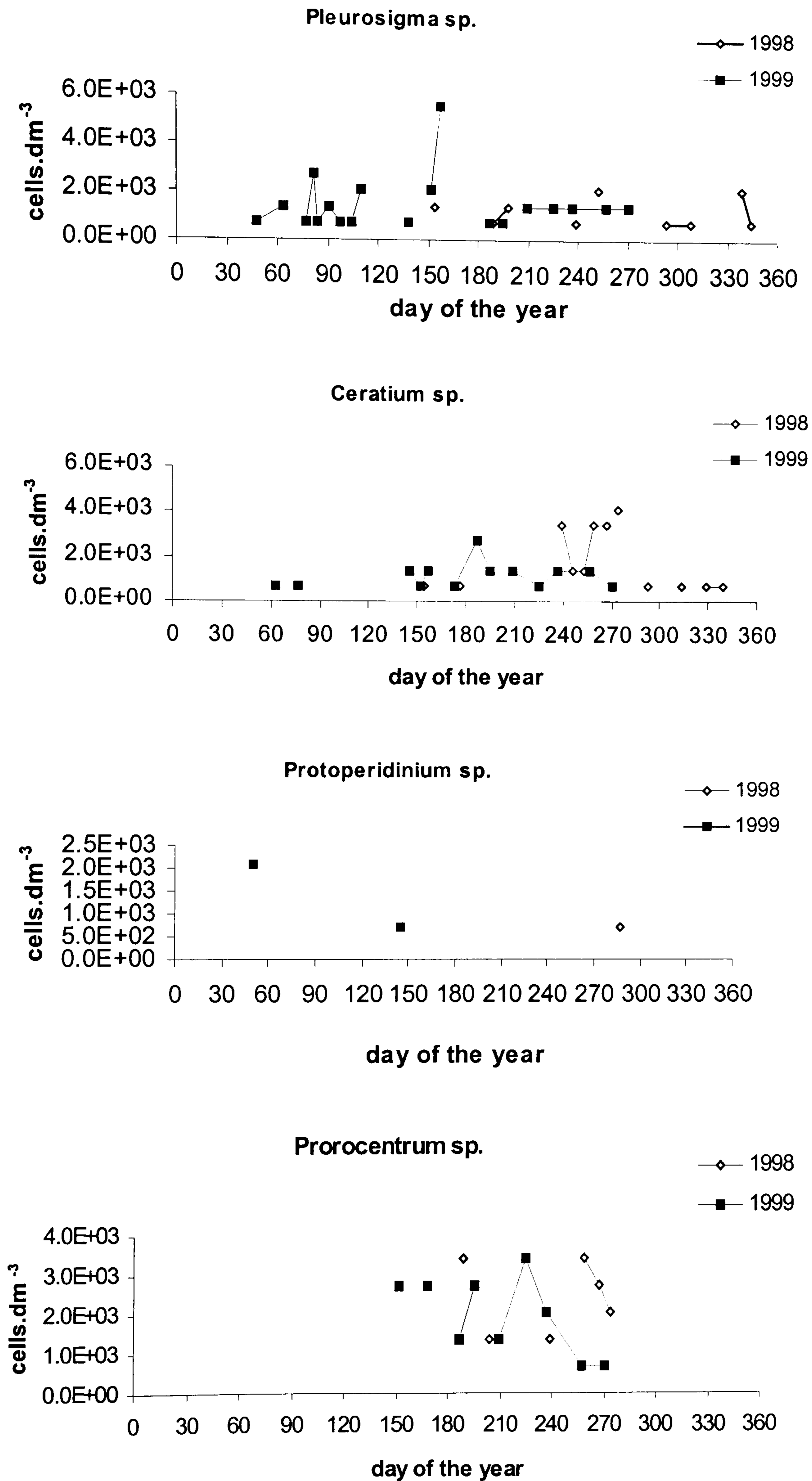
The species found to be restricted to a particular period of the study were *Guinardia sp.*, *Lauderia sp.*, *Stephanopyxis sp.*, *Peridinium sp.*, and *Ditylum sp.*. *Guinardia sp.* occurred in summer and autumn. Maximum cells number in 1998 ( $3.6 \times 10^4$  cells.dm<sup>-3</sup>) occurred in July (day 204) and ( $3.02 \times 10^4$  cells.dm<sup>-3</sup>) in July 1999 (days 195 and 201). *Lauderia sp.* occurred only in 1999 mostly in spring. Maximum cells number ( $1.1 \times 10^4$  cells.dm<sup>-3</sup>) occurred in May (day 124). *Stephanopyxis sp.* occurred mostly during spring with maximum cells number ( $1.03 \times 10^4$  cells.dm<sup>-3</sup>) in May (day 131). *Peridinium sp.* mostly occurred during summer in both years. The maximum cells number in 1998 ( $9.6 \times 10^3$  cells.dm<sup>-3</sup>) occurred in September (day 259). In 1999 this species was more abundant with the maximum cells number ( $2.1 \times 10^4$  cells.dm<sup>-3</sup>) in July (day 187). *Ditylum sp.* was abundant during spring. Maximum abundance ( $2.7 \times 10^4$  cells.dm<sup>-3</sup>) occurred in April (day 117).



**Figure 4.11c2. Annual variation of phytoplankton species abundance in the Menai Strait during 1998&1999. These species were characterised with low abundance and restricted to certain period of the study.**

#### 4.3.3.4. Very low abundance

Phytoplankton species categorised as very low abundance (Figure 4.11d) (up to  $10^3$  cells.dm<sup>-3</sup>) were *Pleurosigma* sp., *Ceratium* sp., *Protoperidinium* sp., and *Prorocentrum* sp. *Pleurosigma* sp. despite its low abundance, was present more or less throughout the year. Maximum cell abundance in 1998 ( $2 \times 10^3$  cells.dm<sup>-3</sup>) occurred in September (day 253). In 1999 the maximum cell abundance ( $5.5 \times 10^3$  cells.dm<sup>-3</sup>) occurred in June (day 157). *Ceratium* sp. occurred during summer and autumn. The maximum cell number in 1998 ( $4.1 \times 10^3$  cells.dm<sup>-3</sup>) occurred in October (day 274) while in 1999 the maximum ( $2.7 \times 10^3$  cells.dm<sup>-3</sup>) occurred in July (day 187). *Protoperidinium* sp. was recorded once 1998 and twice in 1999. Maximum cell abundance  $2.3 \times 10^3$  cells.dm<sup>-3</sup> occurred in spring 1999. *Prorocentrum* sp. was mostly abundant during summer and autumn. Maximum abundance in 1998 ( $3.4 \times 10^3$  cells.dm<sup>-3</sup>) occurred in September (day 259) and in 1999 ( $3.4 \times 10^3$  cells.dm<sup>-3</sup>) occurred in August (day 225).



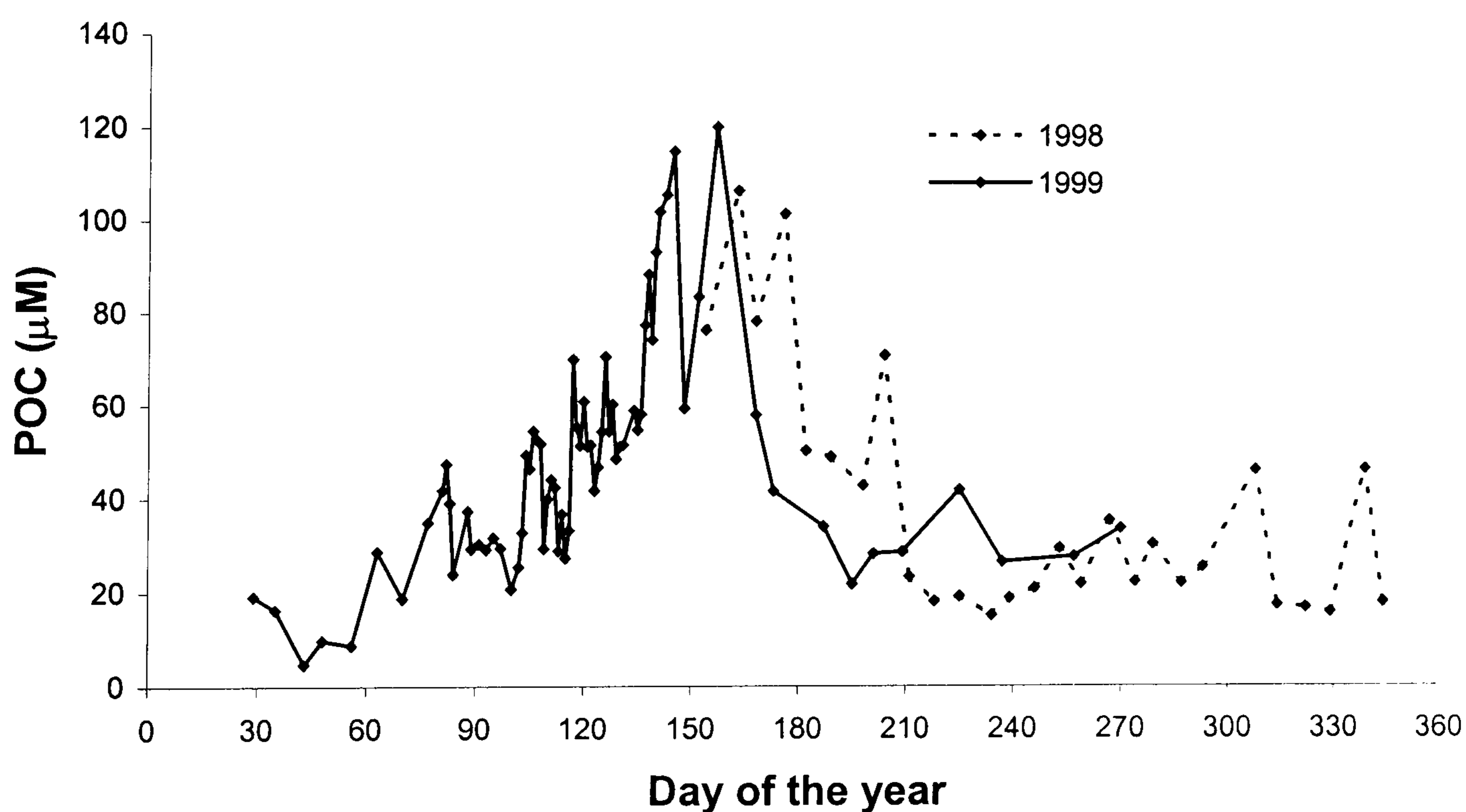
**Figure 4.11d. Annual variation of phytoplankton species abundance in the Menai Strait during 1998&1999. These species were categorised as very low abundance ( $<10^3$  cells.dm<sup>-3</sup>)**

## 4.5. Particulate Organic Matter

### 4.5.1 Particulate Organic Carbon (POC) and Nitrogen (PON)

Seasonal changes in particulate organic carbon in 1998 and 1999 are shown in Figure (4.12a). During 1998, the concentration of POC varied between 15 and 105 $\mu\text{M}$ . The highest concentration of POC (ranging between 77 and 107 $\mu\text{M}$ ) occurred in June.

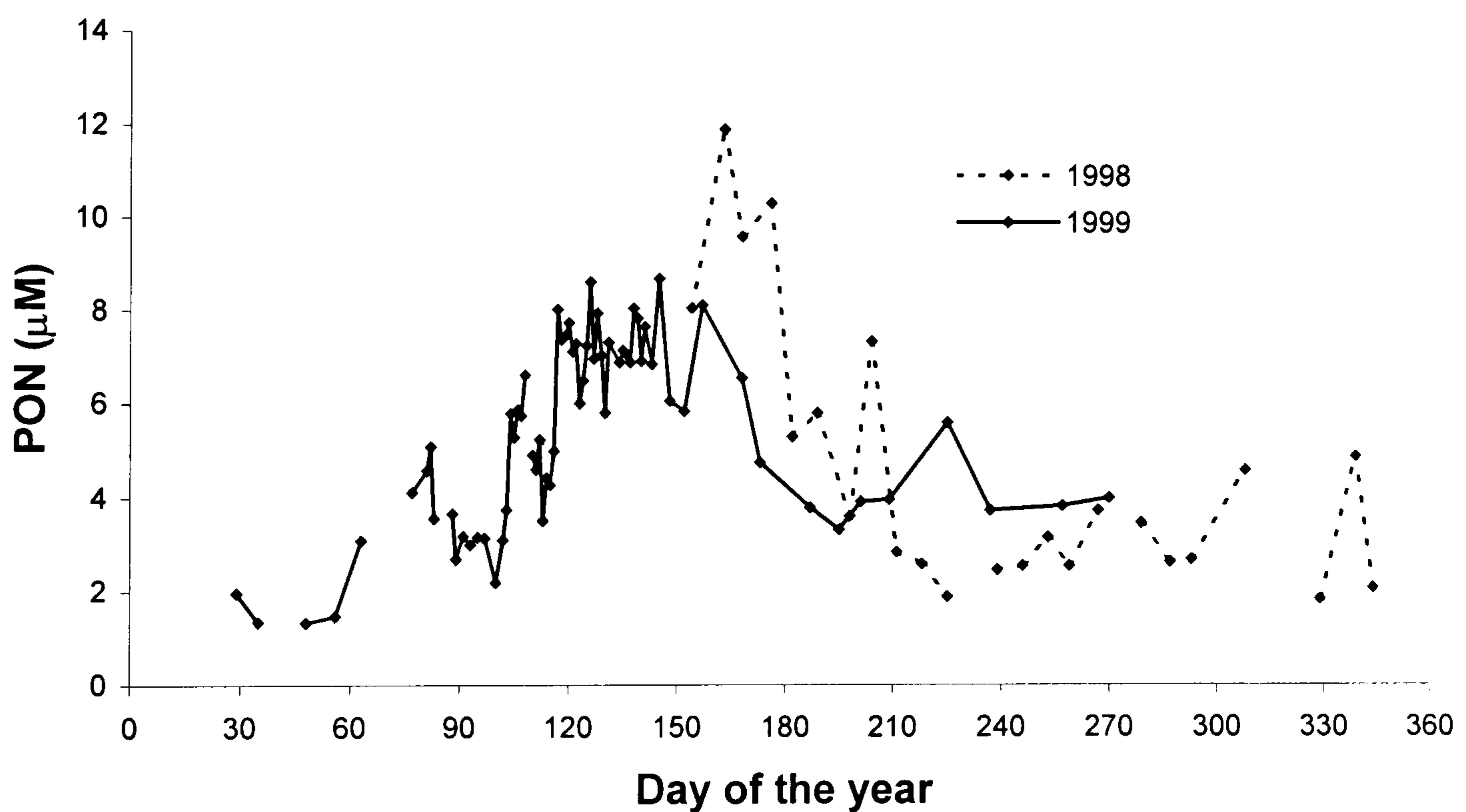
During 1999, POC reached the lowest concentration (5 $\mu\text{M}$ ; day 43). This was followed by an increase despite the fluctuation which appeared to be associated with the development of the spring phytoplankton bloom. This was characterised by a period of fluctuation and linear increase from day 63 to 136 with concentration ranging between 19 to 71 $\mu\text{M}$ . A sharp increase occurred from day 137 (78 $\mu\text{M}$ ) to day 145 (115 $\mu\text{M}$ ). This coincided with the major peak of POC (121 $\mu\text{M}$ ) which occurred almost a week later.



**Figure 4.12a. Seasonal variation of particulate organic carbon in the Menai Strait water during 1998 and 1999.**



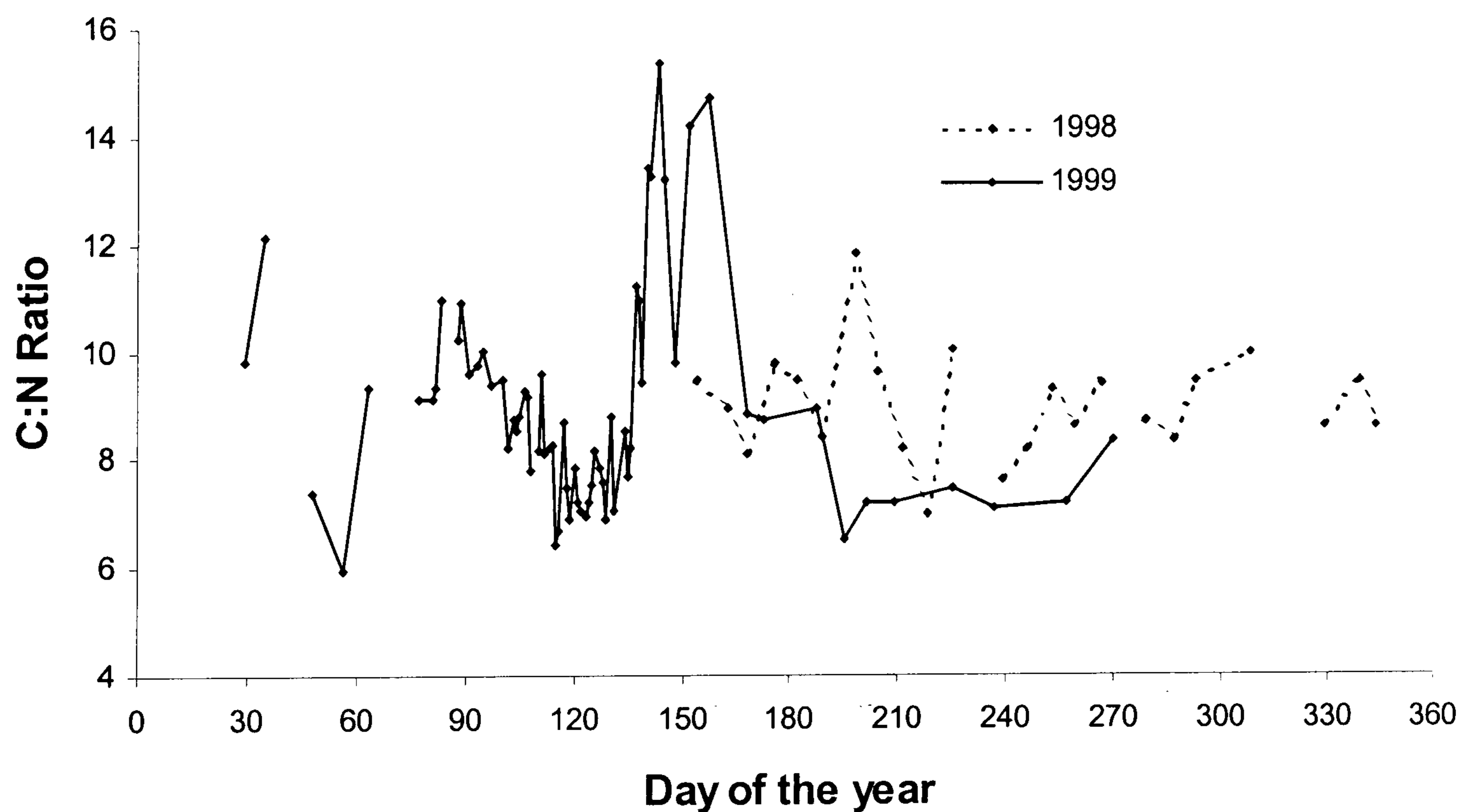
Particulate organic nitrogen (PON) (Figure 4.12b.) followed a similar trend to POC in 1998 and 1999. In 1998 PON concentrations varied from a maximum concentration of  $12\mu\text{M}$  (day 163) to  $2\mu\text{M}$  (days 225, 329 and 344). The concentration decreased during summer, followed by a slight increase in autumn (concentration 3 to  $5\mu\text{M}$ ) and back to low concentration during winter. In 1999 it followed a similar pattern and the concentration varied between minimum  $1\mu\text{M}$  (day 35, 43, 48 and 56) and maximum  $9\mu\text{M}$  (day 126 and 145).



**Figure 4.12b. Seasonal variation of particulate organic nitrogen in the Menai Strait water during 1998 and 1999.**

#### 4.5.2. C:N Ratios of the Particulate Organic Matter:

In 1998 the C:N ratio of the particulate matter (Figure 4.12c) varied between a maximum of 12 (day 198) to a minimum of 7 (day 218) and the mean of 9. The C:N ratio varied slightly during summer and autumn and remained constant during winter. In 1999 the ratio ranged between 6 (day 218) and 15 (day 157). The pattern for summer and autumn was similar for both years except during spring phytoplankton bloom in 1999 when the ratio increased to its maximum (15). This increase coincided with major peaks of chlorophyll and phytoplankton abundance.



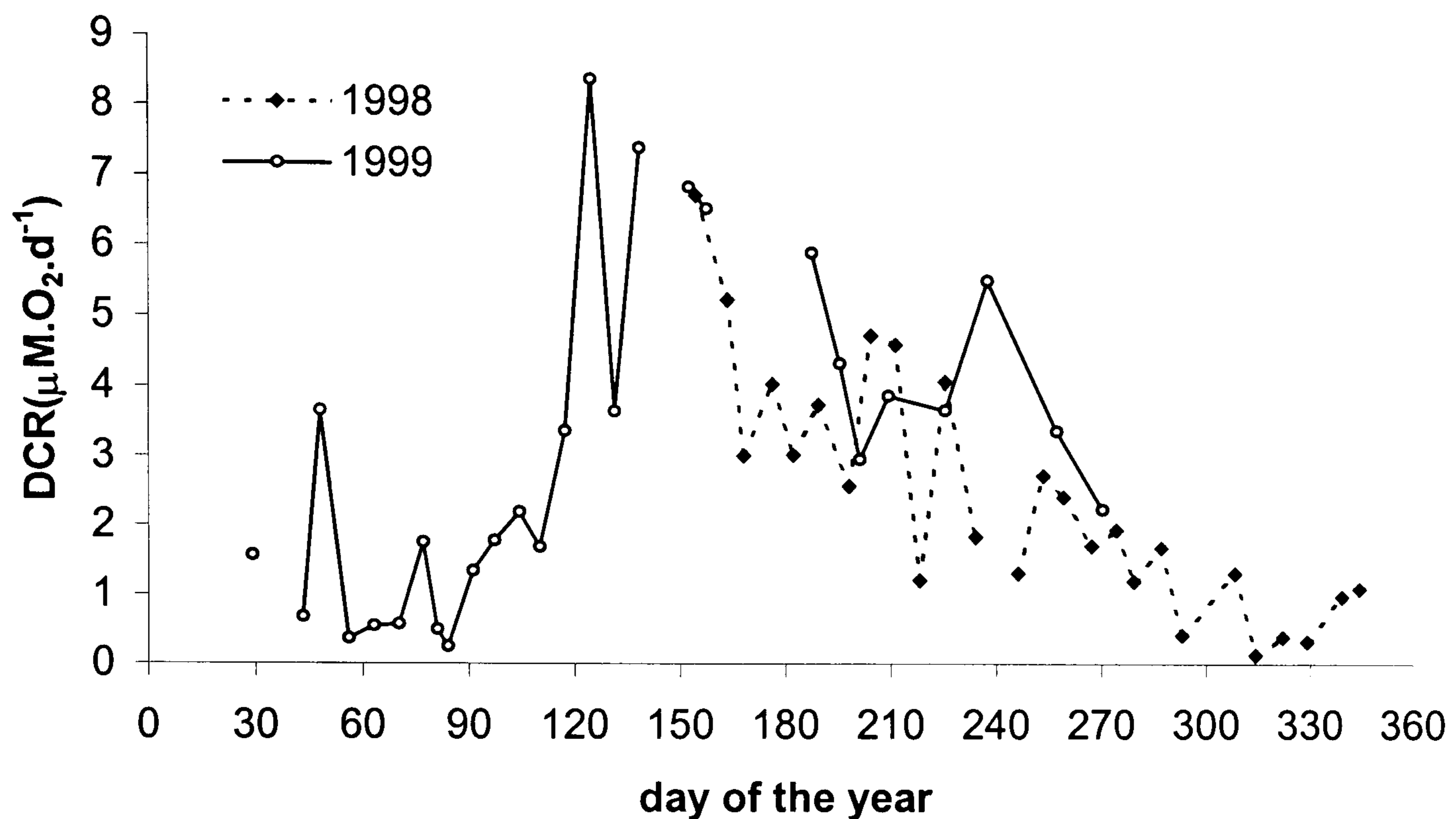
**Figure 4.12c. Seasonal variation of C:N ratio of the particulate organic matter in the Menai strait water during 1998 and 1999.**

## 4.6. Primary Production

### 4.6.1. Respiration

The seasonal variation of respiration in 1998 and 1999 (Figure 4.13a.) was characterised by major peaks during spring and summer. One peak was observed in June 1998 (day 154,  $6.65\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ) followed by fairly constant rates (ranging between  $1.8$  and  $5.3\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ) during summer and sharp decline during autumn and winter. The maximum respiration rate coincided with the negative net primary production and high bacterial abundance. In 1999, low respiration rates occurred from January (day 29) to May (day 117). Despite the sharp increase in net community production from day 91, respiration remained fairly constant (ranging between *ca.*  $1$  to  $3\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ). A sharp increase in respiration rate occurred from day 124 to day 145 (maximum  $9\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ) which coincided with the maximum bacterial and phytoplankton abundance (almost two weeks from the maximum gross and net community production rates).

During summer and autumn, respiration rate gradually decreased followed by a slight increase ( $5.6\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ) on day 237. The variation in respiration rates did not follow closely that of the net community production. For example the increase in gross and net production on day 225, did not necessary increase the respiration, similarly high respiration rate on day 157 coincided with negative net community production.



**Figure 4.13a. Seasonal variation of community respiration in the Menai Strait water during 1998 and 1999.**

#### **4.6.2. Gross and Net Community Production (GCP and NCP)**

The seasonal variation of gross and net community production in 1998 and 1999 (Figures 4.13.b&c), followed patterns similar to those observed in previous studies (Blight 1996; Rodriguez 1998). This pattern was characterised by major peaks during spring and summer declining towards winter.

Gross production in 1998 were characterised by two peaks ( $17.4\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ) day 168 and ( $22.2\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ) day 239. The two peaks coincided with high chlorophyll *a* and phytoplankton abundance. The gross community exhibited low values following the decrease of phytoplankton abundance during winter.

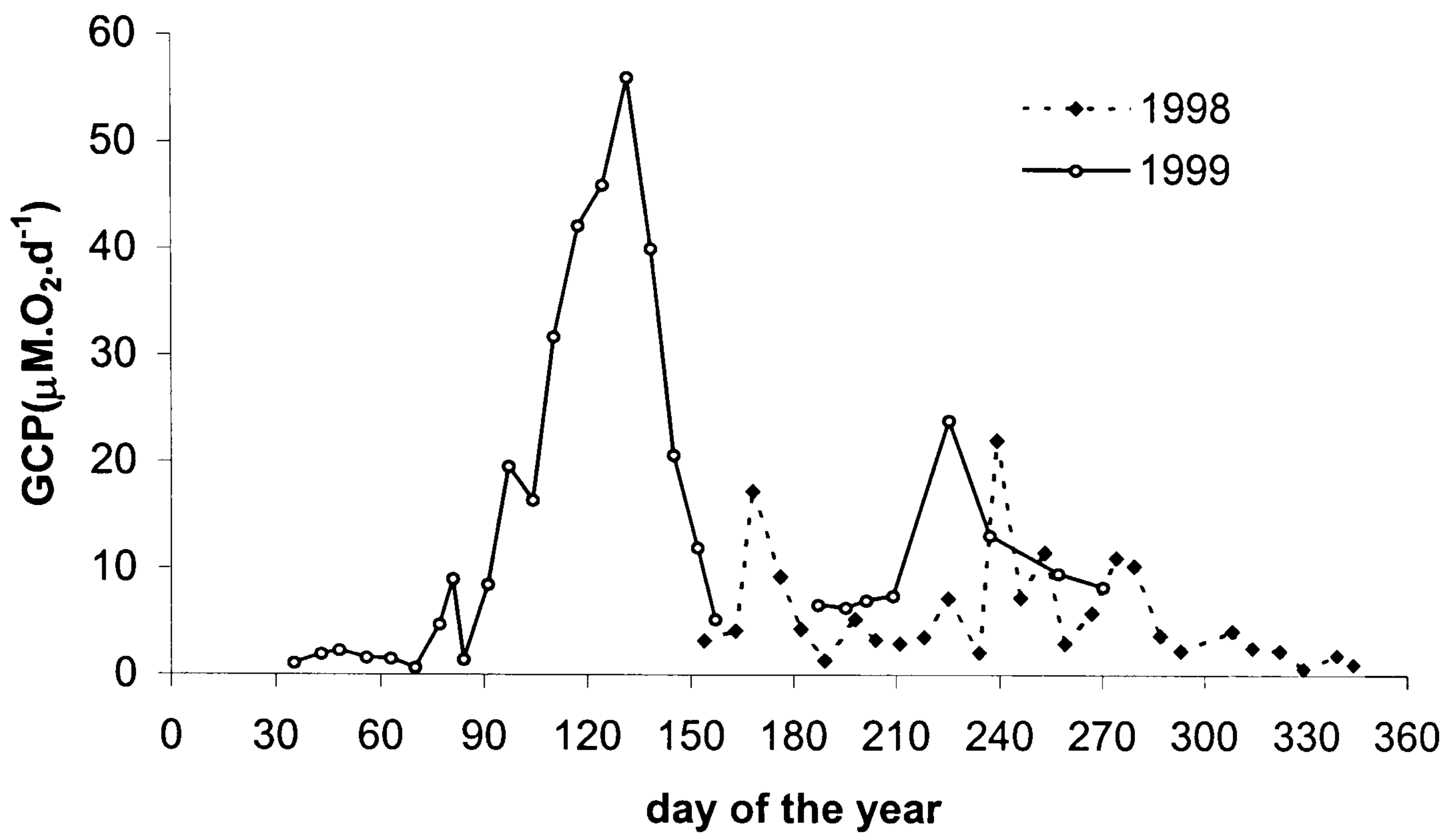


Figure 4.13b. Seasonal variation of gross production in the Menai Strait water during 1998 and 1999.

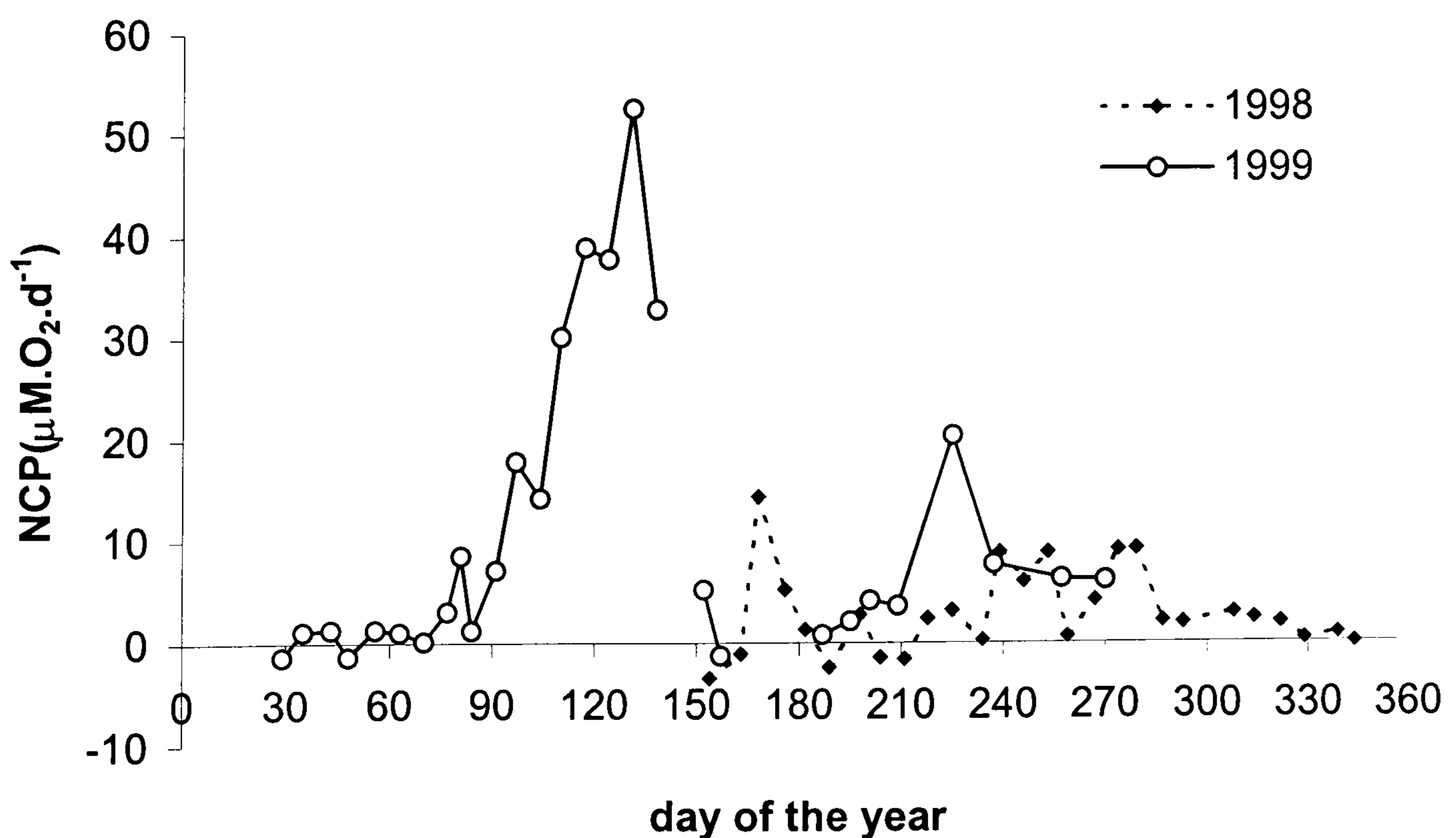


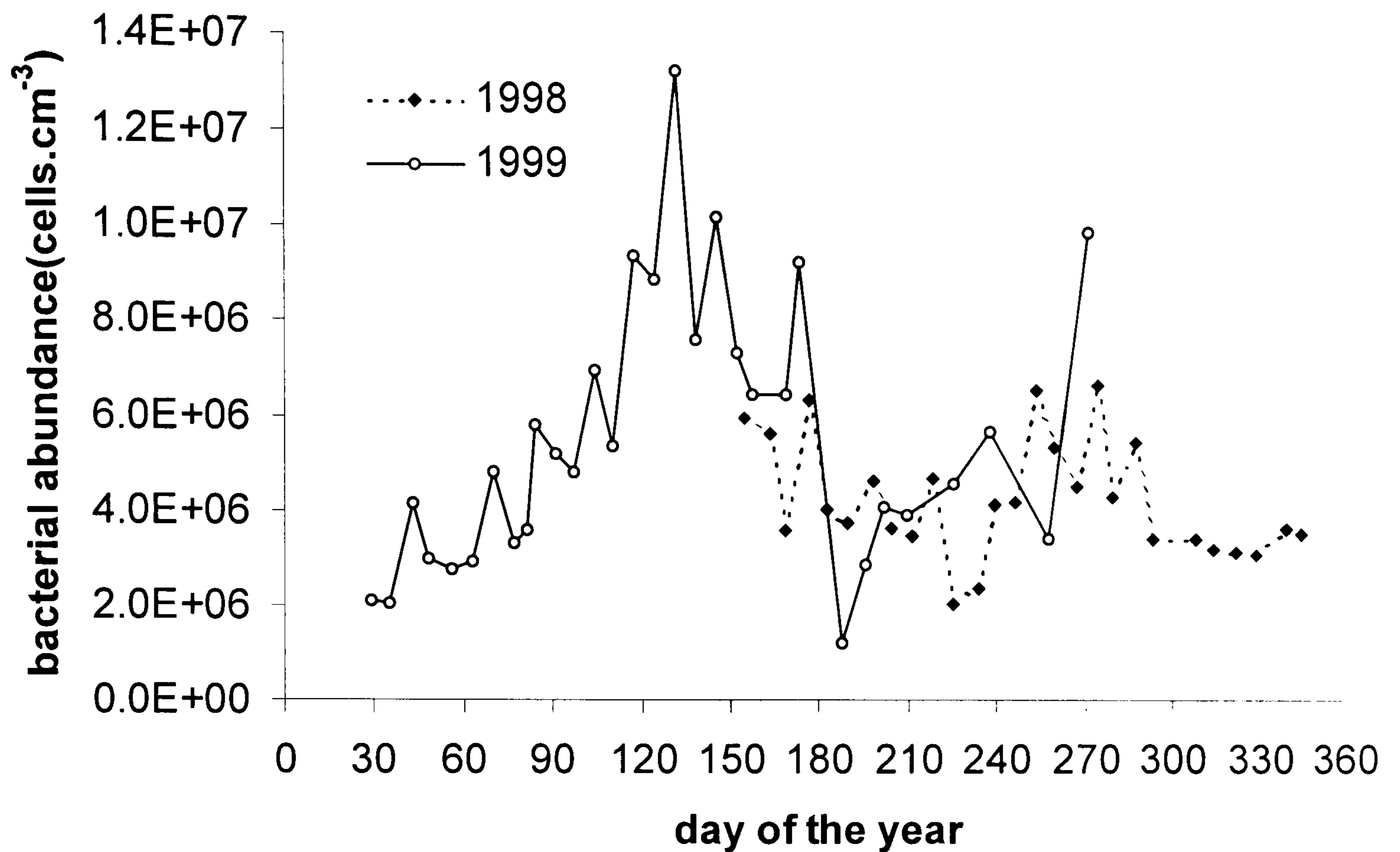
Figure 4.13c. Seasonal variation of net production in the Menai Strait water during 1998 and 1999.

Net community production followed similar patterns as gross community production. Two peaks on days 168 ( $14.4\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ) and 279 ( $9.2\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ). Negative rates of net community production occurred at the beginning of summer (days 154 and 163). During 1999, gross and net community production exhibited low rates from January (day 29) to March (day 89). This was followed by a period of sharp increase from day 91 to 131 leading to maximum rates of gross and net community production ( $56$  and  $53\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$  respectively). There followed a period of decline in gross and net community production especially from day 145. Summer was characterised by a sharp decline of gross and net production with rates as low as  $5\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$  (day 152) and negative rates of net community production ( $-1\mu\text{M O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ ) on day 157.

## 4.7. Bacterial Abundance

The seasonal variation of bacterial abundance in 1998 and 1999 (Figure. 4.14) exhibited short-term fluctuations. Despite this a seasonal trend was observed, featuring high abundance in spring and summer and low abundance in winter. Two peaks occurred in 1998, one in June ( $6.38 \times 10^6 \text{ cells.cm}^{-3}$ ) on day 176 and another ( $6.67 \times 10^6 \text{ cells.cm}^{-3}$ ) on day 274. Both peaks coincided with low net community production and low chlorophyll. Bacterial abundance varied during summer, with the lowest abundance during this period ( $2.07 \times 10^6 \text{ cells.cm}^{-3}$ ) occurred in August (day 225). This was followed by lowest abundance during winter.

In 1999, despite low chlorophyll and net community production in early spring, a sudden increase in bacterial abundance occurred from day 63 reaching the maximum abundance ( $1.3 \times 10^7 \text{ cells.cm}^{-3}$ ) on day 131. This coincided with high chlorophyll and maximum net community production.



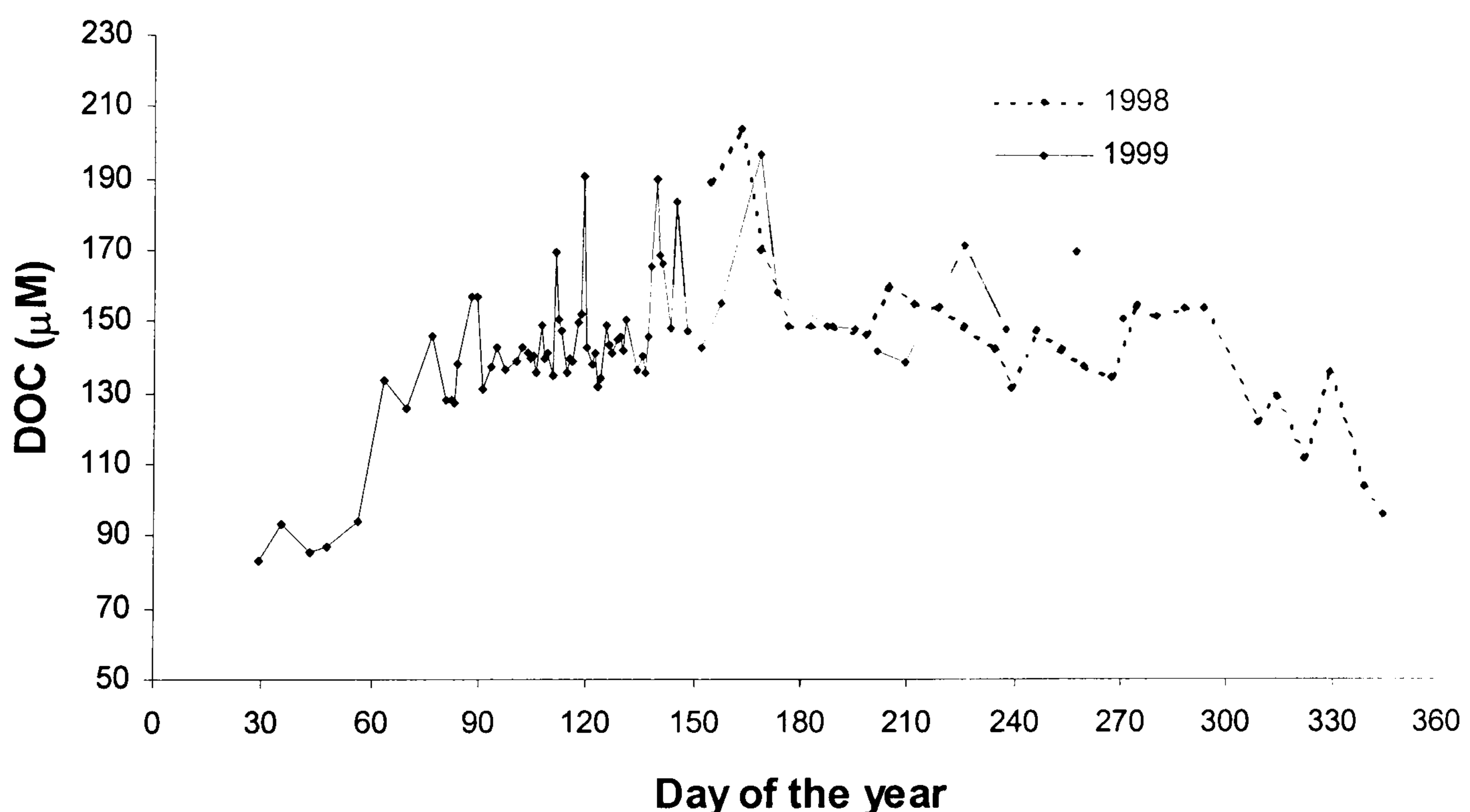
**Figure 4.14. Seasonal variation of bacterial abundance in the Menai Strait water during 1998 and 1999.**

Nutrient reached their lowest level after the second highest abundance ( $1.0 \times 10^7$  cells.cm<sup>-3</sup>; day 145) which coincided with maximum chlorophyll and phytoplankton abundance. The magnitudes of the bacteria peaks in 1998 were small compared to those in 1999. This could be as result of the high primary production in 1999 that enhanced bacterial growth and abundance

## 4.8. Dissolved Organic Matter (DOM)

### 4.8.1 Dissolved Organic Carbon (DOC)

A similar annual pattern of dissolved organic carbon (DOC) (Figure 4.15a) was found for both years 1998 and 1999. Despite the fluctuation, highest concentrations occurred during the major phytoplankton bloom, followed by fairly constant concentration during summer and an increase during autumn. Lowest concentration occurred during winter.



**Figure 4.15a. Seasonal variation of dissolved organic carbon in the Menai Strait water during 1998 and 1999.**

Highest concentration in 1998 ( $205\mu\text{M}$ ) occurred during the second week of June (day 163). This coincided with negative net community production, but with high chlorophyll and phytoplankton abundance. In addition, DOC during summer declined (concentrations between  $132$  and  $205\mu\text{M}$ ) followed by a slight increase in autumn ( $156$ ,  $152$  and  $155\mu\text{M}$ ; day 274, 279, 287 and 293 respectively) and a decrease towards the lowest concentration ( $97\mu\text{M}$ ) in December (day 344).

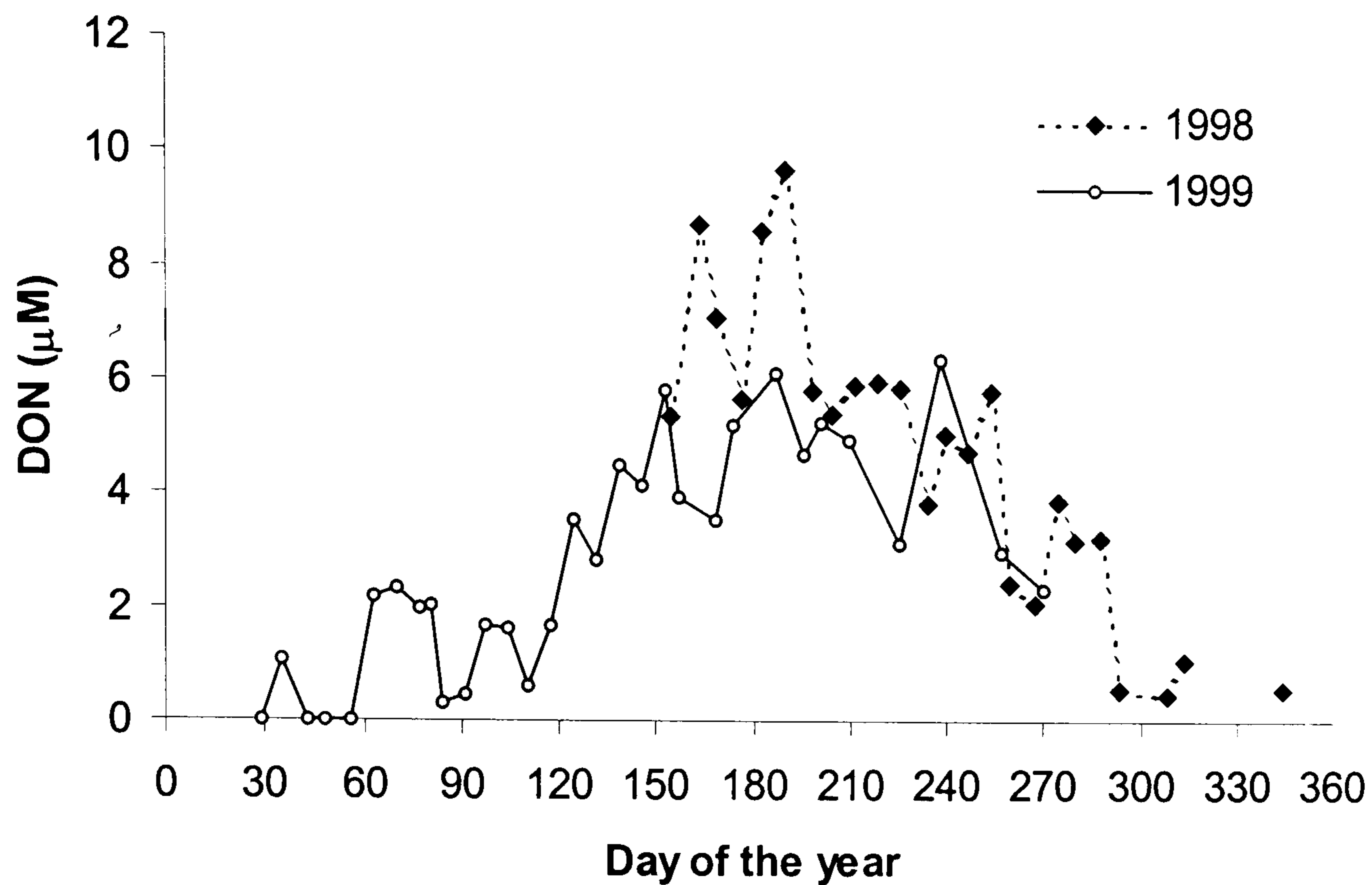


In 1999, the DOC concentration was characterised by low but constant concentration from day 29 to 56 (concentrations ranging between 83 to 94 $\mu$ M). This period was characterised by low bacterial and phytoplankton abundance. In addition the negative community production was at its lowest (negative rates; day 20 and 48). A sudden increase occurred from day 63 towards the major DOC peak (198 $\mu$ M) during the third week of June (day 168). This occurred 23 days later than the major peaks of chlorophyll/phytoplankton abundance and second highest concentration of yellow substance.

DOC concentration declined following the major peak during summer with slight increase on day 225 (172 $\mu$ M) and 257 (171 $\mu$ M). The increase on day 225 coincided with the increase in chlorophyll *a* and net community production, while no increase in chlorophyll *a* or production was evident on day 257. DOC concentrations in both years were fairly within the same range.

#### **4.8.2. Dissolved Organic Nitrogen (DON)**

Dissolved organic nitrogen (DON) (Figure 4.15.b) showed a similar pattern to that of DOC. Higher concentrations were encountered in the period May-June and lower concentrations during winter. In 1998, the concentration of DON ranged from below detection to 9.72 $\mu$ M on day 189. Concentrations varied around 6.0 $\mu$ M during summer but were followed by a decline during autumn and winter. Low concentrations persisted in early 1999, followed by an increase during spring. The maximum DON concentration (6.4 $\mu$ M) occurred in August (day 237). The other two high concentrations (5.86 and 6.14 $\mu$ M) occurred on days 152 and 187 respectively. The DOC&DON sequence i.e. the increase of DOC followed by DON or vice versa, was not clear in this study due to great fluctuation of the two parameters.



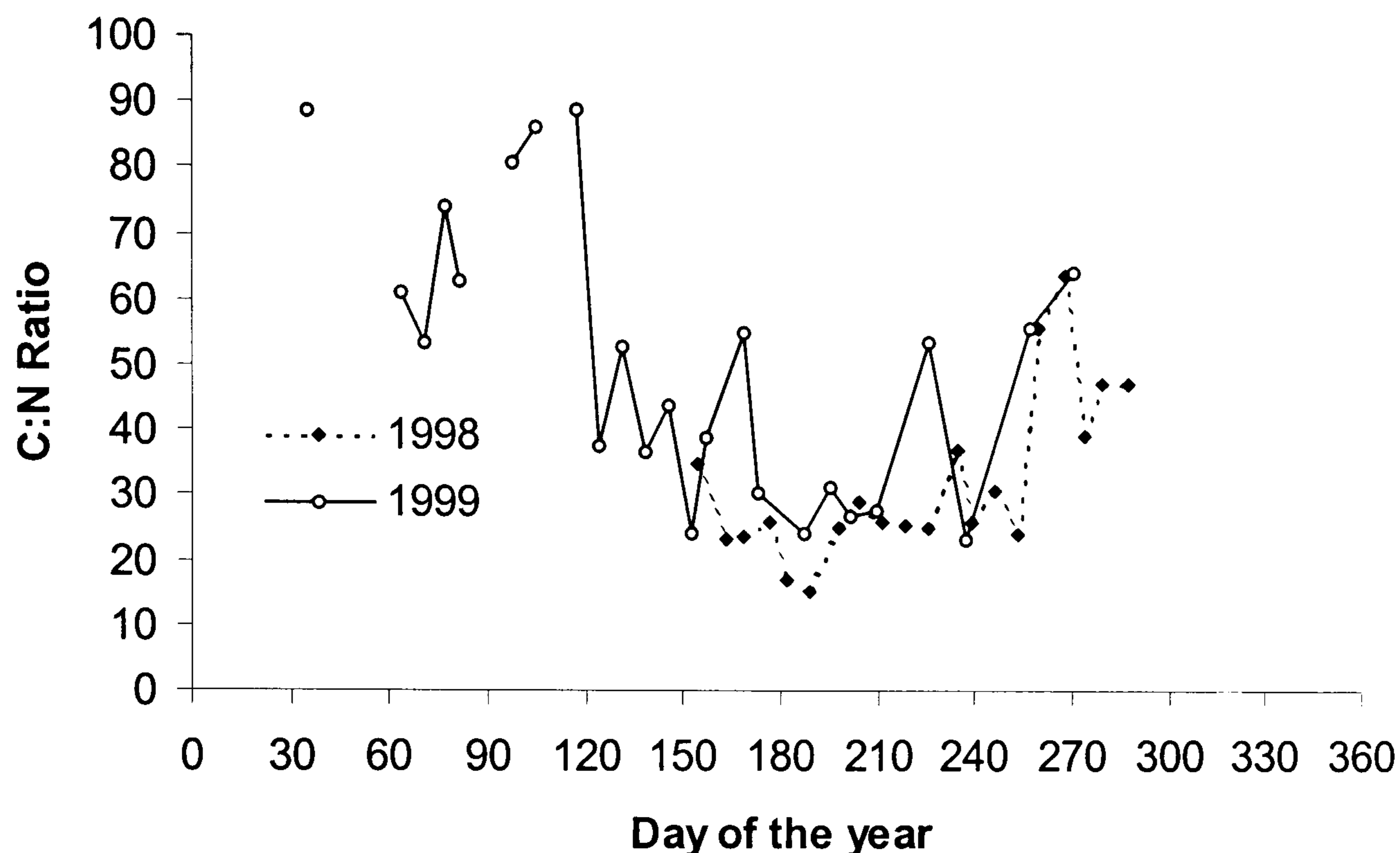
**Figure 4.15b. Seasonal variation of dissolved organic nitrogen in the Menai Strait water during 1998 and 1999. Measurement of DON were on weekly basis.**

### 4.8.3. DOC:DON Ratios

The seasonal variation of C:N of the dissolved organic matter in 1998 and 1999 (Figure 4.15.c) exhibited high ratios. In 1998, the C:N ratio showed great fluctuation with values ranging between 17 and 35 during summer, followed by the increase in ratio during autumn. The maximum ratio (64) occurred in September (day 267). This ratio coincided with low DON concentration. During autumn and winter high C:N ratios reflected very low DON concentrations.

During 1999, high ratios coincided with the period of low nitrate and high DON concentration. The seasonal C:N ratio in this study showed high values which could have resulted from low DON concentration due to metabolic processes. The high ratios of the DOC and DON could be interpreted as the result of the non-proportional increase in DOC relative to DON especially during winter where DON was at its lowest concentration (below detection). Nevertheless, a seasonal pattern could be

distinguished, low ratio during summer perhaps due to nitrogen depletion and increase in DON and high ratio during autumn and winter due to low DON.

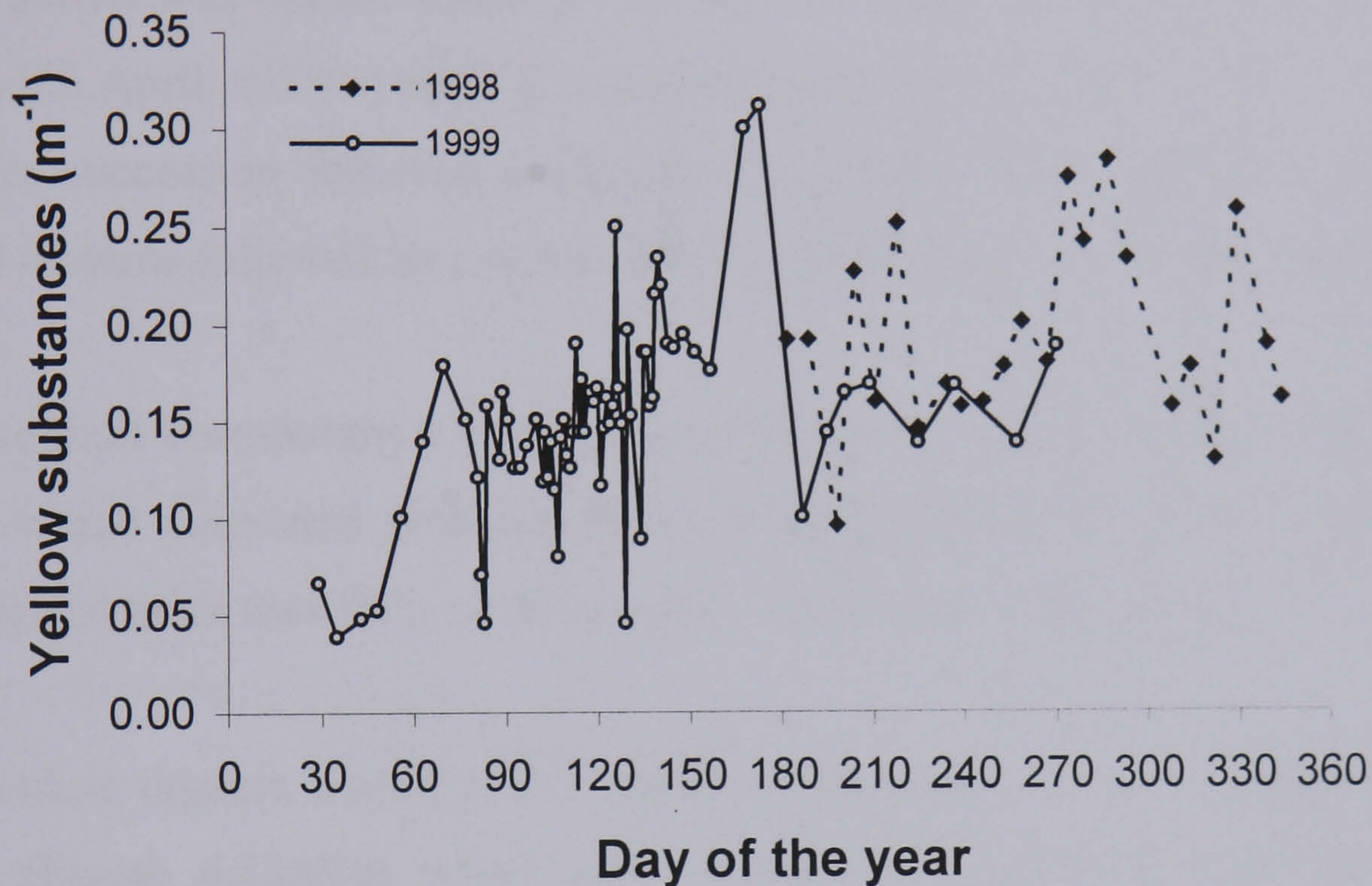


**Figure 4.15c. Seasonal variation of C:N ratio of dissolved organic matter in the Menai Strait water during 1998 and 1999. High ratios are clear during winter and decrease during spring and summer.**

#### 4.8.4. Yellow Substances ( $g_{440}$ )

The seasonal variation of yellow substance in 1998 and 1999 (Figure 4.16) had highest values during summer and autumn, declining towards lowest values during winter. The annual variation in 1998 ranged between  $0.10$  and  $0.29m^{-1}$ . The maximum value occurred in October (day 287) and minimum ( $0.10 m^{-1}$ ) in July (day 198). There was no clear direct link between yellow substance and chlorophyll *a* or with DOC. Despite short time fluctuation, there was a period of low values (day 29 to 48) followed by a sharp increase reaching a value of  $0.18m^{-1}$  (day 70). During this period, chlorophyll/phytoplankton and bacterial abundance were low. This was followed by a period of fairly high concentration (ranging between  $0.10$  and  $0.25 m^{-1}$ ). The major peak ( $0.30 m^{-1}$ ) occurred on days 168 and 173 coinciding with the major peak of DOC (day 168). Lowest values followed the major peak and followed

by a slight increase in autumn. The mean values of yellow substance for July, August and September 1998 (0.17, 0.18 and 0.26  $\text{m}^{-1}$  respectively) and 1999 (0.15, 0.16, and 0.17  $\text{m}^{-1}$  respectively) were within the same range. I therefore assume that the major peak of yellow substance in 1998 may have occurred during the major peak of DOC or a week after as in 1999, however there is no proof for that.



**Figure 4.16. Seasonal variation of yellow substance in the Menai Strait water during 1998 and 1999.**

# CHAPTER V

## CHARACTERISATION OF THE PHYTOPLANKTON BLOOM

### 5.1. Summary

This period was characterised by the season's main phytoplankton growth, which started in April and increased to a maximum by the end of May 1999 (day 145). The species succession followed a pattern which was characterised by a dominance of small diatoms followed by a combination of large diatoms and flagellates in May.

Chlorophyll concentration gradually reached a maximum in May (day 145; Figure 4.8), which coincided with maximum phytoplankton abundance. *Phaeocystis sp.* comprised more than 90% of the species composition of the peak.

Particulate organic matter (POC and PON) increased to a maximum in May (day 157) (Figure 4.12a&b) which also coincided with the time of chlorophyll and phytoplankton maxima. The C:N ratio fluctuated, decreasing until mid May (day 120) and reaching the maximum at the beginning of June (day 143).

Gross and net community production increased following the development of the phytoplankton bloom with the maximum gross and net production (day 131) occurring one week before the chlorophyll and phytoplankton abundance maxima. A respiration peak occurred in May one week before the bacterial maximum (day 124).

The predominant form of inorganic nitrogen taken up by the whole community was ammonium uptake in early spring (March day 81). This coincided with the dominance of small diatoms species. Nitrate uptake increased in mid April (day 111) with a decrease in ambient ammonium and increase in phytoplankton abundance. Nitrite uptake fluctuated with maximum uptake occurring in mid May (day 126) following the decline of nitrate uptake. Phosphate followed more or less the same pattern as nitrate uptake.

## 5.2. Discussion

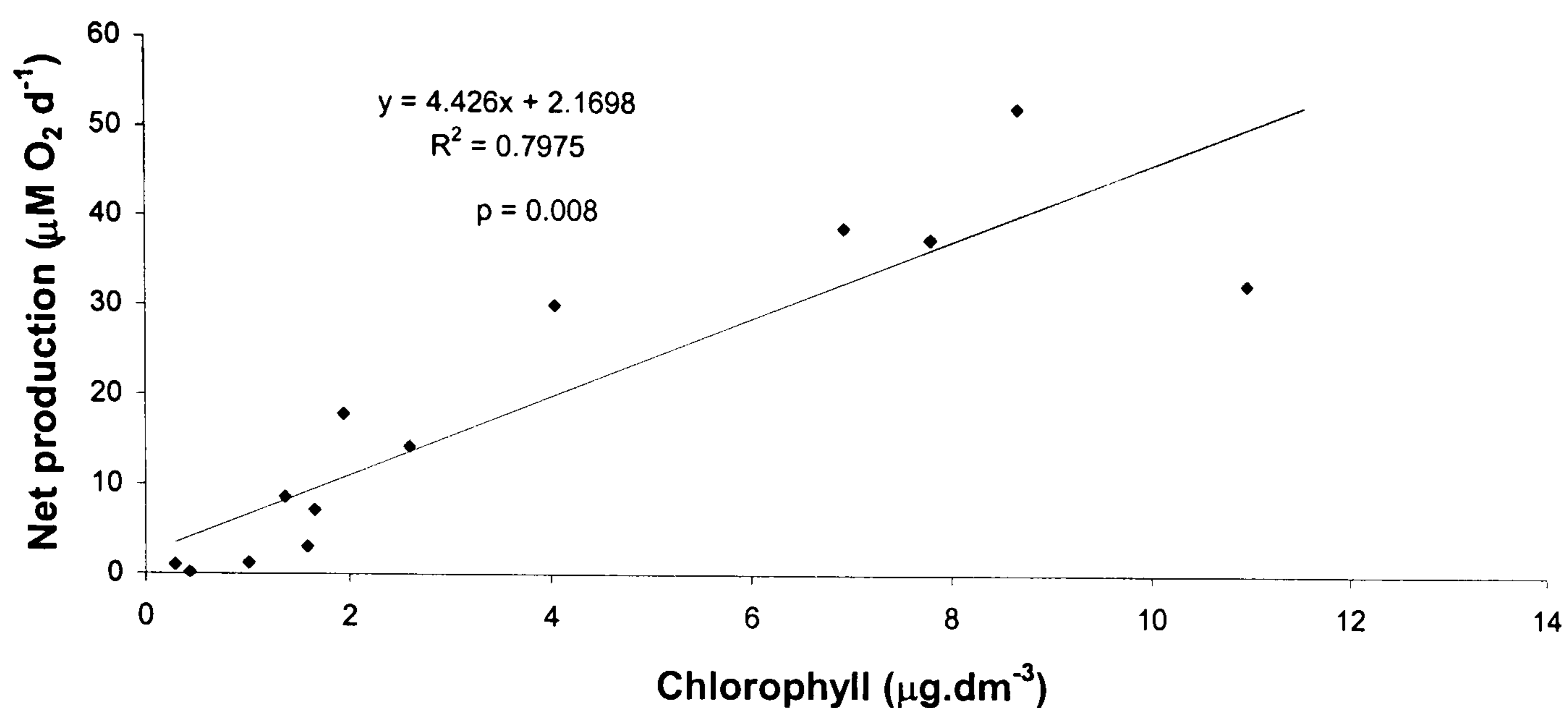
### 5.2.1. Primary production, respiration and bacteria

The general pattern of gross (GCP) and net community production (NCP) during spring showed exponential growth with an increase in phytoplankton abundance. NCP was significantly correlated with chlorophyll *a* and bacterial abundance (Figure 5.1a&b). This indicates that during spring, phytoplankton production is a vital source of organic matter required for bacterial growth.

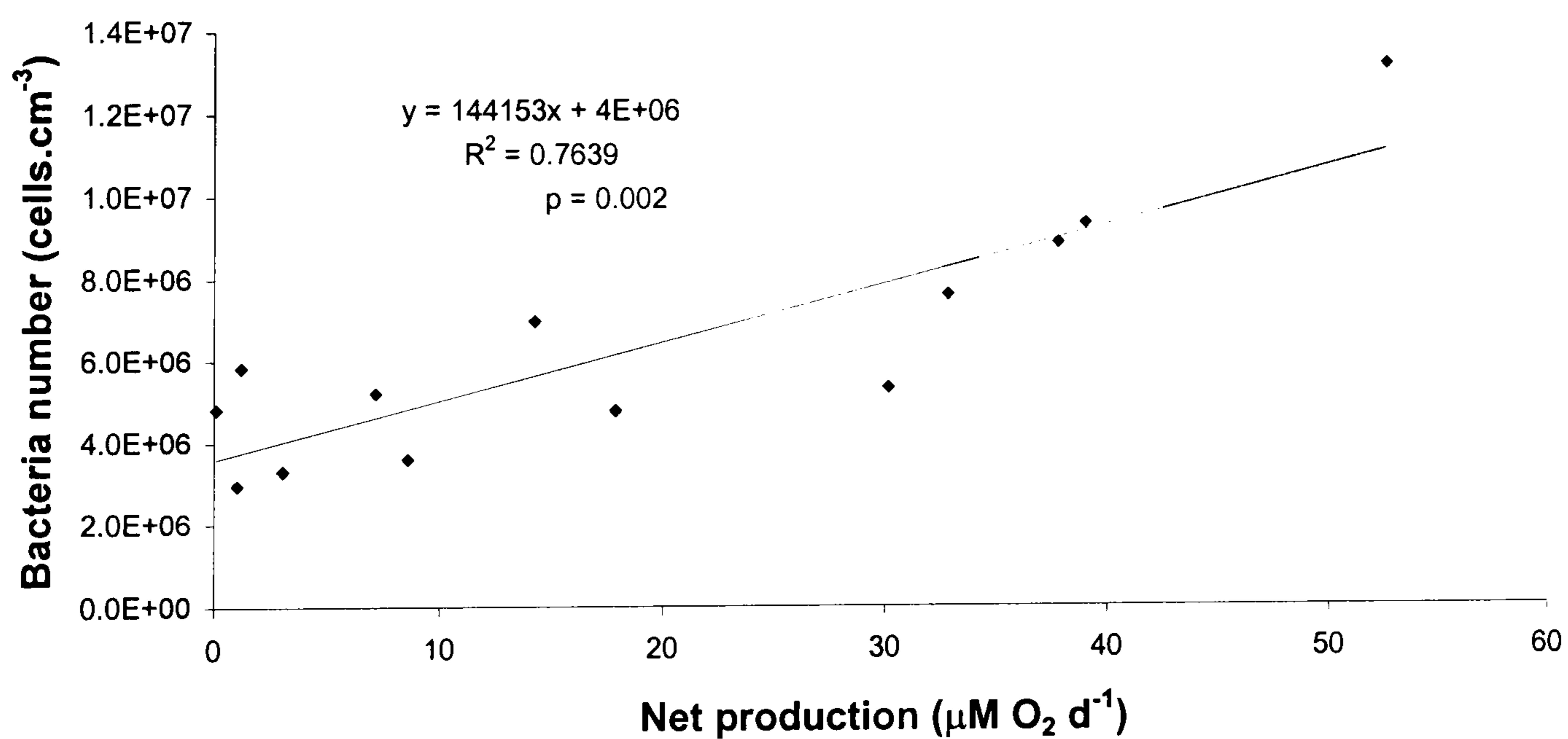
Even though bacteria production and growth most often seem to be resource limited, specific growth rates generally appear to be density independent (Hanson et al., 1986). It seems that bacteria production is limited by phytoplankton production in many coastal waters, as it is shown through correlation between bacterial production and phytoplankton biomass (Fuhrman & Azam 1980; Cole *et al.* 1988). However it may be sometimes difficult to see or interpret these kinds of correlations. Although bacterial production was not estimated in this study, bacteria abundance could still provide some indication of their role with respect to primary production dynamics.

#### 5.2.1.1. Trends in production and respiration in relation to phytoplankton and bacterial dynamics

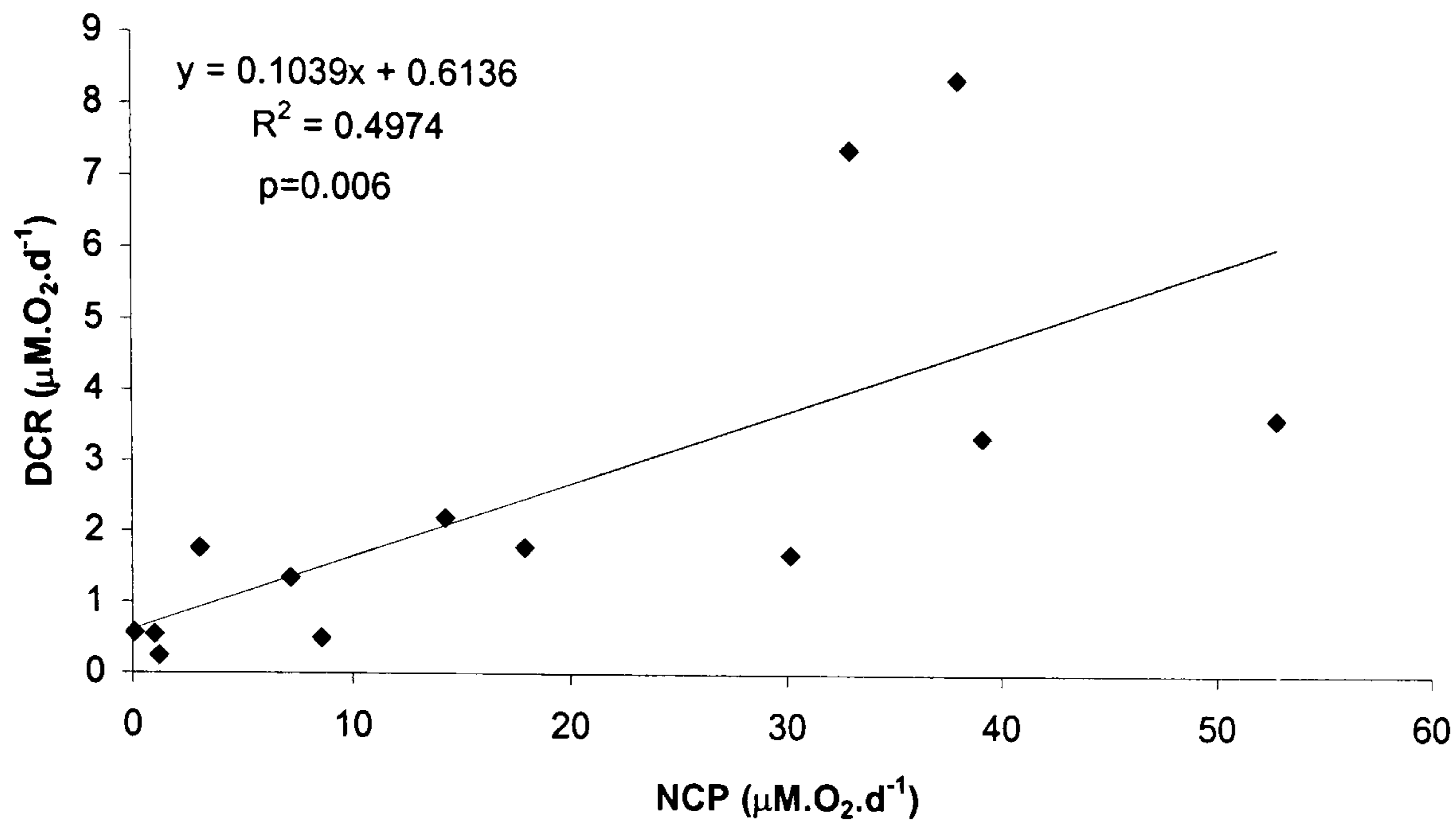
The bacterial response to the intense growth of phytoplankton was clear, however the difference in the time lag between the maxima in phytoplankton biomass and bacterial maxima was not clear in this study. The second bacterial abundance maxima coincided with the maximum phytoplankton abundance and respiration. However the maximum bacterial abundance occurred almost two weeks before the maximum phytoplankton abundance and coincided with the maxima GCP and NCP and low respiration. Furthermore respiration was significantly correlated with NCP (Figure 5.2;  $p < 0.05$ ). The positive y-intercept of the regression was significantly different from zero. This implies that a background respiration rate of about  $0.61 \mu\text{M.O}_2.\text{dm}^{-3}.\text{d}^{-1}$  is not associated with the *in situ* planktonic production.



**Figure 5.1a. Correlation between net community production and chlorophyll *a* during spring 1999.**



**Figure 5.1b. Correlation between net community production and bacterial abundance during spring 1999.**



**Figure 5.2. Correlation between respiration and net community production during spring 1999.**

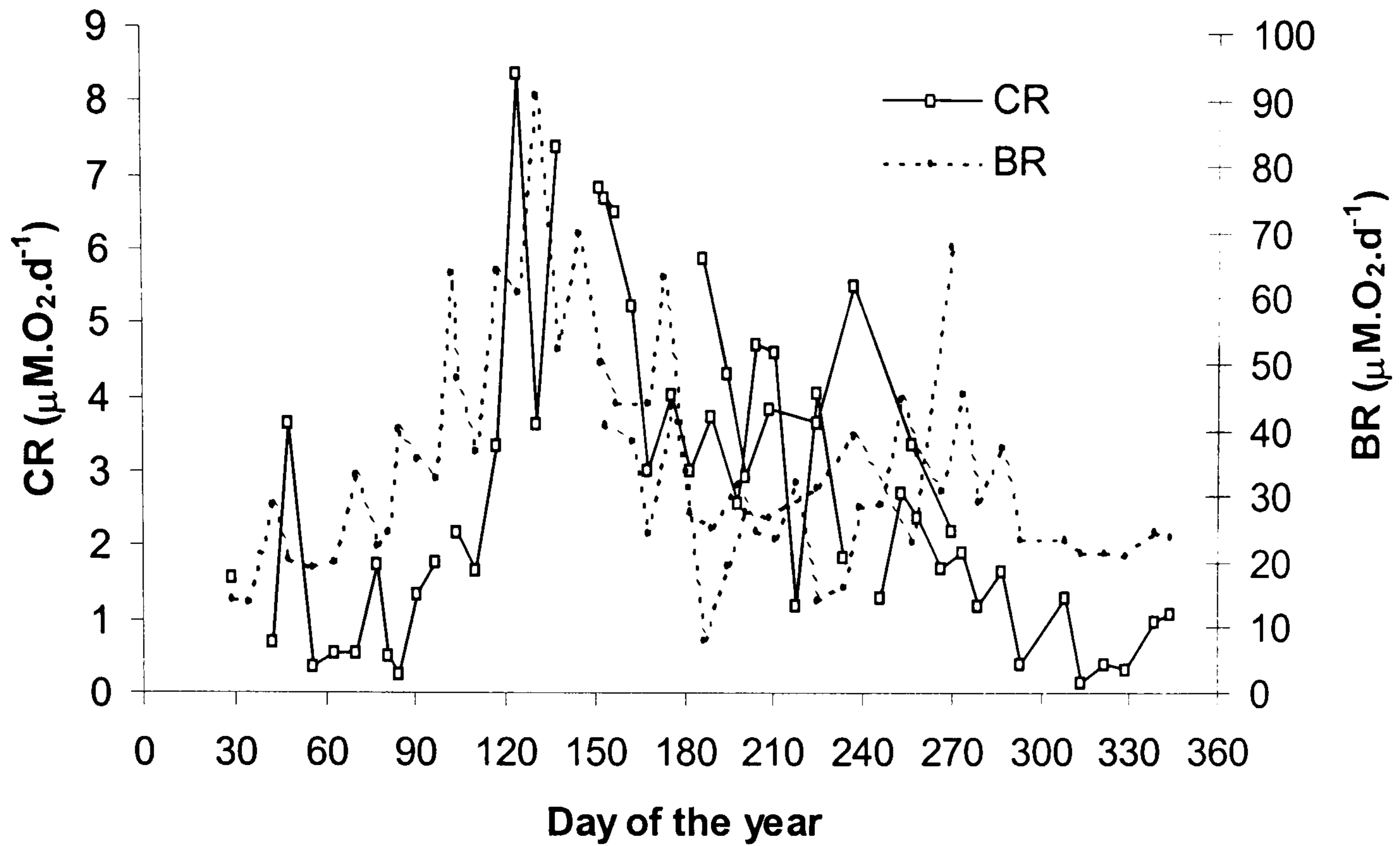
Bacteria are considered to be the major respirers and their metabolism and change in abundance influence the phasing of GCP and DCR (Blight *et al.* 1995). The phasing of GCP and DCR revealed in this study was characterised by positive net production changes to negative production during summer. This pattern was reported in previous studies in Menai Strait (Blight *et al.* 1995; Rodrigues 1998). This pattern was explained as being associated with high rates of respiration that occur during the *Phaeocystis sp.* bloom. Indeed the maximum respiration rate occurred within the period of *Phaeocystis sp.* dominance. This species produces a high molecular pool of organic matter that is difficult for heterotrophic organisms to break down (Thingstad & Billen 1994), causing a delay between GCP and DCR. Thingstad & Billen (1994), suggest that the low biodegradability of *Phaeocystis* material is due to nutrient limitation, such as inorganic nitrogen and phosphate, and low temperature. Inorganic nitrogen and phosphate were at their lowest level during the *Phaeocystis* bloom. Osinga *et al.* (1997), reported that the breakdown time of 50% of the degradable organic matter produced by *Phaeocystis sp.* takes 2 to 3 days, whilst Janse *et al.* (1999) suggest that it could take up to 11 days. The maximum respiration in this study occurred almost two weeks from the maximum GCP. In addition DCR was significantly correlated with NCP indicating a loose coupling between plankton production and respiration (Figure 5.2). However this coupling is



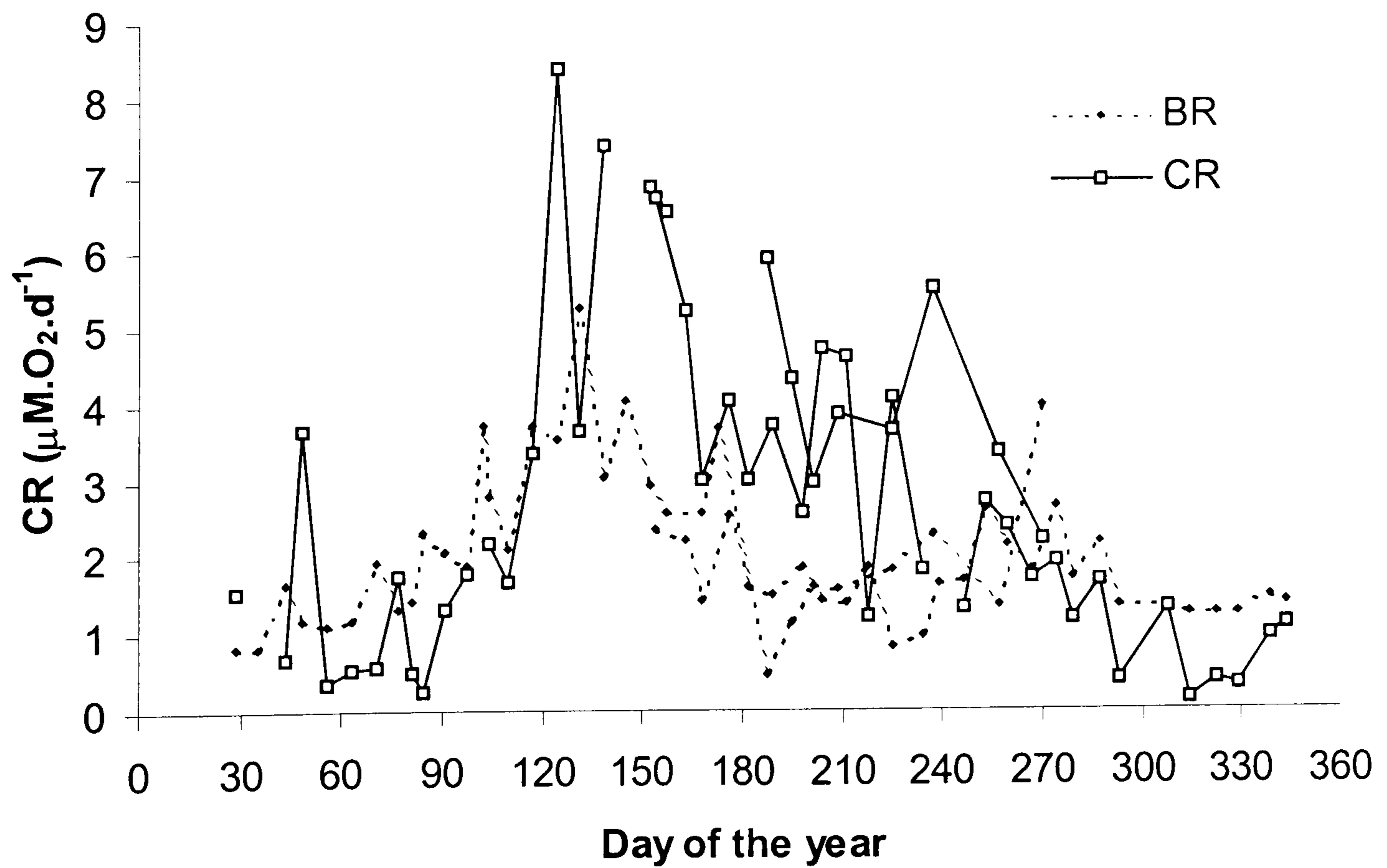
not always the response of either primary production or respiration (Smith & Kemp 1995).

### **5.2.1.2. Relative influence of phytoplankton respiration (PR) and bacterial respiration (BR) on community respiration (CR).**

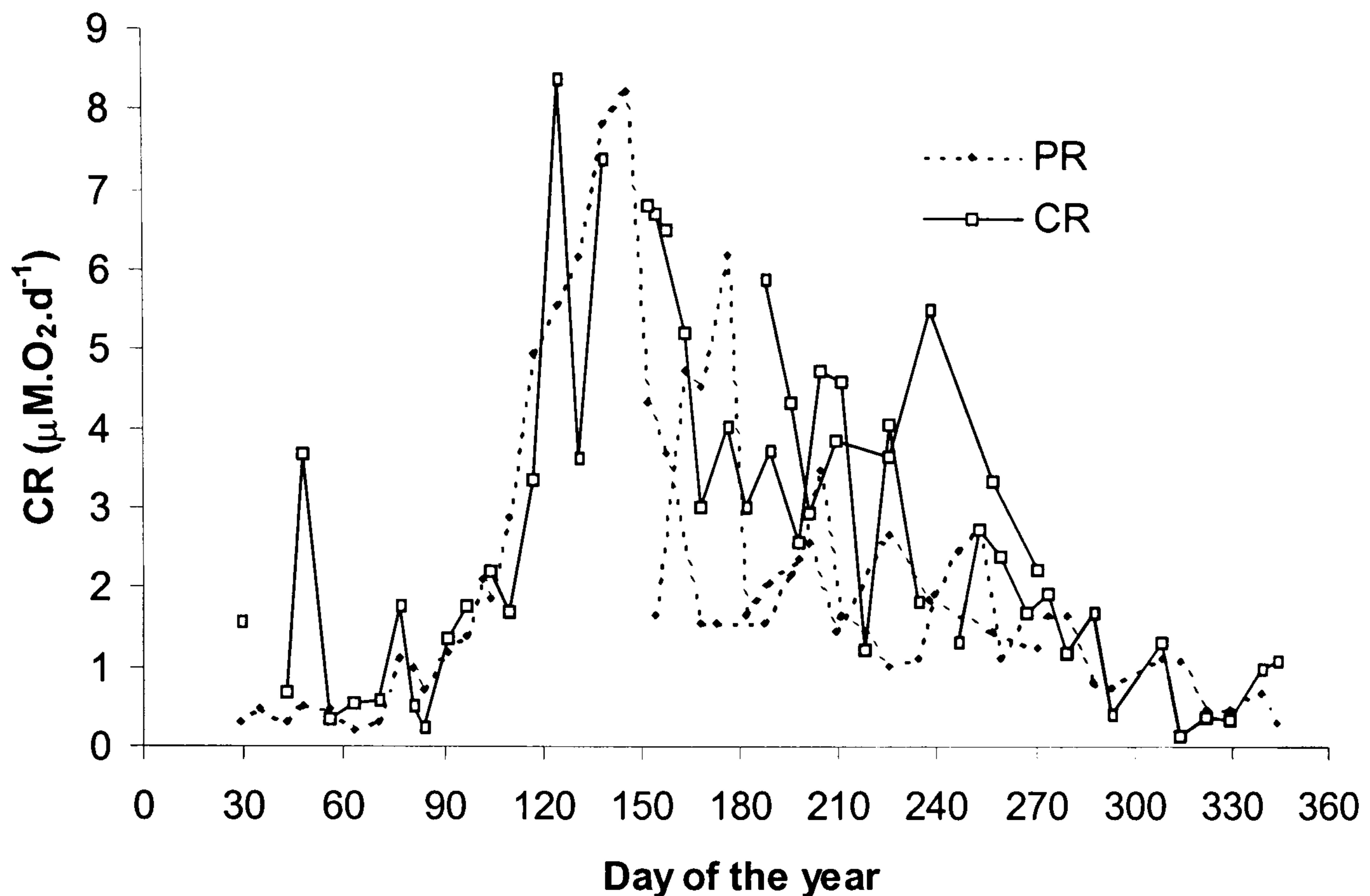
Unlike photosynthesis, respiration is common to all aerobic organisms and occurs at all depths in the ocean (Biddanda & Benner 1994). Among the microorganisms, heterotrophic bacteria are known to be the major consumers of dissolved and particulate organic carbon (Cho and Azam 1988; Simon *et al.* 1992) and dissolved oxygen (Griffith *et al.* 1990). Bacterial respiration is important in overall community respiration because nearly half of marine primary production is estimated to flow through bacteria each day (Cole *et al.* 1988; Williams & Benner 1994). An attempt was made in this study to estimate both bacterial and phytoplankton respiration in relation to community respiration. The contribution of phytoplankton (PR) and bacteria respiration (BR) to community respiration (CR) is shown in Figure (5.3a,b&c). The PR was calculated using the mean chlorophyll specific respiration rate  $0.030 \text{mmole.mg.chl}^{-1}.\text{hr}^{-1}$  (Langdon, 1993) and changes in chlorophyll concentrations. The BR was estimated using the bacterial cell specific rate ( $0.4\text{-}6.8 \text{fmol.O}_2.\text{cell}^{-1}.\text{d}^{-1}$ ) (Blight 1996) and bacterial abundance. While these calculations may not provide absolute values, they give a useful indication of changes in the relative magnitudes of phytoplankton and bacterial respiration. The seasonal variation of PR and BR followed the changes in primary production. Major peaks occurred during the period of the major phytoplankton bloom indicating that the major contribution to community respiration occurred during spring and summer. However the estimation of PR and BR using the above aforementioned techniques could provide overestimated rates of respiration as shown in this study. Nevertheless bacterial respiration using the minimum factor at least showed that the contribution of bacterial respiration to community respiration were within the reported ranges in other studies (>40%). Biddanda and Benner (1994) reported from their studies in the Louisiana shelf that bacterial respiration was about 49% of community respiration. Other workers (Griffith *et al.* 1990; Chin-Leo and Benner 1992) reported that the overall contribution of plankton community respiration usually exceeds 40% in



**Figure 5.3a. Seasonal variation of bacterial respiration (BR) in relation to total community respiration (CR) in the Menai Strait during 1998&1999. Using converting factor for BR ( $6.8 \text{ fmol.O}_2\text{.cell}^{-1}\text{.d}^{-1}$ ).**



**Figure 5.3b. Seasonal variation of BR in relation to CR in the Menai Strait during 1998&1999. Using converting factor for BR ( $0.4 \text{ fmol.O}_2\text{.cell}^{-1}\text{.d}^{-1}$ ).**



**Figure 5.3c. Seasonal variation of phytoplankton respiration (PR) in relation to total community respiration (CR) in the Menai Strait during 1998&1999.**

different marine environments. This is an indication that bacteria contribute to a large extent to the flow of carbon through utilisation of primary production, especially during the phytoplankton bloom.

### **5.2.1.3. DOM dynamics in relation to phytoplankton and bacterial activity.**

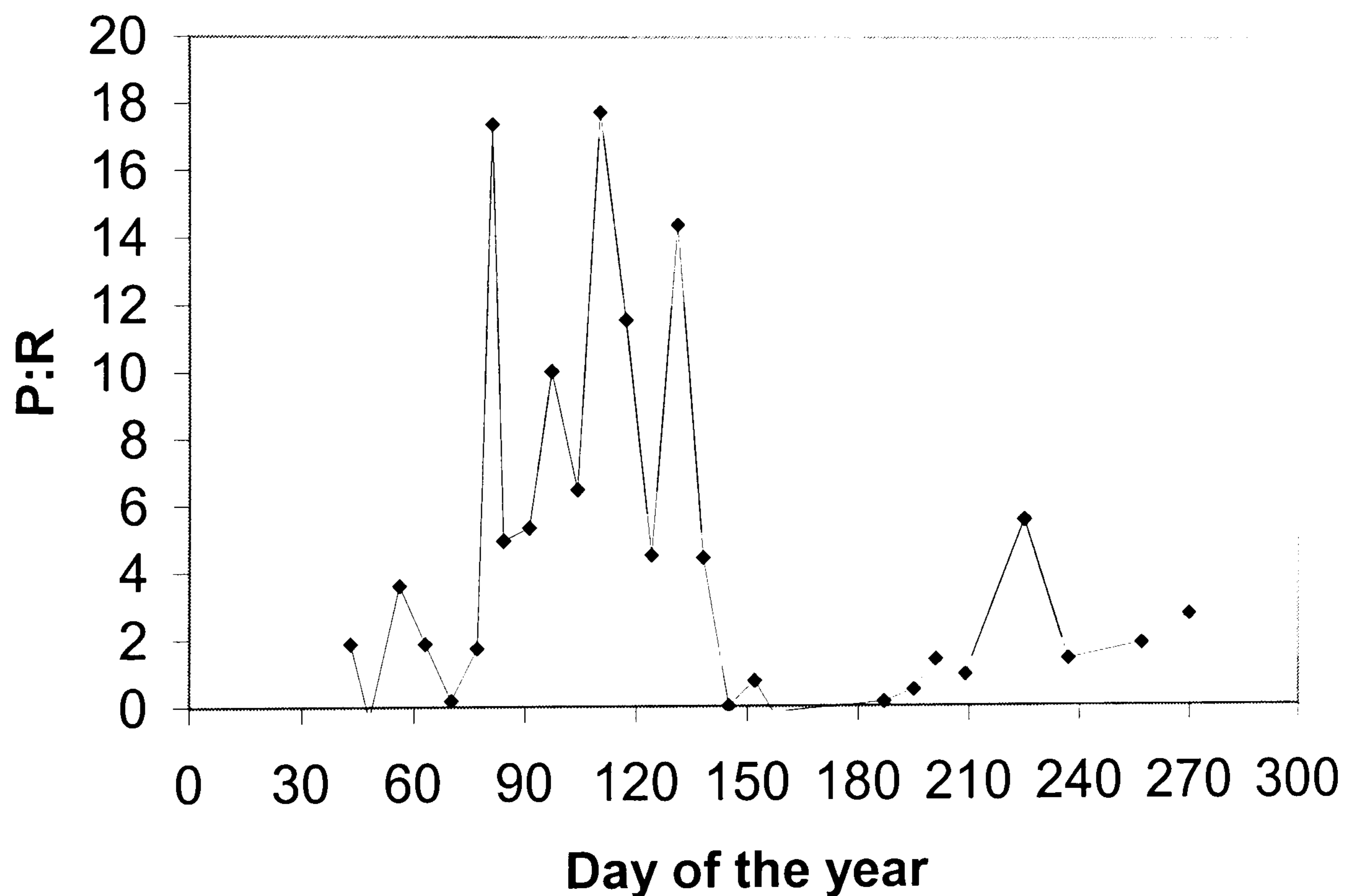
The amount and type of organic matter from phytoplankton production can limit the respiration rate. This could be because the organic matter supply from phytoplankton to bacteria is not sufficient depending on the rate of primary production. Consequently autotrophic production can depend on nutrients regenerated by heterotrophic organisms, as was the case in the Menai Strait during summer. In addition, the amount of organic matter can depend on the dominance of species. All these relationships make the relation between production and respiration difficult to explain as cause and effect.

For example Blight *et al.* (1995) hypothesised that the increase in bacterial abundance during spring varied according to primary production. During the period when small diatoms dominated (March and April), the amount of organic matter from primary production passed to bacteria was insufficient for high bacterial growth. This was clear in current study. In early spring (days 63 to 120), the species composition comprised of small diatoms and primary production exhibited a slight increase whilst respiration remained fairly low. This was followed by an increase in the amount of organic matter produced by large diatoms (*Rhizosolenia delicatula*). Consequently, it can be assumed that the organic matter produced during this period was assimilated quickly by bacteria therefore increasing the bacterial abundance. During the *Phaeocystis* bloom, more organic matter was probably passed to bacteria thus increasing their abundance to the maximum. However a time lag between primary production and respiration occurred indicating a loose coupling between the two processes. As explained earlier, this may have been due to the refractory nature of the organic matter produced by *Phaeocystis sp.*

A sudden increase in primary production coincided with dominance of *Skeletonema costatum* whilst respiration was still low. During this period bacterial abundance and respiration were low. This could be an indication that there are factors affecting bacterial growth such as the amount of organic matter passed to bacteria and the complex type of organic matter produced (dominated by *Skeletonema costatum*) (Sanders & Purdie 1998) which requires more time to be assimilated by bacteria. In accordance with Blight *et al.* (1995) there was a difference between phasing of GCP and DCR for the diatom blooms and that of *Phaeocystis*. During the maximum diatom bloom, there was no obvious DCR maximum. During the major peak (dominated by *Asterionella sp.*, *Peridinium sp.*, *Nitzschia sp.*, and *Phaeocystis sp.*) there was an increase in primary production and respiration. This reached a maximum on day 145. This indicates that both bacteria and phytoplankton contributed significantly to the total community respiration.

The ratio of net community production and respiration (P:R) can also be used as an index in tracking the relative importance of autotrophy and heterotrophy within the aquatic ecosystems (Odum 1956). In this study the plankton community metabolism was distinctly autotrophic in nature during the springtime period. P:R ratios reached

a maximum of almost 18 and possibly reflecting the uncoupled nature of production and respiration during this period (Figure 5.4). This ratio decreased during late spring and summer as a result of respiration increase. This pattern has been reported in several studies (Kenney *et al.* 1988; Hoppema 1991; Kemp *et al.* 1992). This could be an indication that during spring in temperate regions the input of nutrient is vital to stimulate primary production while respiration rates are controlled by water temperature, whilst in summer both production and respiration rates are enhanced by rapid recycling of organic matter and high temperature resulting in low P:R.



**Figure 5.4.** Seasonal variation of the ratio of primary production and community respiration (P:R) in the Menai Strait in 1999.

### 5.2.2. Nitrogen sources supporting primary production

A more complete understanding of how phytoplankton and bacteria compete for nutrients, which nutrients may be limiting and how nutrient availability, either through physical transport processes or biologically regenerations processes, control the growth and primary production rates in marine populations is vital. Various nitrogen species in the sea contribute to biological processes in the sea directly or indirectly. Thus the ambient concentration of inorganic nitrogen represents the balance between rates of supply and consumption.

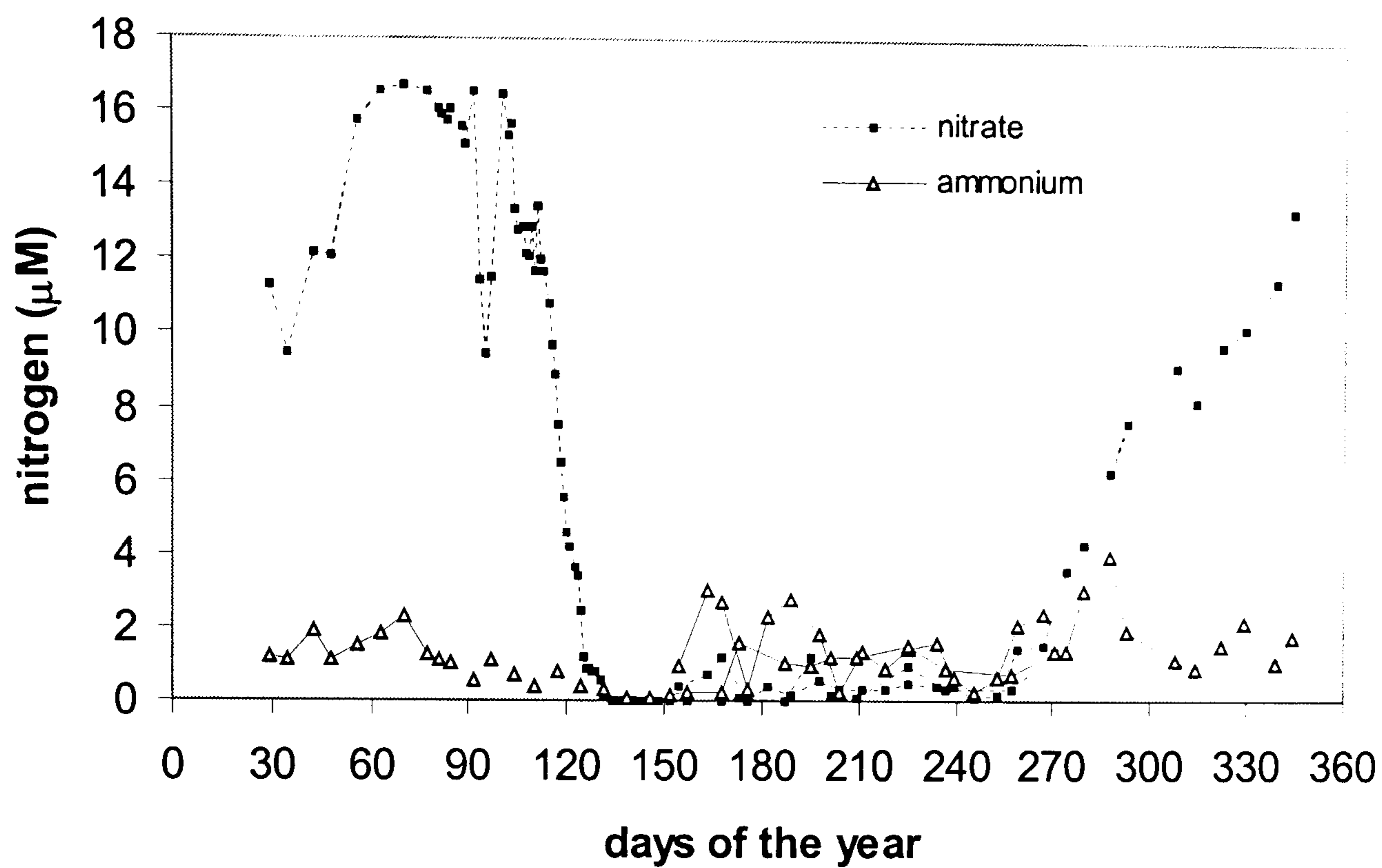
In general it has been reported that the order of preference of phytoplankton for inorganic nitrogen in several offshore, coastal and freshwater environments is ammonium, urea, nitrite, and nitrate (McCarthy *et al.* 1977; Glibert *et al.* 1982; Parson & Parker 1993; Eleskens *et al.* 1997). This preference involves a number of processes reducing the forms of nitrate to nitrite and yielding ammonium as an end product. These reducing reactions require a lot of energy. Therefore algae will take up preferentially those forms of nitrogen already present in the environment in a more reduced state which do not require a lot of energy to be converted. Initially it was believed that little or no nitrate was taken up at high ammonium concentrations ( $>1\mu\text{M}$ ) (Glibert *et al.* 1982). Dortch (1990) showed that there is a variation in the degree to which ammonium affects the nitrate uptake, and the reduction of nitrate in the presence of ammonium is rarely complete.

The mechanisms of supply and utilisation of inorganic nitrogen are believed to play a critical role in determining the productivity, size structure and species succession of phytoplankton communities in marine environment. The seasonal cycle of primary production is accompanied by changes in the rates of utilisation of inorganic nitrogen. The quantification and inhibition of nitrate and ammonium is only possible when investigations on uptake rates of both nutrients alone are conducted without any kind of interference from other (Elskens *et al.* 1997). This is difficult if not impossible to achieve with field samples. McCarthy *et al.* (1977) suggests the use of Relative Preference Index (RPI) to estimate the preference of the phytoplankton population for the inorganic nitrogen forms in relation to their availability.

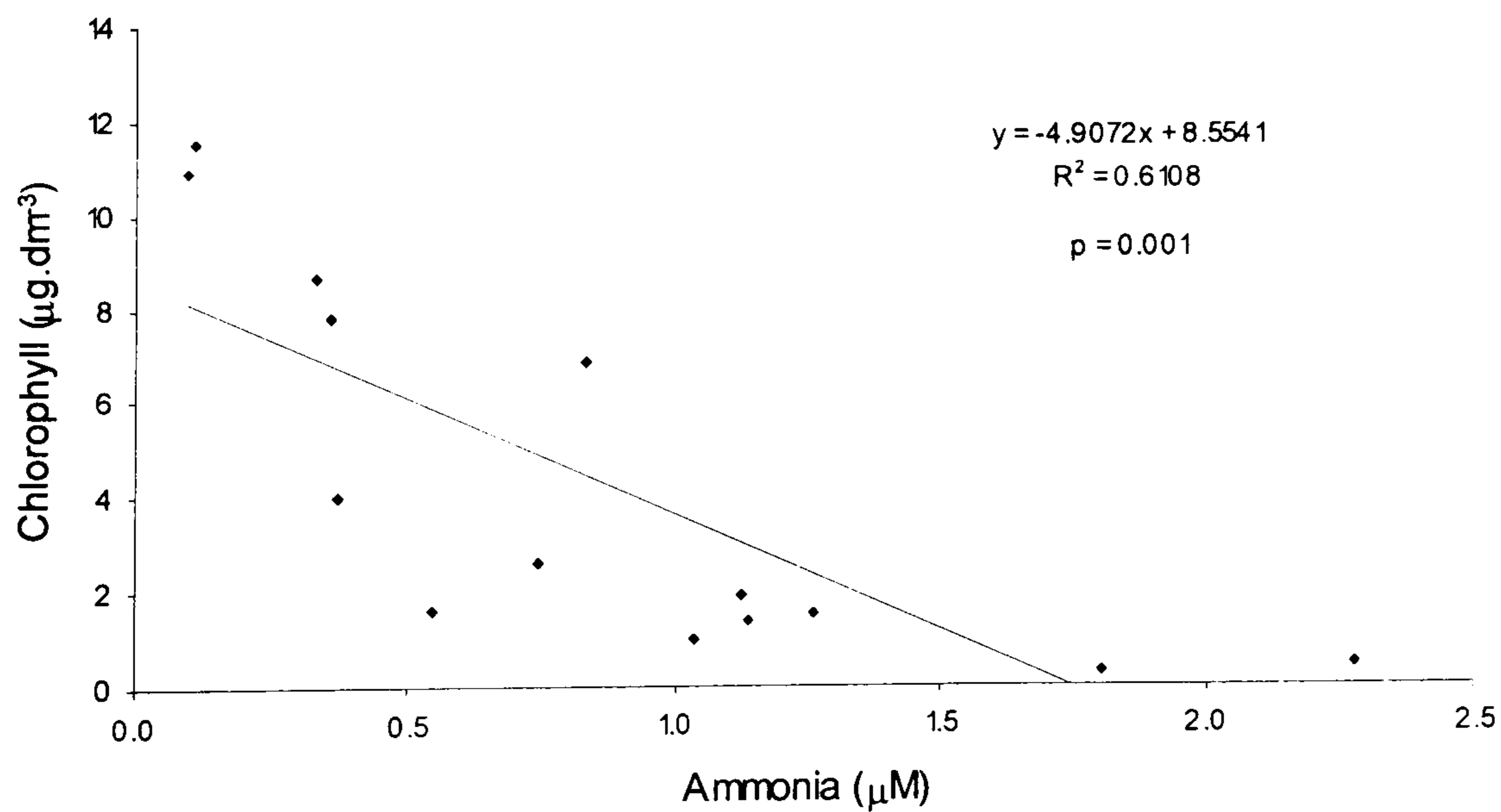
The seasonal variation of ammonium and nitrate RPI were reported in the Menai Strait (Rodrigues 1998). Despite the high concentration of nitrate during spring, phytoplankton showed clear preference for ammonium. This pattern has been observed by a number of studies (Eppley *et al.* 1973; McCarthy *et al.* 1977; Glibert *et al.* 1982).

Due to the limitation of the field and experimental data in this study, it was impossible to calculate the Relative Preference Index (RPI), the *f*-ratio, and the seasonal net uptake of nitrogen by different phytoplankton species. Information such as the uptake rates of ammonium and nitrate in experiments are missing along with information such as the half-saturation constant and other determined constants used to calculate the *f*-ratio for nitrate and ammonium. Nevertheless, the inorganic nutrient uptake in the Menai Strait exhibited differences in the sequence and timing of uptake. This sequence was characterised by early utilisation of ammonium followed by nitrite, nitrate and phosphate. This was indicated by a decrease in ambient ammonium concentration from day 70 to 91 (Figure 5.5). During this period of ammonium decline, no change in nitrate occurred. In addition chlorophyll showed a significant correlation with ammonium but not with nitrate (Figures 5.6). Thus indicating that ammonium was the major source of nitrogen for phytoplankton production in early spring. The utilisation of ammonium during the pre-bloom period has been reported in different environments. Clarke & Leakey (1996) observed the pattern in a near shore Antarctic system. A delay of about two weeks between the uptake of ammonium and that of nitrate was reported in the North Sea (Radach & Lenhart 1995). This agrees with the delay of about three weeks observed in the Menai Strait. This pattern was not registered in recent studies (Blight *et al.* 1995; Rodrigues 1998). This could be explained as a result of ammonium inhibition of nitrate uptake. However more investigation is required to assess the importance of physiological processes, environmental effects and taxonomical differences on the nature of the nitrate-ammonium interaction.

Several studies have reported the inhibitory effect of ammonium on nitrate uptake by phytoplankton (McCarthy *et al.* 1977; Conway 1977; Radach & Lenhart 1995; Clarke & Leakey 1996). However the concentration at which this inhibition takes place appears to vary widely.



**Figure 5.5.** Seasonal changes of nitrate and ammonium in the Menai Strait in 1998&1999. Notice the decrease of ammonium from day 70 and no change in nitrate.



**Figure 5.6.** Correlation between chlorophyll *a* and ammonium in early spring from days 70 to 91.



For example, McCarthy *et al.* (1977) suggest that an ammonium concentration of 0.5 to 1  $\mu\text{M}$  would allow nitrate uptake, while Carpenter & Dunham (1985) suggest a lower range (0.2 to 0.5  $\mu\text{M}$ ). A strong negative correlation between inhibition and chlorophyll concentration was reported (Wheeler & Kokkinakis 1990). This relation indicates that when ammonium concentration is at least 0.5  $\mu\text{M}$ , which inhibits nitrate uptake, the phytoplankton biomass is low. By contrast the low concentration of ammonium (<0.5  $\mu\text{M}$ ) will allow high nitrate uptake and results in high phytoplankton biomass. This explains the impact of nutrient assimilation sequence and changes in phytoplankton biomass in the Menai Strait. The concentrations of ammonia observed in this study suggest that inhibition of nitrate uptake is likely to have occurred especially from days 29 to 91 when ammonia concentrations were  $\sim 1 \mu\text{M}$ . The low concentration of ammonia (<1  $\mu\text{M}$ ) observed from day 91 may have allowed nitrate to become the alternative source of inorganic nitrogen for phytoplankton production.

This is supported by the strong correlation between chlorophyll *a* and nitrate and phytoplankton abundance and nitrate (Figures 5.7a&b) during spring compared with that of ammonium. In addition Rodrigues (1998) reported from the estimation of “*f*-ratios” that about 87% of the primary production recorded in the Menai Strait was nitrate-based. Furthermore, other studies (McCarthy *et al.* 1977; Paasche & Kristiansen 1982; Boyer *et al.* 1994) have shown that ammonium plays a greater role in the annual cycle of primary production than nitrate as a nitrogen source, but that nitrate is only important during spring. Horrigan *et al.* (1990) suggest that nitrite can also be the preferred source of nitrogen. Nitrite showed significant correlation with chlorophyll *a* and phytoplankton abundance (Figures 5.8a&b). However it is available in very low concentrations which makes it difficult to establish its impact on phytoplankton abundance.

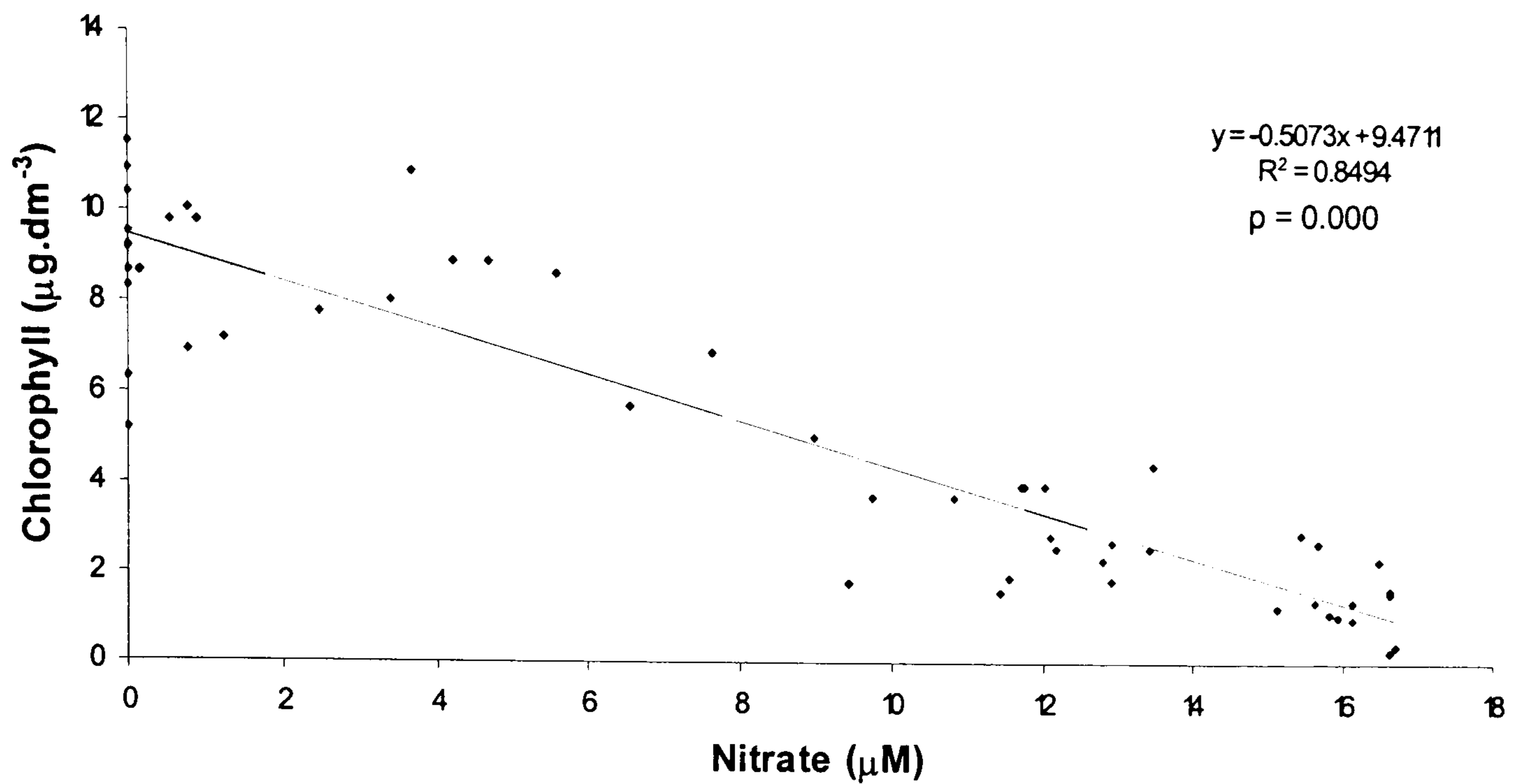


Figure 5.7a. Correlation between chlorophyll *a* and nitrate during spring 1999 (days 63 to 148) in the Menai Strait.

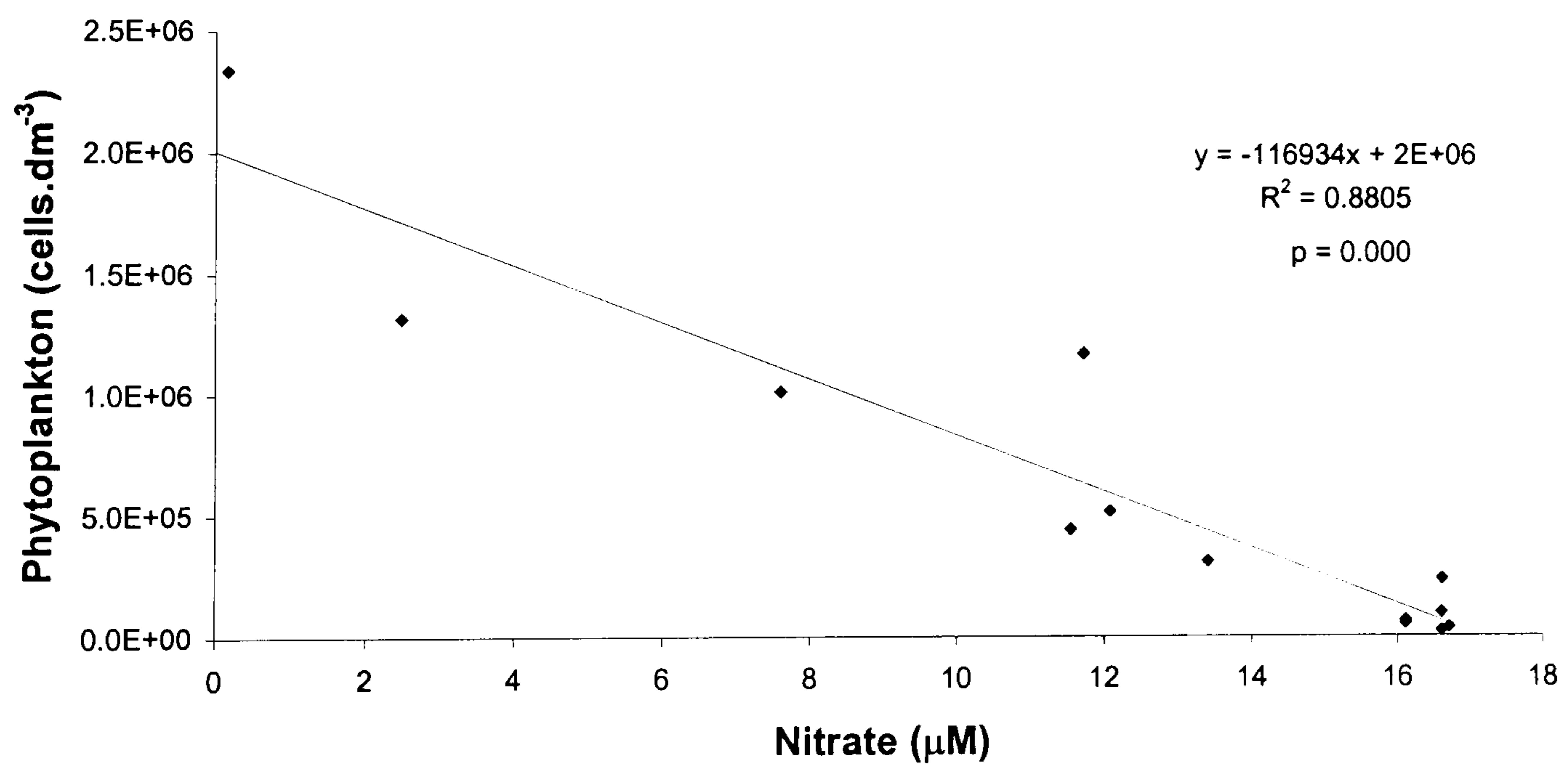


Figure 5.7b. Correlation between phytoplankton abundance and nitrate during spring 1999 (days 63 to 148) in the Menai Strait.

Therefore it appears that preference for reduced forms of inorganic nitrogen over each other is influenced by many factors such as light, temperature and phytoplankton composition (McCarthy *et al.* 1977; Glibert *et al.* 1982; Glibert *et al.* 1995; Rees *et al.* 1995). For instance McCarthy *et al.* (1977) and Rees *et al.* (1995) suggest that this preference is species related and is relative to the availability of the nutrient itself.

The seasonal cycle of primary production is accompanied by changes in the rates of utilization of inorganic nitrogen. The maximum primary production occurred during spring following the onset of inorganic nitrogen uptake. Although the data in this study was limited to the calculation of net nitrogen uptake to particular periods, it can be hypothesized that high net uptake rates of nitrate and ammonium take place during the spring bloom. By contrast during summer and autumn months these rates are characterized by low nitrate uptake with ammonium continuing to be utilized at relatively high rates supporting the primary production during this period of low inorganic nitrogen.

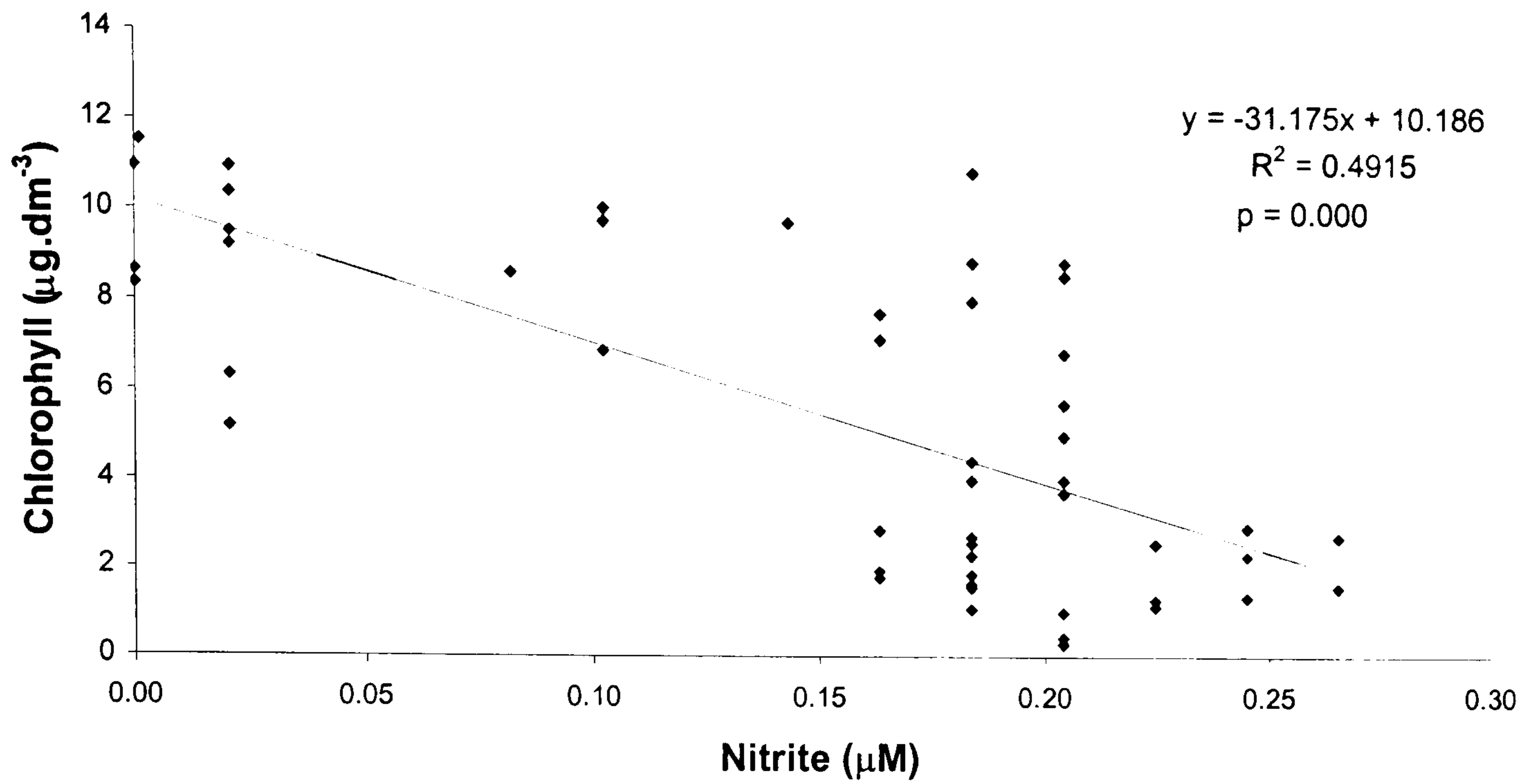


Figure 5.8a. Correlation between chlorophyll *a* and nitrite during spring 1999 (days 63 to 148) in the Menai Strait.

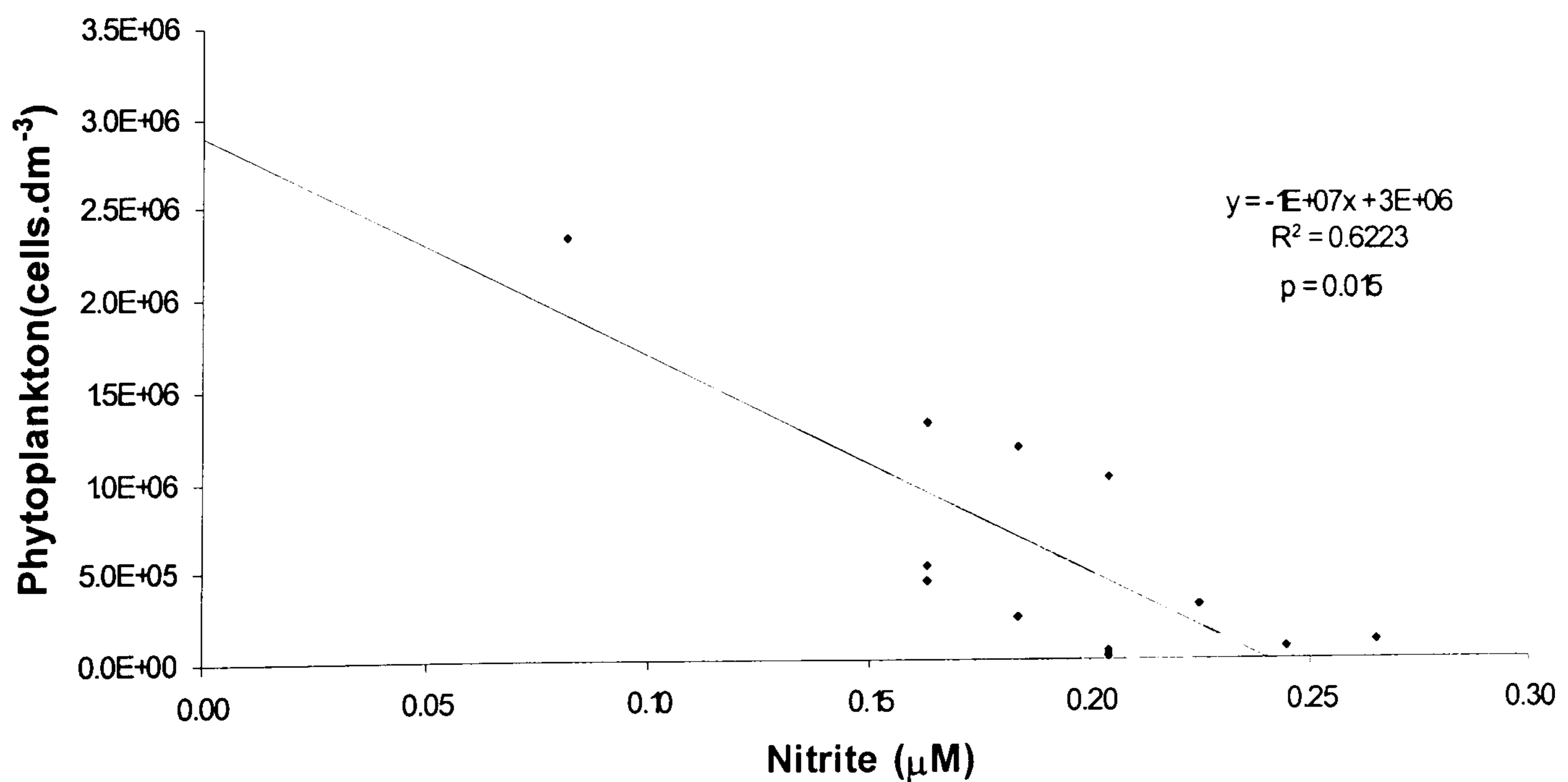
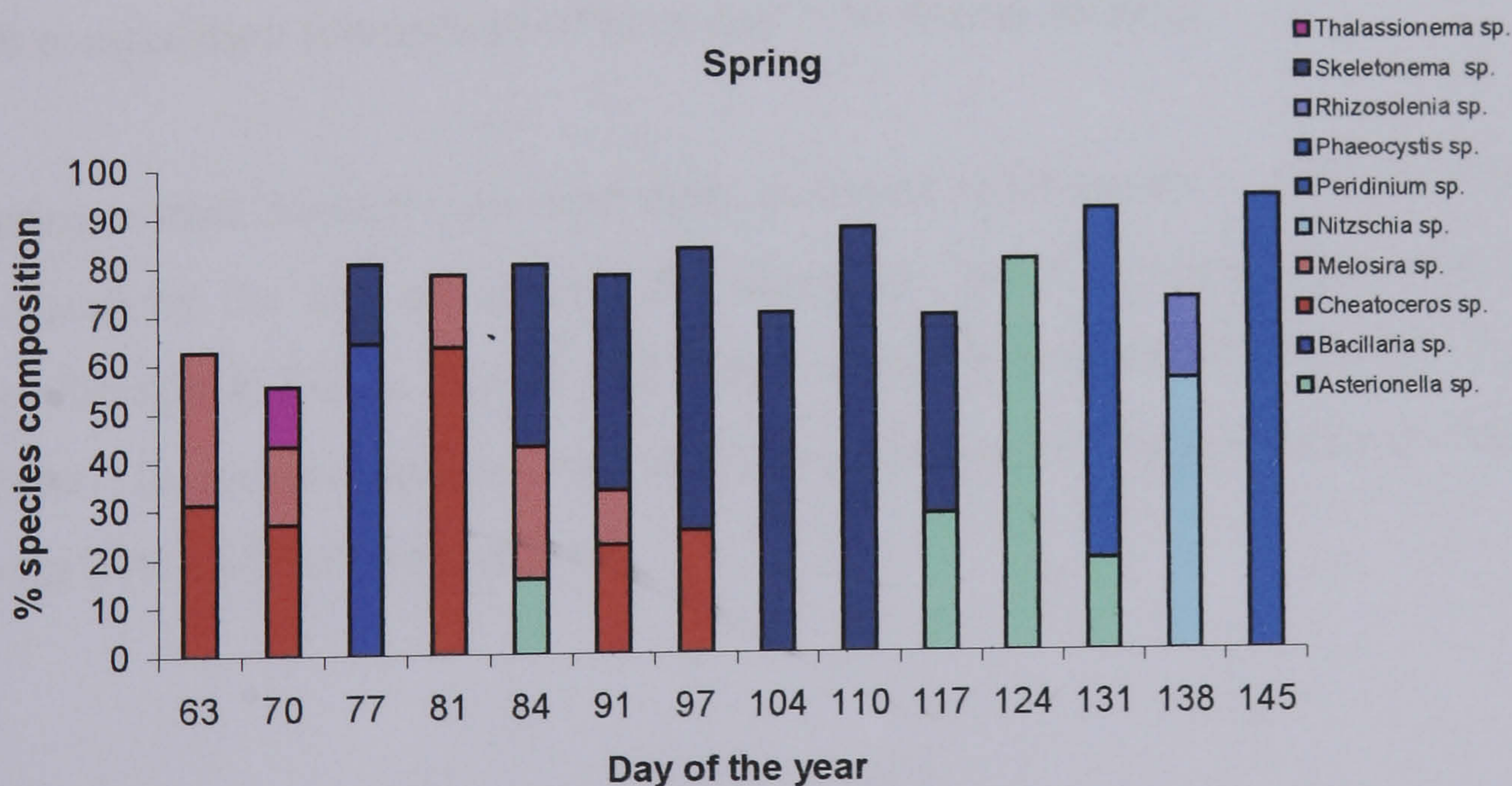


Figure 5.8b. Correlation between phytoplankton abundance and nitrite during spring 1999 (days 63 to 148) in the Menai Strait.

### 5.2.3. Nitrogen sources and the regulation of phytoplankton succession

The pattern of seasonal succession of phytoplankton species in the Menai Strait is shown in figures 5.9a, b, c, d, and e expressed as percentage of total cell number. The species percentages represent species which comprised at least 10% of the total cell number. During spring (day 63-145), *Melosira sp.* occurred, however it formed a relatively small percentage. *Chaetoceros sp.* dominated at the beginning of spring reaching 63% (day 81). From day 84 *Skeletonema sp.* persisted for about a month and formed the major component of the spring bloom. The maximum percentage (88%) occurred on day 110. *Asterionella sp.* occurrence followed the disappearance of *Skeletonema sp.* and reached the maximum (81%) on day 124. This species persisted in the water for about two weeks and disappeared. Species such as *Bacillaria sp.*, *Peridinium sp.*, *Nitzschia sp.*, and *Phaeocystis sp.*, despite their short occurrence in this study but they comprised a significant percentage of total cell number (*Bacillaria sp.* (64%); *Peridinium sp.* (94%); *Nitzschia sp.* (56%); *Phaeocystis sp.* (94%)) at specific times.

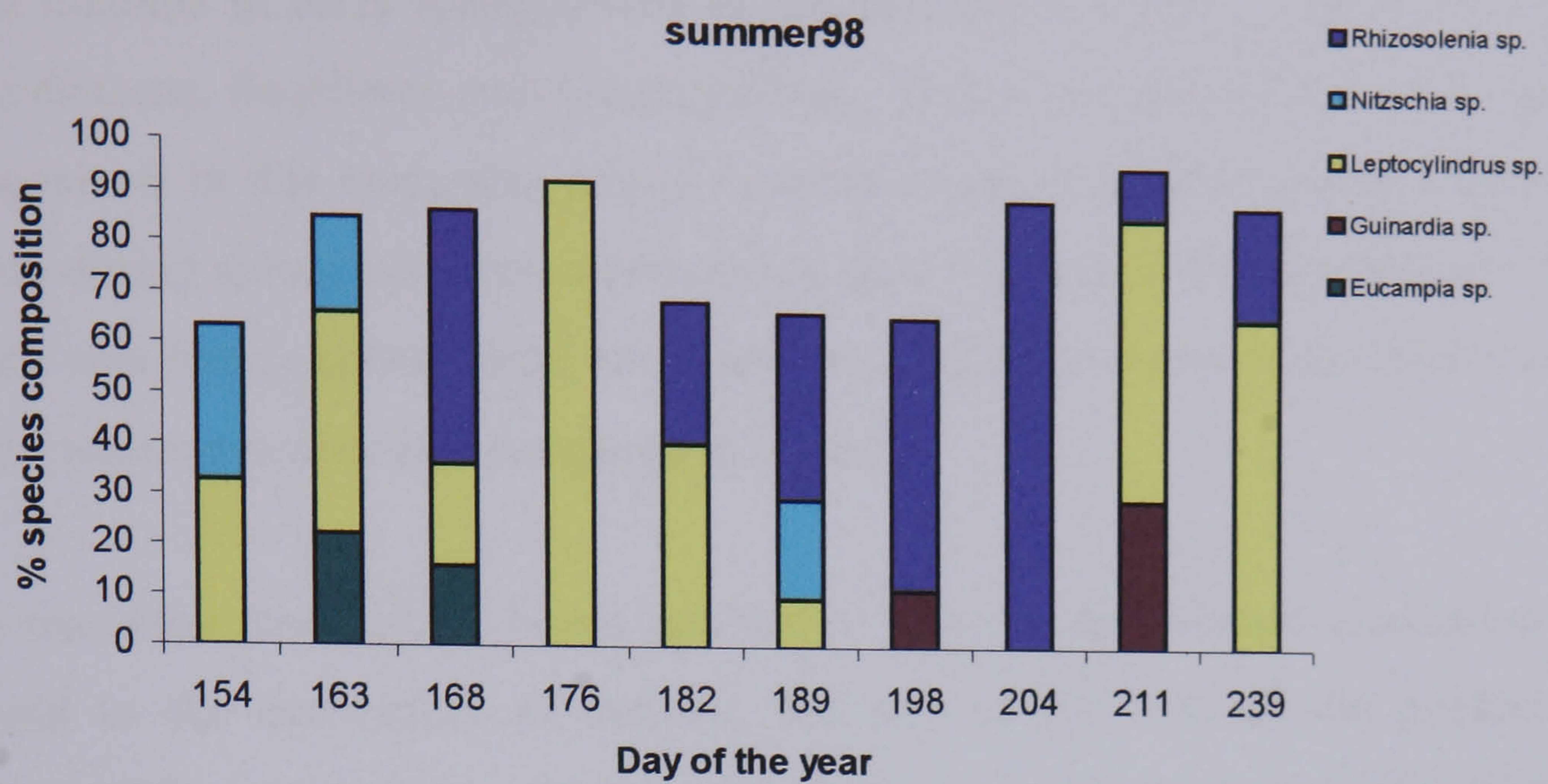


**Figure 5.9a.** Phytoplankton species succession during spring (days 63 to 145) in 1999 in the Menai Strait.

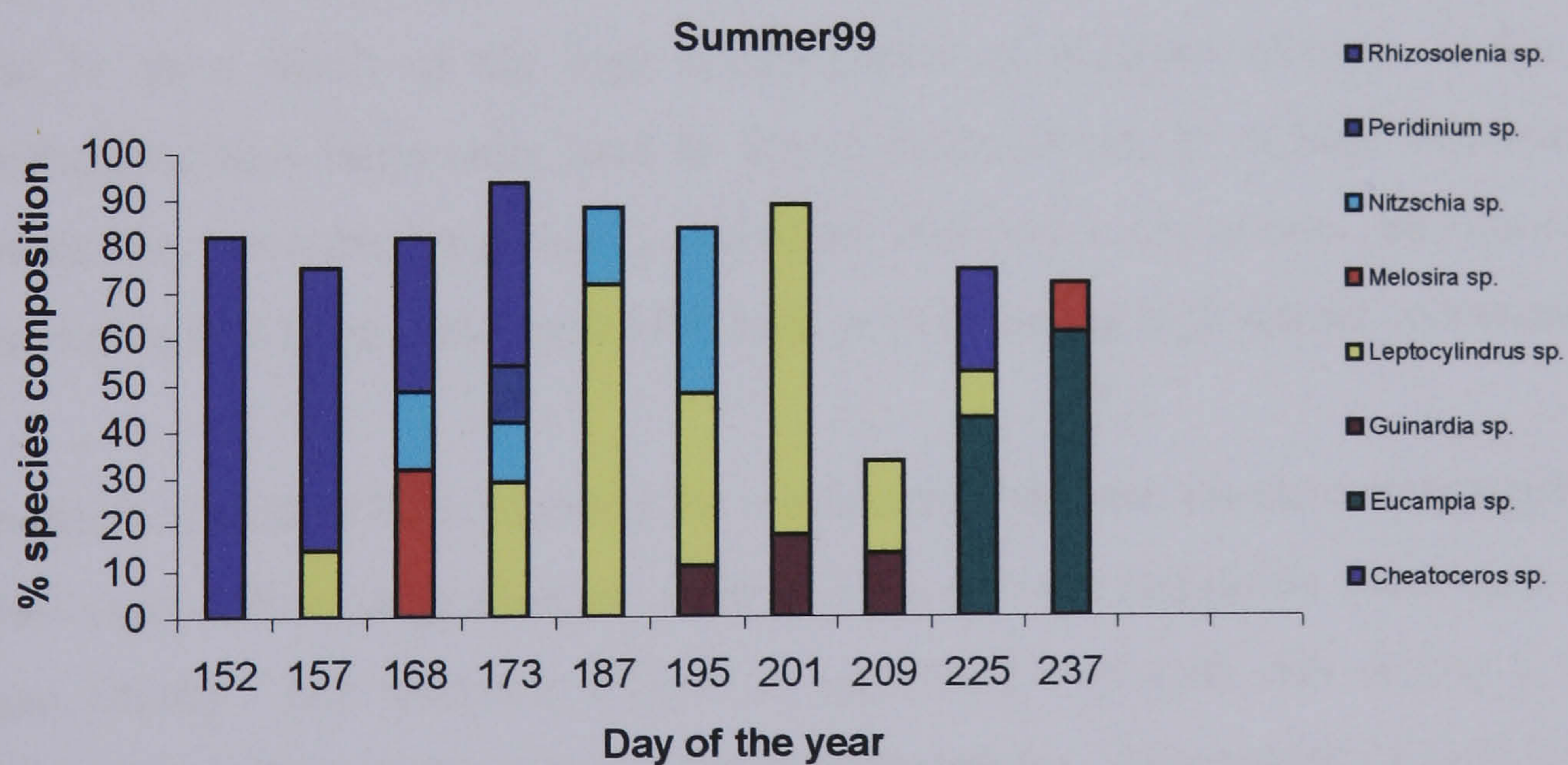
During summer 1998 (days 154 to 239), *Leptocylindrus sp.* and *Rhizosolenia sp.* were the most abundant. *Leptocylindrus sp.* occurred throughout summer when it reached the maximum abundance (92%) on day 176. The maximum abundance of *Rhizosolenia sp.* (80%) occurred on day 204. *Leptocylindrus sp.* continued to dominate the species composition until early autumn (day 246 and 253) and disappeared in winter. During summer 1999, *Rhizosolenia sp.* persisted for about three weeks and maximum cell percentage (82%) occurred on day 152. This species disappeared and reappeared again towards the end of summer following the decline of *Leptocylindrus sp.* *Leptocylindrus sp.* persisted for about seven weeks with a maximum of 72% observed on day 187. *Eucampia sp.* followed the decline of *Leptocylindrus sp.* and maximum percentage (62%) occurred on day 237. Species such as *Nitzschia*, *Melosira*, *Peridinium* occurred sporadically but formed relatively small percentage.

During autumn (days 246 to 293) *Leptocylindrus sp.* was the most abundant (87% and 66%) in early autumn (days 246 and 253 respectively). *Melosira sp.* occurred throughout and also was abundant (49% and 46% on days 274 and 279 respectively). Despite the short appearance, *Biddulphia sp.* formed a significant percentage of total cell composition (comprised 43% on day 279) during autumn.

During winter *Melosira sp.* was most abundant reaching up to 80% on day 29 and declined by the end of winter. *Bacillaria sp.* and *Chaetoceros sp.* also occurred significantly (52% on day 35 and 56% on day 56 respectively) towards the end of winter. In addition species such as *Coscinodiscus* and *Thalassiosira sp.* represented about 27% and 20% respectively.



**Figure 5.9b. Phytoplankton species succession during summer (days 154 to 239) in 1998 in the Menai Strait.**



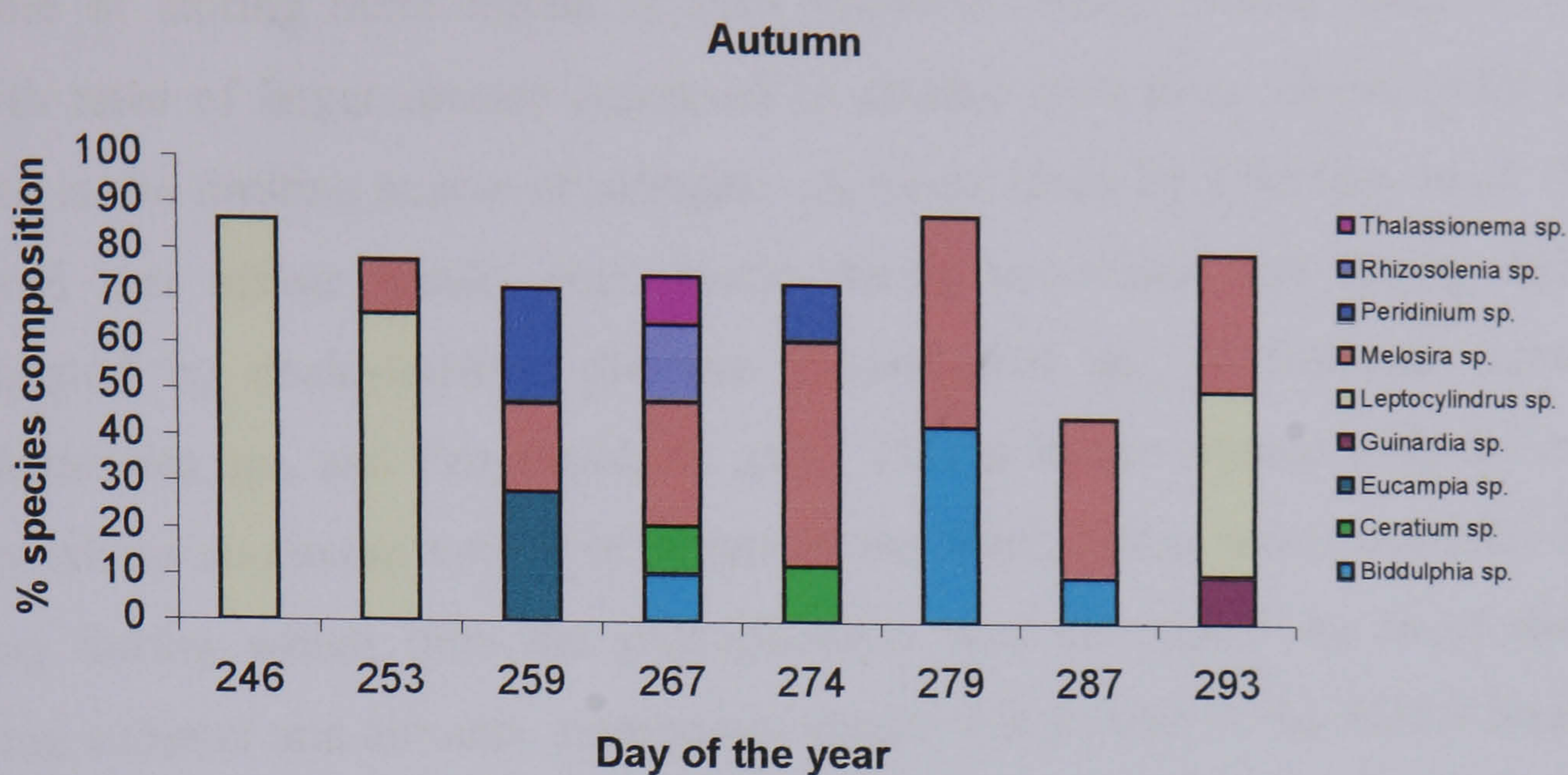
**Figure 5.9c. Phytoplankton species succession during summer (days 152 to 237) in 1999 in the Menai Strait.**

As it was described previously the phytoplankton succession is characterised by small diatoms in early spring whilst in late spring (May-June) it is dominated by large diatoms, flagellates and dinoflagellates. This is an indication that the species composition in this study was related to some extent to nutrient changes especially nitrate during spring and ammonium during summer. Large diatoms occurred when nitrate was highly concentrated and small ones during summer when ammonia and nitrite were more abundant compared to nitrate.

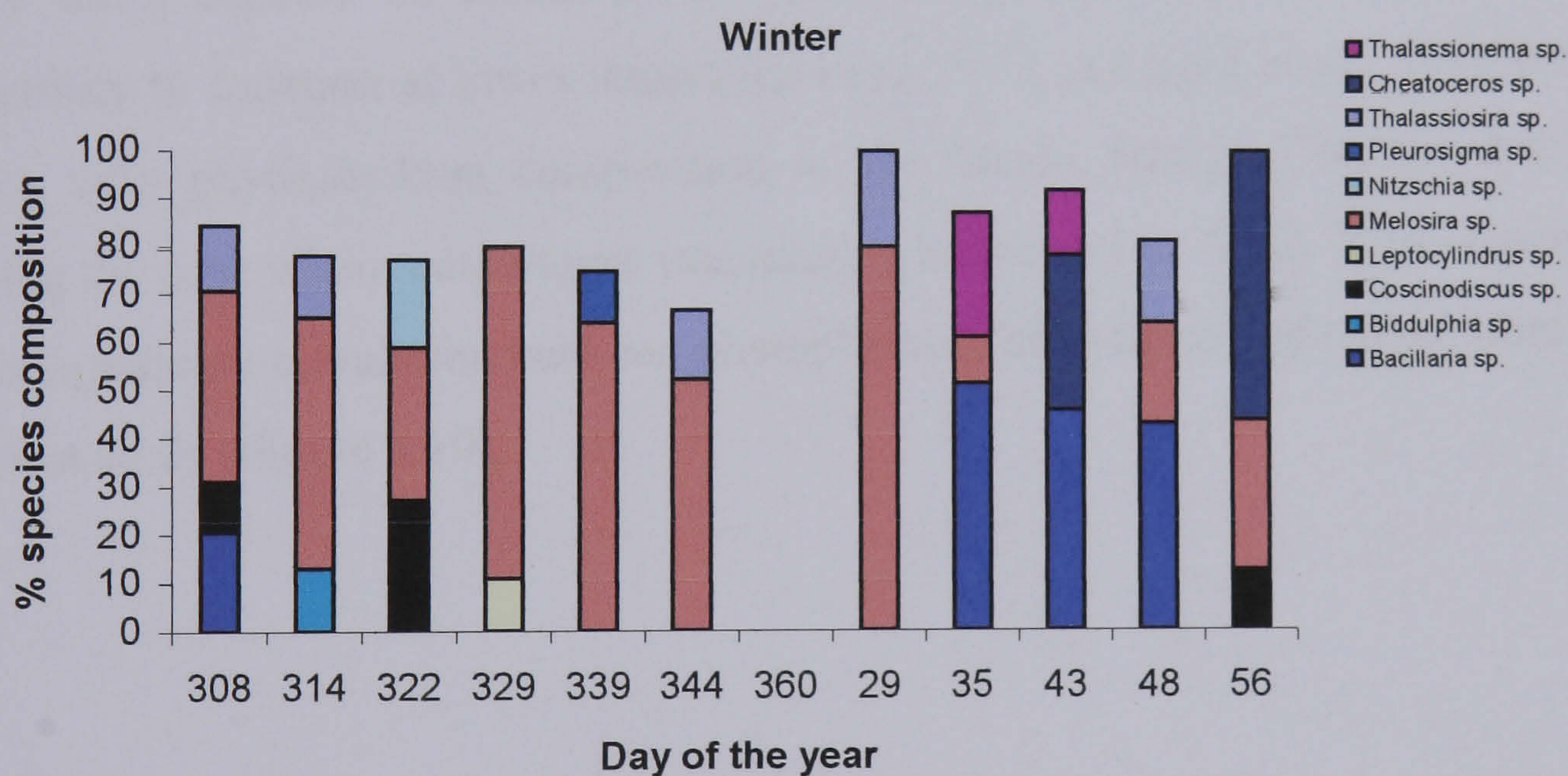
The transition from nitrate-based production to ammonium-based production may depend to the availability of nutrient, the size of the cells of the predominant phytoplankton assemblage and their preference for the available inorganic nitrogen. In addition other processes may lead to the recycling of inorganic nitrogen. The high nitrate utilization during spring compared to other period of the year could be linked to the predominant phytoplankton species. As described previously the species composition in this study was characterized by microplankton during spring while during summer it was assumed that it was characterised by nanoplankton as reported in previous studies (Blight *et al.* 1995; Rodrigues 1998) in the Menai Strait. This could be as a result of the high concentration of ambient nitrate. It has been hypothesised that large cells tend to bloom when nitrate is in high concentration because they have the capability to assimilate and store more nitrate. Several reports have shown that large diatoms exhibit high growth during high nitrate concentration.

A number of studies have reported the relationship between nitrate concentration and *Phaeocystis* and/or large diatoms such as *Rhizosolenia delicatula* (dominant in the Menai Strait). For instance, Glibert *et al.* (1982) reported that during a winter bloom, *Rhizosolenia delicatula* favoured nitrate uptake. Riegman *et al.* (1992) tested the ability of *Phaeocystis sp.* to compete for nutrients and reported that this species showed poor competition under P-limitation. However it was capable of competing with other phytoplankton (*Thalassiosira sp.*, *Dytilum sp.*, *Lauderia sp.*, and *Stephanopyxis sp.*), under nitrate limitation. Muggli & Smith (1993) suggested that *Phaeocystis sp.* was capable of growing in low nitrate concentration. Furthermore Glibert *et al.* (1995) reported that diatoms were more capable of assimilating high concentrations of nitrate due to their capability to function at lower temperature and light level.





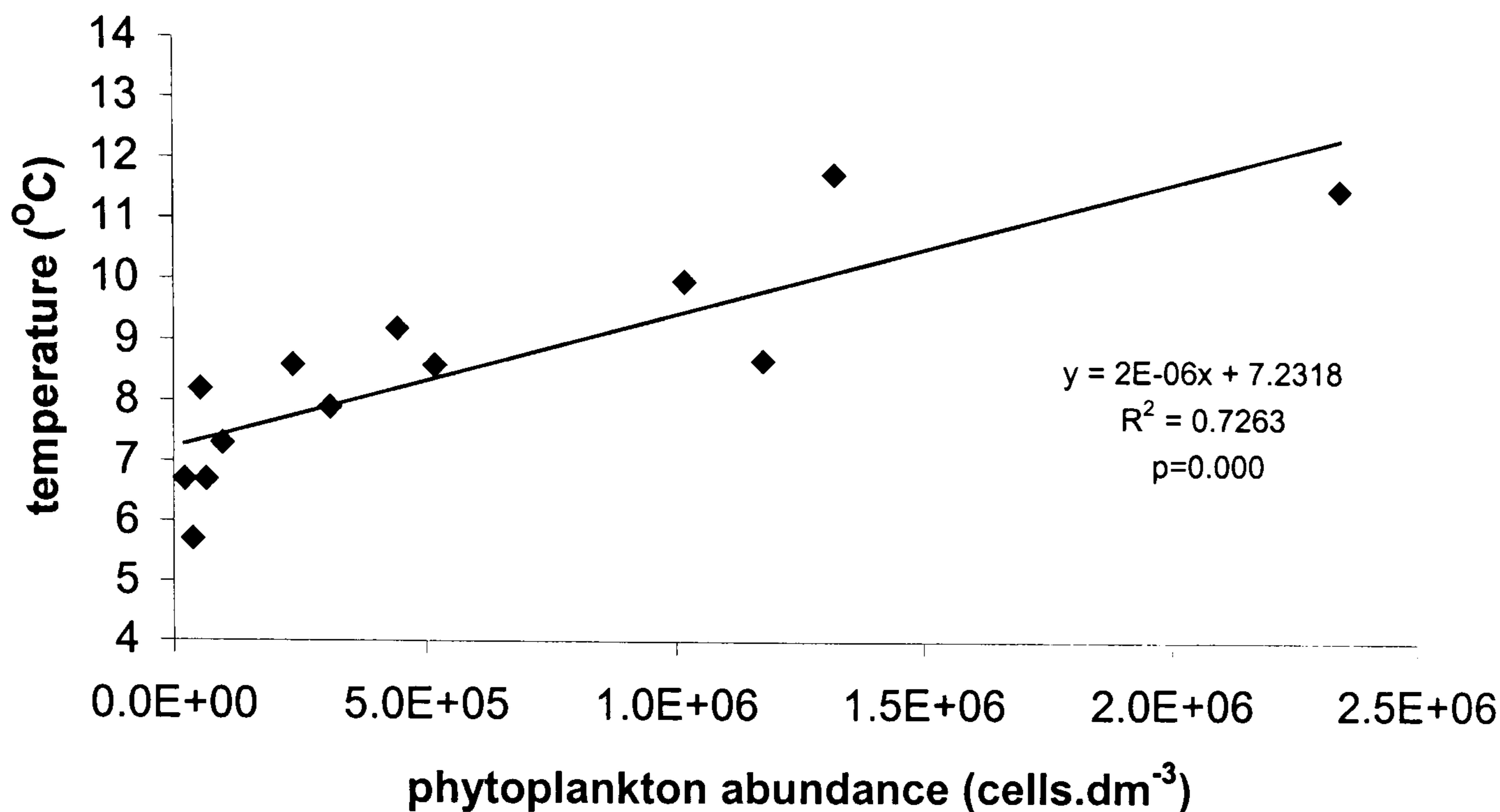
**Figure 5.9d. Phytoplankton species succession during autumn (days 246 to 293) in 1998 in the Menai Strait.**



**Figure 5.9e. Phytoplankton species succession during winter (days 308 to 56) covering the period from November 1998 to February 1999 in the Menai Strait. Notice the period from day 345 in 1998 to day 28 in 1999 no phytoplankton samples were collected.**

Consequently Riegman & Noordeloos (1998) suggested that large diatoms were capable of storing more nitrate in their vacuoles, which would result in higher growth rates of larger species compared to smaller ones in an environment where nitrate is the limiting source of nitrogen. A recent study by Tremblay *et al.* (2000) showed that nitrate uptake was greater during pre-winter and spring and was dominated by chain-forming diatoms (*Chaetoceros sp.*, *Skeletonema costatum*, *Thalassiosira sp.*, and *Fragilariopsis sp.*). This is in accordance with the current study where maximum uptake of nitrate in the Menai Strait water occurred during spring during which time the phytoplankton was dominated by large diatoms. During summer and autumn, ammonium uptake was greater in the Menai Strait and dominated by small-sized phytoplankton.

Other environmental factor such as temperature and light appear to influence the inorganic nitrogen uptake. For example Glibert *et al.* (1995) reported that diatoms were more capable of assimilating high concentrations of nitrate due to their capability to function at lower temperature (*ca.* 5°C) and light level. This seems to agree with phytoplankton composition in the Menai Strait. Diatoms dominated during the time when temperature was ranging between 5 to 10°C. This is supported by a significant correlation between phytoplankton abundance and temperature in the current study (Figure 5.10).



**Figure 5.10.** Correlation between phytoplankton abundance and water temperature during spring 1999 (days 63 to 145).

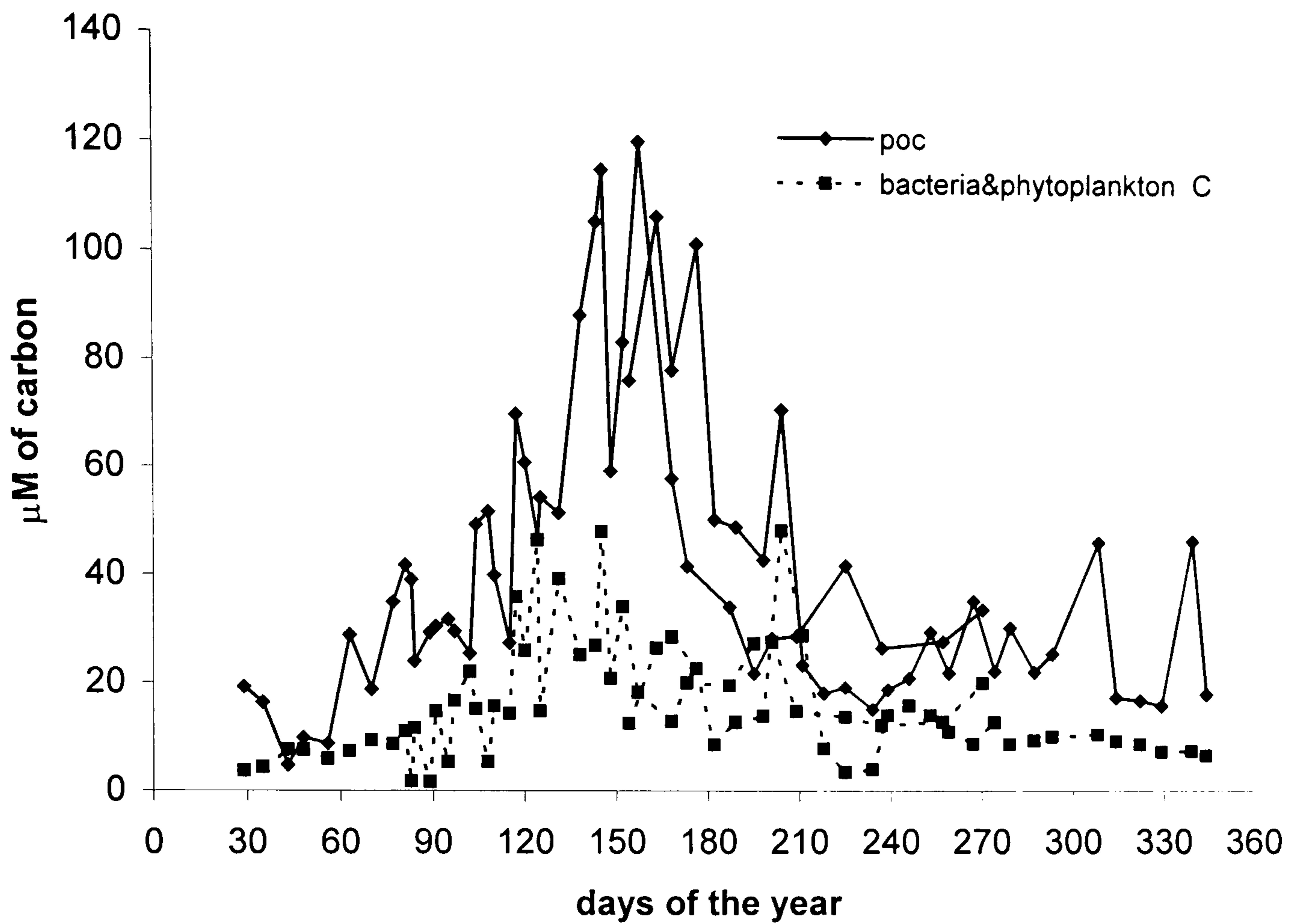
#### **5.2.4. The influence of phytoplankton blooms on the magnitude and composition of particulate organic matter.**

A large increase in particulate organic carbon (POC) is associated with the bloom but how this biogenic carbon is partitioned among the autotrophic and heterotrophic biomass and the detritus and how this changes during the development of the bloom are not well known.

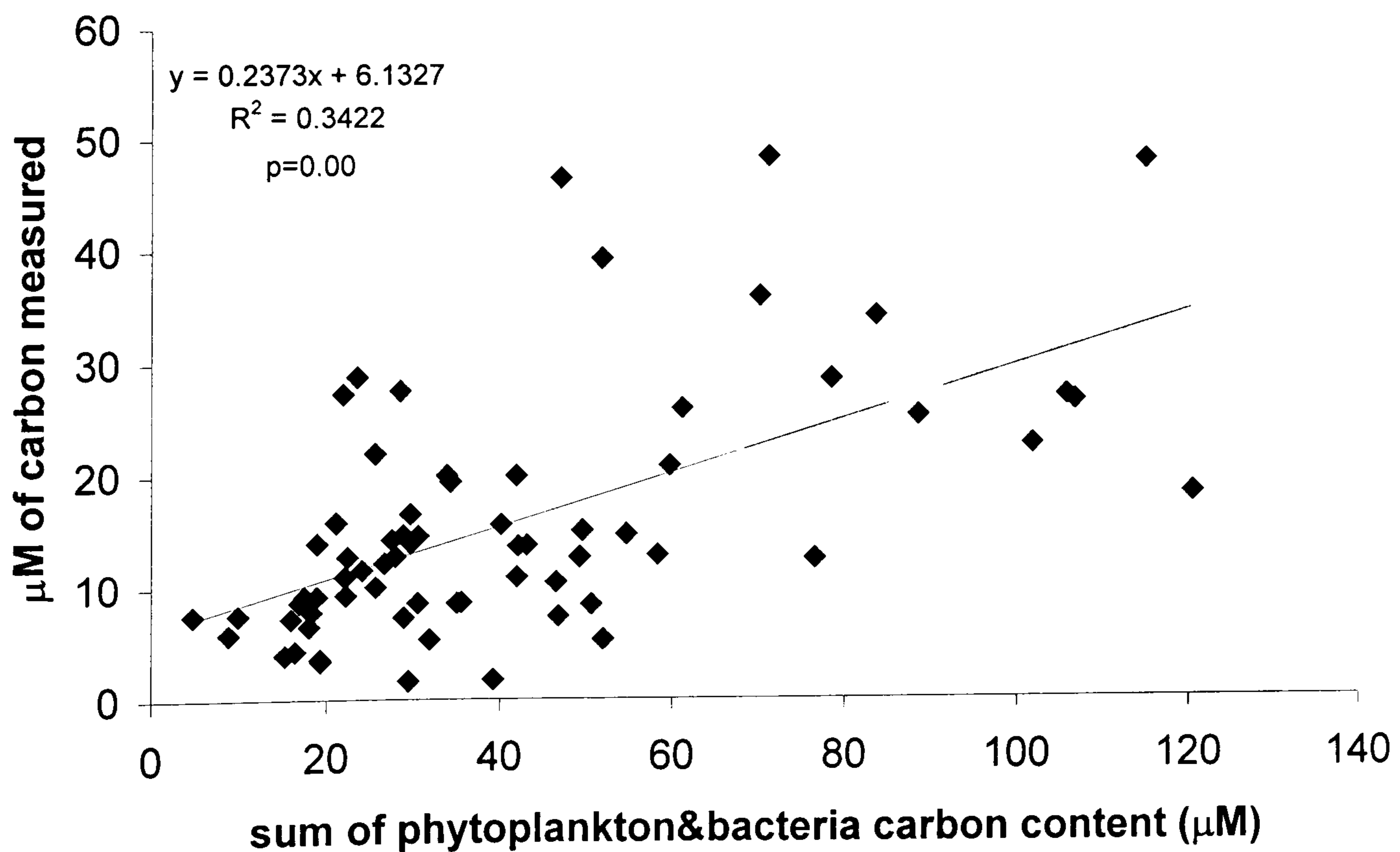
As reported previously, the increase in particulate organic matter appeared to be associated to some extent with the increase in chlorophyll *a*. However in a well-mixed environment such as Menai Strait, there are several sources of organic matter contributing to the particulate organic matter pool. The phytoplankton carbon content was estimated using the method described by Mullin *et al.* (1966) and bacteria carbon content using the technique described by Blight (1995). The phytoplankton carbon content was estimated as a function of cell volume (see Appendix 4) and the cell carbon content was estimated using the equation  $\log_{10}C=0.76\log_{10}V-0.29$  (Mullin *et al.* 1966). Subsequently, the bacteria carbon content was estimated using the average value of 20fg C cell<sup>-1</sup> and assuming that the

cells were cocci and with diameters of roughly 0.4 – 0.5 $\mu$ m (Blight 1995). Both phytoplankton and bacterial carbon content were converted to  $\mu$ M C. As shown in Figure 5.11 phytoplankton and bacteria contributed *ca.*45% to the total POC and this contribution is supported by a significant correlation between measured carbon and phytoplankton and bacteria carbon (Figure 5.12). In addition, the contribution of different phytoplankton classes is shown in Figures (5.13) indicating the diatom dominance in carbon content in all seasons. No measurements were made for zooplankton biomass in order to estimate its contribution to the total POC. Plankton biomass (phytoplankton and bacteria) comprised less than half of the total POC, indicating that there is a high contribution to the POC even during bloom period from other sources such as detritus and sediments.

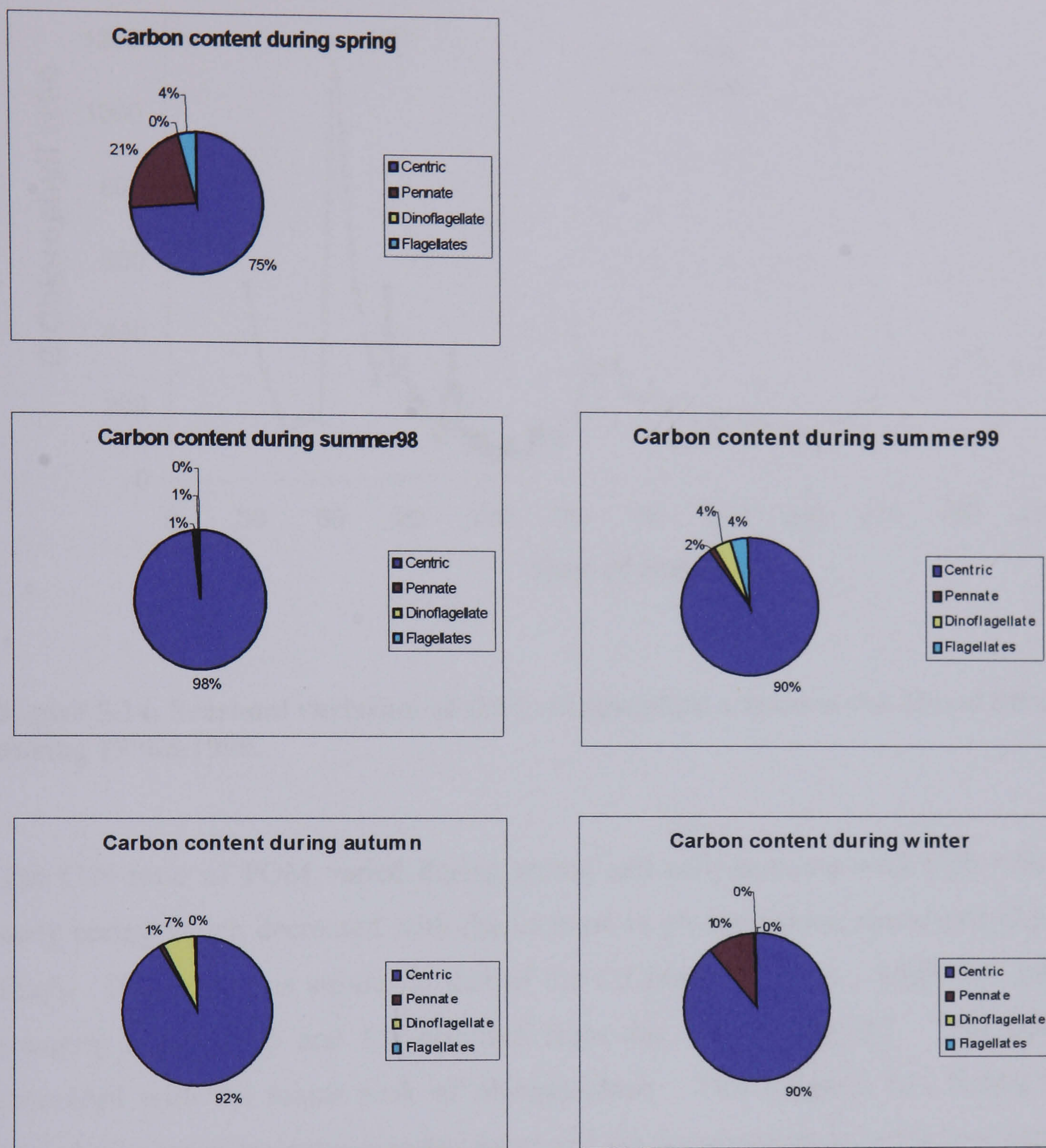
This contribution is observed from the seasonal variation of C:Chlorophyll ratio by the whole community (Figure 5.14). A high ratio occurred at the time when chlorophyll was at its lowest concentration, followed by a decrease in C: Chl *a* with an increase in phytoplankton abundance. Cifuentes *et al.* (1988) suggested that C:Chlorophyll ratios of more than 200 indicated that organic matter comprise two types: terrestrial and detritus and /or planktonic. Therefore it seems that the contribution of terrestrial and detritus material dominated in Menai Strait water until April. From April to May, organic matter from phytoplankton contributed a large proportion to the total particulate organic matter pool. Despite this assumption some caution should be given that C:Chl *a* ratios will be erroneous in winter when levels are low.



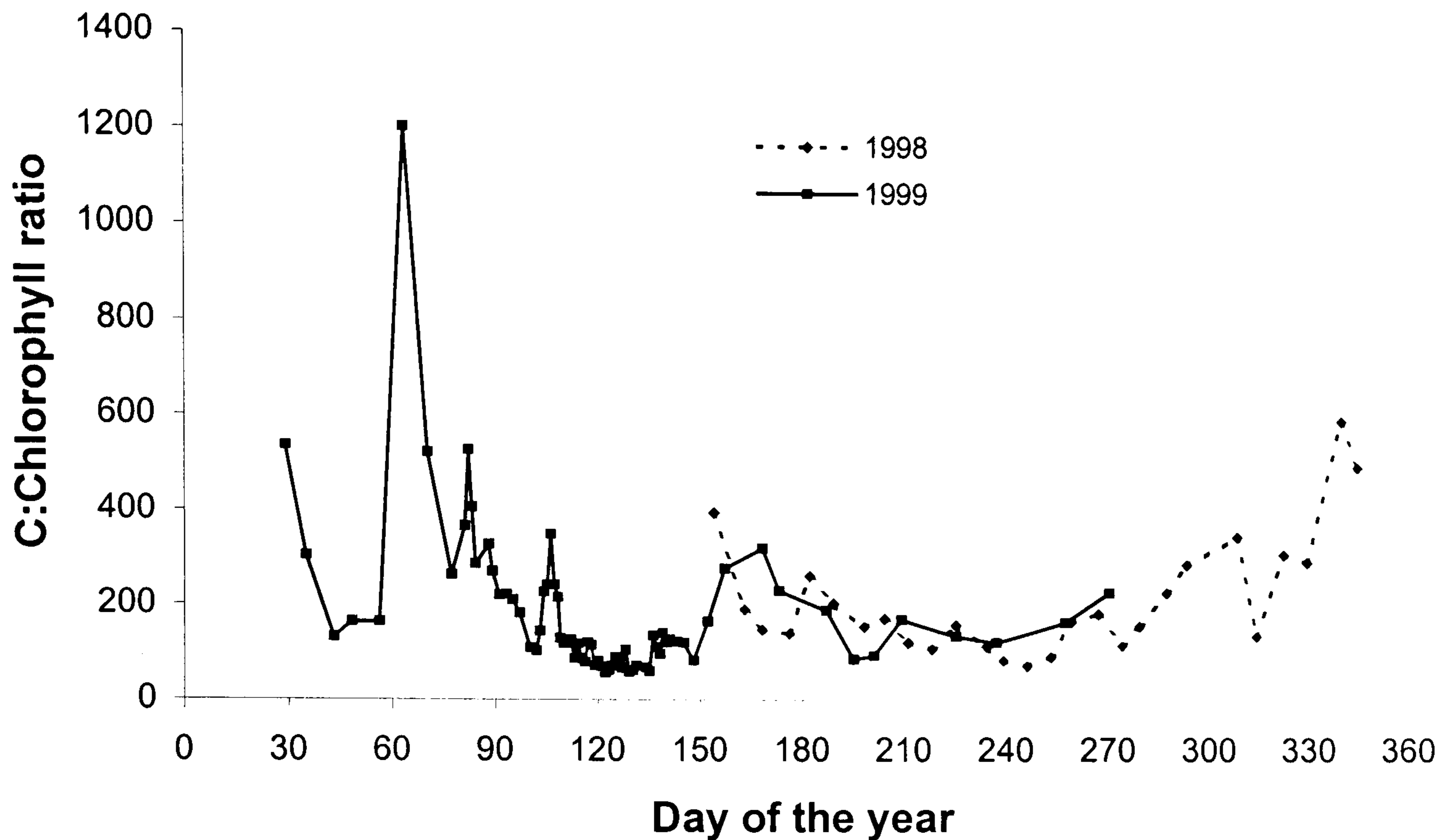
**Figure 5.11. Seasonal variation of phytoplankton and bacterial biomass in relation to total particulate organic carbon in the Menai Strait in 1998 and 1999.**



**Figure 5.12. Correlation between measured carbon and sum of phytoplankton and bacteria carbon in the Menai Strait during 1998 and 1999.**



**Figure 5.13. Seasonal variation of carbon content of phytoplankton taxonomical classes in the Menai Strait during 1998&1999.**



**Figure 5.14. Seasonal variation of the C:Chlorophyll *a* ratio in the Menai Strait during 1998&1999.**

The C:N ratio of POM varied during spring and early summer with high ratio in early spring which decreased with the increase in phytoplankton abundance (April-May). This pattern is similar to that of the C:Chlorophyll ratio. High C:N ratios (ranging between 13 and 15) occurred from day 140 to day 157. This period coincided with the major peak of phytoplankton. This suggests that during this period nitrogen was depleted in the water and the disproportional increase of carbon relative to nitrogen resulted in high C:N ratio. This is in agreement with a previous study (Rodrigues 1998).

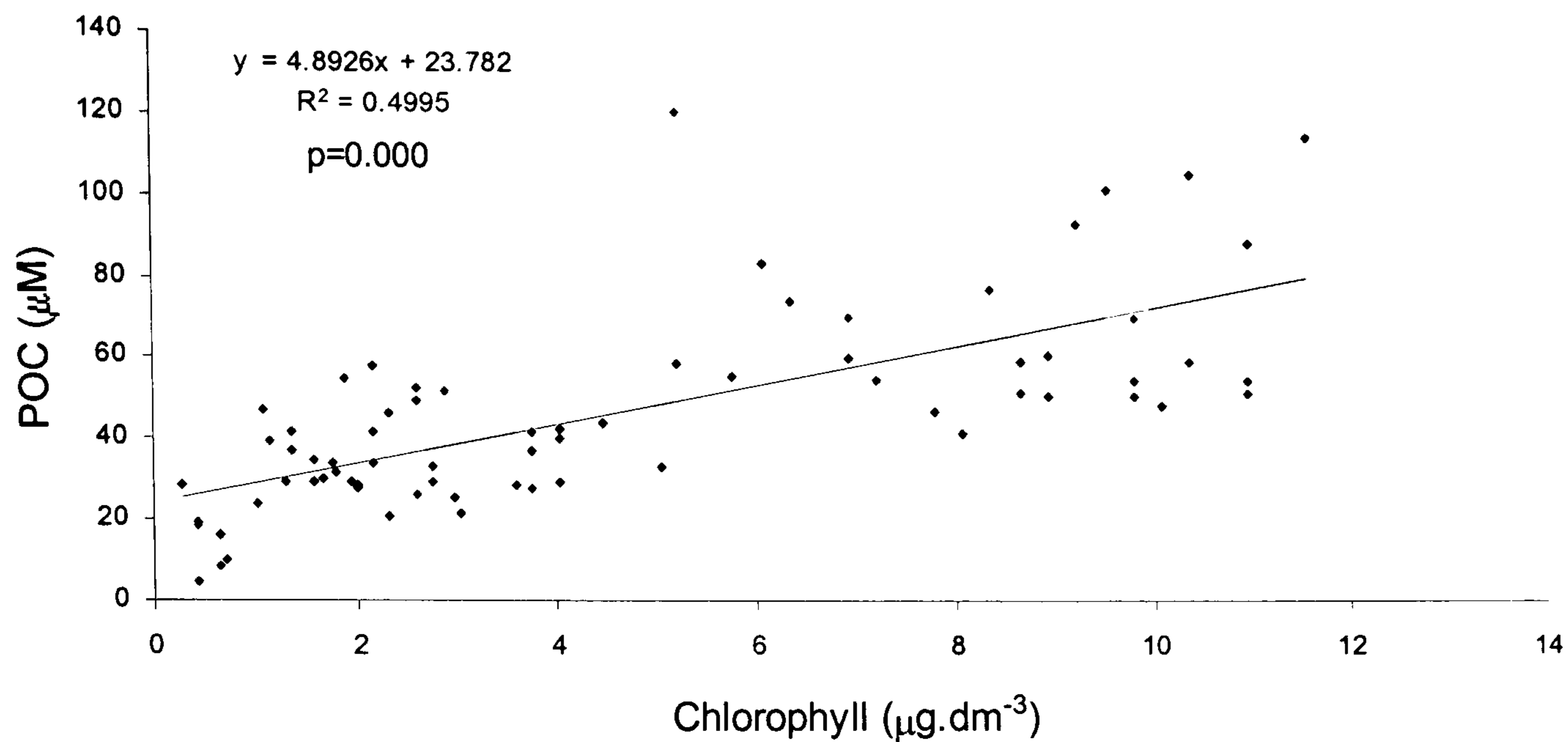
Rodrigues (1998) reported a seasonal variation of the C:N ratio of the particulate organic matter ranging from 5.2 to 15.8 with a seasonal mean of 7.9. The percentage contribution of phytoplankton biomass to the total POC and PON was estimated using the relationship between chlorophyll and POC as well as between chlorophyll and PON. It was reported that 65% of POC and 76% of PON could originate respectively from phytoplankton biomass and the rest from detritus material.

A similar attempt was made to estimate the percentage of POC and PON from phytoplankton in this study (Figure 5.15a&b). About 50% of POC and 67% of PON were contributed by phytoplankton. These percentages were lower than those reported by Rodrigues (1998), indicating that other sources of organic matter played a role in the seasonal variation of C:N ratio in the Menai Strait during this study. The percentage of phytoplankton contribution to the total POC estimated using phytoplankton volumes are considerably lower (20%) than the percentage contribution using the POC/chlorophyll relationship (50%). This difference can be associated with errors in the calculations of phytoplankton volumes and abundance.

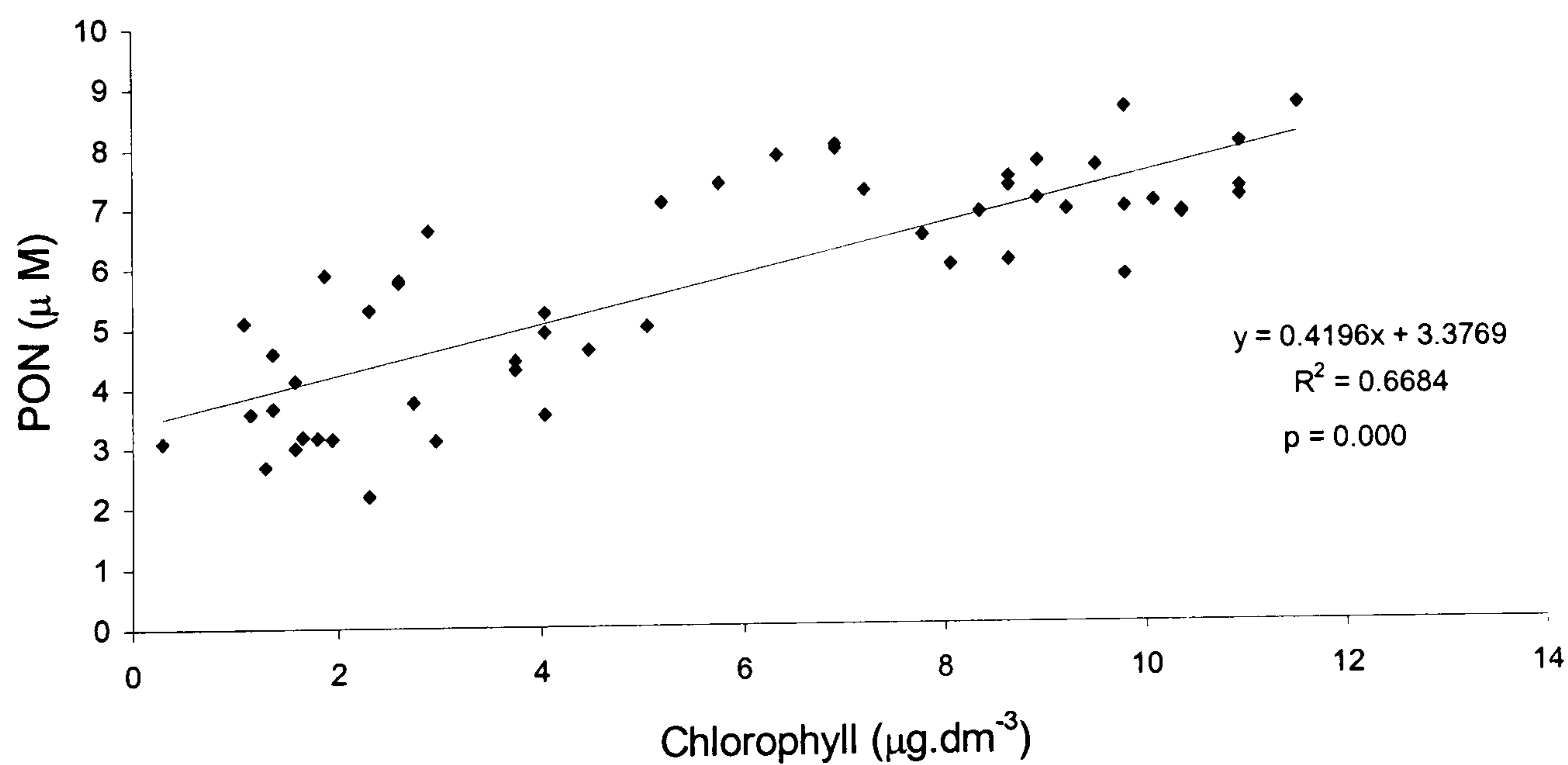
The mean C:N of 9 found in this study is higher than that reported by Rodrigues (1998) and Blight *et al.* (1995) (7.9 and 6 respectively). In addition the percentage contribution of non-phytoplanktonic matter in the POC and PON was slightly higher than reported by Rodrigues (1998). This indicates the influence of seasonal variation in the load of inorganic and organic suspended matter in the Menai Strait.

In conclusion the data in this study show that the contribution of phytoplankton to particulate organic matter is significant during spring, but that there is also a significant contribution of organic matter from other sources which occurs throughout the year.





**Figure 5.15a. Relationship between POC and chlorophyll *a* during spring 1999 in the Menai Strait. Notice intercept was different from zero.**



**Figure 5.15b. Relationship between PON and chlorophyll *a* during spring 1999 in the Menai Strait. Notice intercept was different from zero.**

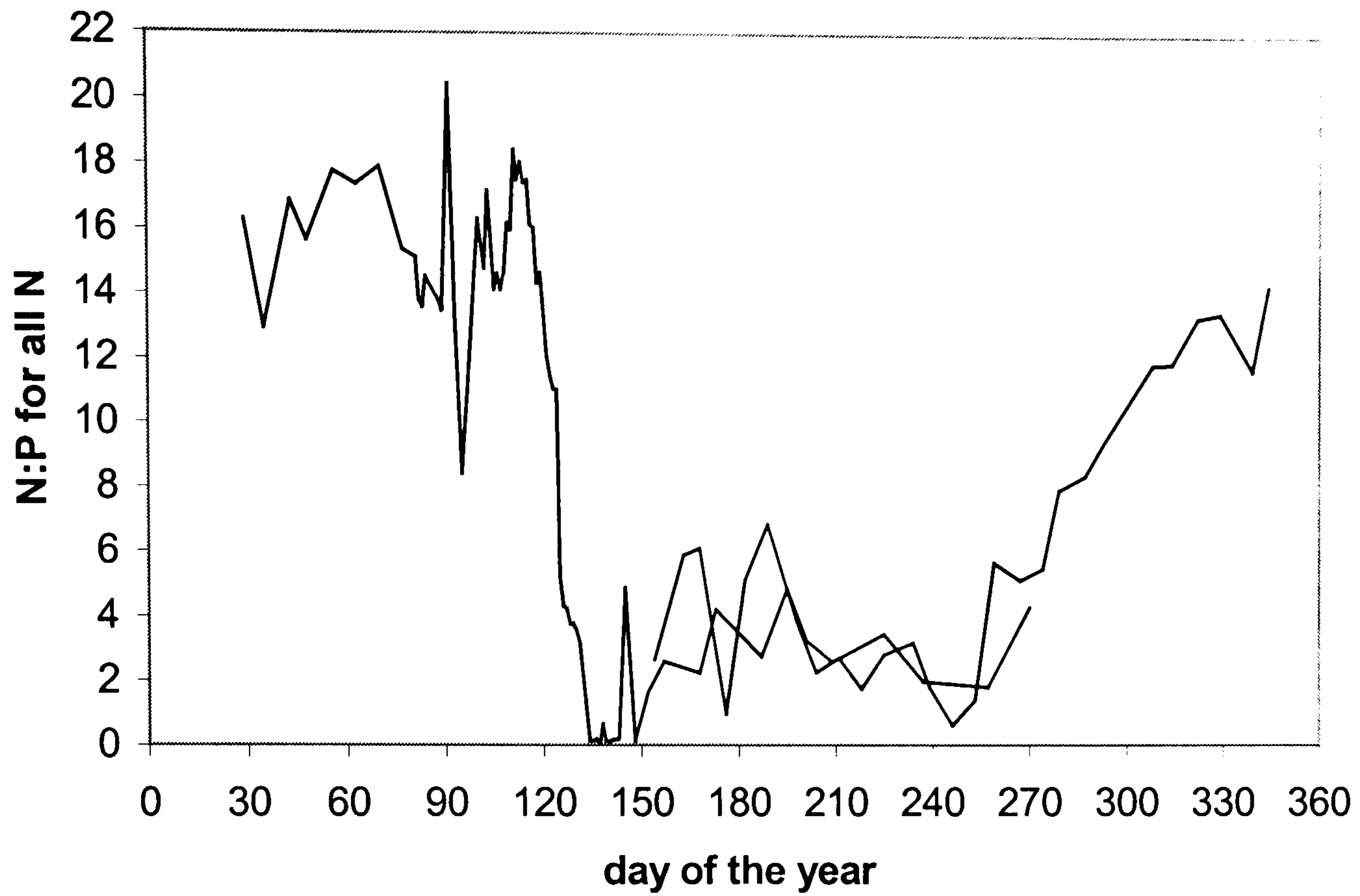
### 5.2.5. Seasonal variation of nitrogen: phosphate ratios and nutrient regeneration

The data presented in this study show that the N:P ratio exhibited a seasonal trend (Figure 5.16) characterized by values close to the Redfield ratio during late winter and early spring. The lowest ratio occurred during summer. This could be explained as a result of nitrate decrease and a quick regeneration of phosphate. In addition a regression analysis of all data gave a slope of 15 (Figure 5.17) indicating that the N:P of the Menai Strait water is generally close to the Redfield ratio (16).

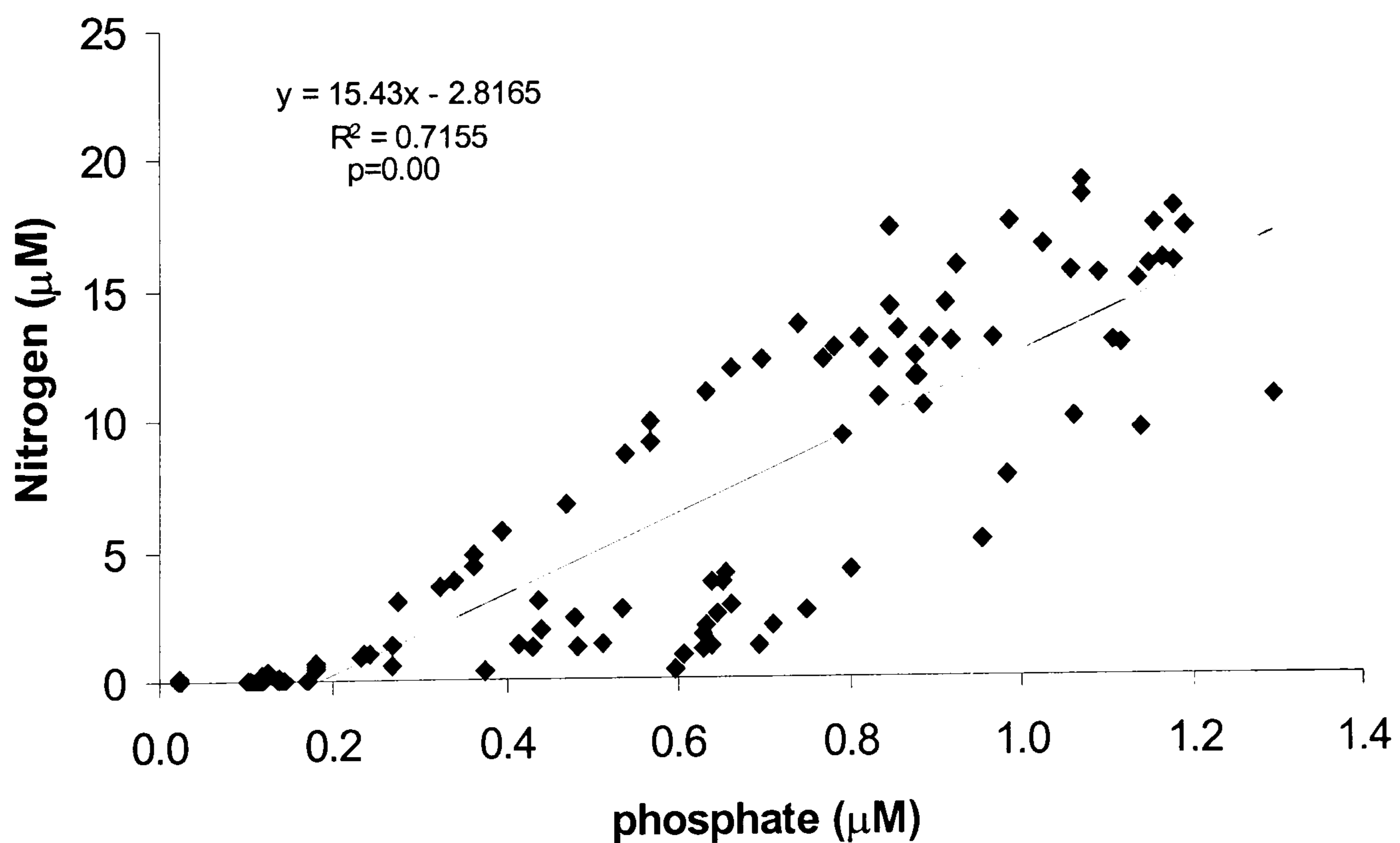
From their study, Karl *et al.* (1996) reported a quick regeneration of phosphate from particles in the deep water. It has been reported that the rapid regeneration of phosphate is due to the fact that phosphate release doesn't require oxygen consumption (Jordan & Joint 1998). Pingree *et al.* (1977) also reported a low N:P ratio during summer due to rapid regeneration of phosphate. The major factors controlling the variation of the N:P ratio are assimilation of nutrients by phytoplankton and heterotrophic processes involved in nutrient recycling (Jordan & Joint 1998).

The seasonal variation of N:P ratio was characterized by the highest ratio occurring during early spring when nitrate and phosphate were at their maximum concentrations. The nitrate and phosphate started to decrease with the development of the spring bloom. It should be expected that the N:P should not change and remained close to Redfield ratio however other processes contributed to this change such as regeneration. Other sources of nutrient input could have been in place such as the resuspension of sediments caused by the turbulence in Menai Strait water.

The decay of organic matter could also be a vital source of these nutrients especially phosphate and ammonia. Although it is not clear which mechanism dominated the input of phosphate and ammonia during summer, it seems most likely that heterotrophic activity was responsible. During this time of the year more organic matter is released from phytoplankton blooms and is remineralised by heterotrophic



**Figure 5.16. Seasonal variation of the nitrogen and phosphorus ratio (N:P) in the Menai Strait during 1998&1999.**



**Figure 5.17. Correlation between nitrate and phosphate for all data observation in 1999 in the Menai Strait.**

activity, thus regenerating ammonia and phosphate. This is supported by the rapid regeneration of phosphate and ammonia, but not nitrate, recorded in this study during summer. Despite the contribution ammonia to the total N:P pools, the N:P ratio remained low since nitrate was at its lowest concentration.

# CHAPTER VI

## 6. DISSOLVED ORGANIC MATTER (DOC, DON, and YS) DYNAMICS.

### 6.1. Introduction

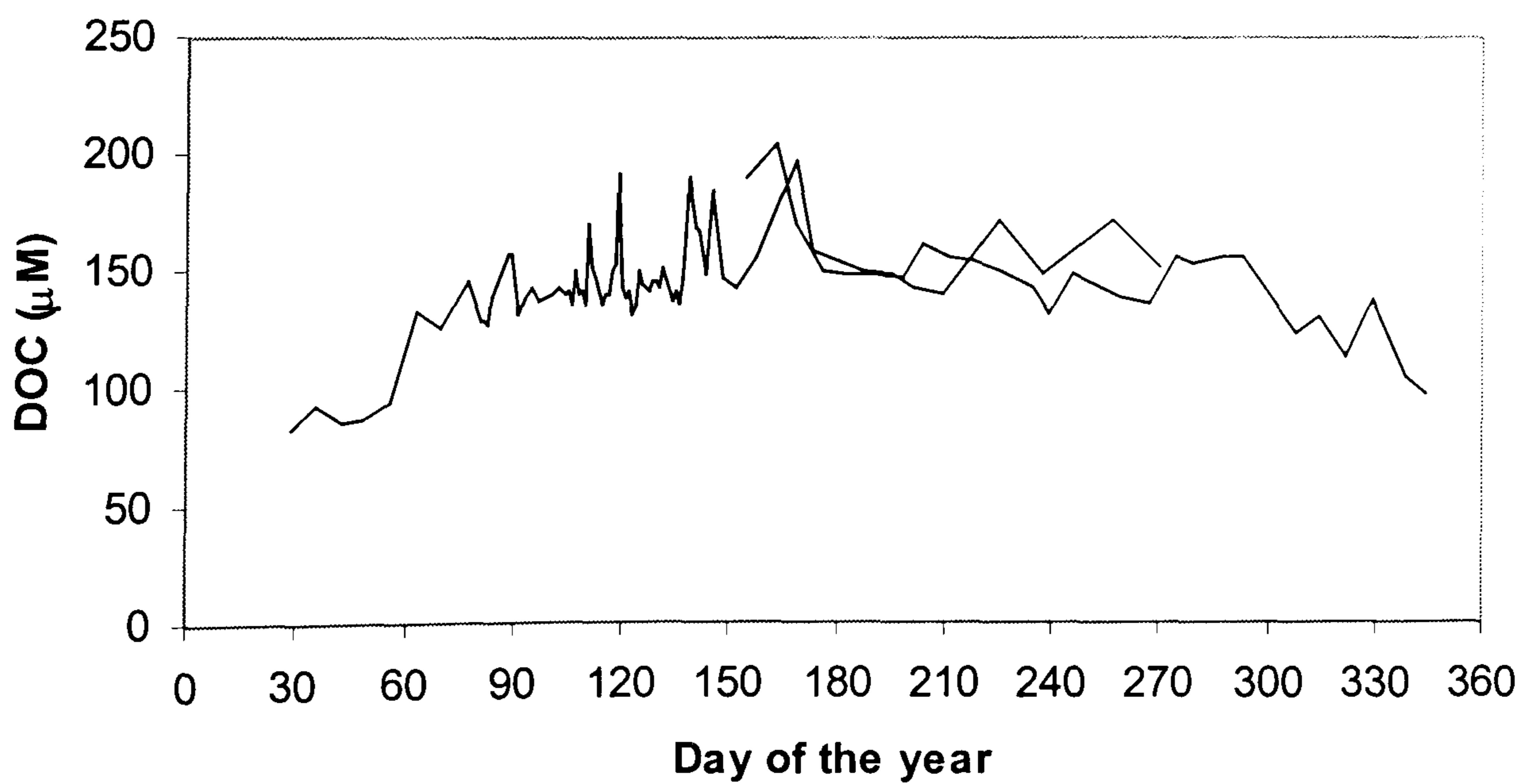
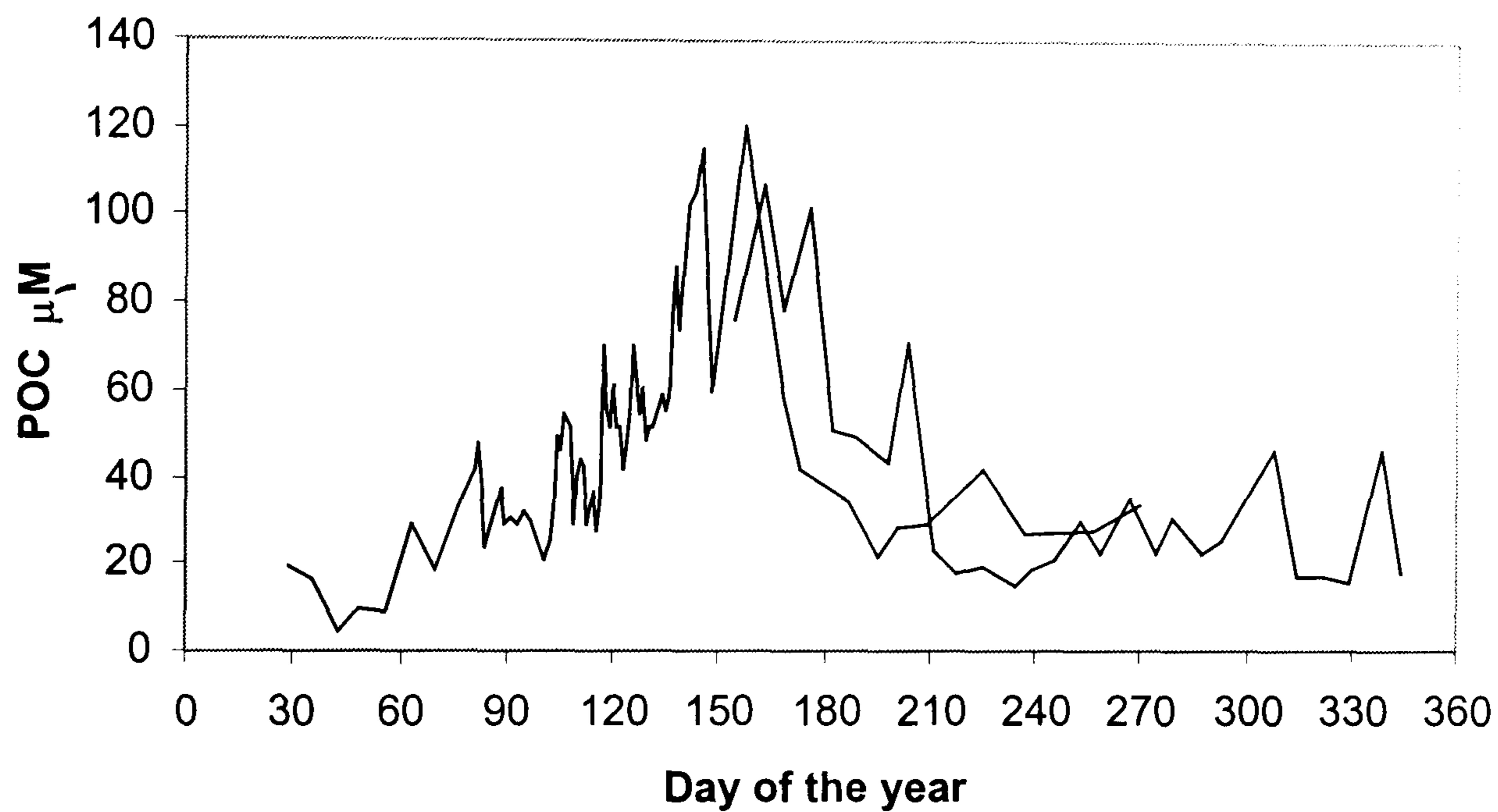
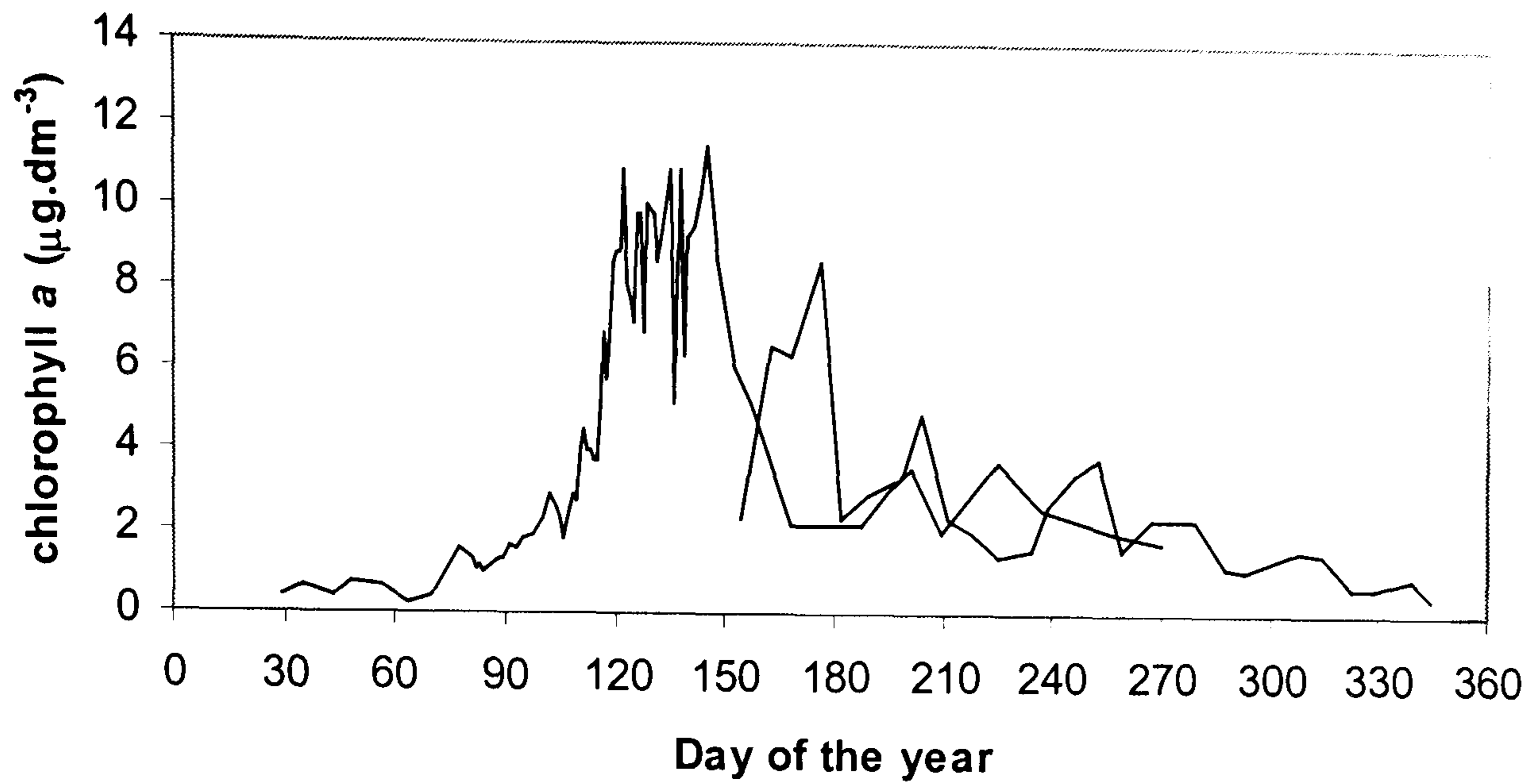
In order to contribute to a better understanding of a complete global cycle in the marine environment, consideration is necessary of the nature and seasonality of organic matter. This includes particulate and dissolved fractions. This could be achieved by investigating the role of phytoplankton as supplier of DOM to the marine environments, either directly by releasing dissolved organic matter to marine environment or indirectly through exudation, sloppy feeding by grazers and degradation of particles. In addition the role of bacteria as primary DOM consumers should be examined (Azam *et al.* 1983; Kirchman 1990), especially in coastal waters.

The Menai Strait has been extensively studied covering different aspects of the pelagic system; however it has been poorly investigated in terms of the role of primary production and loss processes on the seasonal variation of dissolved organic matter (DOC, DON and yellow substance). It is the aim of this study to try to partially fill the gaps from previous studies, especially aspects concerning the relationships between phytoplankton dynamics and dissolved organic matter.

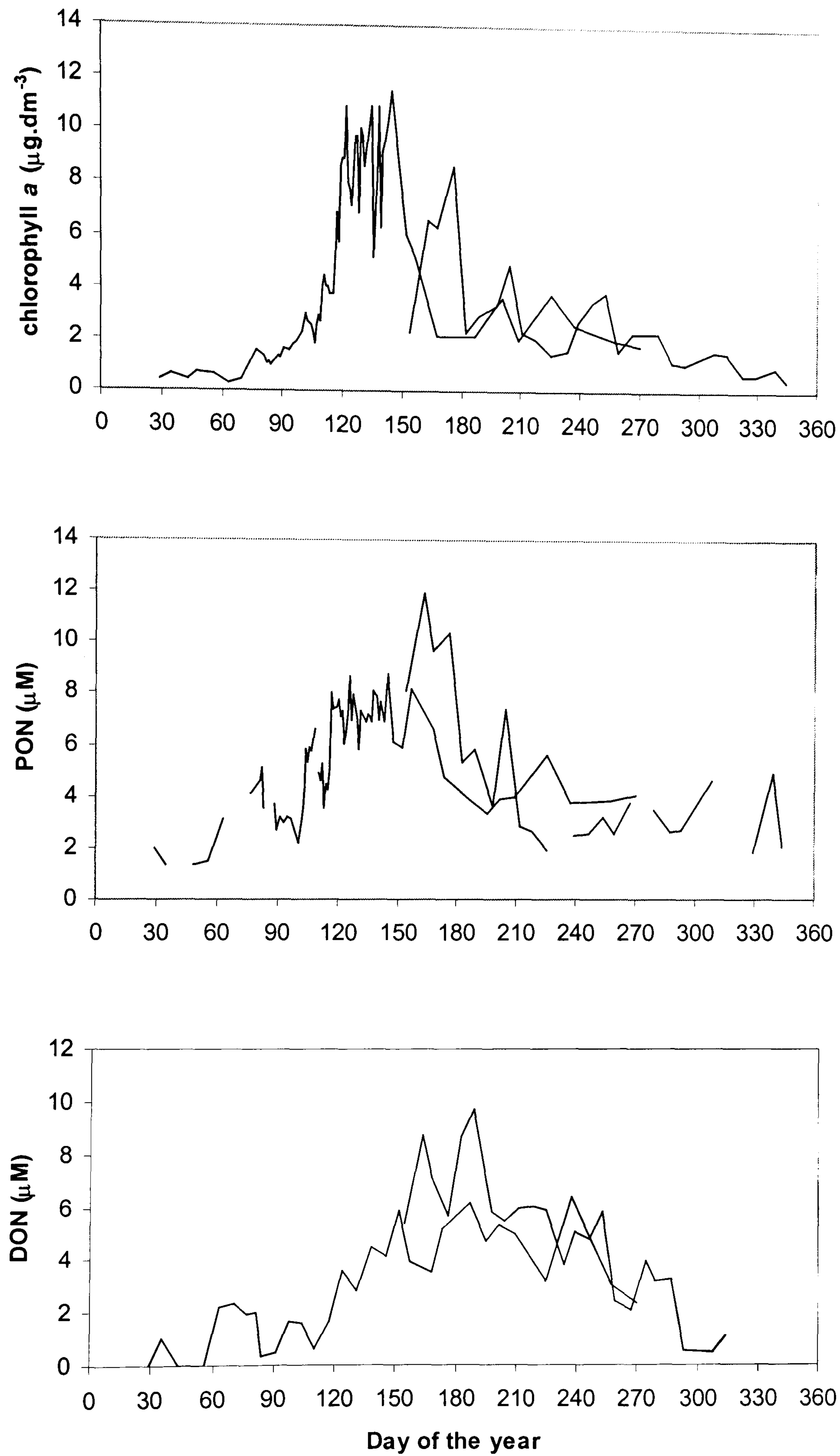
### 6.2. Discussion

#### 6.2.1. Dissolved organic carbon and nitrogen

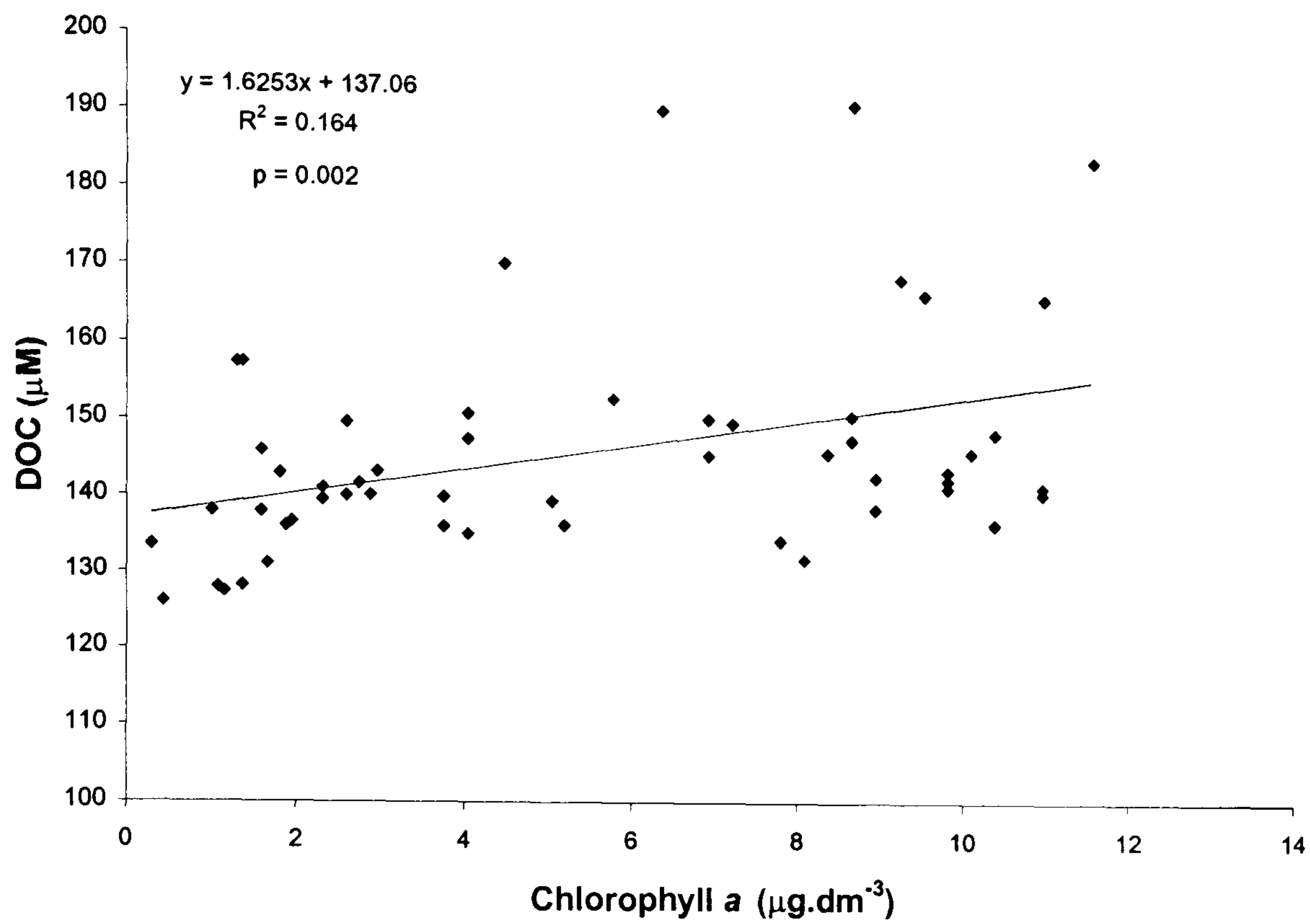
The general seasonal variability of DOC, POC, DON and PON seem to follow the variation of chlorophyll *a* (Figure 6.1 and 6.2). This is supported by significant correlations of DOC with both chlorophyll *a* and phytoplankton abundance (Figures 6.3a & 6.3b.). The correlation coefficients were low indicating a weak but significant relationship between the primary producers and organic matter.



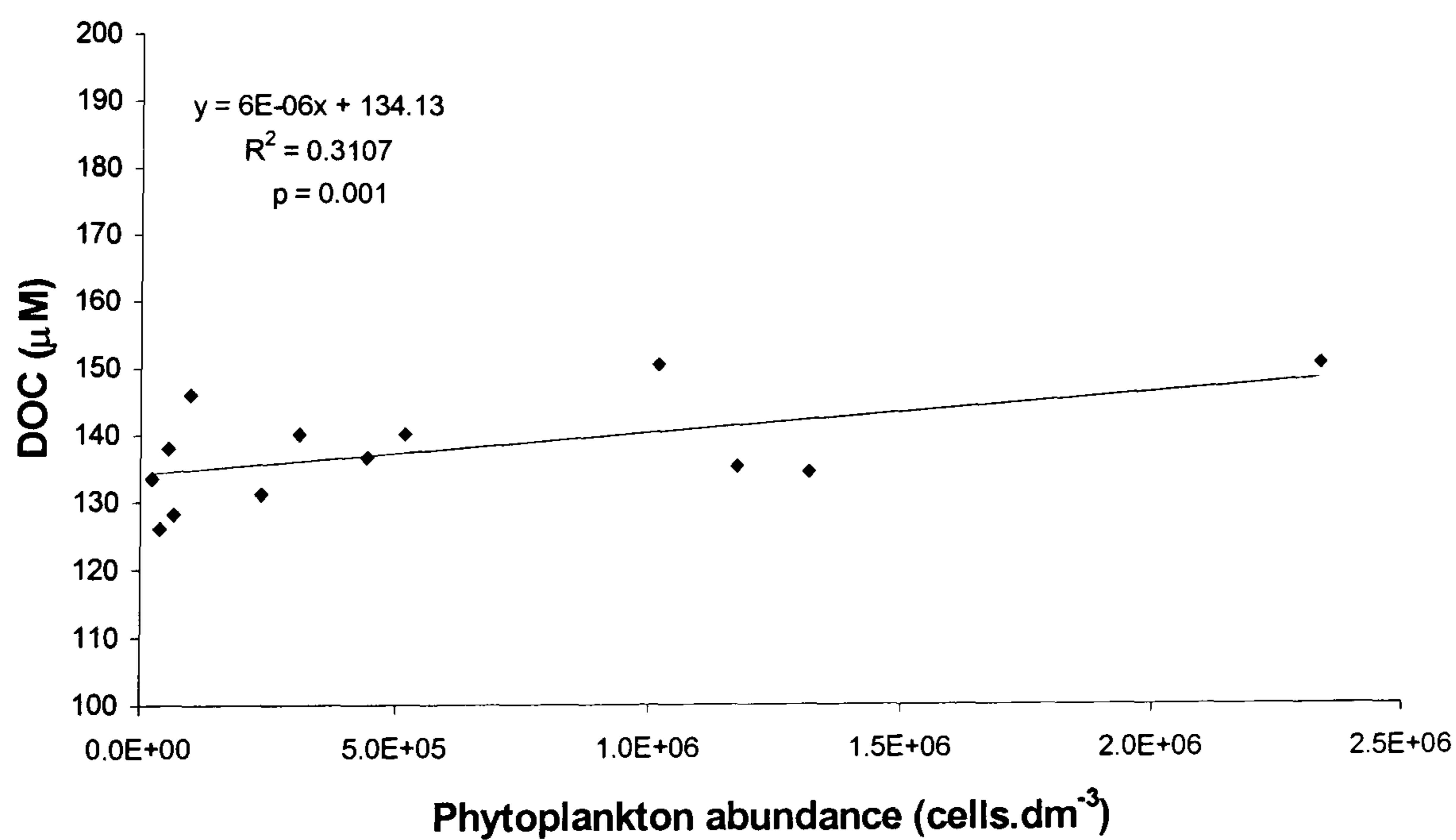
**Figure 6.1. Seasonal variation of DOC, POC and chlorophyll *a* in the Menai Strait. Showing overlapping data in 1998&1999.**



**Figure 6.2. Seasonal variation of DON, PON and chlorophyll *a* in the Menai Strait. Showing overlapping data in 1998&1999.**



**Figure 6.3a. Correlation between DOC and chlorophyll *a* during spring 1999 in the Menai Strait.**



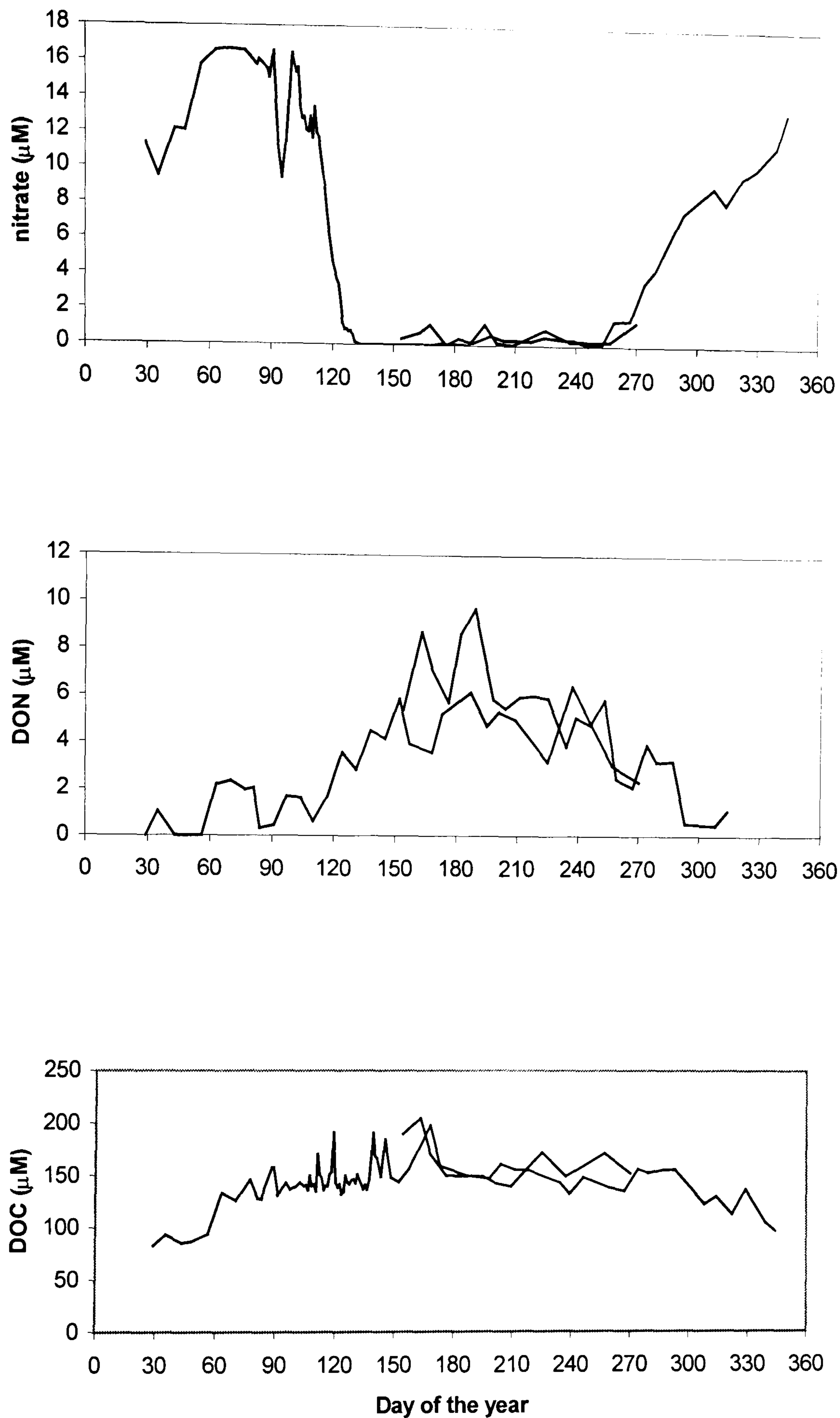
**Figure 6.3b. Correlation between DOC and phytoplankton abundance during spring 1999 in the Menai Strait.**



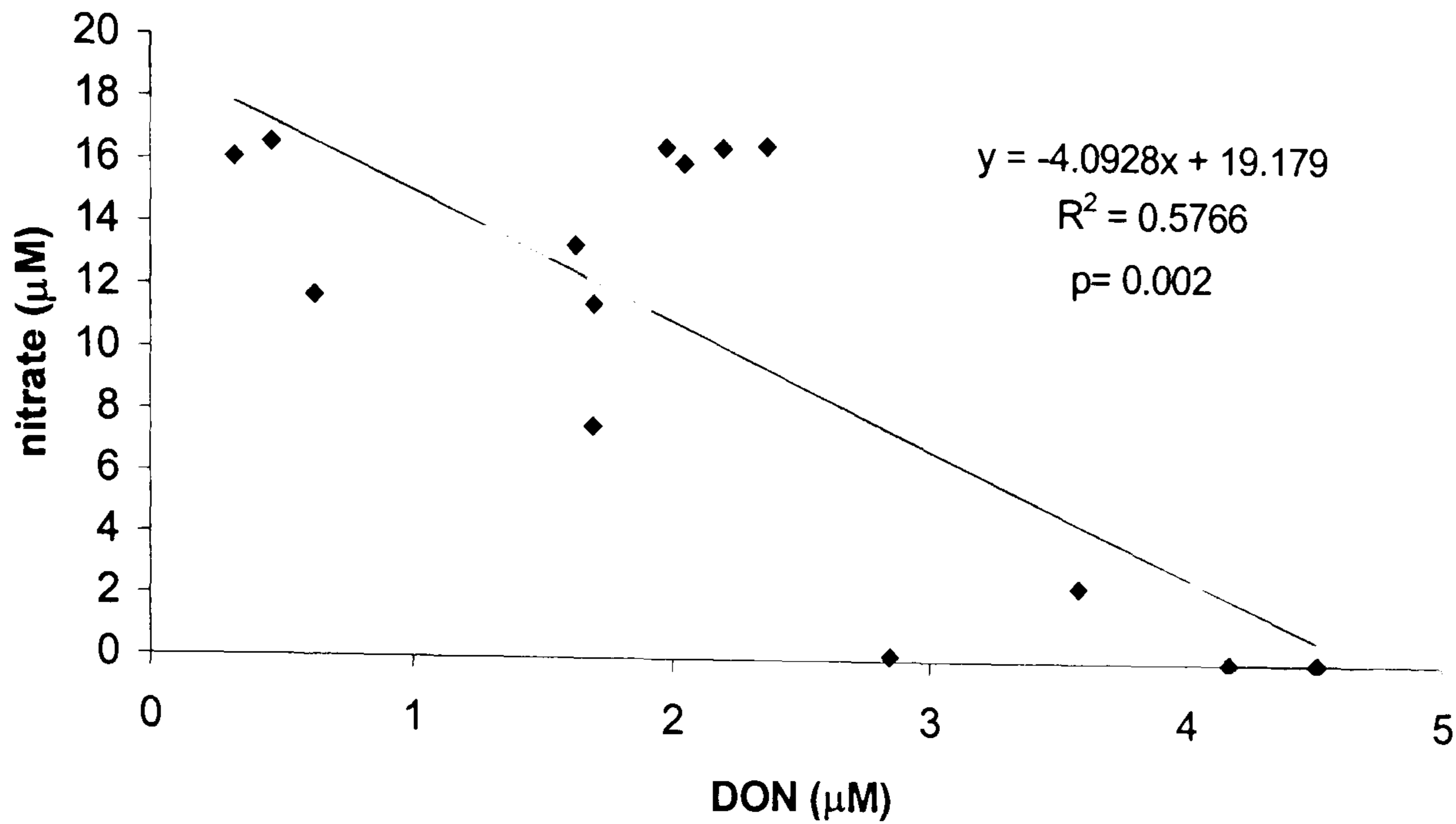
The accumulation of DOC reached a maximum during summer while DON reached maximum almost three weeks later. During this period the inorganic nitrogen was depleted in the water column (Figure 6.4) and a significant inverse correlation between DON and nitrate (Figure 6.5) was found. As a result this could have affected the consumption and production of DOC relative to DON. When nitrogen is the limiting nutrient, the dissolved organic material produced will be rich in carbon relative to nitrogen (Lancelot 1983). In addition bacteria require inorganic nitrogen to assimilate the nitrogen poor DOM (Anderson 1992). As a result the accumulation and production of DOC and DON is affected. While these correlations may suggest that the DOC produced comes from biological activities, they do not provide information on the type of dissolved organic matter produced. Alternatively information on the biolability of DOC and the production mechanisms can be gained by studying the carbon utilisation by bacteria and DOC accumulation (Carlson *et al.* 1998).

Several studies have shown the accumulation of DOC during and after phytoplankton blooms (Copin-Montegut & Avril 1993; Williams 1995; Zweifel 1999). In addition DOC accumulation has been linked to nutrient depletion (Anderson 1992; Goldman *et al.* 1992). For instance Goldman *et al.* (1992) suggest that large production of DOC results from nutrient depletion. However other studies have demonstrated increases of DOC coinciding with the increase in *Phaeocystis sp.* abundance (Billen & Fontigny 1987; Eberlein *et al.* 1985).

There was a time lag of 23 days between the DOC peak and the major peak of chlorophyll *a*, phytoplankton abundance, and second highest bacterial abundance (i.e. DOC and Chlorophyll *a*/phytoplankton/bacteria). At the same time correlation was significant between DOC and bacteria abundance and respiration and bacteria abundance as shown in Figures 6.6a&b. The significant correlation between DOC and phytoplankton abundance indicates that part of the DOC during this time of the year could have resulted from phytoplankton excretion, while the significant correlation between DOC and bacteria may indicate decomposition i.e. production by bacteria. It may also indicate response of bacteria to DOC production i.e. consumption by bacteria. Another possible source of DOC could have been from terrestrial run-off especially in coastal waters such as the Menai Strait.

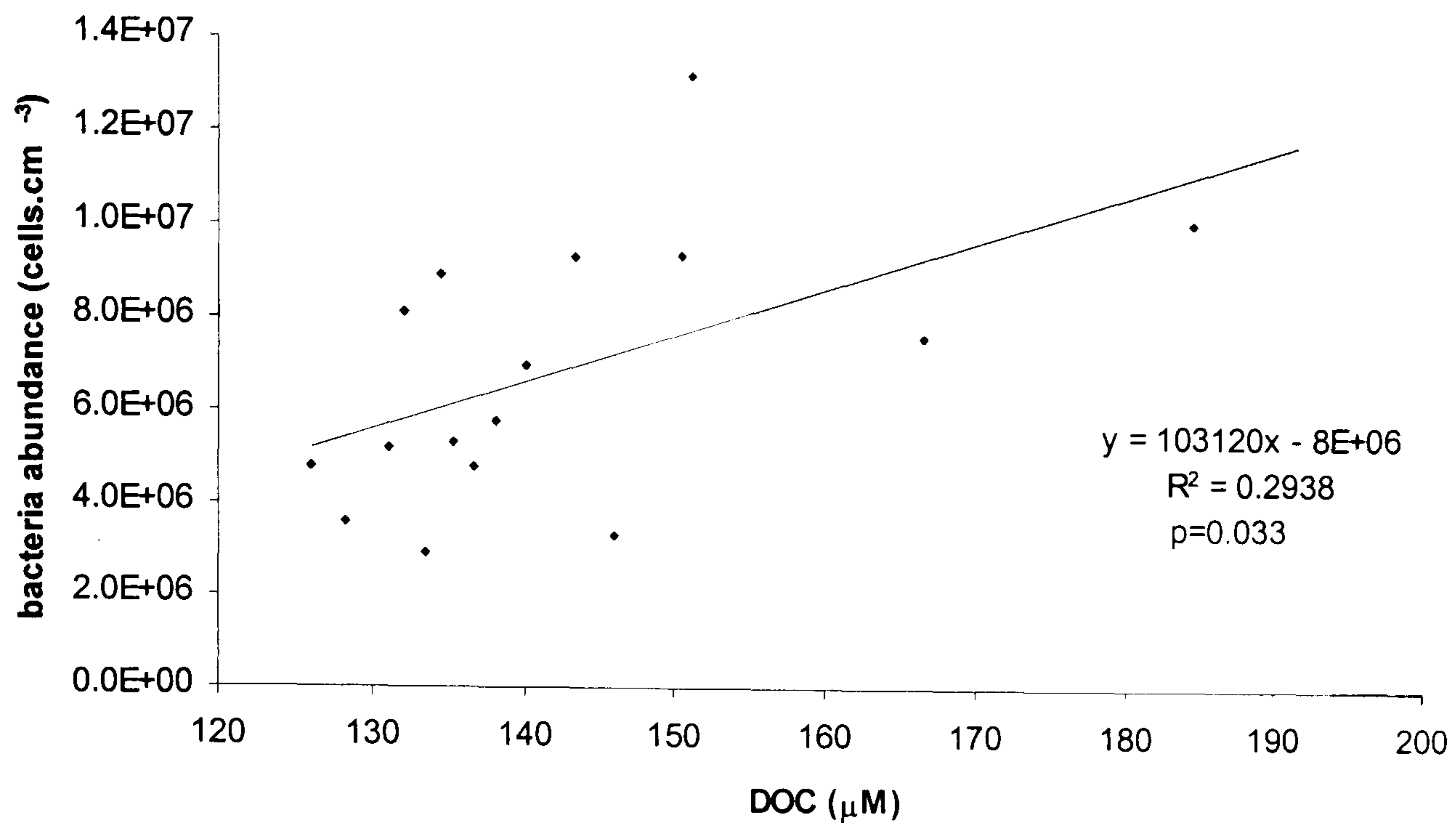


**Figure 6.4. Seasonal variation of DOC, DON and nitrate in Menai Strait. The overlapping lines represent the two years data 1998&1999. DOC&DON maxima occurred during nitrate depletion.**

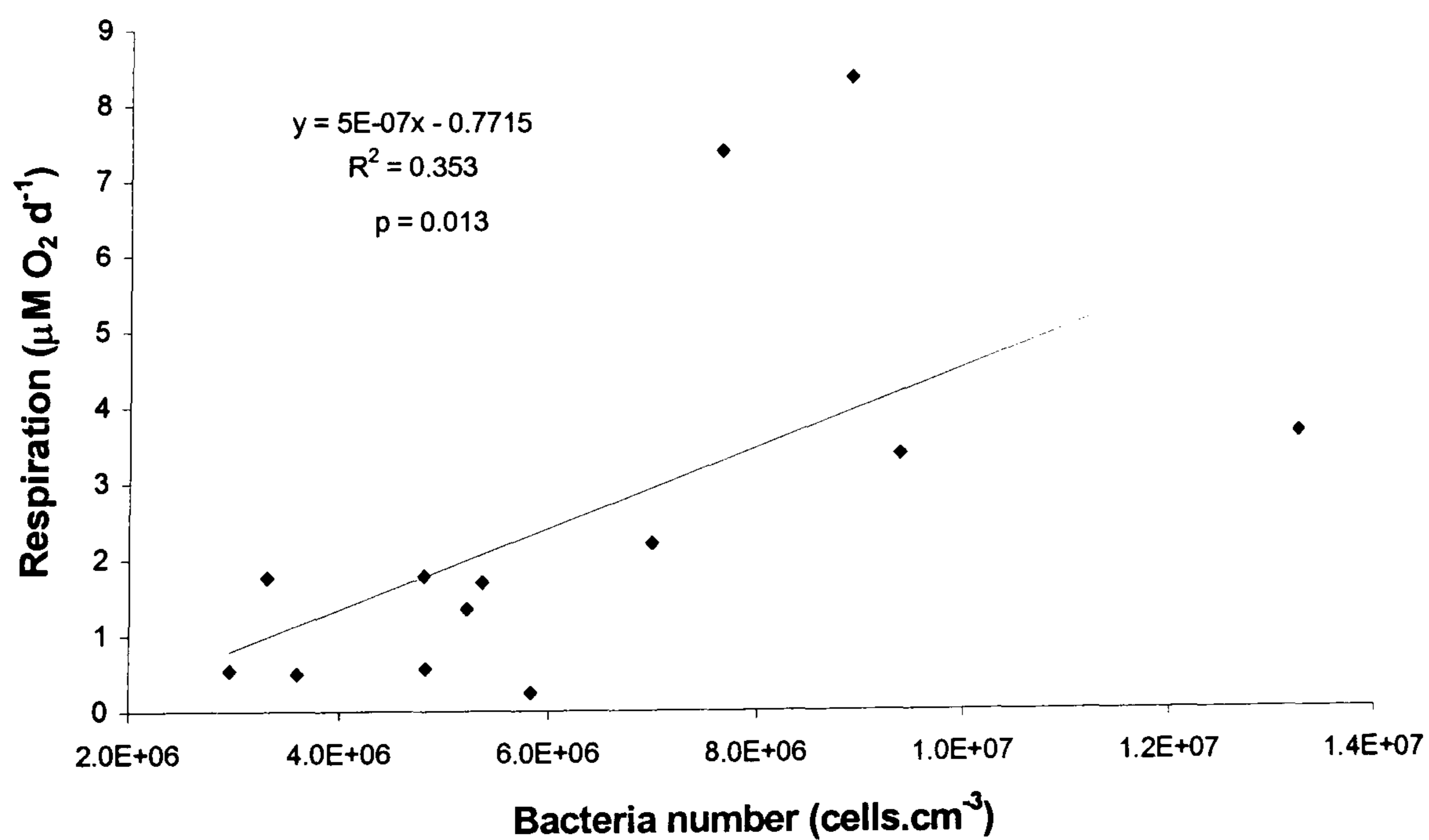


**Figure 6.5. Correlation between DON and nitrate during spring 1999 in the Menai Strait.**

No correlation was found between DOC and salinity; therefore terrestrial runoff as an important source of DOC in the Menai Strait was disregarded. Nevertheless, identifying the sources of DOC in coastal regions is complex due to the different processes involved. For example, Sondergaard *et al.* (2000) suggested that the seasonal variation and accumulation of DOC depended on biotic and abiotic mechanisms such as biological production, input of DOM from terrestrial origin and also the biodegradability of the newly-produced DOC. In addition the newly-produced DOC is comprised of a labile and refractory DOC pool. However the fraction that each of these pools represents in the total DOC pool is still debatable. A number of studies (Ogura 1972; Fry *et al.* 1996; Carlson & Ducklow 1996) suggest that at least part of the labile DOC is degraded in a very short time (days to weeks). This fraction represents one fifth of the total DOC pool in seawater (Sondergaard & Middelboe 1995). Zweifel (1999) suggests that the accumulation of DOC in estuarine and coastal waters is mainly influenced by input from rivers which indicates that the refractory fraction of DOM dominates in the coastal region. However the influence of river input in the Menai Strait proved to be weak through correlation between DOC and salinity, indicating that the major source of DOC originates from biological activity within the Menai Strait. The possibility cannot be ruled out that river input could influence the DOC in the Menai Strait through the water coming from Liverpool Bay and Irish Sea.



**Figure 6.6a. Correlation between DOC and bacterial abundance during spring 1999 in the Menai Strait.**



**Figure 6.6b. Correlation between bacterial abundance and respiration during spring 1999 in the Menai Strait.**

As mentioned previously (chapter V), the phytoplankton composition and succession in the Menai Strait is characterised by the dominance of small-sized diatoms at the beginning of spring followed by a peak of large diatoms and flagellates in early summer. This could have an influence on the seasonal accumulation and assimilation of DOC in this study. As shown previously (Figure 6.3a&6.6a), there was a close coupling between primary production and consumption from the correlation of DOC with both phytoplankton and bacterial abundance. Blight *et al.* (1995) suggested that during the diatom-dominated peak, the organic matter produced was assimilated quickly thus increasing bacterial abundance. Carlson *et al.* (1998) and Sondergaard *et al.* (2000) suggested that the most labile DOC originated from autotrophic communities dominated by phytoplankton. This could be the case in the Menai Strait during this study. A possible scenario is: at the beginning of spring, labile DOC from diatom-dominated communities accumulated with the increase in phytoplankton biomass, and could be influenced by nutrient availability and bacterial uptake. The increase in phytoplankton abundance depleted nutrients and triggered *Phaeocystis* bloom at the end of the spring season. This species is known to release polysaccharides (HMW organic matter). The production of polysaccharides could also be associated with nutrient depletion in diatom-dominated communities (Guerrini *et al.* 1998). A loose coupling occurred during the period of *Phaeocystis* blooms indicating that organic matter (HMW) was not immediately metabolised, due to its resistance to degradation, and accumulated reaching a major peak during summer.

Bacterial numbers also decreased after the *Phaeocystis* bloom. This could be the result of low nitrogen or chemicals released by *Phaeocystis* which inhibit bacteria metabolism. In fact, several studies have reported the effect of *Phaeocystis* blooms on bacterial growth. Sieburth (1960) reports that *Phaeocystis* release acrylic acid which has antimicrobial properties. Eberlein *et al.* (1985) suggested that the accumulation of dissolved organic substances during a *Phaeocystis* bloom in the North Sea may have been due to low decomposition by bacteria whose activity could have been reduced by acrylic acid. Lancelot (1984) and Thingstad & Billen (1994) suggested that the high molecular weight organic matter produced by *Phaeocystis* could limit the bacterial growth.

The presence of the acrylic acid has been reported in the Menai Strait (Al-Hasan *et al.* 1975). Therefore it is possible that the *Phaeocystis* bloom could have inhibited bacterial nitrogen uptake in order to minimise competition for inorganic nutrients since at this time nitrogen was almost below detection. However other studies (Putt *et al.* 1994; Slezak *et al.* 1994) suggest that acrylic acid concentrations are too low to inhibit phytoplankton growth. Therefore it seems that nutrient limitation is the major factor effecting bacterial activities during the *Phaeocystis* bloom and accumulation of DOC. This could be possible since the major peak of DOC in this study occurred after the major peak of phytoplankton indicating the decline in bacterial uptake of DOC or possibly an increase in the rate of DOC production.

It could be assumed that the bacterial demand of inorganic nitrogen was easily met in early spring since the organic matter produced by diatoms was possibly mainly LMW (Amon & Benner 1996). During summer a number of mechanisms may contribute to the production of HMW. These include cell lysis, sloppy feeding by grazers and HMW organic matter produced by *Phaeocystis* (Kirchman 1994). The LMW pool is N-rich thus the C:N ratio is relatively low and the pool is turned over in a matter of hours. In contrast the HMW pool is C-rich compounds with high C:N ratios and can take days to weeks to be turned over (Lancelot 1984; Amon & Benner 1996). The LMW materials support high bacterial growth efficiencies, while HMW compounds support high rate of bacterial respiration (Amon & Benner 1996; Gardner *et al.* 1996).

Bacteria need to assimilate nitrogen from the environment in order to utilise the HMW. On the contrary the LMW materials produced at the earlier stages of phytoplankton growth may not require extensive utilisation of nitrogen by bacteria since these compounds are already N-rich and have C:N ratios close to that of these organisms. In addition heterotrophic bacteria are thought to be rich in nitrogen compared with phytoplankton (Kirchman 2000). The C:N ratios of marine bacteria, which varies between 3.8 to 9.9 (Fukuda *et al.* 1998), are important in order to understand the contribution of bacteria in assimilating and releasing ammonium (Kirchman 2000).

Amon & Benner (1994) reported the nutrient dynamics between HMW and LMW DOM. These authors found that in an incubation of HMW DOM, inorganic nitrogen rather than organic nitrogen sources were used as the primary source of nitrogen for bacterial growth. By contrast in LMW DOM incubation there was net regeneration of inorganic nitrogen indicating that LMW DOM was relatively rich in organic nitrogen. In addition, nitrate was the major determinant of bacterial growth efficiencies and could result in relatively low bacterial growth because of the high-energy demand associated with nitrate assimilation. In contrast, organic nitrogen supported relatively high bacterial growth in LMW DOM.

This may explain the situation found in the Menai Strait. In early spring the phytoplankton bloom was dominated by diatoms, there was an increase in primary production associated with low respiration. This period is characterised by low nitrogen uptake (Rodrigues 1998) and increase in bacterial abundance, indicating that the organic matter passed to bacteria appeared to be LMW DOM. During the late stage of the spring bloom, respiration increased, as did bacterial number indicating that the pool of organic matter available for bacteria was HMW. This was followed by a decline in bacterial number which could be due to the depletion of inorganic nitrogen and also other factors such as grazing. During summer ammonium regenerated rapidly and primary production, along with other non-living organic matter, might have supported bacterial growth. In addition, the high nitrogen uptake during summer (Rodrigues 1998) indicates that the HMW DOM was the dominant source of organic matter available for bacteria metabolism. No size fractionation of the phytoplankton community was conducted in this study. However the contribution of pico- and nanoplankton could have influenced the total DOC accumulation in the Menai Strait. Bjornsen (1988) suggested that the release of DOC by phytoplankton is significantly correlated with biomass and ratio of the surface area versus the volume of alga. Therefore, the smaller the alga, the higher the production of DOC. Huang *et al.* (1999) also reported high DOC production in association with the dominance of nano- and picoplankton in the Taiwan Strait. This DOC production accounted for 24.8% of the total.

### 6.2.2. Contribution of DOC&POC to TOC

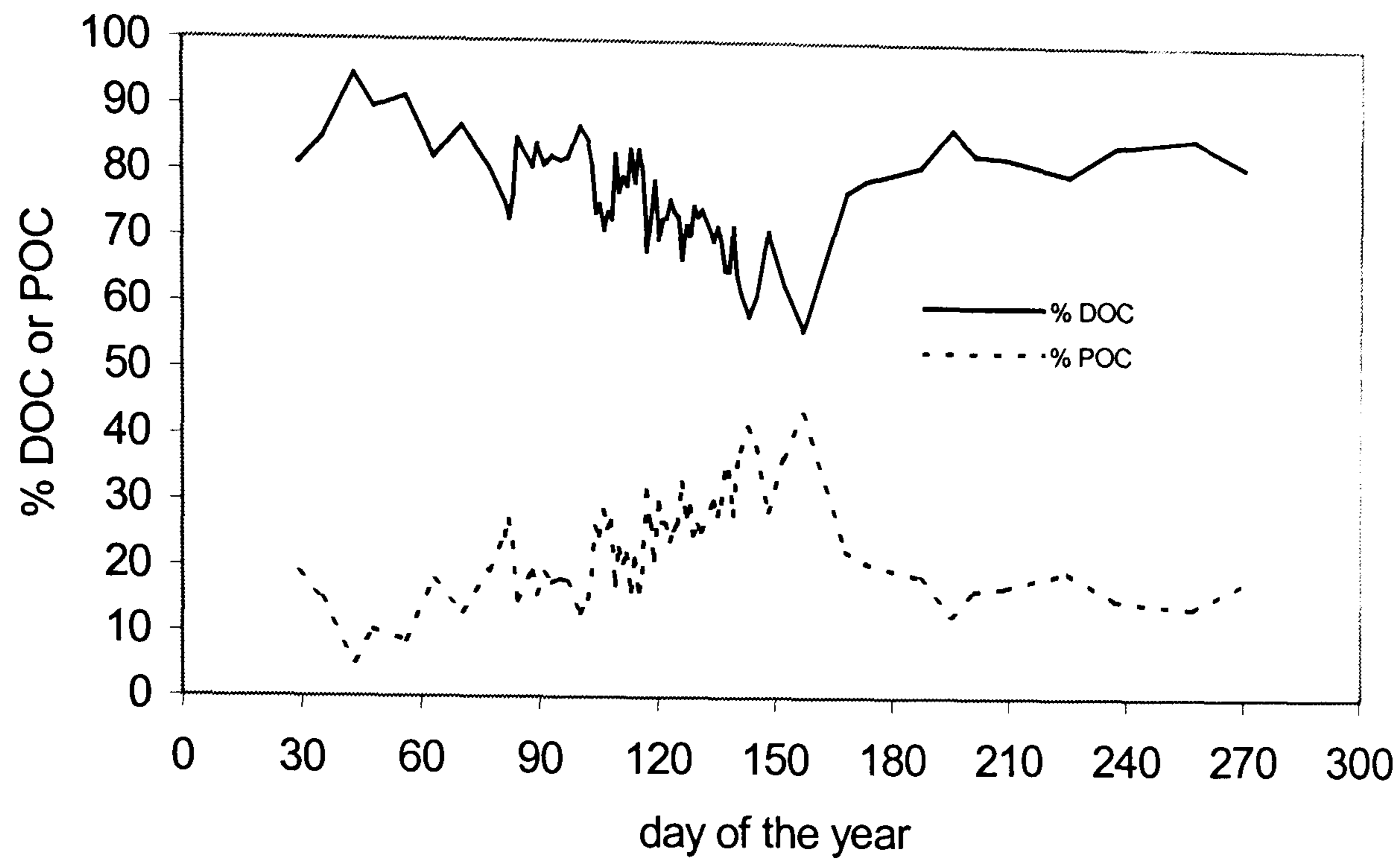
Although the size fractionation of DOC was not conducted in this study, the low molecular weight and colloidal fraction accounted between 56% and 95% of total organic carbon. In contrast particulate organic carbon (POC) contributed with a relatively small fraction < 45% of TOC (Figure 6.7). This could be an indication that bloom productivity is sometimes underestimated when it is defined from the measurements of POC. On the other hand the contribution of dissolved organic nitrogen (DON) and particulate organic nitrogen (PON) exhibited different pattern from that of TOC. During the spring bloom PON contributed about 60 to 90% of the TON (Figure 6.8), whilst during the post bloom DON contributed more than PON (35 to 76%).

### 6.2.3. Respiration and size-fractionated C:N ratio

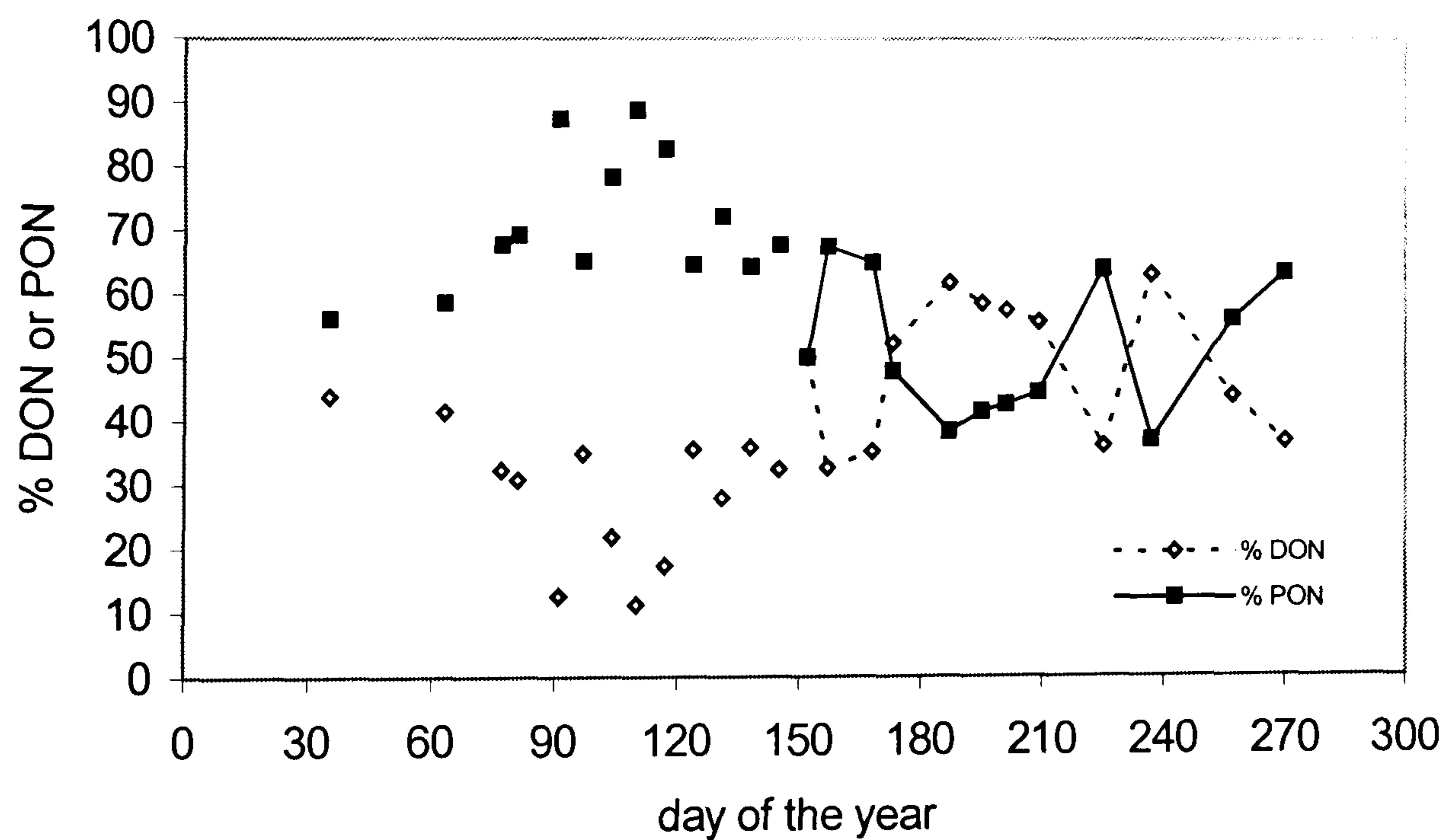
During the bloom the C:N of the DOC and POC appeared to be unrelated to the community respiration (Figure 6.9a&b). However the two parameters were significantly correlated,  $R^2=0.28$ ,  $p=0.02$  and  $R^2=0.20$ ,  $p<0.021$  respectively (Figure 6.10a&b). The correlation between C:N of TOM and respiration ( $R^2=0.25$ ,  $p=0.01$ ) appeared to be the function of both DOM and POM despite the differences in the timing of the peaks in relation to community respiration. These weak correlations may indicate the indirect relationship between the production of DOC and POC and bacteria consumption. This is influenced by the fate of organic matter produced as well as other parameter such temperature and nutrients which could have significant impact on the production and consumption of organic matter.

The data in Figures 6.9a&b and the correlations in Figures 6.10a&b may indicate that respiration somehow contributed to the changes of the C:N of POM and DOM. This could be through mineralization of organic matter produced by phytoplankton. The POM and DOM showed high C:N ratios during the study period. The C:N of POM was higher than the Redfield ratio especially before the bloom, followed by a decline during the bloom. This could be an indication that other sources of organic





**Figure 6.7. Percentage contribution of dissolved and particulate organic carbon to total organic carbon (TOC) in 1999.**



**Figure 6.8. Percentage contribution of dissolved and particulate organic nitrogen to total organic nitrogen (TON) in 1999.**

matter contribute to the carbon content during this period thus decreasing the C:N ratio.

The particulate organic matter produced by phytoplankton is broken down to DOM which in turn is respired to CO<sub>2</sub> and the inorganic nitrogen is released. Input of inorganic nitrogen from remineralisation increases the inorganic nitrogen content in the water thus reducing the C:N ratio. The processes of breaking down organic matter and remineralisation increase the carbon and inorganic nitrogen content in the water column thus reducing the C:N ratio. This is supported by the increase in respiration and decrease of C:N ratio of different fraction of organic matter. This is also supported by the significant correlation between respiration and C:N ratios.

During the bloom the heterotrophic organisms are not necessarily relying on the carbon produced by decomposition. In addition the increase in DOC was not reflected immediately in the bacterial abundance. However a significant correlation between DOC and bacterial abundance (Figure 6.6a) and bacteria and respiration (Figure 6.6b) was found.

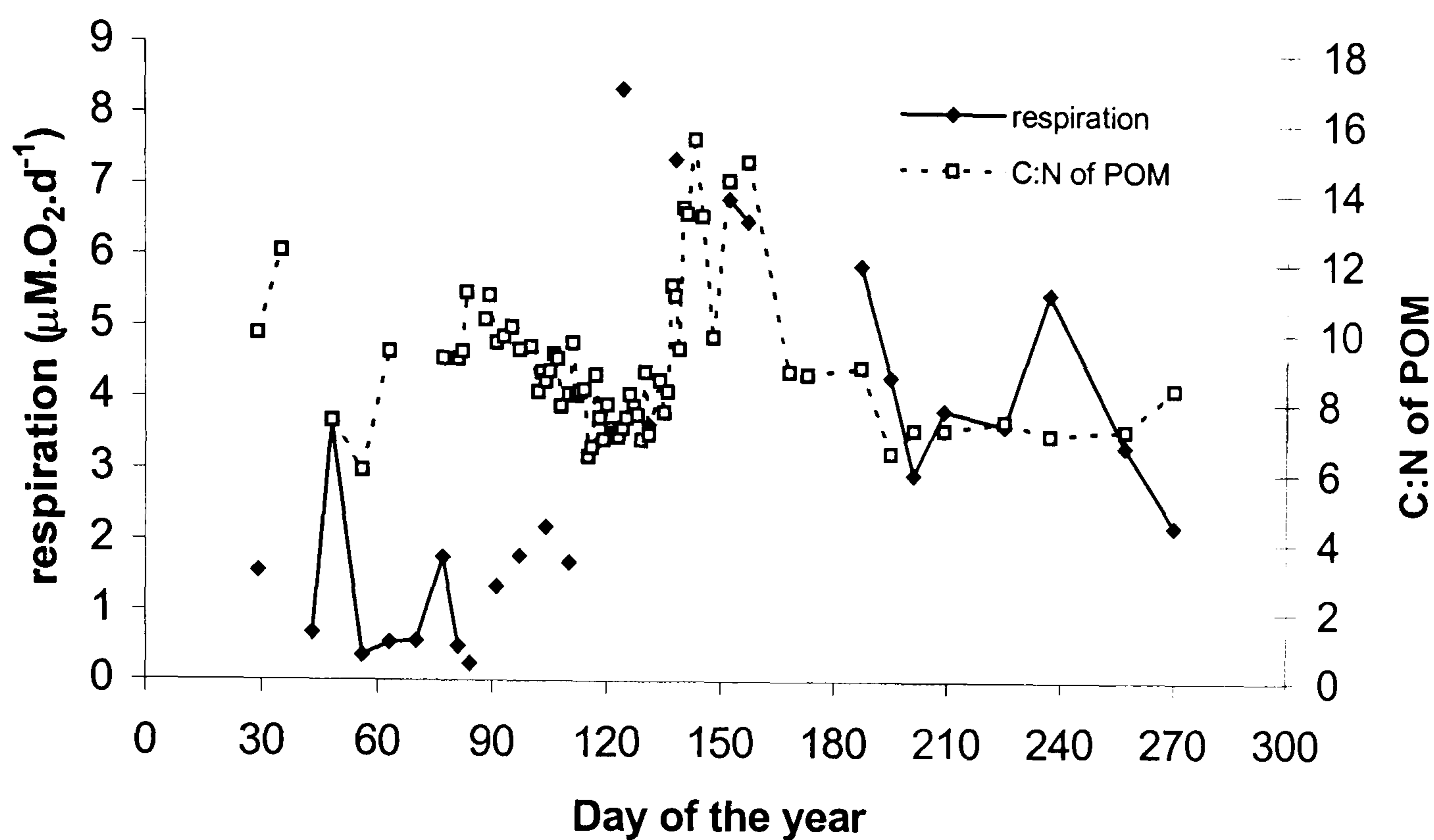


Figure 6.9a. . Seasonal variation of C:N of particulate organic matter (POM) in relation to respiration in the Menai Strait in 1999.

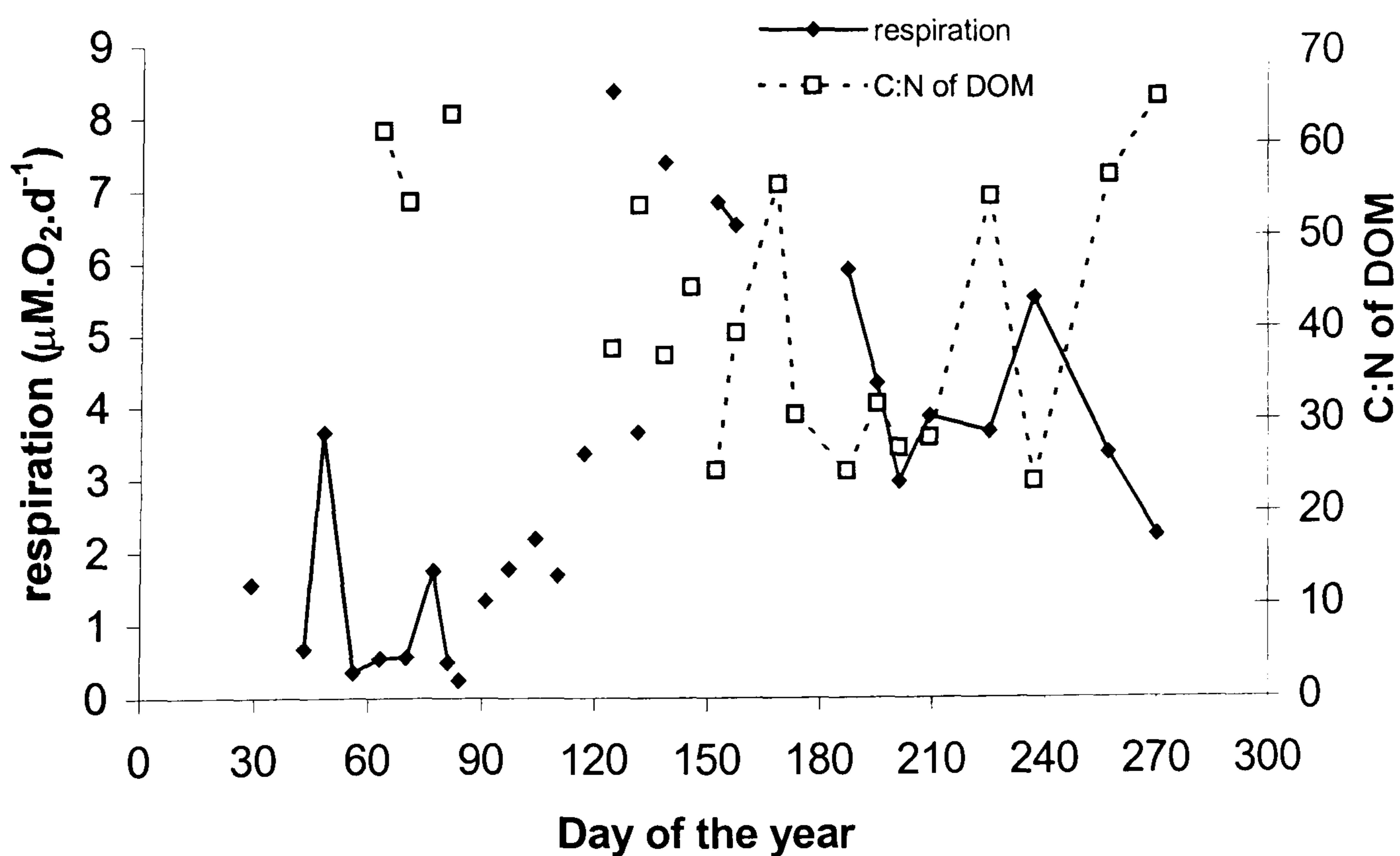
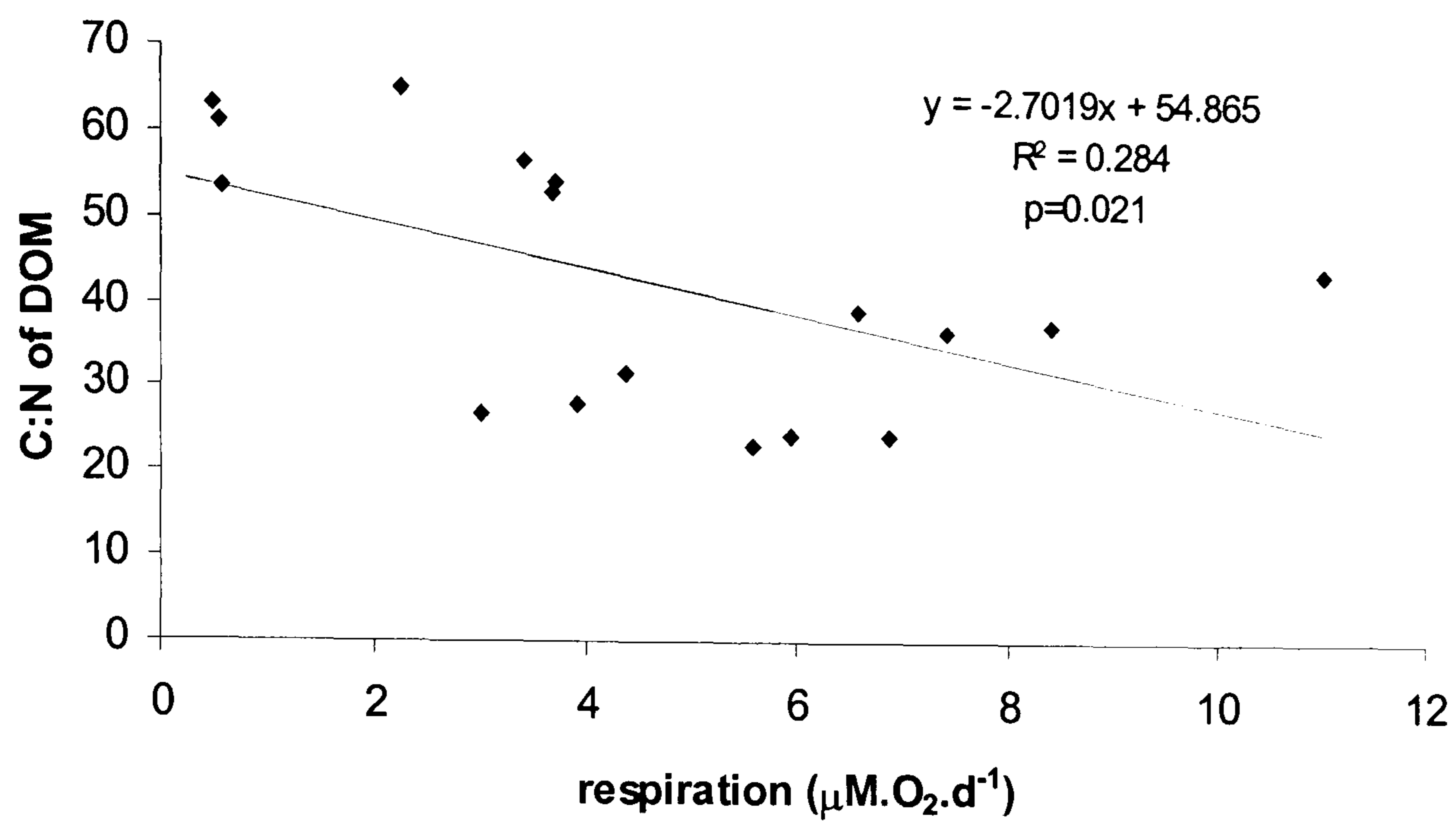
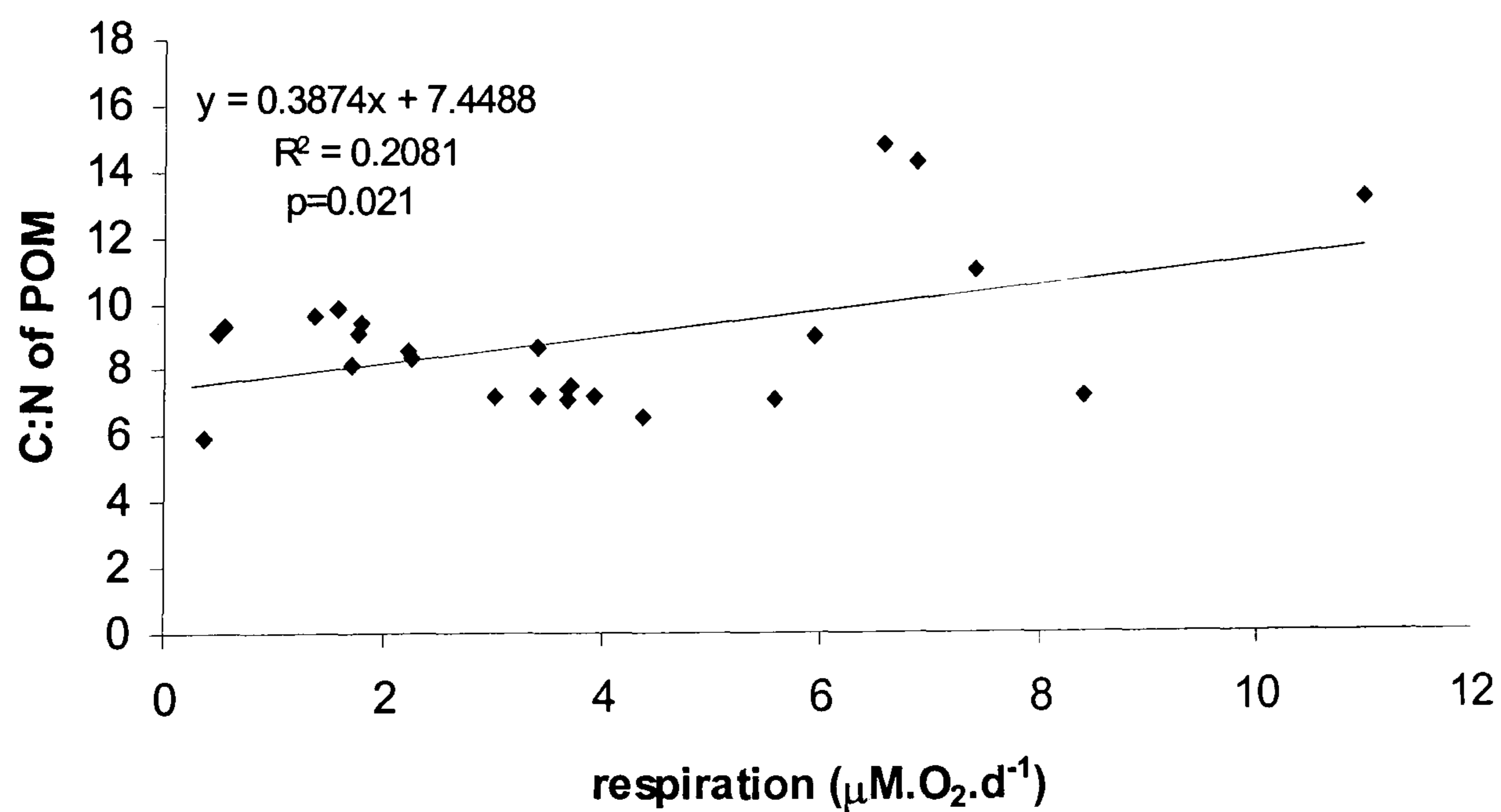


Figure 6.9b. Seasonal variation of C:N of dissolved organic matter (DOM) in relation to respiration in the Menai Strait in 1999.



**Figure 6.10a. Correlation between C:N of DOM and respiration in the Menai Strait in 1999.**



**Figure 6.10b. Correlation between C:N of POM and respiration in the Menai Strait in 1999.**

For example the input of organic matter from macroalgae and sediments was not assessed in the Menai Strait. Khailov & Burlakova (1969) reported that the total release of DOM by live seaweeds is about 37% of gross production and the chemical nature of the macrophyte is diverse (molecular weight from 200 to 5000). In addition the organic matter produced by seaweeds may be directly used by bacteria populations. Hough (1976) reported that macroalgae release significant amount of DOC and the rate of release tended to be lower in high oxygen rather than in low oxygen condition.

Despite the fact that the measurement of particulate and dissolved organic matter in this study provides a picture of DOM in relation to primary production, it is difficult to be sure which fraction of the DOM contributed to the high C:N ratios. Consequently it is also difficult to know to what extent other organisms contribute to primary production and degradation of organic matter during the phytoplankton bloom. Bacteria showed a significant correlation with respiration (Figures 6.6b). However the bacteria were not the only micro-organisms involved. Zooplankton, protozoa (Kepkay & Jonhson 1989) and phytoplankton (Langdon 1993) are also important components of total community respiration.

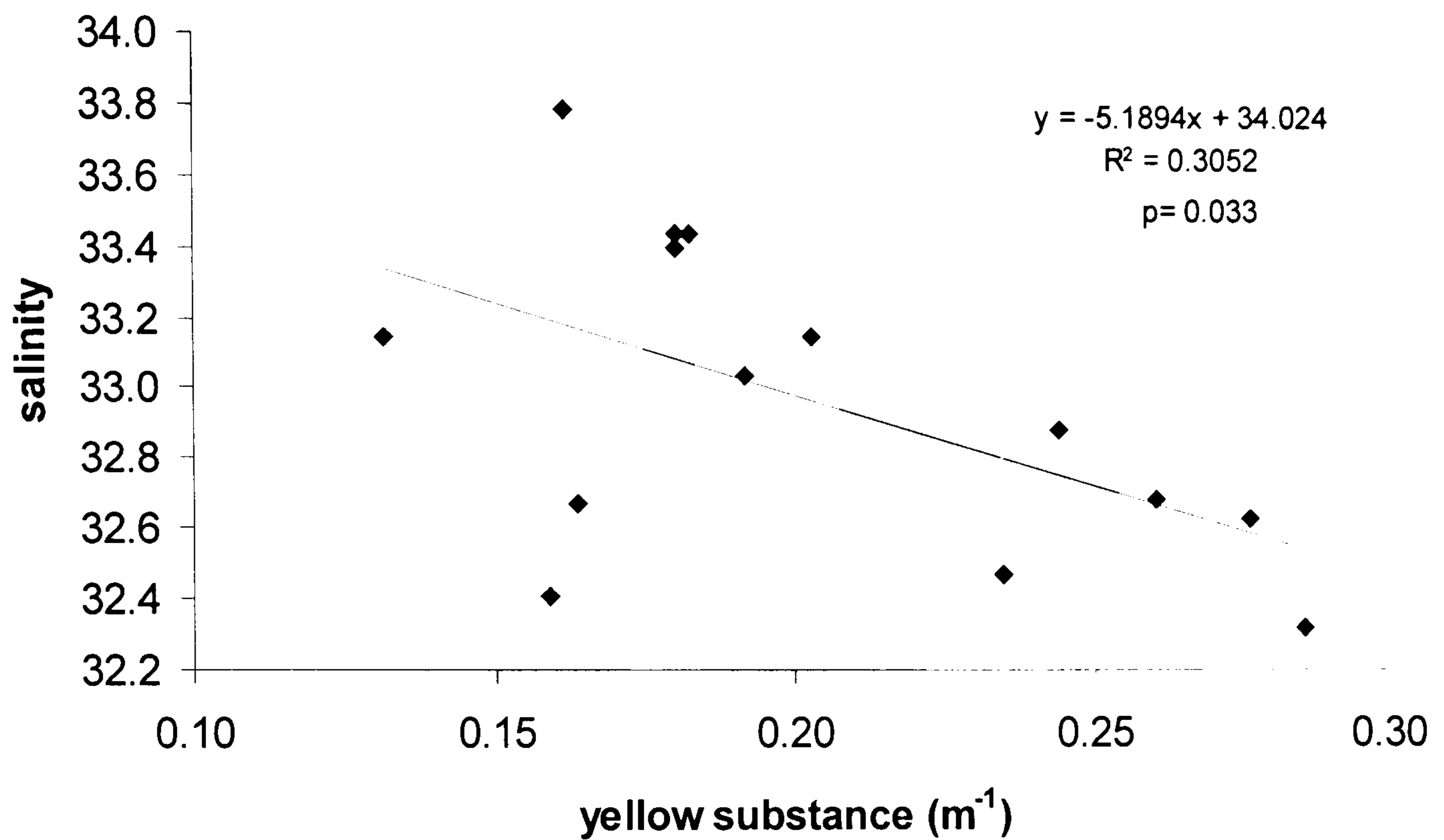
#### **6.2.4. Yellow substance**

There are a number of hypotheses related to the source and distribution of yellow substances. For instance, in coastal areas this fraction of dissolved organic matter is known to originate from terrestrial run-off (Fogg & Boalch 1958; Harvey *et al.* 1983). It is thought also to originate from primary production as a by-product (Bricaud *et al.* 1981) with the remainder of terrestrial origin (Meyers-Schulte & Hedges 1986). In addition it is difficult to differentiate between the two types of yellow substance.

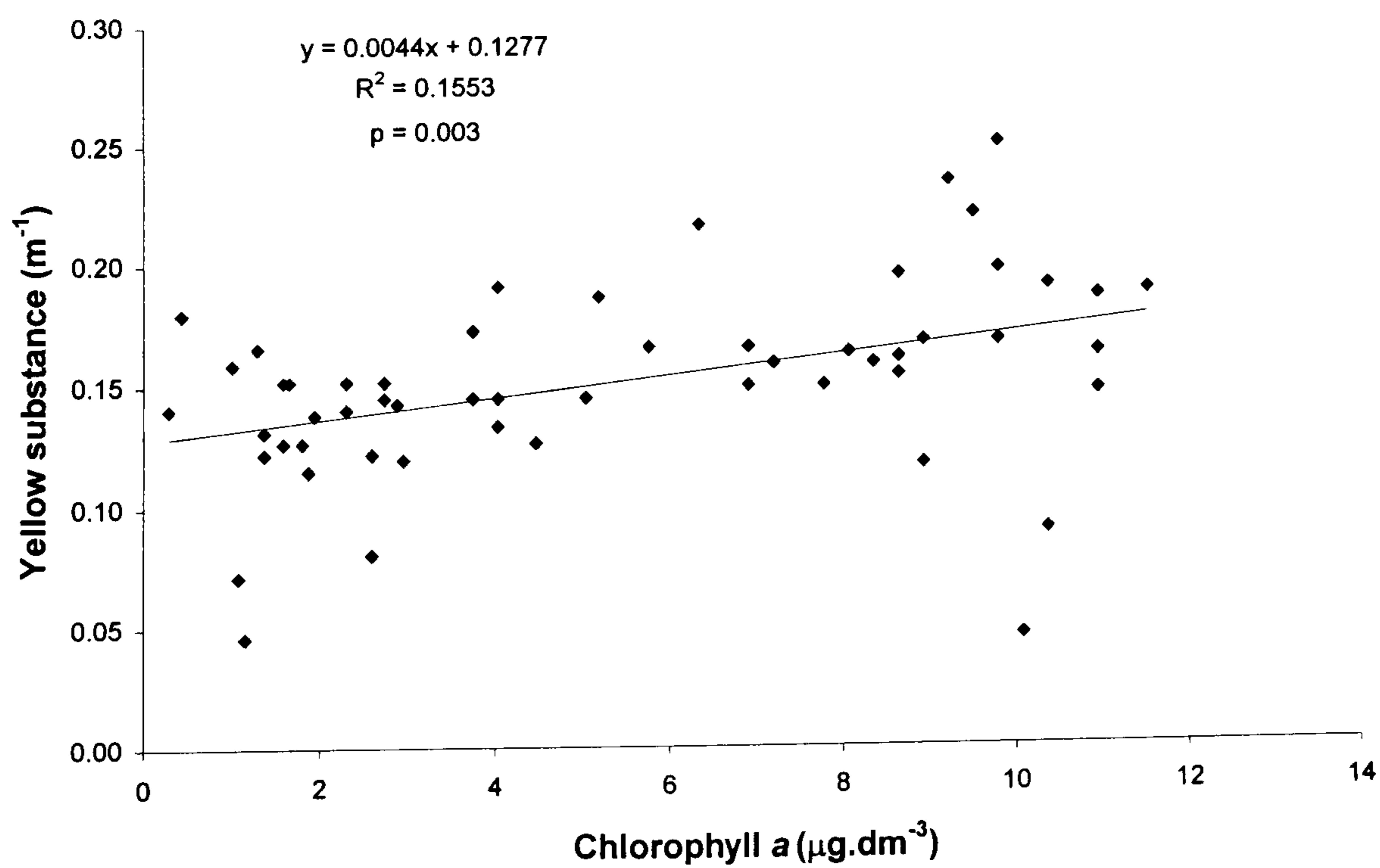
A coastal area such as the Menai Strait could be influenced by both sources. However no correlation was found between salinity and yellow substance (for the entire data) to indicate the influence of freshwater input. However a significant

correlation between the two parameters was found in autumn (Figure 6.11) indicating that freshwater input may have influenced yellow substance concentration during autumn.

Looking at the seasonal pattern of yellow substance and chlorophyll *a* it seems that the increase of yellow substance followed that of chlorophyll *a*. However a lack of correlation between yellow substance and chlorophyll *a* and/or net community production (entire data) and a weak significant correlation during spring (Figure 6.12) suggests that yellow substance is not directly produced by phytoplankton during spring bloom. Similar observations were reported by Kratzer *et al.* (2000). Their study showed an increase in chlorophyll along with yellow substance in Menai Strait waters. However the two parameters did not correlate.



**Figure 6.11. Correlation between yellow substance and salinity in autumn 1998 in the Menai Strait.**



**Figure 6.12. Correlation between yellow substance and chlorophyll *a* during spring.**

Rochelle-Newall *et al.* (1999) reported a lack of correlation between chlorophyll and yellow substance in their mesocosm experiment. Carder & Steward (1989) observed a lack of correlation between phytoplankton biomass and yellow substance and suggested that an increase in phytoplankton does not have an immediate influence on yellow substance concentration. This could be the case in the present study. There was a time lag between the chlorophyll a maximum and/or bacterial abundance and yellow substance. In early spring, yellow substance showed an increase from winter values while chlorophyll a remained low. From April, a sharp increase in chlorophyll a did not necessarily have direct influence on yellow substance. However the high concentration of yellow substance during summer could still be explained as a result of phytoplankton production and bacterial activity.

The maximum bacterial abundance and respiration occurred almost two weeks after the maximum NCP and coincided with the major phytoplankton abundance. The increase of bacterial abundance may have been influenced by the labile DOM mostly originated from diatoms production. Subsequently the *Phaeocystis* bloom coincided with inorganic nitrogen depletion. It is assumed that the organic matter produced during this time is HMW and nitrogen poor. This fraction of yellow substance (refractory) remained in water for sometime before it degraded, thus allowing the maximum accumulation during summer. A link between microbial activity and yellow substance distribution has been found in other studies (Carlson & Ducklow 1996).

From their bacterial culture experiment Carlson & Ducklow (1996) reported that a decrease of about  $19\mu\text{M}$  of DOC occurred over a two week period, while yellow substance showed an increase of about  $0.3\text{m}^{-1}$  in the first 48 hours followed by a decrease of about  $0.2\text{m}^{-1}$  over the next 30 days. This suggests a direct link between microbial activities and yellow substance. During summer, yellow substance in this study exhibited fluctuation and a general decrease occurred. The decline of yellow substance during summer could have been associated with various factors. For example, the exposure to high irradiance during this time of the year could have resulted in changing the DOM from high molecular weight to low molecular weight with the latter assimilated by bacteria (Kouassi & Zika 1990; Doney *et al.* 1995;



Siegel & Michaels 1996). In addition it has been suggested that during summer the fraction of yellow substance (refractory) which resisted microbial breakdown could be adsorbed to smaller inorganic particles which bind together and sink to the bottom (Kratzer *et al.* 2000). This could result in a decline of yellow substance in mid-summer.

Looking at the seasonal trends of yellow substance and bacterial abundance, especially during summer, it seems that the accumulation of yellow substance followed that of bacterial abundance (Figure 6.13). Consequently yellow substance and DOC did not co-vary in this study (Figure 6.14) as might be expected if total DOC is a substrate for microbial production of yellow substance. This observation is in accordance with that of Nelson *et al.* (1998).

This suggests that either the proportion of the yellow substance to the total DOM pool is small and its production is not limited by the total DOM (Siegel & Michaels 1996) or there are other processes which reduce the yellow substance more rapidly than its production. Kopelevich & Burenkov (1977) suggested that yellow substance had a labile component that originated from the recent decomposition of organic matter, and a refractory component which resisted microbial breakdown. Carlson *et al.* (1996) suggested that the production of yellow substance was a function of the semi-labile or labile fraction of DOM.

During summer, a period of high irradiance, photooxidation may be responsible for low concentrations of yellow substance (Siegel & Michaels 1996), thus changing the HMW DOM to LMW DOM which is assimilated by bacteria (Kouassi & Zika 1990; Doney *et al.* 1995; Siegel & Michaels 1996). A significant correlation was found between bacterial abundance and yellow substance but not with DOC during summer (Figure 6.15). It is clear from Figure 6.14 that yellow substance declined during summer while DOC remained fairly stable.

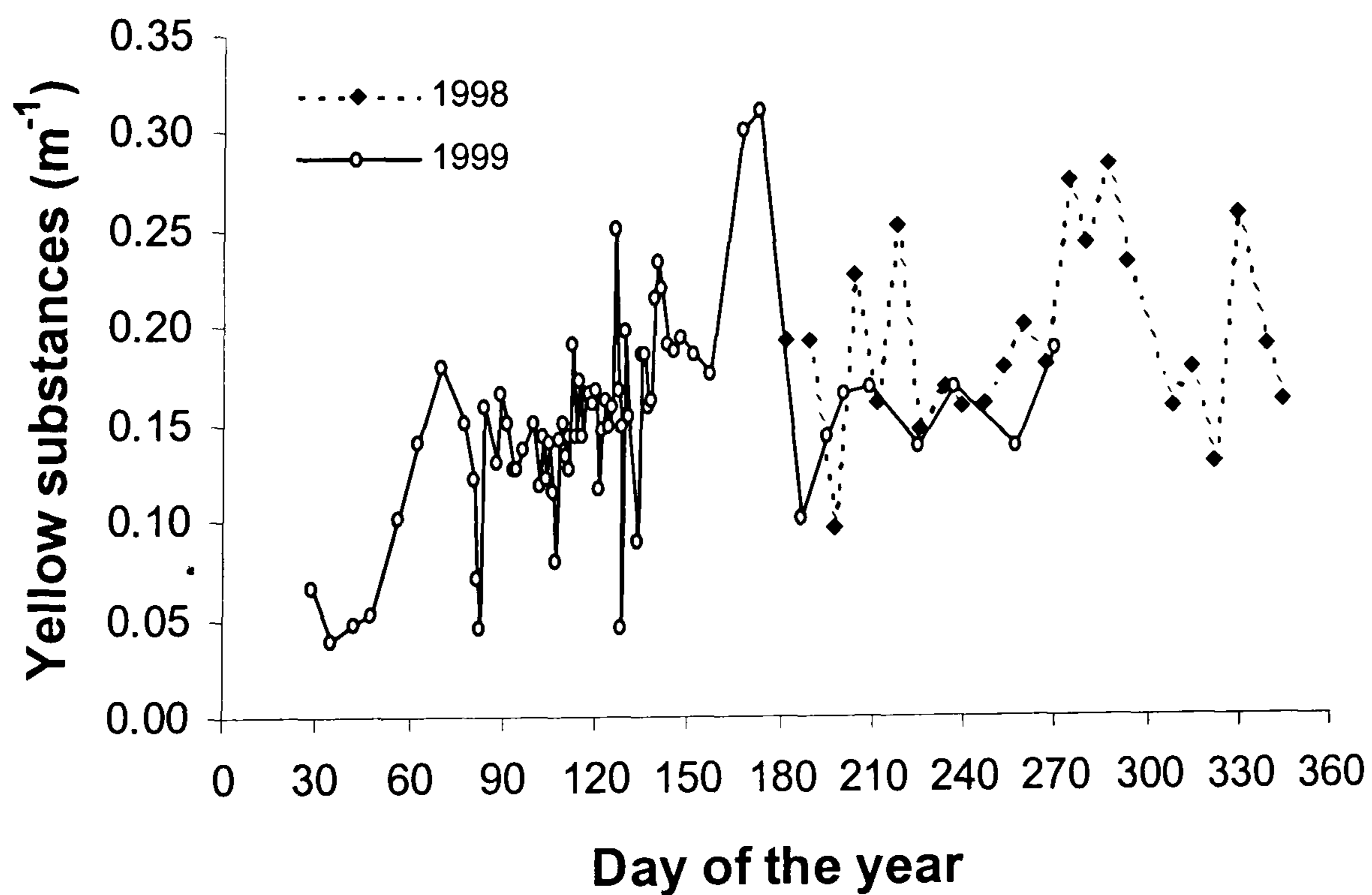
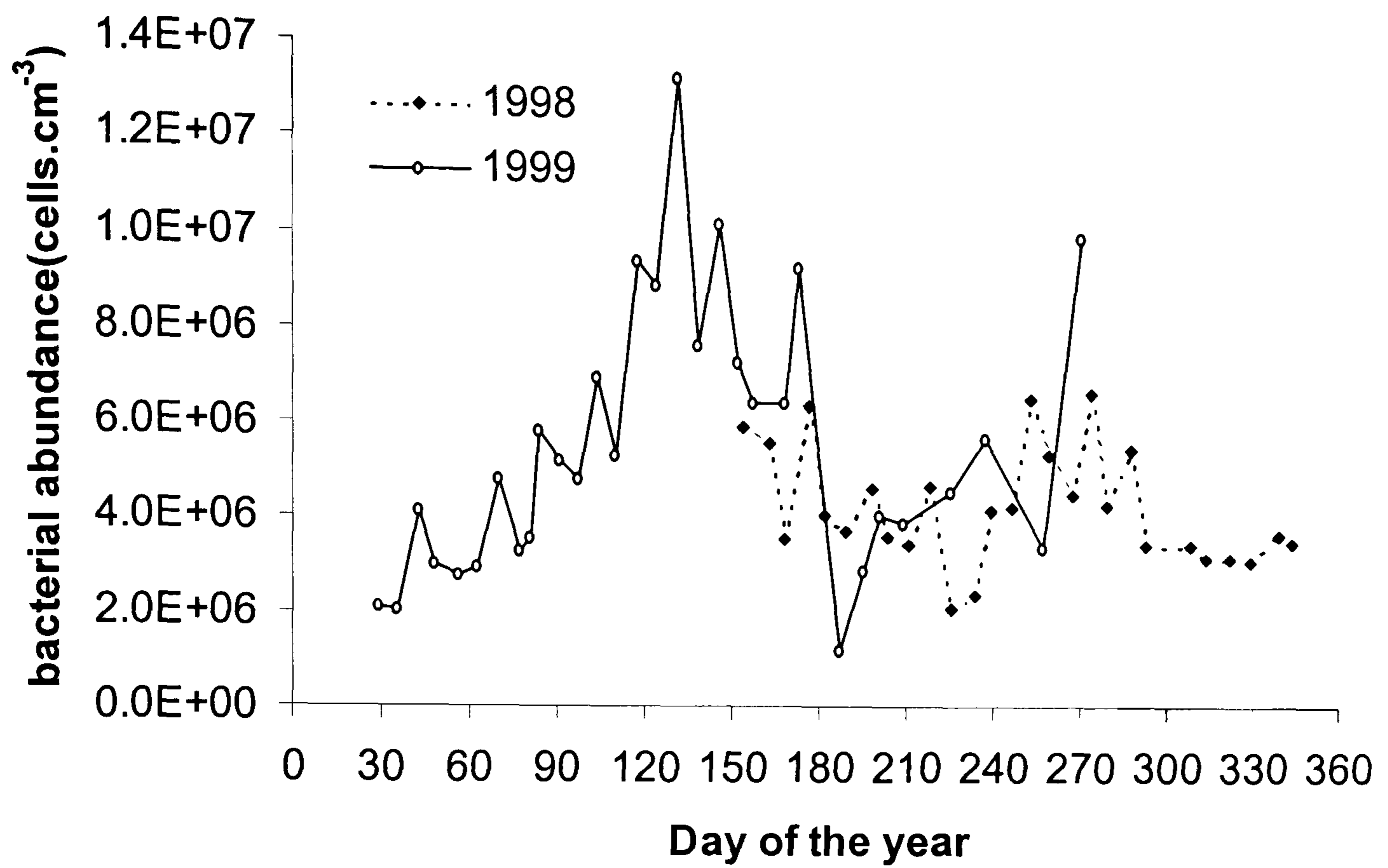
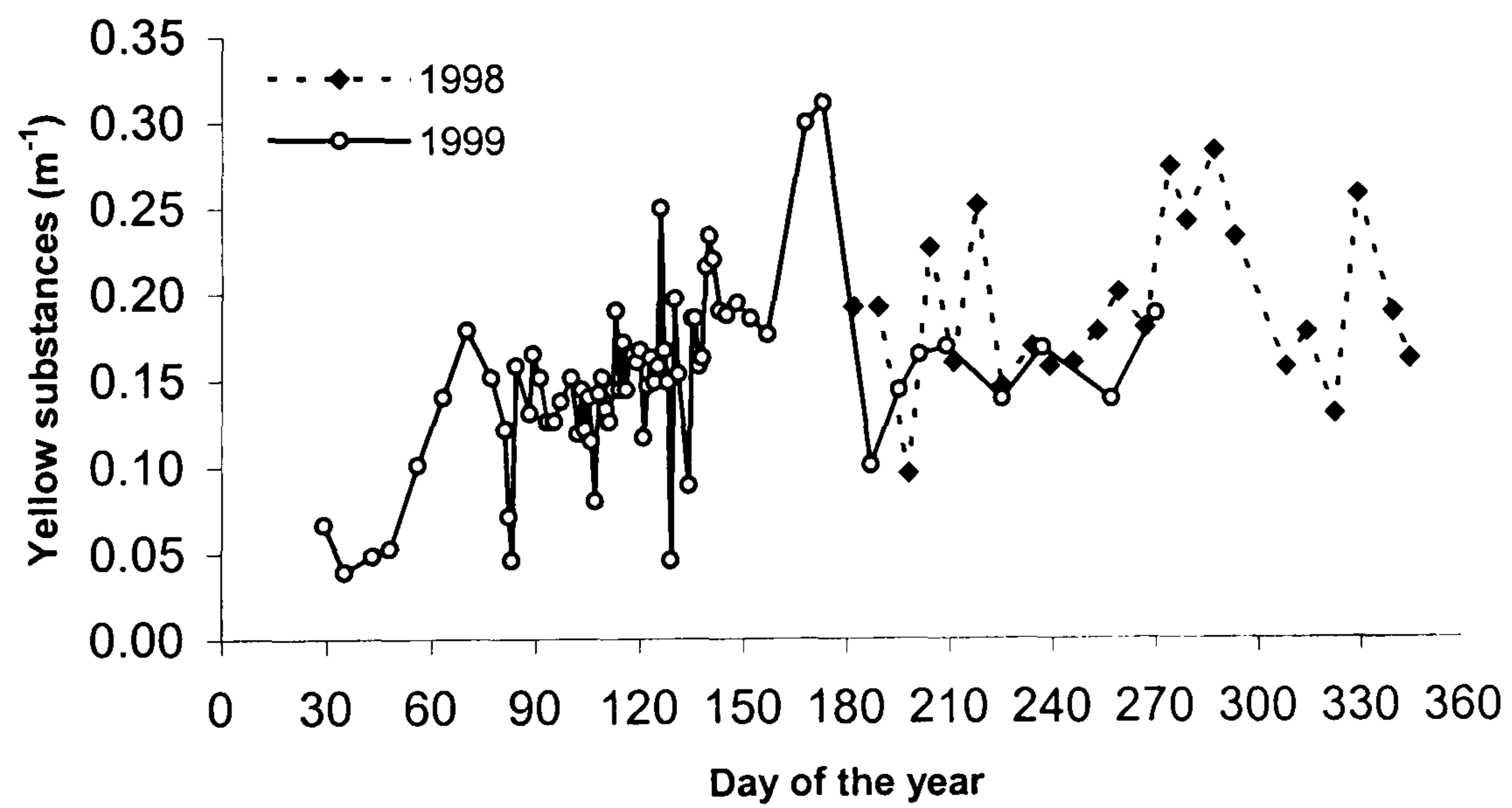
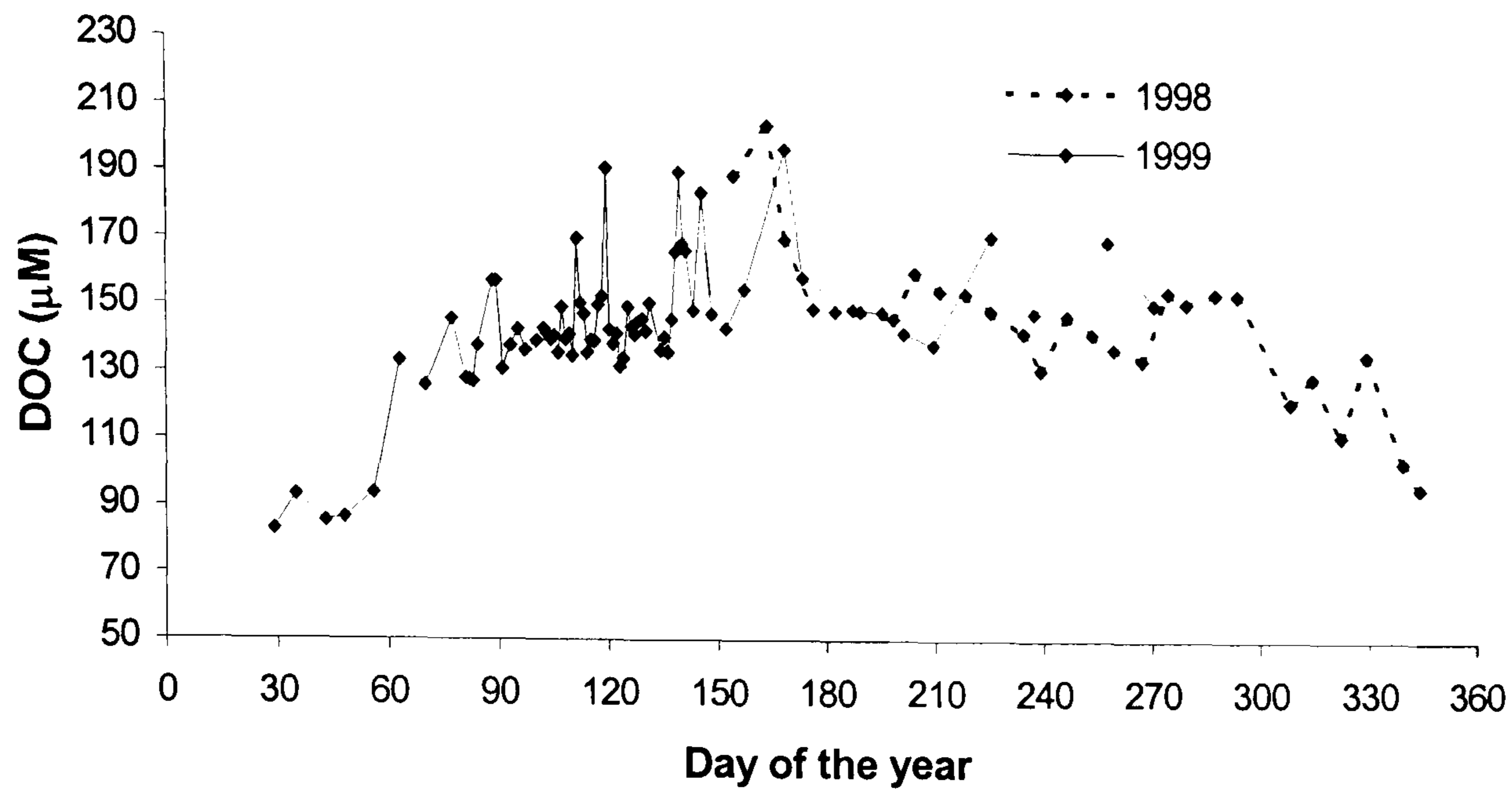
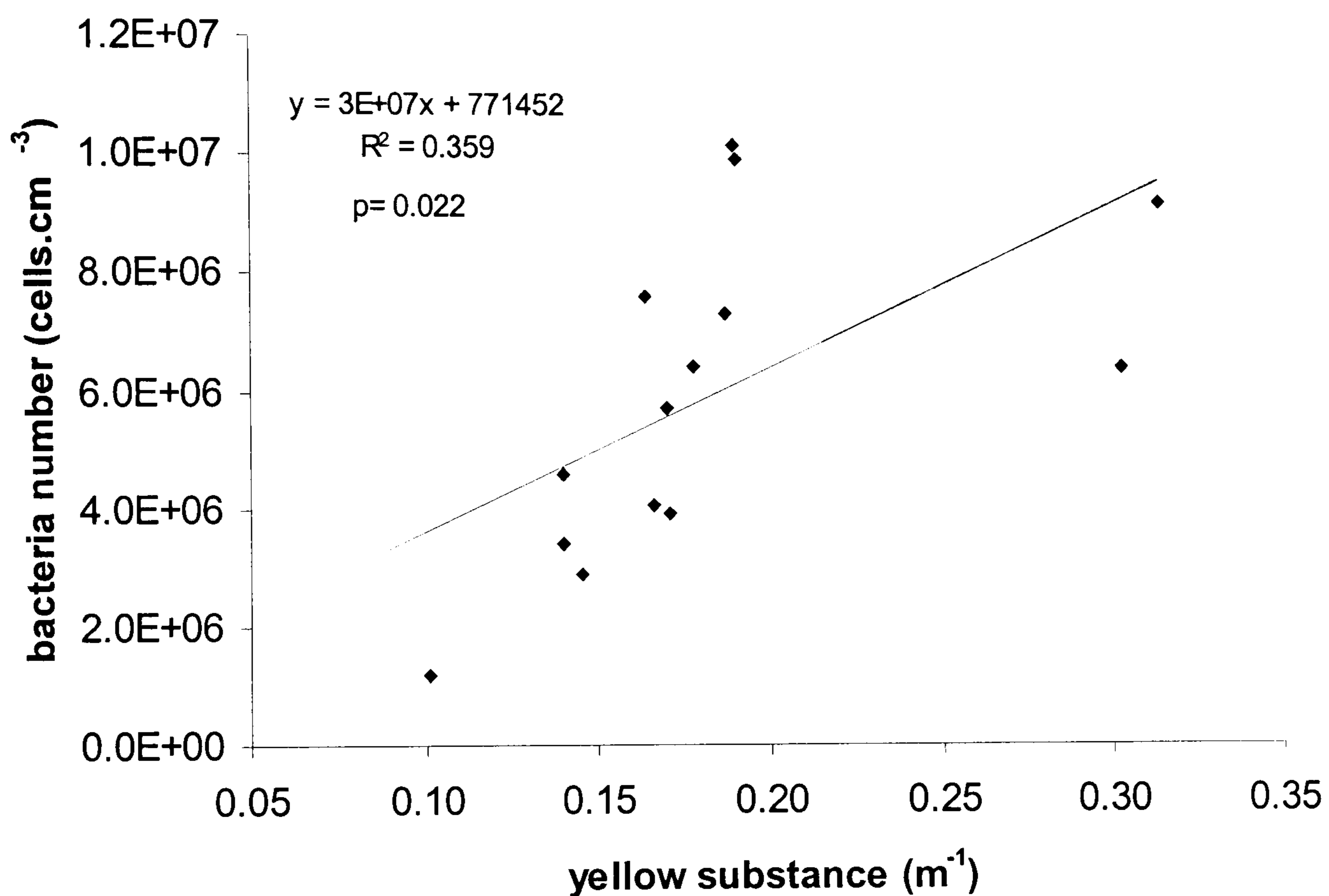


Figure 6.13. Seasonal variation of bacterial abundance and yellow substance in the Menai Strait during 1998&1999.



**Figure 6.14. Seasonal variation of DOC and yellow substance in the Menai Strait during 1998&1999.**

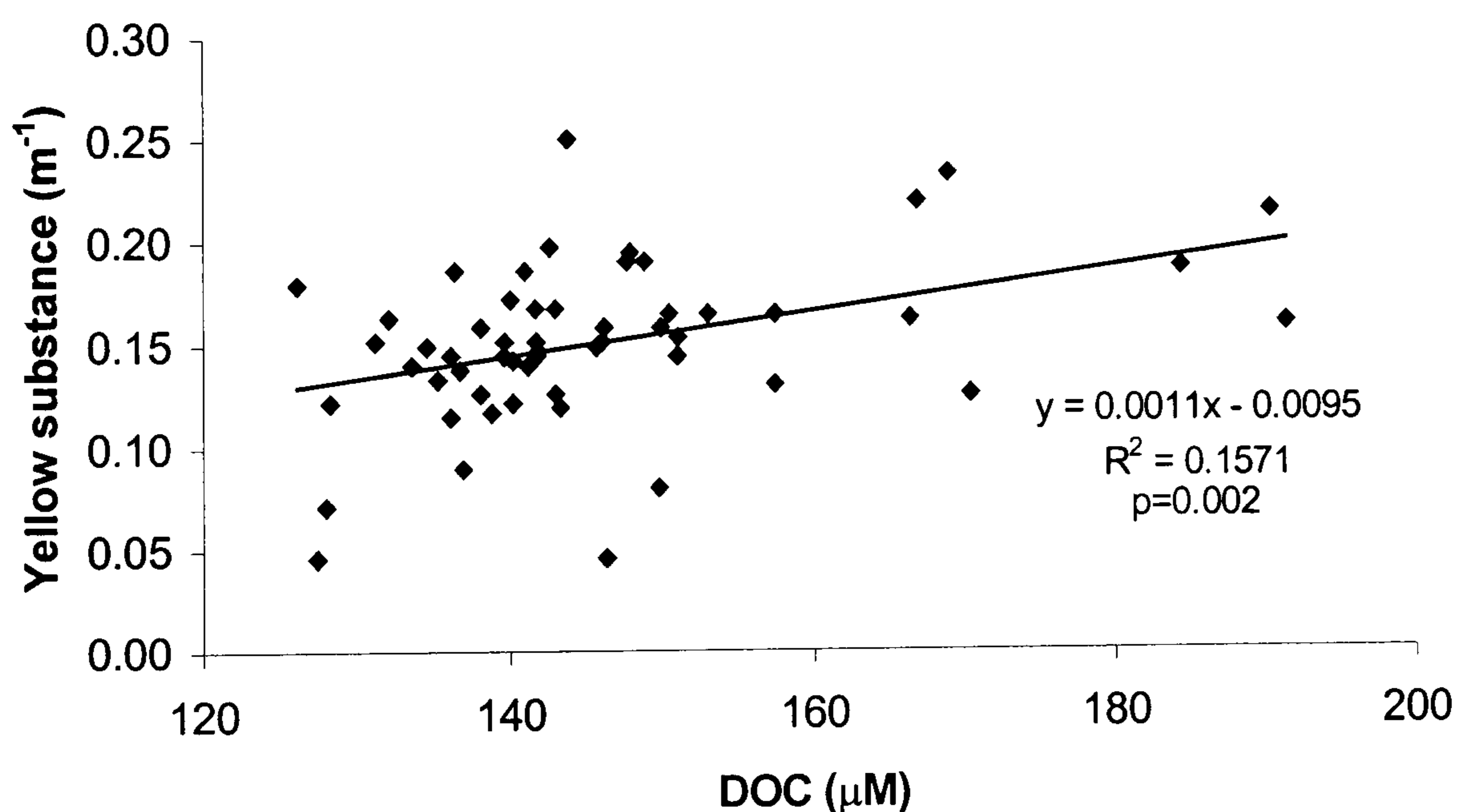
Adsorption and sedimentation may be other factors which cause yellow substance decline during summer in the Menai Strait. The refractory fractions which resist microbial breakdown are adsorbed together and sink to the bottom (Kratzer *et al.* 2000) thus influencing the decline in mid-summer.



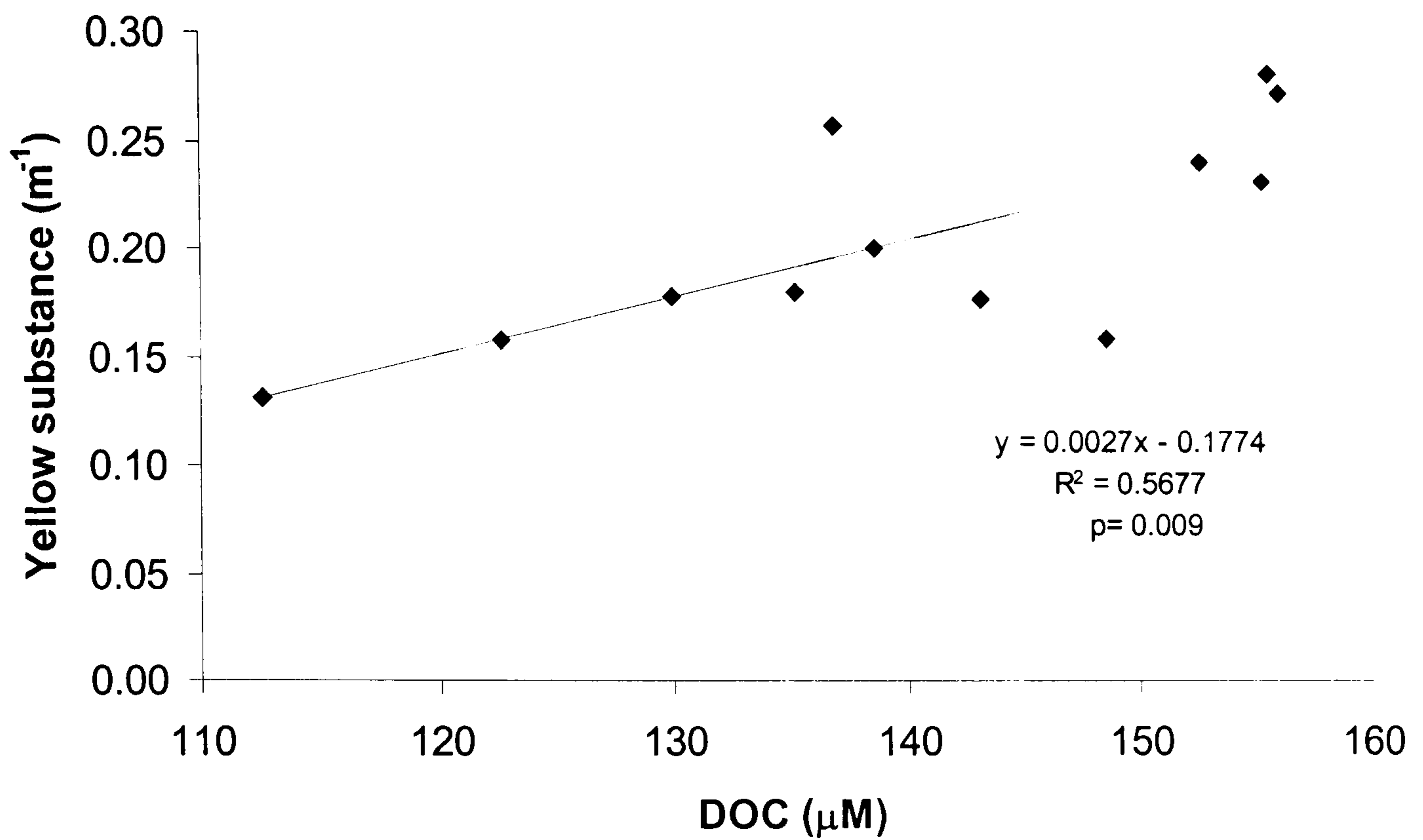
**Figure 6.15. Correlation between bacteria number and yellow substance during summer.**

During autumn, yellow substance showed slight increase which may have been associated with the autumn phytoplankton bloom and a possible input of terrestrial material from rainfall and runoff. The maximum yellow substance during autumn occurred almost two weeks after the highest concentration of chlorophyll *a*. In addition to river input, strong winds may have influenced the re-suspension of particles and increased the yellow substance concentration in autumn. The decline, which occurred during winter, may have been associated with low biological activity regulated by limited light and temperature.

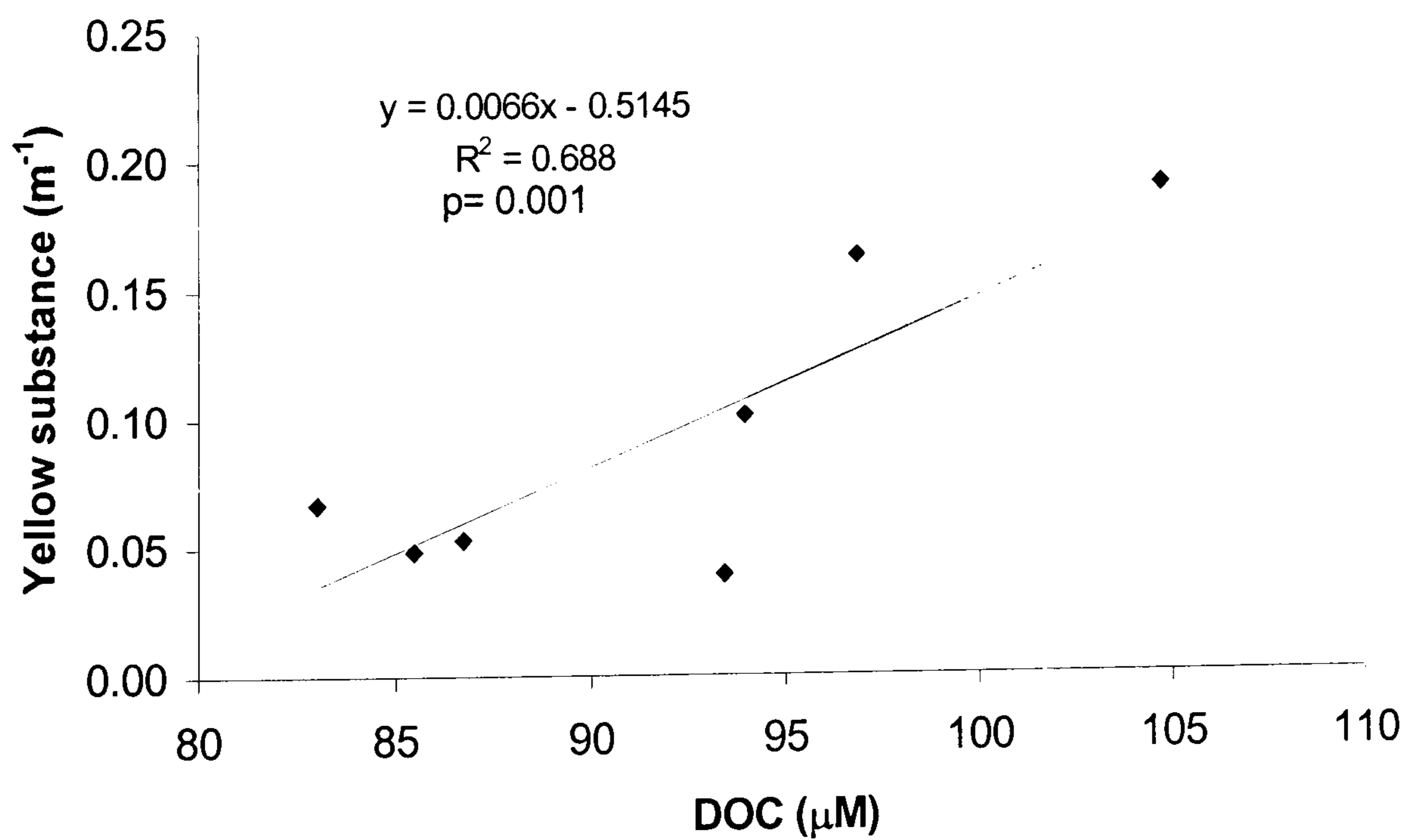
In conclusion the seasonal cycle of yellow substance in the Menai Strait is not directly linked to phytoplankton biomass or primary production. Consequently microbial activities may play a vital role in the concentration and distribution of yellow substance. Photo-oxidation may have transformed HMW to LMW DOM but this did not necessarily change the DOC concentration in the water. Yellow substance and DOC showed significant correlation during spring (Figure 6.16), autumn (Figure 6.17), and winter (Figure 6.18) but not in summer. Observation in this study agrees to a certain extent with that of Frost (1973) who suggested that yellow substance concentration in the Menai Strait is influenced by the labile portion of the organic matter (DOC). However further investigation is required to establish the nature of this relationship.



**Figure 6.16. Correlation between yellow substance and DOC during spring 1999.**



**Figure 6.17. Correlation between yellow substance and DOC during autumn 1998 in the Menai Strait.**



**Figure 6.18. Correlation between yellow substance and DOC during winter 1998 in the Menai Strait.**

# CHAPTER VII.

## 7. Dissolved Organic matter experiments.

### 7.1. Introduction:

#### 7.1.1. The extracellular release of dissolved organic matter by phytoplankton in laboratory experimental studies.

The production of dissolved organic matter (DOM) involves a number of mechanisms the most evident is related to phytoplankton blooms. In the marine environment, phytoplankton are considered to be the most reliable source of dissolved organic matter (Kirchman 2000). Nevertheless, there are several mechanisms responsible for DOM production including extracellular release by phytoplankton, grazing and excretion by zooplankton, release via cell lysis (viral and bacterial), bacterial transformation and release. The type of DOM produced and factors influencing the production are still debatable.

The seasonal accumulation of DOC is influenced by number of chemical, physical and biological parameters. One of the ways to understand the seasonal accumulation of DOC is to design laboratory (or mesocosm) experiments in which several key parameters are controlled and their influence on DOC release and accumulation measured. Culture experiments have demonstrated that marine phytoplankton are capable of producing DOM in different forms such as carbohydrates, organic acids, nitrogenous compounds, and lipids (Hellebust 1965; Fogg 1983, Williams & Egge 1998). Phytoplankton extracellular release (PER) is considered to be an important factor to estimate the quantitative role of DOM release (Nagata 2000). It has been reported that the average phytoplankton extracellular release in culture is about 5% and its variability is related to factors such as nutrient depletion, irradiance, and species composition (Ignatiades & Fogg 1973). Therefore by investigating different variables in experimental studies and their role in the extracellular release, would contribute to the understanding of DOM dynamics.

### 7.1.2. The quality and quantity of dissolved organic matter produced by phytoplankton.

In the sea phytoplankton, along with other types of algae are the main producers of organic matter from inorganic compounds. In addition, natural phytoplankton populations are known to release extracellular dissolved organic matter (Kirchman 2000). DOM can be classified into two major categories, either by size or by molecular weight. The classification of DOM by molecular weight consists of two categories, the low molecular weight (LMW) and the high molecular weight (HMW) or colloidal material which are both measured using reverse osmosis and ultrafiltration. The LMW consists of compounds < 1000 Daltons (Da) while the HMW compounds have a molecular weight > 1000 Da. (Jensen 1983). Some studies have reported that 20-30% of the DOM is HMW (Benner *et al.* 1992) and 65-80% is LMW (Amon & Benner 1994), resulting in two types of pools: a carbon rich HMW fraction and a nitrogen rich LMW fraction (Biddanda & Benner 1997). It is believed that LMW material is rapidly remineralized, while HMW compounds are refractory (Amon & Benner 1994).

The most biologically reactive compounds in seawater include dissolved free compounds such as sugars or amino acids. These compounds have a rapid turnover (minutes to hour) and are available in low concentrations in the open ocean (Rich *et al.* 1997). It is suggested that these compounds can be found in high concentration in the marine environment despite the fact that they have rapid turnover especially in coastal waters (Furhman & Ferguson 1986; Keil & Kirchman 1999). In other oceanic systems, the production and consumption of DOM is close coupled so that the accumulation of labile DOM is not measurable (Carlson & Ducklow 1996). The biologically reactive compounds also represent the reliable sources for bacterial carbon and nitrogen requirements (Kirchman 2000). Labile DOM concentration is characterised by a decrease from coastal regions towards open oceans (Kirchman 2000).

The HMW or refractory pool is found in high concentrations in deep oceans (Williams & Druffel 1987). It is suggested that the HMW DOM pool is uniformly distributed in the water column and represents approximately 70% of the surface



DOC (Carlson & Ducklow 1996). The HMW DOM is biologically resistant but it has been suggested that it can be broken down into labile DOM via photooxidation (Kieber *et al.* 1989). Hansell & Carlson (1998) reported that deep ocean DOC is removed over time but the mechanism responsible for this process are still not well understood. There are hypotheses that a combination of microbial removal and photooxidation could be responsible (Kirchman 2000), also degradation by attached bacteria to sinking particles (Williams 2000).

The compositional differences between different molecular size fractions produced by phytoplankton have an impact on the rates at which organic matter can be broken down and returned to DOM pools (Amon & Benner 1994), i.e the HMW DOM supports high rates of bacterial respiration, while the LMW DOM supports higher bacterial growth (Gardner *et al.* 1996). This has been reported by Amon & Benner (1994), in an experiment where rates of carbon consumption were examined by measuring changes in dissolved oxygen. DOC concentrations in HMW incubations decreased rapidly being mineralized to carbon dioxide by bacteria; on the other hand low consumption was observed in LMW DOM incubation indicating that bacteria used only a small fraction of LMW DOM. This has a big impact on the microbial loop, as differences in the composition of HMW and LMW DOM seem to be reflected in microbial growth and respiration and contributed to the variations of the dissolved organic matter (DOM) in the surface water of oceans. Subsequently, the mechanisms by which these compounds are produced needs some consideration in order to fully understand their role in the microbial loop.

### **7.1.3. The mechanisms of release of DOM by phytoplankton.**

There are two mechanisms proposed for the release of DOM by phytoplankton in order to understand the extracellular release. They are the overflow and leakage mechanisms (Fogg 1983; Bjornsen, 1988; Nagata 2000). Under optimum light and low nutrient concentrations phytoplankton production may be produced faster than it is incorporated resulting in extracellular release (the overflow mechanism) (Fogg 1983; Wood & Van Valen 1990). It has been suggested that it would be less costly for a cell to release carbon-rich dissolved organic matter than store it under the nutrient depletion conditions (Wood & Van Valen 1990).

The leakage, or passive diffusion, mechanism is explained as the continuous release of low molecular weight DOM across the cell membrane because of the passive permeation through the membrane (Bjornsen 1988). Neutral sugars and nitrogenous compounds such as dissolved free amino acids are suggested to be released through the leakage mechanism (Hellebust 1965; Bronk & Glibert 1993). Bjornsen (1988) suggested that the leakage mechanism is a process that continues through the night and the estimate of daily loss by leakage of the intracellular pool of a 10 $\mu$ m phytoplankton cell is about 50%.

The overflow and leakage models, seem to be supported by a number of reports. For example, extracellular release at night (Mague *et al.* 1980) and the release of low molecular weight DOM (Mague *et al.* 1980; Lee and Rhee, 1997) support the leakage model. In contrast, the release of HMW DOM (Lancelot, 1983; Lancelot and Billen 1984; Biddanda & Benner 1997) and the increase of phytoplankton extracellular release (PER) during the nutrient limitation (Wood & Van Valen 1990; Goldman *et al.*, 1992; Smith *et al.* 1998; Alcavero *et al.* 2000; Staats *et al.* 2000) support the overflow model. It seems however that, despite the support for the two models, there are other parameters such as the local environmental conditions, the species composition and growth conditions that may control which model is dominant at a particular time and environment.

#### **7.1.4. The factors affecting phytoplankton extracellular release.**

Phytoplankton extracellular release (PER) can be affected by several factors such as nutrient deficiency, temperature, light intensity, and community structure. However the effect of these factors could also be influenced by other environmental parameters such species composition and the physical and chemical parameters in the local environment. Culture experiments have demonstrated that the most common factors influencing phytoplankton extracellular release are high irradiance, change in temperature (Ignatiadea & Fogg 1973; Zlotnik & Dubinsky 1989), the depletion of nutrients (Obermosterer & Herndl 1995), and the transition of phytoplankton growth to stationary stage (Obermosterer & Herndl 1995). The variability of PER may be also related to phytoplankton species composition (Wolter 1982; Lancelot 1983). Moreover, the chemical and physical characteristics of the

organic matter released may also vary in response to different conditions (Smith & Underwood 2000).

Extracellular release of dissolved organic carbon might be less than 5% under optimum light and temperature conditions but can increase to up to 40% when exposed to a combination of low temperature and light (Collins & Boylen 1982). In addition light can also influence the production and degradation of organic matter, i.e. the increase or decrease of light could influence the amount of DOM produced. For instance high light intensity could cause stress to phytoplankton cells and result in extracellular release. Geider and Osborne (1992) reported that the net photosynthesis and the assimilation of carbohydrates occurs during the illuminated part of the day, whereas protein synthesis may continue in darkness.

#### 7.1.4.1. Light

Many studies have investigated the effect of light on phytoplankton, either in the laboratory or in the field (Dohler 1984;Dohler & Bierman 1987;Cullen & Lesser 1991; Smith *et al.* 1992;Schofield *et al.* 1995;Buma *et al.* 1996; Davidson *et al.* 1996; Goes *et al.* 1996, among other). These authors have investigated the effect of light on the physiology and biochemical composition of phytoplankton, and the structure of phytoplankton communities. However the focus of this study is to investigate the role of light in cultures. It has been reported that the exposure to UV-B radiation can effect the production of amino acids in phytoplankton cultures. Dohler (1984) observed an increase in amino acids in a culture of marine diatoms exposed to a short UV-B radiation (5h per day) for 2 days. Subsequently, Buma *et al.* (1996) observed either constant or increasing in cellular protein content when three temperate marine diatoms were exposed to UV-B radiation. Zlotnik and Dubinsky (1989), also reported the influence of light on DOC excretion. From their experiment, the effect of light on DOC excretion was investigated in three phytoplankton species *Chlorella vulgaris*, *Isochrysis galbana*, and *Synechococcus sp.* In all cases light had showed a significant influence on DOC excretion. The increase in the DOC excretion was explained as a result of photoinhibition. The studies discussed above show that the effect of light on phytoplankton is species

related. This makes it even more complicated to understand the specific effect of light on dissolved organic matter released by phytoplankton in culture.

#### **7.1.4.2. Inorganic nutrients.**

It is known that the production and release of polysaccharide matter is increased by nutrient depletion. Subsequently the type of nutrient limitation can also influence the type of organic matter released. Myklestad and Haug (1972) found from the culture of *Chaetoceros affinis* that the depletion of nitrate led to an increase in the amount of carbohydrates stored in the cells. Therefore, physiological state might affect the excretory behaviour of the algae. In contrast the depletion of inorganic phosphate caused the high production of extracellular polysaccharides. Production of a high amount of polysaccharide was also found in the culture of the diatom *Cylindrotheca fusiformis* grown under inorganic phosphate limitation (Guerini *et al.* 1998). This behaviour was considered to be a result of the metabolic switch between protein production, which is limited by a low phosphate concentrations to high carbohydrate production (Guerini *et al.* 1998).

The effect of nutrient depletion is not limited only to marine species. Several studies (Rhee 1978; Healey & Hadzel 1979; Harrison *et al.* 1990; La Roche *et al.* 1993; Larson & Rees 1996; Kilham *et al.* 1997) have shown that nutrient depletion has an impact on the biochemical composition of freshwater algae and diatoms. Subsequently the changes in biochemical composition might have impact on the excretory behaviour and types of dissolved organic matter excreted. For instance, Kilham *et al.* (1997) observed an increase in carbohydrate production under phosphate limitation; however a significant increase occurred when nitrogen was limiting. Subsequently, Shifrin and Chisholm (1981) found an increase in lipids in some nitrogen-limited diatoms.

#### **7.1.4.3. Taxonomical composition of phytoplankton.**

The taxonomic composition of phytoplankton assemblages is influenced by the individual growth rates. This in turn influences quantitative and qualitative changes in the utilization of nutrients thus influencing the quantity and quality of extracellular release (Clark 2001). Myklestad (1974) reported variation in the production of

extracellular polysaccharide in particular species, leading to differences in the ratio of extracellular polysaccharide to cellular carbohydrate. Moreover, Myklestad (1977) reported that the extracellular carbohydrates in *Chaetoceros affinis* culture were dependent on the N:P ratio of the culture medium, but this was not observed in *Skeletonema costatum* culture. Guerrini *et al.* (2000) found an increase of total carbohydrates production occurred under both N and P limitation whilst the extracellular releases in general increased only under P limitation. In addition, Kiorboe and Hansen (1993) found the exopolymers produced by *Skeletonema costatum* and *Chaetoceros socialis* in batch culture are different in quality and quantity. *Chaetoceros socialis* produced a large amount of colloids during the stationary stage whilst *Skeletonema costatum* produced large amount during the exponential growth. These findings confirm the influence of taxonomical composition of phytoplankton on extracellular release although more investigation are required.

#### 7.1.4.4. Temperature.

Phytoplankton have an optimum temperature where growth rate is maximal. The growth rate of phytoplankton generally increases with temperature but only within a certain temperature range, and the temperature dependency of the growth rate differs among species (Eppley 1972; Goldman & Carpenter 1974). Suzuki & Takahashi (1995) investigated the relationship between temperature and maximum growth in eight diatom species isolated from different temperatures. They found that individual species showed maximum growth rate at the temperature near to the upper limit of growth. Kristiansen (1983) reported an optimum temperature for *Skeletonema costatum* growth ranging between 10°C and 25°C, with maximum growth occurring at 17°C and lowest growth at 10°C. It was suggested that the variation of water temperature has a significant impact on the rate of nitrate uptake with this particular species, as the decrease in temperature reduces the cell's growth (Kristiansen 1983). Therefore the influence of temperature on phytoplankton growth rate is important for the rates of extracellular release of different phytoplankton species. This influence could be indirect, through growth and storage of dissolved organic matter, or direct by affecting the rate at which phytoplankton populations release the dissolved organic matter.

In his experiment on the effect of temperature on DOC excretion, Watanabe (1980) found that the phytoplankton population excretion percentages increased whenever temperatures were either too low (5°C) or too high (35°C-40°C) for optimal photosynthesis (20°C -30°C). Temperature was found to have an influence on the rate of DOC excretion when studying dinoflagellates in culture. McLaughlin *et al.* (1961) reported that carbohydrate excretion by the dinoflagellate *Katodinium dorsalisulcum* was inhibited by low temperatures. In addition, Dohler (1972) reported that glycollate excretion by the unicellular rhodophyte *Phorphyridium cruenutum* reached a maximum at 15°C and was totally inhibited at 27°C. These observations confirm the influence of temperature on DOM excretion and could provide some insights on *Skeletonema costatum* growth and extracellular release in the Menai Strait.

#### **7.1.4.5. Interaction with other organisms.**

The interaction between different size groups of marine organisms (algae, bacteria and zooplankton) is one of the factors affecting extracellular release. Zooplankton and protozoa play an important role in removing phytoplankton production and bacterial production in the marine environment. Nagata (2000) hypothesized that when there is a balance between phytoplankton growth and protozoan grazing, the rate at which the particulate matter could be transformed to DOC being about 10-30%. Subsequently, bacterivory could influence nutrient regeneration within the microbial food web by increasing or decreasing the inorganic nutrient available to phytoplankton and bacterioplankton.

Bacteria play a vital role in carbon cycling by oxidizing dissolved organic carbon (DOC) to CO<sub>2</sub> (Reche *et al.* 1998) and by contributing to the carbon transfer through food webs (Zlotnik & Dubinsky 1989). In pelagic food webs, bacteria are preyed on by flagellates and ciliates, and these protozoa are in turn preyed by copepods. These organisms are controlled by a number of factors such as environmental condition, resource limitation and predation (Felip *et al.* 1996). Therefore the changes in the regeneration rate caused by predation could result in the variation of phytoplankton and bacterial production. Subsequently, this will reflect on the production and consumption of the dissolved organic matter.

Virioplankton are believed to be more abundant in the marine environment and they exceed bacterial abundance by 2 to 25 fold (Bergh *et al.*, 1989; Cochlan *et al.*, 1993). It has been reported that 10-50% of bacterial mortality is caused by viral infection (Steward *et al.*, 1996; Fuhrman, 1999). This process has also been linked to declines of primary production (Gobler *et al.*, 1997). Suttle (1994) estimated that approximately 3% of global primary production is lost to viral lysis. Viral lysis can also have effect on the type of dissolved organic matter produced. Gobler *et al.* (1997) reported that 19% of the total cellular C content was released as DOC yet its availability to bacterioplankton was less than that of DOC released from uninfected cultures.

#### **7.1.5. Technical limitations on experimental studies.**

The concept of extracellular release, both its quantitative and qualitative importance and the factors controlling it, have been subject to much controversy because of experimental approaches and technical limitation on experimental studies. For instance, several experiments have been conducted in the past that have generated debate on the differences in the experimental results. The differences could be generated from experimental design. For example, two experiments with similar design, conducted to study primary production and accumulation of DOC in the same environment, may give different results at different times of the year. The differences might be caused by the chemical and biological composition of the water used for the experiment, which varies during the year. For example water samples taken in June in the Menai Strait, depleted in nutrients and rich in bacteria and flagellates, will show less accumulation of DOC relative to water collected in winter or spring. In contrast the water collected in spring when diatoms are blooming might show high accumulation of DOC when inorganic nutrients are added to the experiment.

Studying DOM production and degradation in culture also could be associated with many artifacts, which could results in biased interpretation. Lancelot (1979) suggested that the release rate of DOC in incubation could be underestimated due to heterotrophic uptake of DOC by microorganisms. In addition, high vacuum pressure and overloading cells on the filter, rupturing cells during filtration could cause

overestimation of the extracellular release (Sharp 1977; Goldman & Dennett 1985). Subsequently if the phytoplankton cells used for culture undergo any sort of stress, they can influence the result of the experiment. There are also problems related to source of light used in most experiments. For example, organisms may react differently to artificial and natural light.

Studying bacterial growth efficiency is one way of understanding the ecological and biogeochemical roles of bacteria in the microbial food web (Sherr & Sherr 1996). For instance, for many years radiolabeled substrates were used to study bacterial growth efficiency but they are now regarded as a sources of overestimation of the real bacterial growth efficiency due to the use of model compounds (del Giorgio & Cole 2000). Culturing phytoplankton and introducing bacteria in order to investigate phytoplankton dynamics may be associated with several problems such as cross contamination, grazing activity, i.e. whether the microbial web in the culture functions as it does in the natural environment and whether long incubations can introduce bottle effect (del Giorgio & Cole 2000). Given the lack of a direct method to study the quantity and type of DOM in the natural environment, estimation of bacterial production and growth efficiency are used as alternatives. However conversion factors are required to convert measured parameters into carbon units, thus resulting in overestimation of the measured parameters (Carlson & Ducklow, 1996; del Giorgio *et al.* 1997).

## **7.2 Aims of experimental studies:**

The interaction of microorganisms and their response to different biogeochemical processes seem to be the most promising avenue for further research. The microbial community seems to play an important role in controlling the accumulation of the dissolved organic matter. Therefore more investigation on specific roles of the microorganisms will bring more insight on the spatial and seasonal accumulation of DOM and its role as key element of nutrient cycles in marine ecosystems. In addition, investigations on the role of photobleaching of refractory DOM may provide explanations of the cycling of marine DOM.



It is therefore the aim of this study to characterise some of the parameters causing variations of dissolved organic matter in the Menai Strait. Also to suggest potential future experimental studies for further investigation based on the results of the limited experimental investigations possible during this study.

A set of experiments was designed to investigate influence of specific environmental factors on the production and destruction of DOM. The relationship between yellow substance and dissolved organic carbon was also examined to understand their dynamics, especially during the phytoplankton bloom.

### **7.3. What is the effect of nutrient and light on the dynamics of dissolved organic matter?**

Investigation of the factors that account for seasonal dynamics and spatial distribution of phytoplankton in marine systems often focus on photosynthetic and growth response. Phytoplankton growth and extracellular release can be influenced by a variety of factors as mentioned previously, and among these factors are nutrient concentration and light intensity. Light intensity plays a significant role in the phytoplanktonic extracellular release (PER).

The effect of light and nutrient on DOC excretion has been investigated mostly by enclosing phytoplankton samples at various depths and comparing excretion rates with variation in irradiance and nutrient levels. However in this type of experiment it is impossible to distinguish between the effect of irradiance and nutrients from other environmental factors such as temperature, salinity, and oxygen in the water column. In addition the microbial organisms may be associated with phytoplankton populations and may contribute to the changes on the DOC concentration. It was therefore important to design experiments in the laboratory in order to investigate the influence of irradiance and nutrients under controlled conditions.

### 7.3.1. Aims of this study:

The aim of this experiment was to investigate the effects of light and nutrient on DOC excretion in order to understand their role in the DOM production and consumption in the Menai Strait. In the field study of the Menai Strait, the system was complex and it was impossible to distinguish between different parameters influencing the production and consumption of DOM. Using cultures in laboratory, it was anticipated that an insight into the effect of the light and nutrients would be gained and contribute to the understanding of the role of the two parameters in the Menai Strait.

### 7.3.2. Experimental design:

During this study, 3 experiments were conducted to investigate the influence of light and nutrients on DOC and yellow substance production and degradation.

#### Experiment 1:

The intensity of light and availability of nutrients (nitrate and phosphate) were investigated. However the initial concentrations of DOC and yellow substances were high and no control was included in the experiment design.

#### Experiment 2:

To investigate effect of light and availability of nutrients through a comparison of experimental manipulations and controls. In addition the initial culture was 200cm<sup>3</sup> of *Skeletonema costatum*, which resulted in high concentrations compared to the values in the Menai Strait.

#### Experiment 3:

This experiment was designed so that the chlorophyll *a* and nutrients added were close to the concentrations in the Menai Strait water during early spring. Details of experimental design and procedures are discussed in the following sections.

### 7.3.2.1 Experiment 1:

#### 7.3.2.1.1 Experimental design:

Menai Strait water was collected by a pumping system and filtered through pre-combusted Whatman GF/F filters ( $\sim 0.45\mu\text{m}$  pore size). The filtrates ( $2\text{dm}^3$ ) were transferred to the culturing vessels, which had been washed thoroughly and bubbled with sterile air in a room of constant temperature ( $15^\circ\text{C}$ ). Standard F/2 nutrient salt solution ( $2\text{cm}^3$ ) was added and mixed thoroughly. Volumes of  $200\text{cm}^3$  of *Skeletonema costatum* culture were transferred into each of the four culture vessels, and were then exposed to different light intensities. The four culture vessels ( $2\text{dm}^3$ ) were treated as follows:

- 1) Culture vessel number one was exposed to high light ( $1 \times 10^{16}$  quanta/sec/cm<sup>2</sup>) and nutrients (nitrate and phosphate; final concentrations  $30\mu\text{M}$  and  $4\mu\text{M}$  respectively) added daily (HL+N).
- 2) Culture vessel number two was exposed to high light and no nutrients added (HL).
- 3) Culture vessel number three was exposed to low light ( $0.4 \times 10^{16}$  quanta/sec/cm<sup>2</sup>) and nutrients (nitrate and phosphate) added daily (LL+N).
- 4) Culture vessel number four was exposed to low light and no nutrients added (LL).

All the cultures were grown for a period of 11 days and subsamples for DOC and yellow substances were collected on days 1,3,5,7,9 and 11. DOC and yellow substances were analysed following the methods described in chapter III.

### 7.3.2.2. Experiment 2:

#### 7.3.2.2.1. Experimental design:

Menai Strait water was collected at high tide during summer and screened through  $53\mu\text{m}$  mesh net and filtered immediately through pre-combusted GF/F Whatman ( $47\text{mm}$ ;  $\sim 0.45\mu\text{m}$  pore size) filters. A total volume of  $4\text{dm}^3$  of filtrate was transferred into each of the six culture vessels (acid washed and sterilised). Standard F/2 nutrient salt solution ( $4\text{cm}^3$ ) was added into culture vessels number 3, 4, 5, and 6. This was followed by  $10\text{cm}^3$  of nitrate ( $\text{KNO}_3$ ) (to make a final concentration of  $20\mu\text{M}$ ) and  $10\text{cm}^3$  of phosphate ( $\text{KH}_2\text{PO}_4$ ) (to make a final concentration of  $4\mu\text{M}$ ).

Then 200cm<sup>3</sup> of *Skeletonema costatum* culture was transferred into culture vessel number 3, 4, 5, and 6. The culture vessels were exposed to light ( $0.4 \times 10^{16}$  quanta/sec/cm<sup>2</sup>), and grown for 10 days. Subsamples for chlorophyll *a*, particulate organic carbon (POC), DOC and yellow substances were collected on days 1, 3, 5, 7, and 9. Nutrients (final concentration 20μM nitrate and 4μM phosphate) were added daily. The details of the experimental design was as follows.

Six culture vessels (5dm<sup>3</sup>) were used in this experiment and were treated as follow:

- a) Culture vessel number 1 was used as control and kept in the dark (C+D).
- b) Culture vessel number 2 was used as control and exposed to light ( $0.4 \times 10^{16}$  quanta/sec/cm<sup>-2</sup>) (C+L).
- c) Culture vessels 3, 4, and 5 were exposed to light and nutrients (nitrate and phosphate) added. (L+N).
- d) Culture vessel number 6 was kept in the dark and nutrients (nitrate and phosphate) added (D+N).

Methods used for chlorophyll *a*, POC, DOC, and yellow substance analyses are described in chapter III.

### 7.3.2.3. Experiment 3:

#### 7.3.2.3.1. Experimental design:

Water was collected from Menai Strait at high tide during summer and screened through 53μm mesh nets and filtered immediately through pre-combusted GF/F Whatman (47mm; ~0.45μm pore size) filters. A volume of 4dm<sup>3</sup> of filtrate was transferred into each of the four culture vessels. This was followed by 4cm<sup>3</sup> of F/2 nutrient salts solution and 20cm<sup>3</sup> of nitrate and phosphate to culture vessel number 2, 3, and 4. Final concentrations of nitrate and phosphate were estimated at 40μM and 4μM respectively. A total volume of 15cm<sup>3</sup> of *Skeletonema costatum* culture was transferred into culture vessels 2, 3, and 4. The culture vessels were exposed to light ( $0.4 \times 10^{16}$  quanta/sec/cm<sup>2</sup>) and were grown for two weeks and subsampled for bacterial abundance, chlorophyll *a*, DOC, yellow substances, nitrate and phosphate on days 1, 3, 5, 7, 9, and 13.

A total of 4 culture vessels were used in this experiment and were treated as follow:

- a) Culture vessel number 1 was used as control (no treatment but was exposed to light).
- b) Culture vessels number 2, 3, and 4 were exposed to light and nitrate and phosphate added.

Analytical methods used for samples analyses are described in chapter III.

### 7.3.3. Results:

#### 7.3.3.1. Experiment 1:

In the first experiment, the initial concentrations of YS and DOC were far greater than the field observations and this could be as a result of contamination from the pumping system which through the water was pumped from the Menai Strait. This assumption was made because the analysis of separate samples from the same pumping system showed higher concentrations compared to Menai Strait water.

Yellow substances exhibited very similar trends in all culturing vessels (Figure 7.1). This was characterized by a slow increase up to day seven followed by a sharp increase from day seven up to day thirteen. High yellow substance values occurred in cultures 1 and 2 and the values ranged between  $0.46$  and  $2.52\text{m}^{-1}$ , and  $0.49$  and  $2.45\text{m}^{-1}$ , respectively. By contrast in cultures 3 and 4 the concentrations ranged between  $0.39$  and  $2.12\text{m}^{-1}$ , and  $0.42$  and  $2.47\text{m}^{-1}$ , respectively.

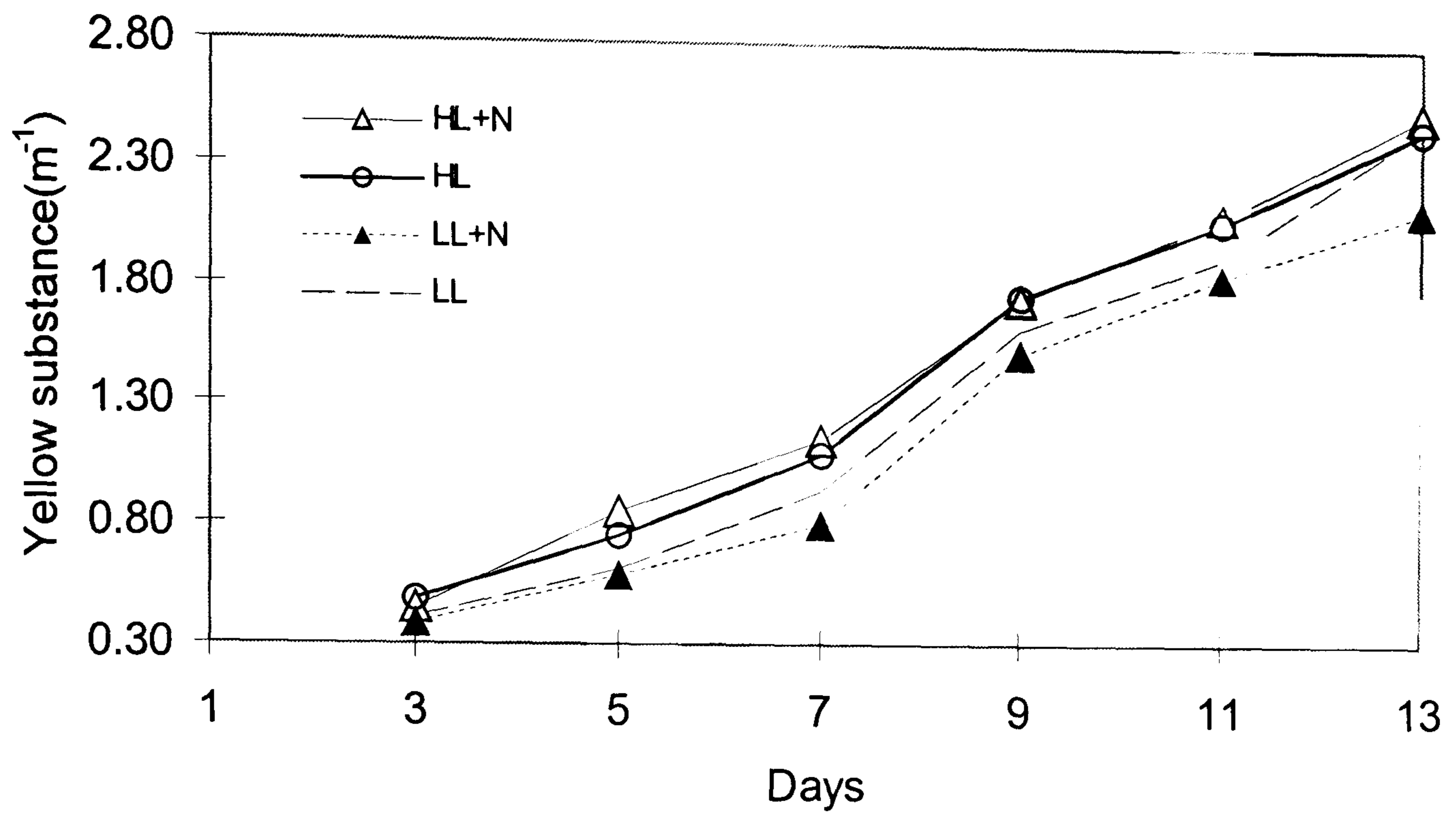
DOC concentration was variable in the four cultures (Figure 7.2) but generally increased during the experiment. Culture vessel 1 showed highest concentrations (ranged between  $1879$  to  $2508\text{ }\mu\text{M}$ ) followed by culture vessel 2 ( $1838$  to  $2474\text{ }\mu\text{M}$ ). Culture vessel 4 showed concentrations higher ( $1882$  to  $2388\text{ }\mu\text{M}$ ) than culture vessel 3 ( $1815$  to  $1898\text{ }\mu\text{M}$ ).

#### 7.3.3.2. Experiment 2:

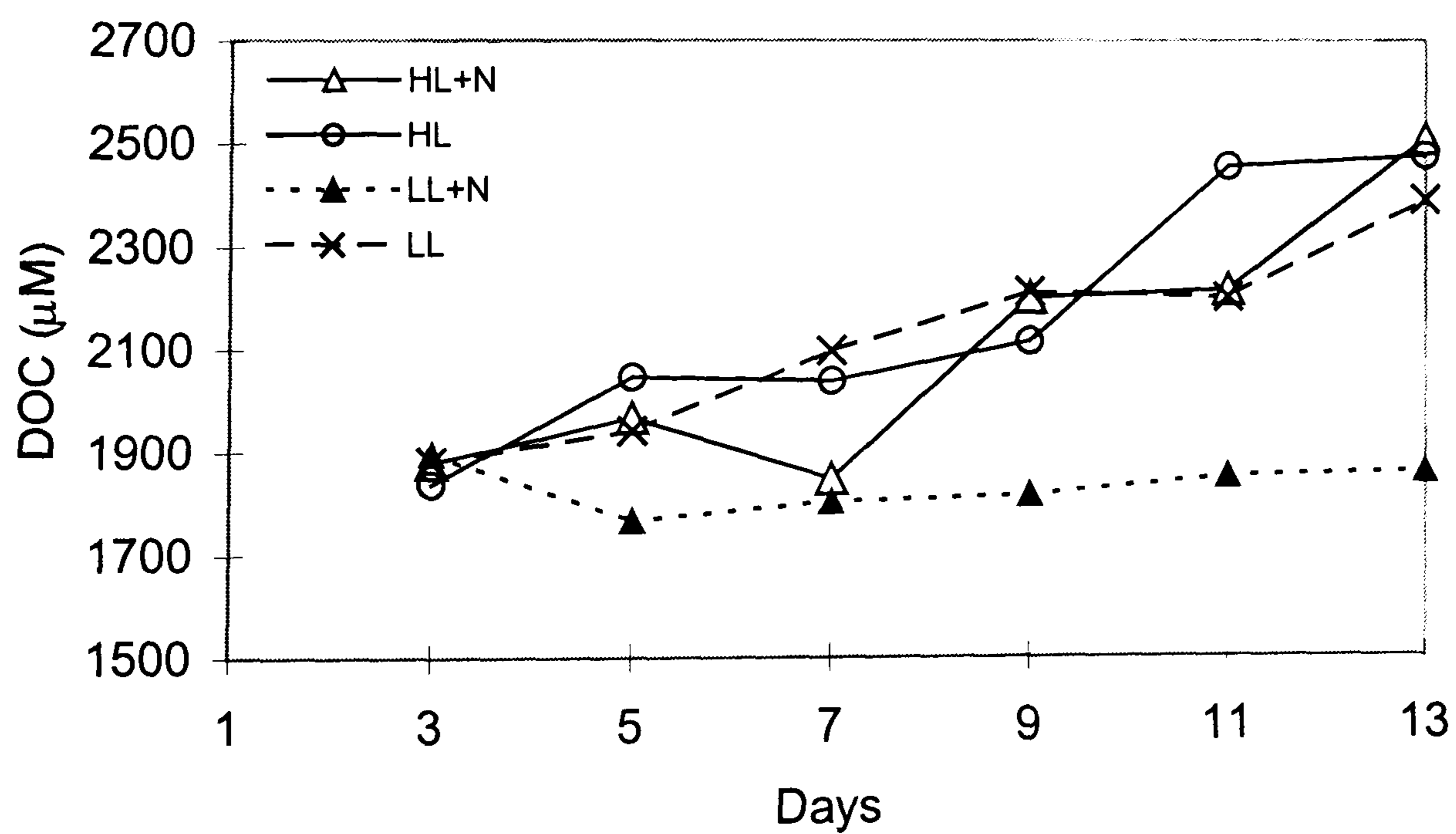
Measurements of all parameters in experiment 2 started one day after the beginning of the culture. The phytoplankton community responded to increased nutrient inputs by increasing its biomass, reaching a maximum chlorophyll *a* concentration of  $40\text{ }\mu\text{g}\cdot\text{dm}^{-3}$  (Figure 7.3). Chlorophyll *a* concentration reached a maximum after one day from the start of the experiment and started to decrease until day 7 when a slight

increase occurred. This increase was associated with contamination, which resulted from the growth of another species in one of the culturing vessels.

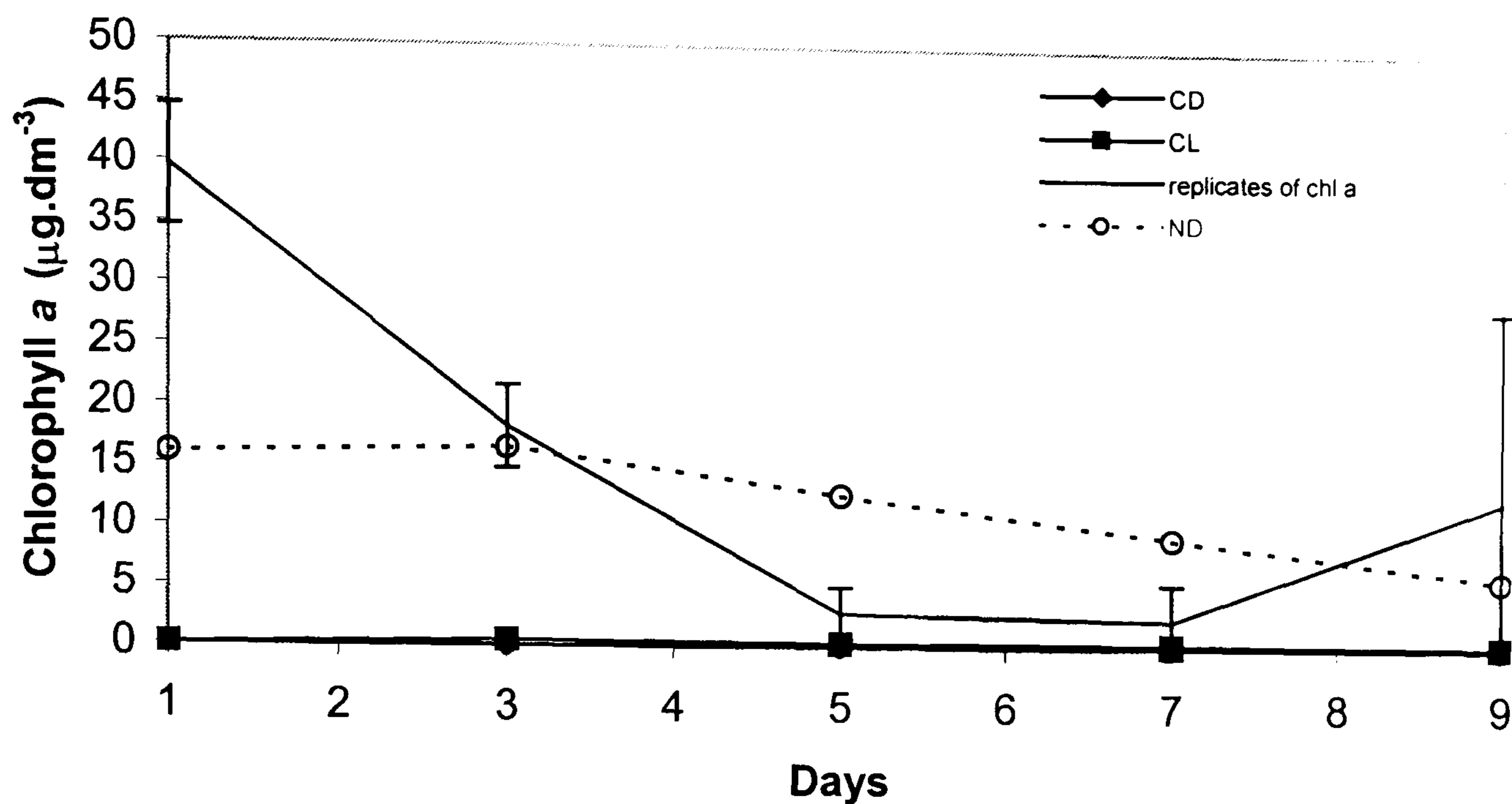
The changes in particulate organic carbon (POC) are shown in Figure 7.4. Particulate organic carbon followed more or less the same trends as that of chlorophyll with an increase from day 1 to maximum concentration on day 3. This was followed by a gradual decrease until day 9 with concentrations ranging between 277 and 186 $\mu$ M. There was a slight variation in concentration among cultures, which resulted in high confidence limits as shown in Figure 7.4. Yellow substances exhibited an exponential growth (Figure 7.5) from the minimum of 0.28 to a maximum of 1.03  $\text{m}^{-1}$  on day 9. The high confidence limit showed on day 9 is as a result of the high value recorded from one of the cultures. Dissolved organic carbon (DOC) followed a similar trend to that of yellow substance (Figure 7.6). The highest increase occurred from day 3 reaching a maximum concentration (685 $\mu$ M) on day 9.



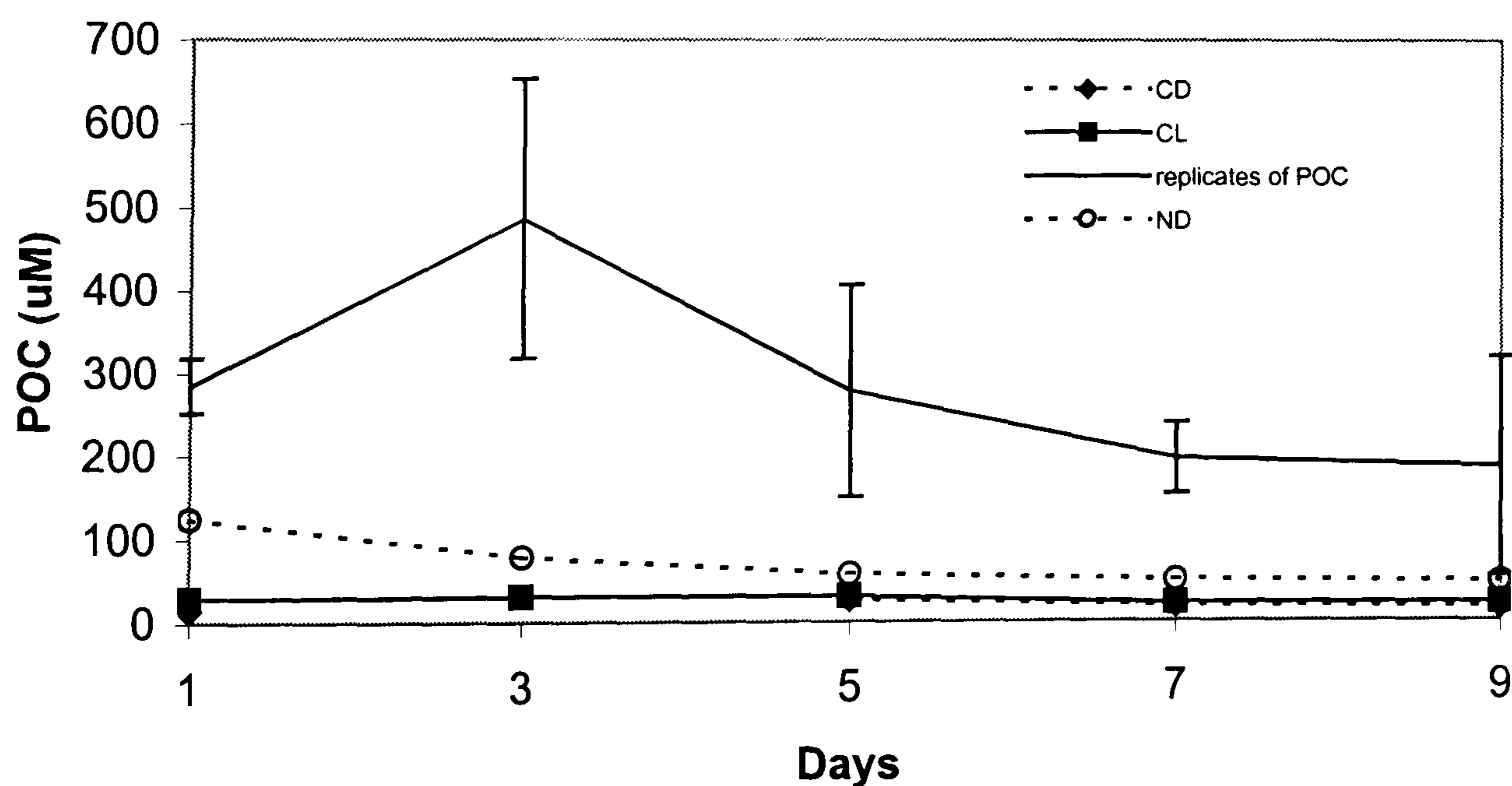
**Figure 7.1. Variations of yellow substance in *Skeletonema sp.* culture under different light intensities and nutrient (HL+N=high light and nutrient added; HL=high light; LL+N=low light and nutrient added; LL=low light).**



**Figure 7.2. Variations of DOC in *Skeletonema sp.* culture under different light intensities and nutrient (HL+N=high light and nutrient added; HL=high light; LL+N=low light and nutrient added; LL=low light).**

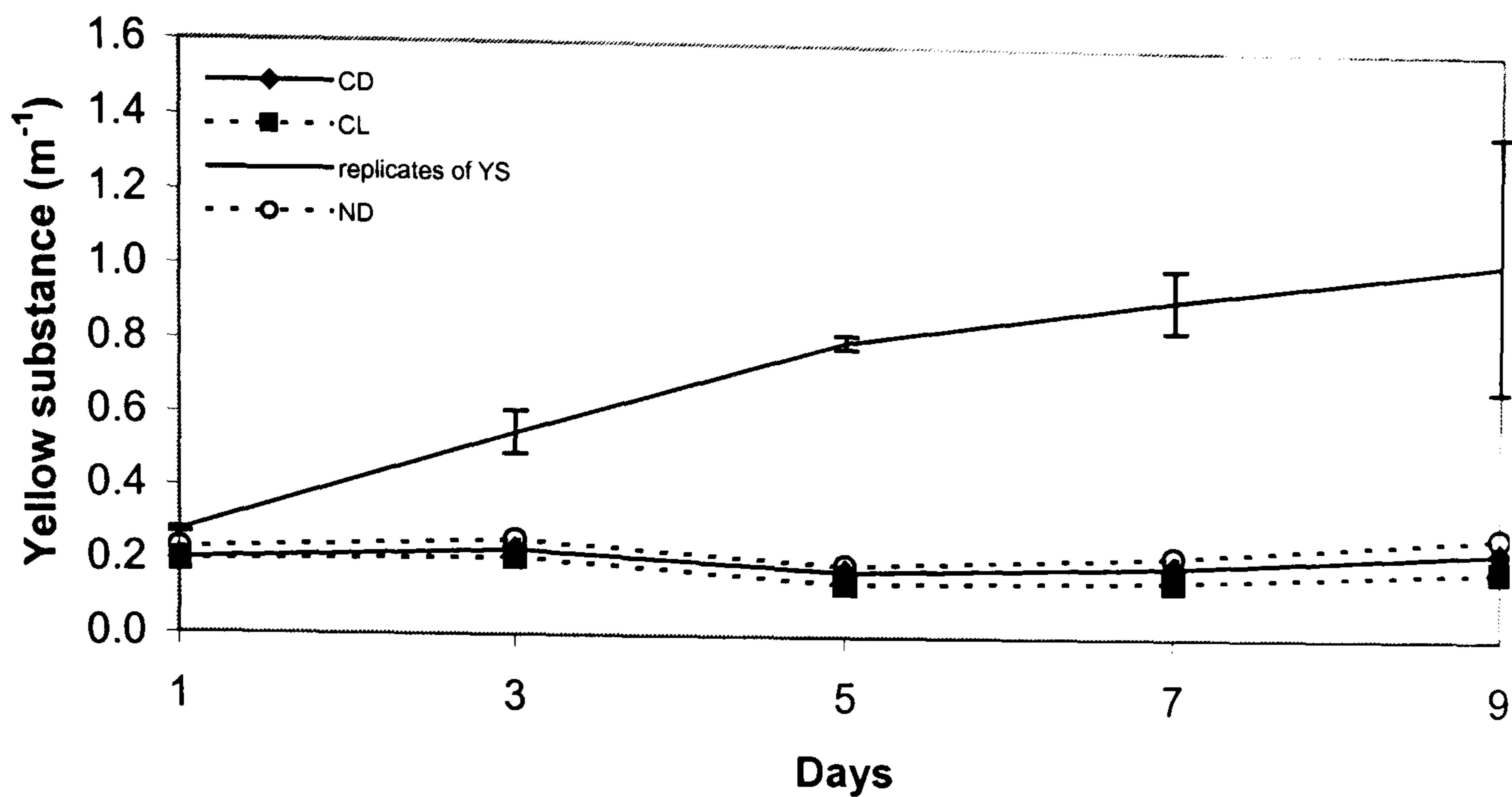


**Figure 7.3. Chlorophyll *a* variations in *Skeletonema* culture under different light intensity and nutrient (CD=control in the dark, CL=control in the light, ND=nutrient added to culture but in the dark).**

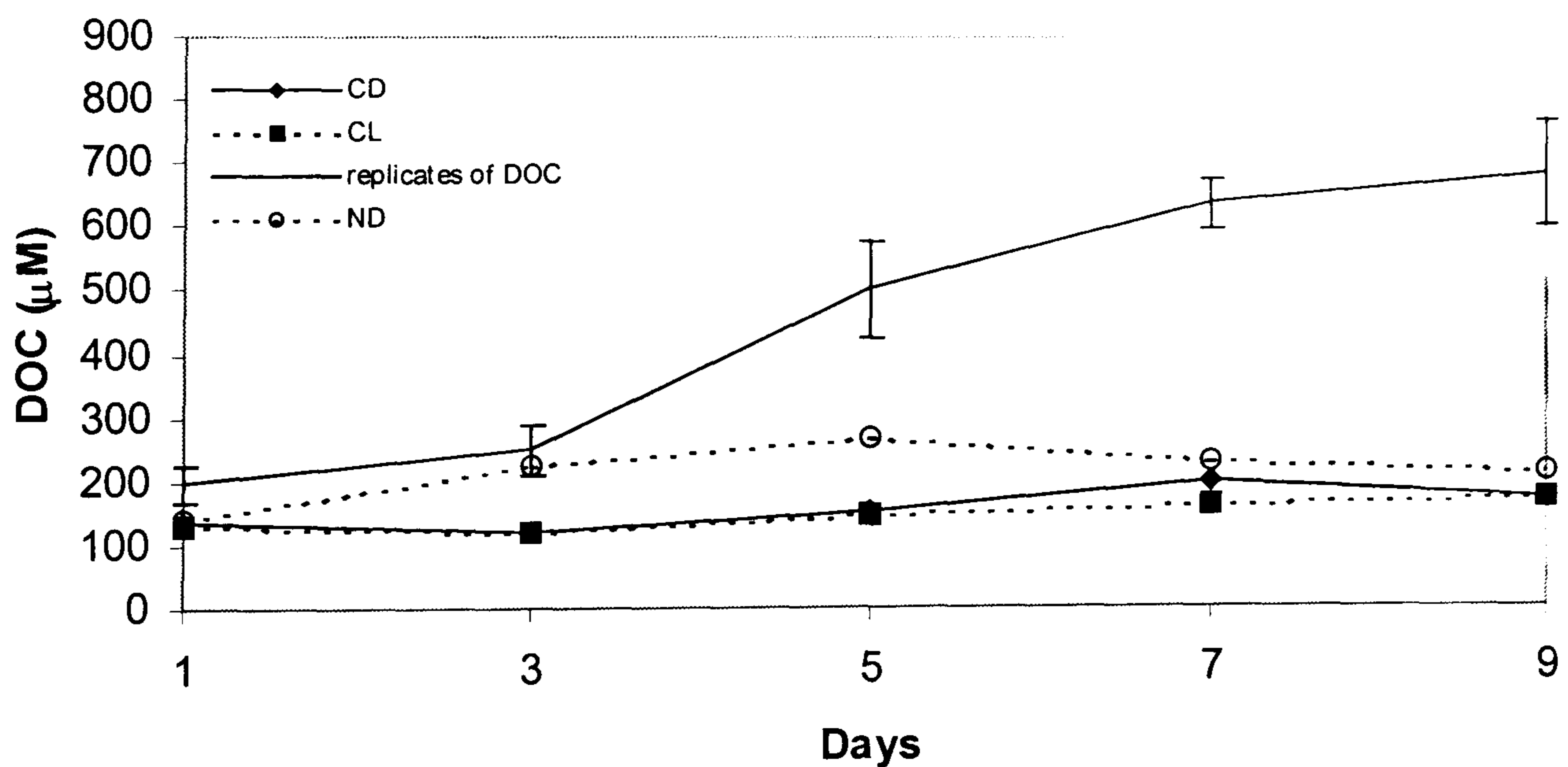


**Figure 7.4. POC variations in *Skeletonema* culture under different light intensity and nutrient (CD=control in the dark, CL=control in the light, ND=nutrient added to culture but in the dark).**





**Figure 7.5. Yellow substance variations in *Skeletonema sp.* culture under different light intensity and nutrient (CD=control in the dark, CL=control in the light, ND=nutrient added to culture but in the dark).**



**Figure 7.6. DOC variations in *Skeletonema sp.* culture under different light intensity and nutrient (CD=control in the dark, CL=control in the light, ND=nutrient added to culture but in the dark).**

### 7.3.3.3. Experiment 3:

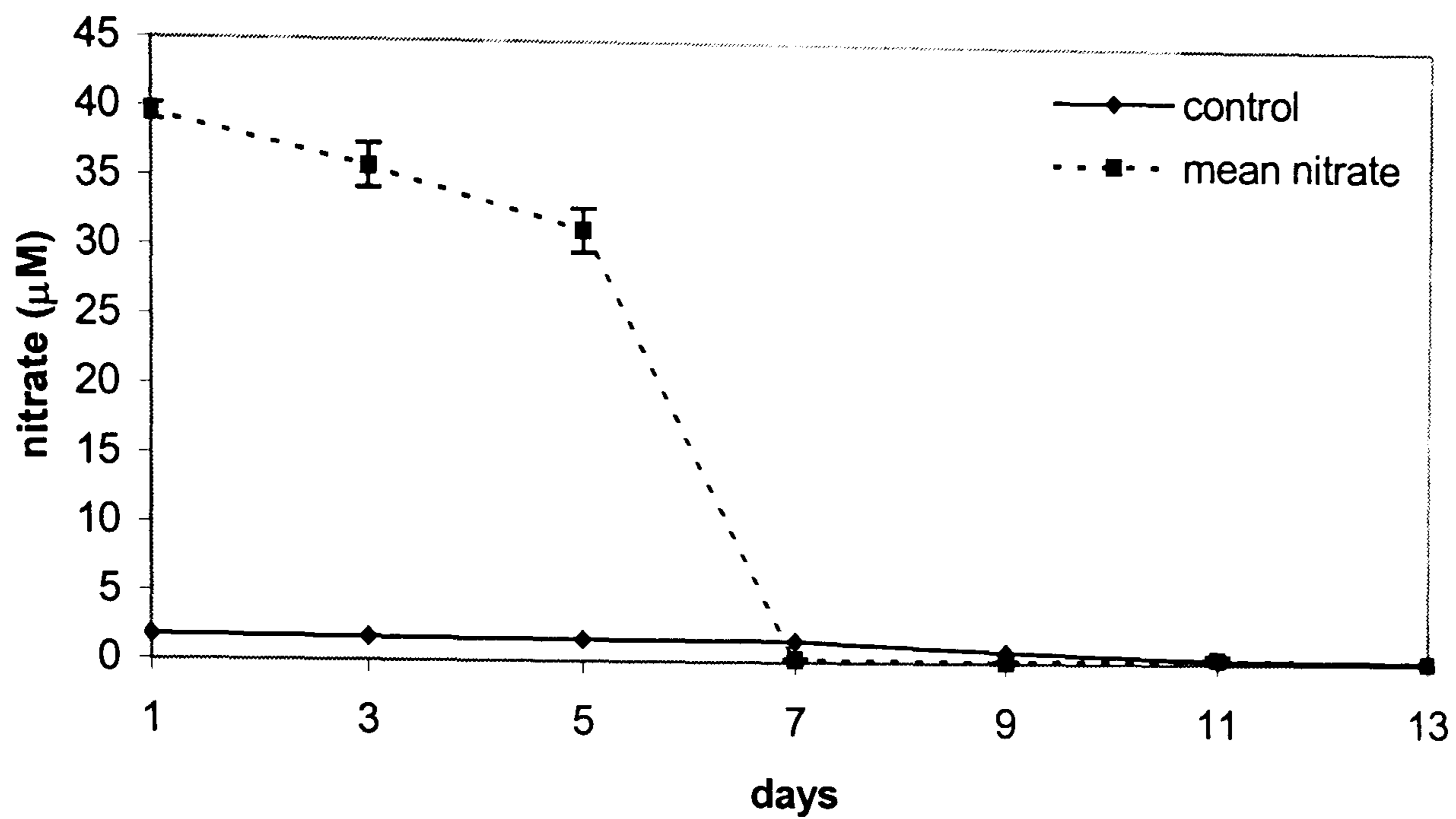
In experiment 3, the initial nitrate and phosphate supply (corresponding to a respective final concentrations of  $40\mu\text{M}$  and  $4\mu\text{M}$ ) in culture vessels 2, 3, 4 were depleted in about 7 days (Figure 7.7 & 7.8). Nitrate and phosphate gradually decreased in the control culture with concentrations of nitrate ranging between  $1.8$  and  $0.17\mu\text{M}$ , and phosphate between  $0.74$  and  $0.07\mu\text{M}$ . A nitrate maximum in the treatments ( $39.6\mu\text{M}$ ) was recorded on day 1, followed by a gradual decline during the first five days and a sharp decrease from day 5, after which concentrations remained below  $1\mu\text{M}$ . Inorganic nitrate in the control culture remained below  $1\mu\text{M}$  and did not differ significantly throughout the experiment. Phosphate followed the same pattern with maximum values occurring on day 1 ( $5.7\mu\text{M}$ ), and a sharp decrease after day 5, leaving concentrations below  $1\mu\text{M}$  for the rest of the study period.

Chlorophyll *a* concentration during the study (Figure 7.9) showed a gradual increase from day 3 reaching a maximum ( $22\mu\text{g}\cdot\text{dm}^{-3}$ ) on day 7 and this was followed by a decrease to a minimum ( $1\mu\text{g}\cdot\text{dm}^{-3}$ ) on day 11. Concentrations in the control remained low.

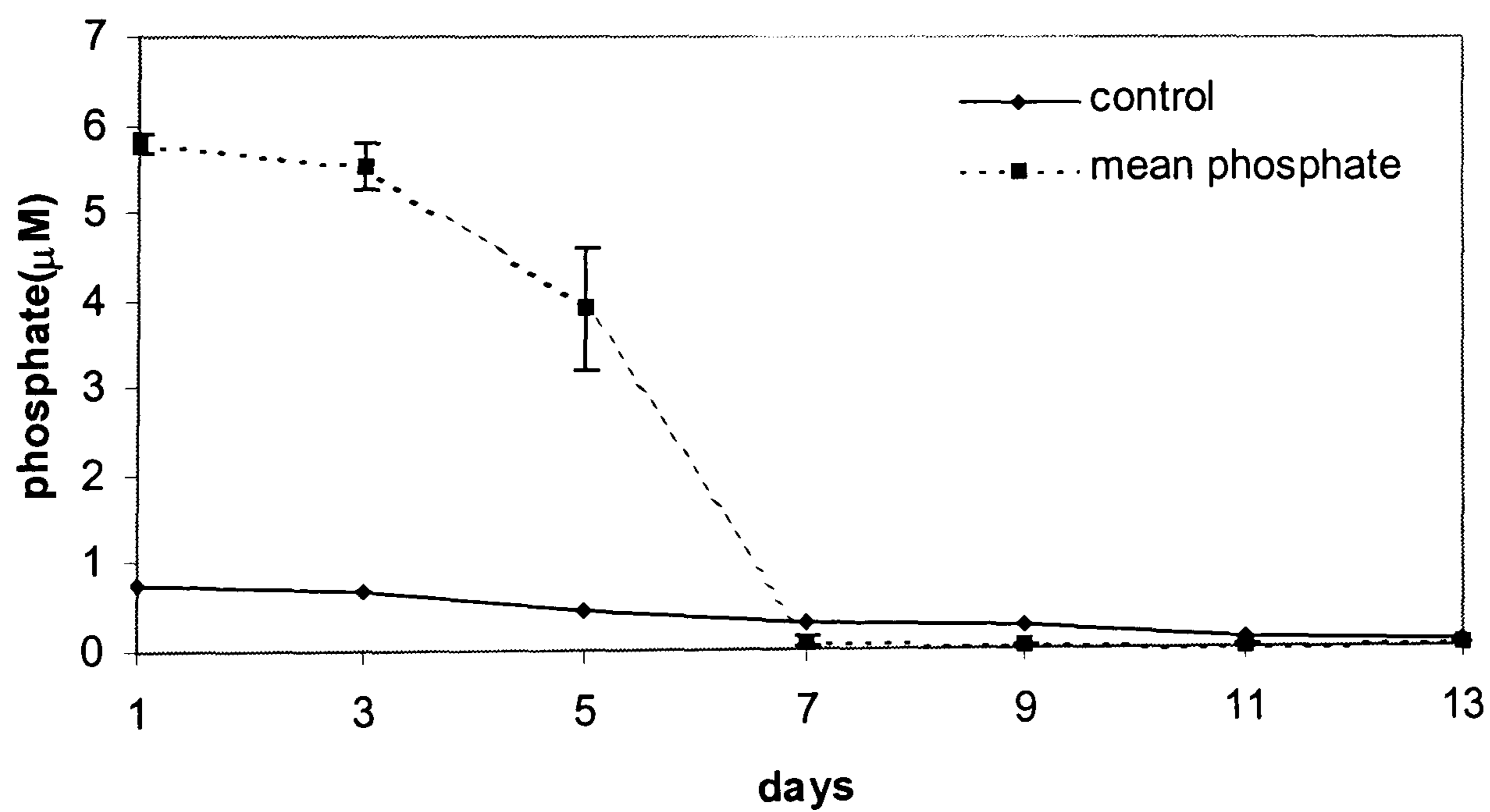
Yellow substance showed slight changes in the control culture while more variation occurred in the treatments (Figure 7.10). For the first 3 days yellow substance remained fairly constant. A gradual increase occurred from day 5 ( $0.20\text{ m}^{-1}$ ) reaching a maximum (ca.  $0.60\text{m}^{-1}$ ) on day 13.

DOC followed similar trend as that of yellow substance (Figure 7.11). Slight variations were observed in control culture ( $149$  to  $167\mu\text{M}$ ). The concentration in the treatments remained constant (ca.  $160\mu\text{M}$ ) for the first three days. A sharp increase occurred from day 3 reaching the maximum concentration ( $352\mu\text{M}$ ) on day 13.

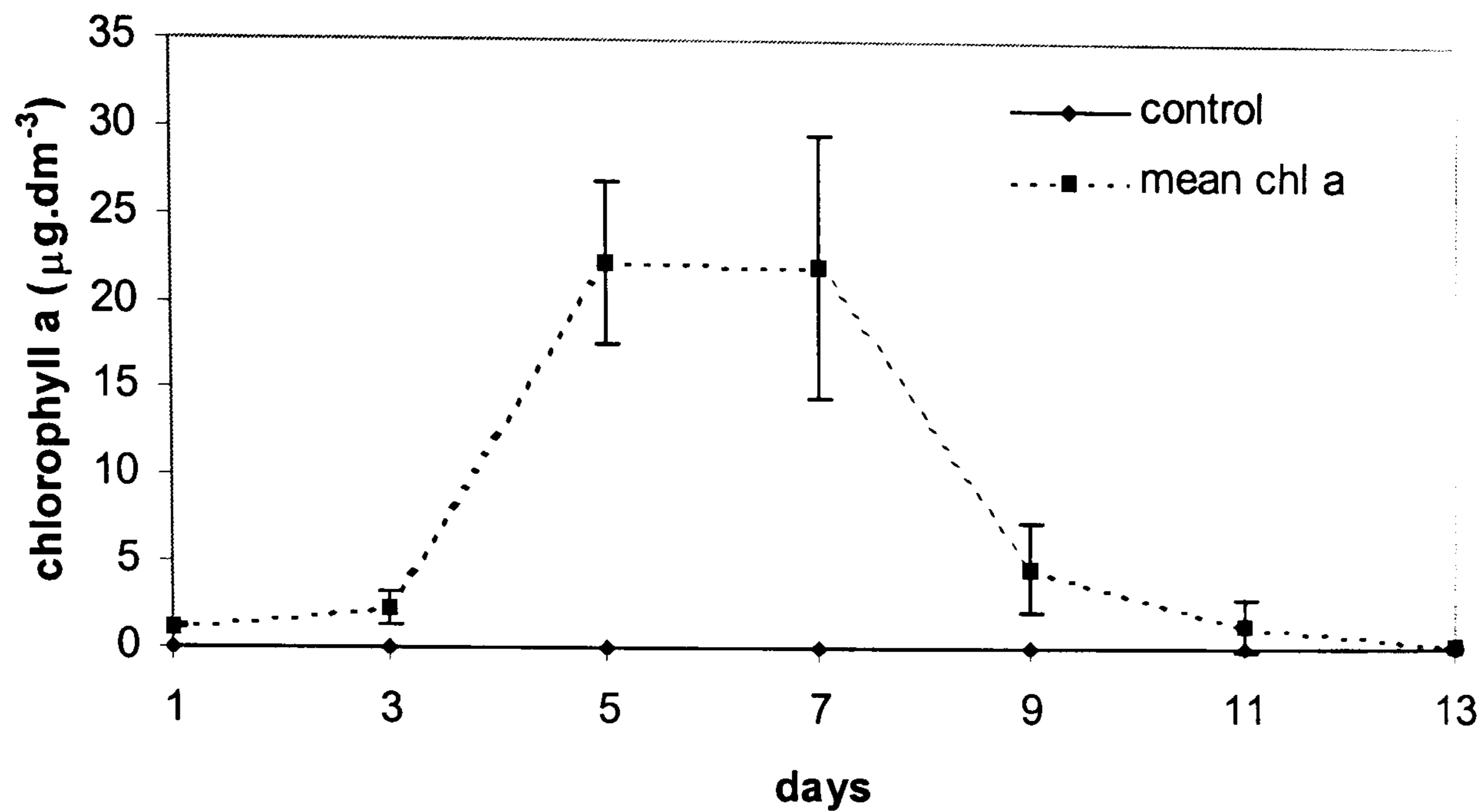
Bacterial abundance (Figure 7.12) decreased over the first 5 days from  $3.6\times 10^6$  to  $2.2\times 10^6\text{ cells}\cdot\text{cm}^{-3}$ . This was followed by an increase reaching a maximum ( $4.9\times 10^6\text{ cells}\cdot\text{cm}^{-3}$ ) on day 9, then a decrease to day 13 ( $3.1\times 10^6\text{ cells}\cdot\text{cm}^{-3}$ ).



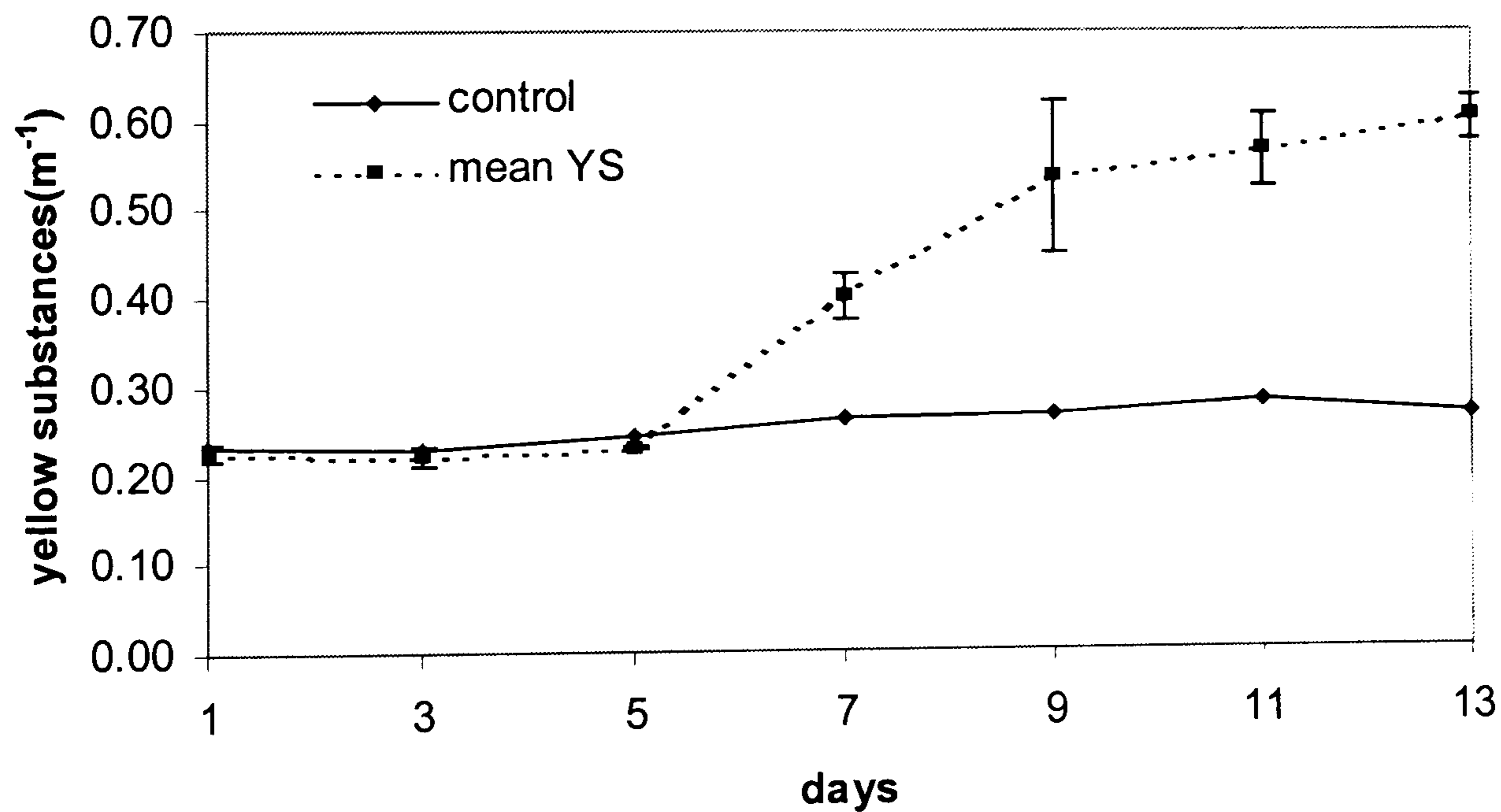
**Figure 7.7.** Influence of growing *Skeletonema sp.* in the culture on nitrate uptake. Slight changes are observed in the control culture.



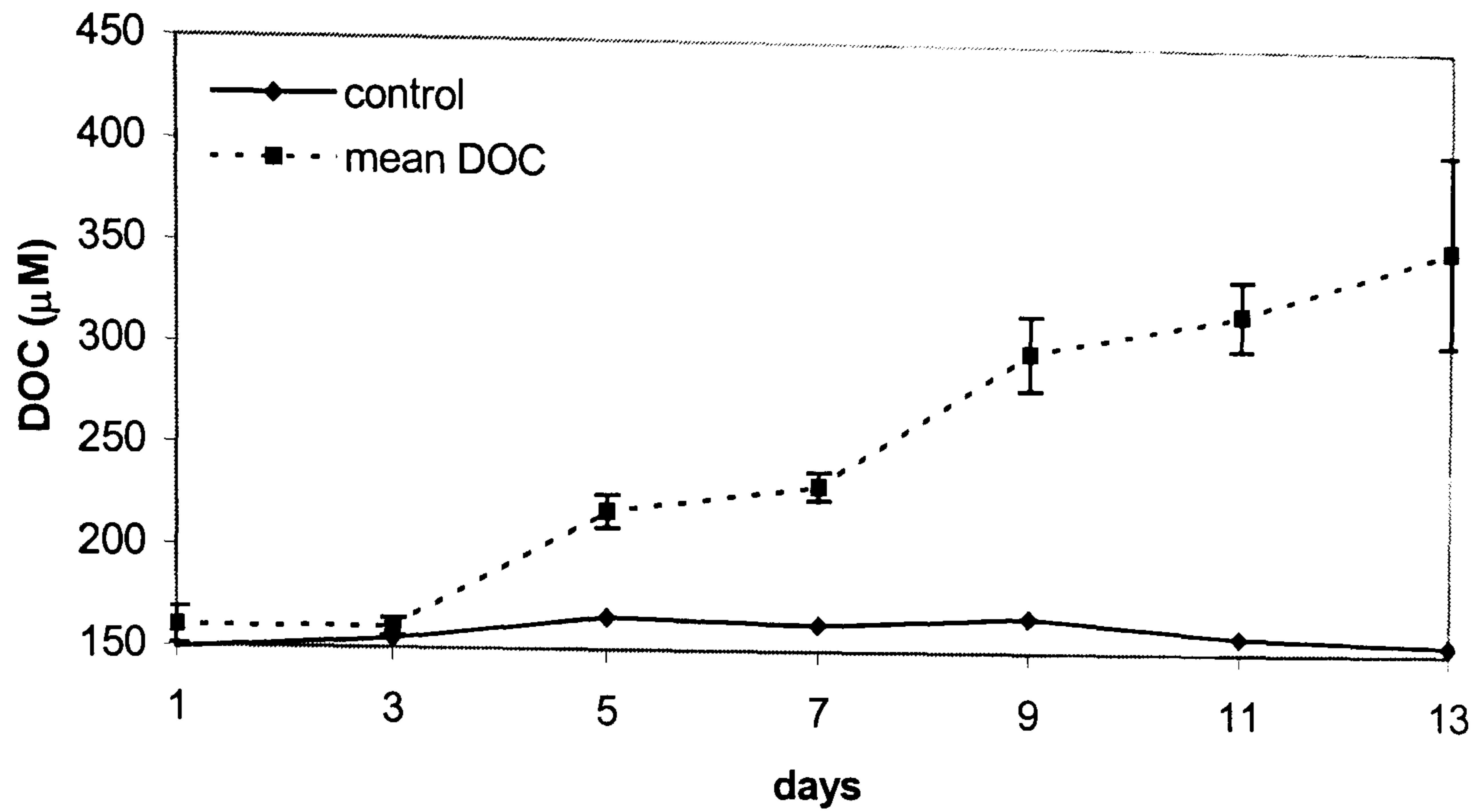
**Figure 7.8.** Influence of growing *Skeletonema sp.* in the culture on phosphate uptake. Slight changes are observed in the control culture.



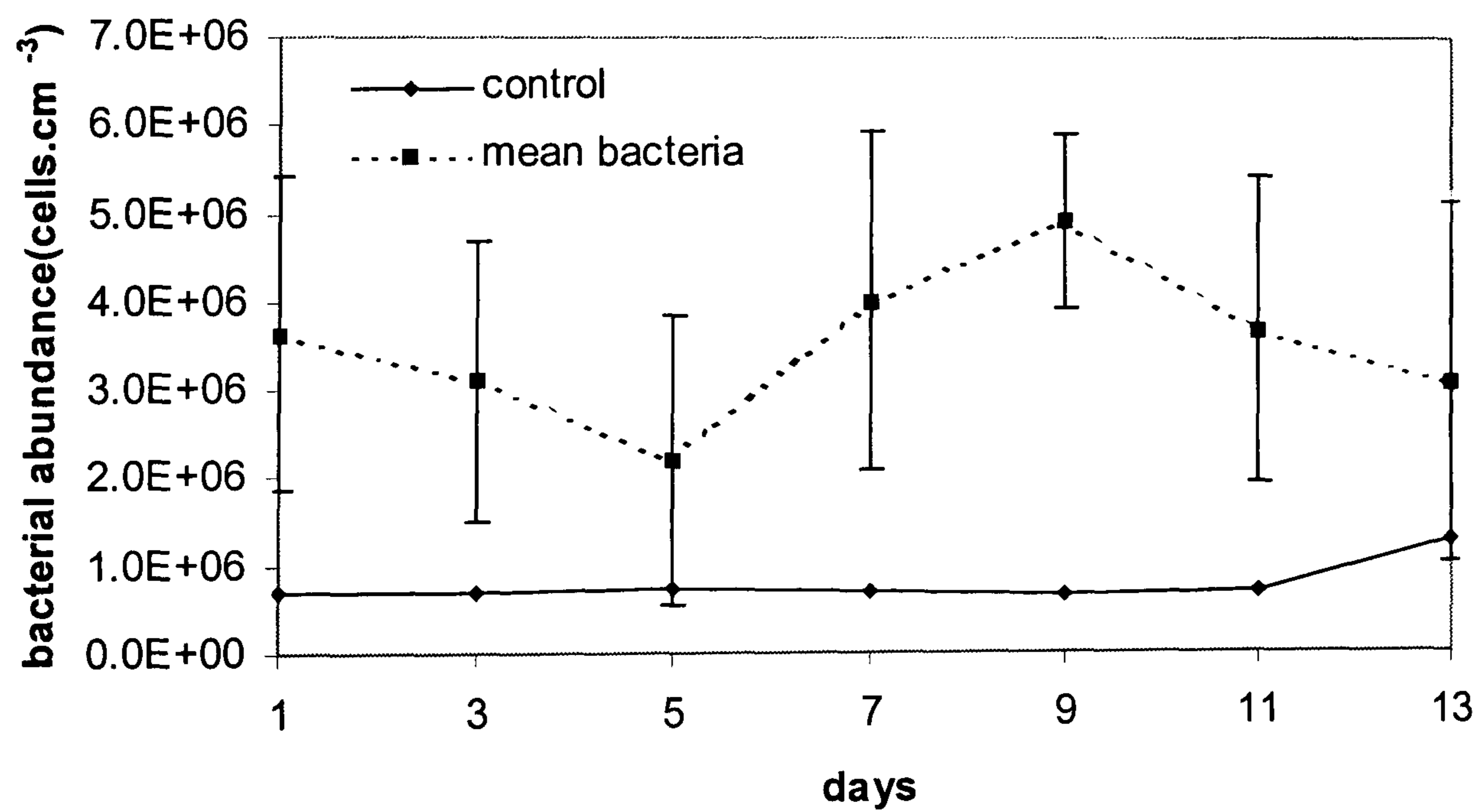
**Figure 7.9.** Changes in chlorophyll *a* in control and treated *Skeletonema* culture. The control shows no changes and maximum chlorophyll *a* on days 5&7.



**Figure 7.10.** Changes in yellow substance in control and treated *Skeletonema* culture. Increase of yellow substance occurred from day 5.



**Figure 7.11.** Changes in DOC in control and treated *Skeletonema sp.* culture. Notice increase of DOC occurred from day 3.



**Figure 7.12.** Changes in bacterial abundance in *Skeletonema sp.* culture. Notice high standard error caused by variations in bacteria number in the cultures.

### 7.3.4. Discussion:

In this set of experiment it was shown that DOC and YS at least in the cultures accumulate throughout the growing phases of the cultures but high accumulation occurred during the stationary phase. Experiment 1 and 2 confirmed the significant correlation between DOC and YS and the effect of light and nutrients. Experiment 3 showed the dynamics of DOC and YS in relation to chlorophyll *a* and nutrients availability.

Experiment 1 was considered as a preliminary experiment since there was missing information in the experimental design. For instance it was assumed that the water used for culture collected via the pumping system would reflect the *in situ* DOC and YS concentration in the Menai Strait. In addition the volume of subculture required to be grown in order to provide a chlorophyll *a* concentration close to that of the Menai Strait water was not known. Nevertheless, this experiment provided information on the continuous accumulation of both DOC and YS throughout the experiment and the different responses to nutrient and light intensity. In addition DOC and YS showed a significant correlation ( $R^2=0.87$ ;  $p=0.006$ ).

The design of experiment 2 was essentially the same as experiment 1 but lower initial DOC concentrations were obtained by filtering rather pumping the water. Experiment 2 confirmed a significant correlation between DOC and YS ( $R^2=0.94$ ;  $p=0.006$ ) and high accumulation during the stationary phase.

In experiment 3, an increase in chlorophyll *a* was followed by decreases in nitrate and phosphate (Figure 7.13) in all culture vessels except the control. Both nitrate and phosphate became exhausted by day 7 of phytoplankton growth. Most of the cell growth occurred before the onset of nutrient depletion. Therefore it is assumed that in this study the production of organic matter occurred under non-nitrogen limiting conditions.

The accumulation of DOC was evident throughout the study period (Figure 7.14) indicating high rates of release during the exponential growth and stationary phase, despite the lack of correlation between DOC and chlorophyll *a*. When production

exceeds consumption it is expected that the accumulation of DOC will be higher at the end of the growth phase. In the current study, the highest concentrations of DOC (352 $\mu$ M C) were observed at the end of the growth of phytoplankton. Similar observations were reported in cultures (Norrman *et al.* 1995; Biddanda & Benner 1997) and at sea at the end of phytoplankton bloom (Carlson *et al.* 1994; Williams 1995).

Biddanda & Benner (1997) reported the production of DOM by different marine phytoplankton in cultures. In their study similar patterns were observed in cultures of *Skeletonema* and *Phaeocystis* species where accumulation of DOC prevailed throughout the experiment, by contrast in *Synechococcus* and *Emiliana* cultures, DOC concentrations were highest during the stationary phase. The accumulation of DOC towards the end of phytoplankton growth has also been observed in this experiment and the field study of the Menai Strait. This indicates the influence of freshly produced phytoplankton DOM, as well as the DOM produced from decay and degradation, on the total DOM accumulation in the oceans.

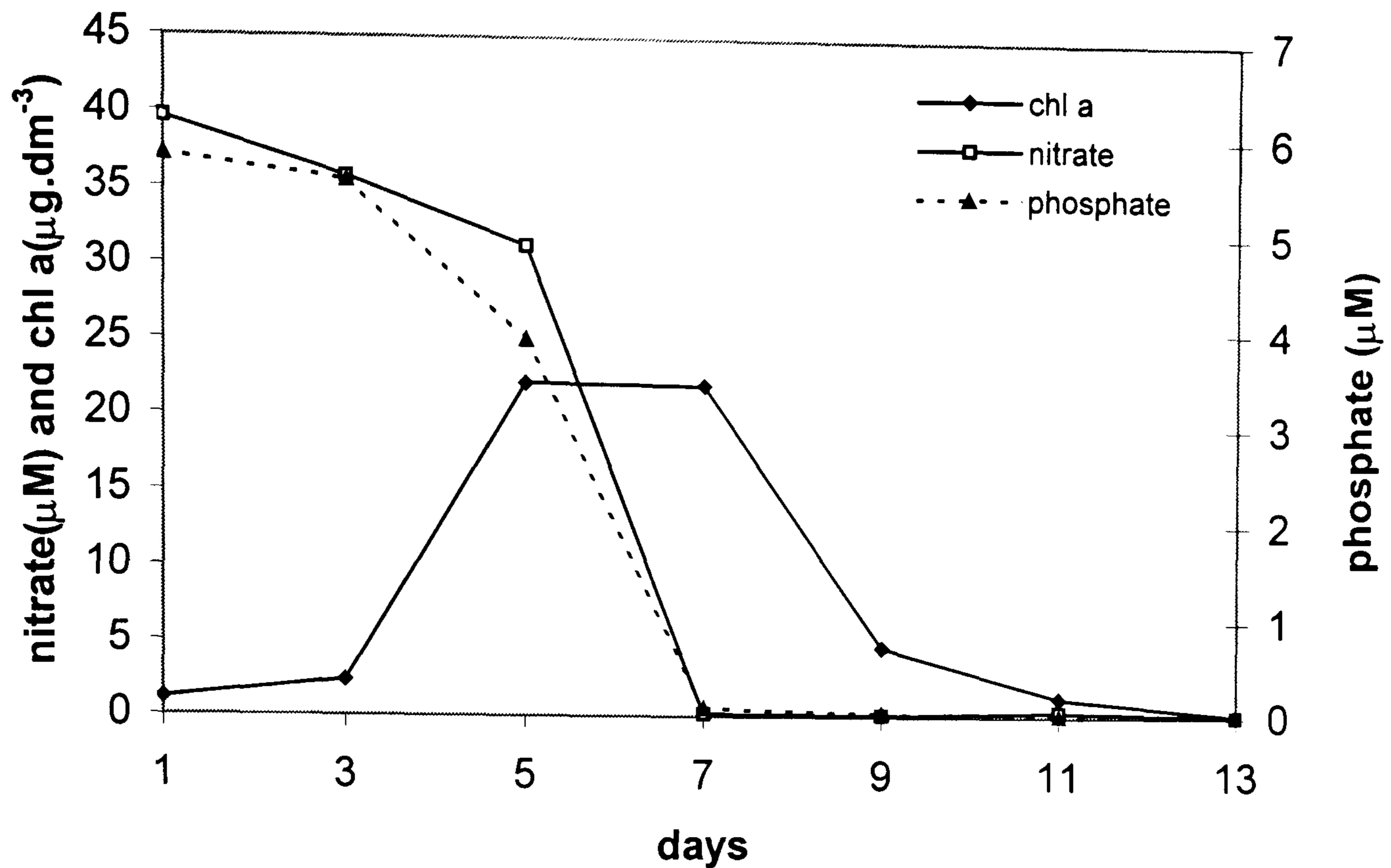


Figure 7.13. Nutrients and chlorophyll *a* variations in *Skeletonema sp* (y-axis represent nitrate ( $\mu\text{M}$ ) and chlorophyll *a* ( $\mu\text{g}\cdot\text{dm}^{-3}$ ), 2<sup>nd</sup> y-axis represents phosphate ( $\mu\text{M}$ )).

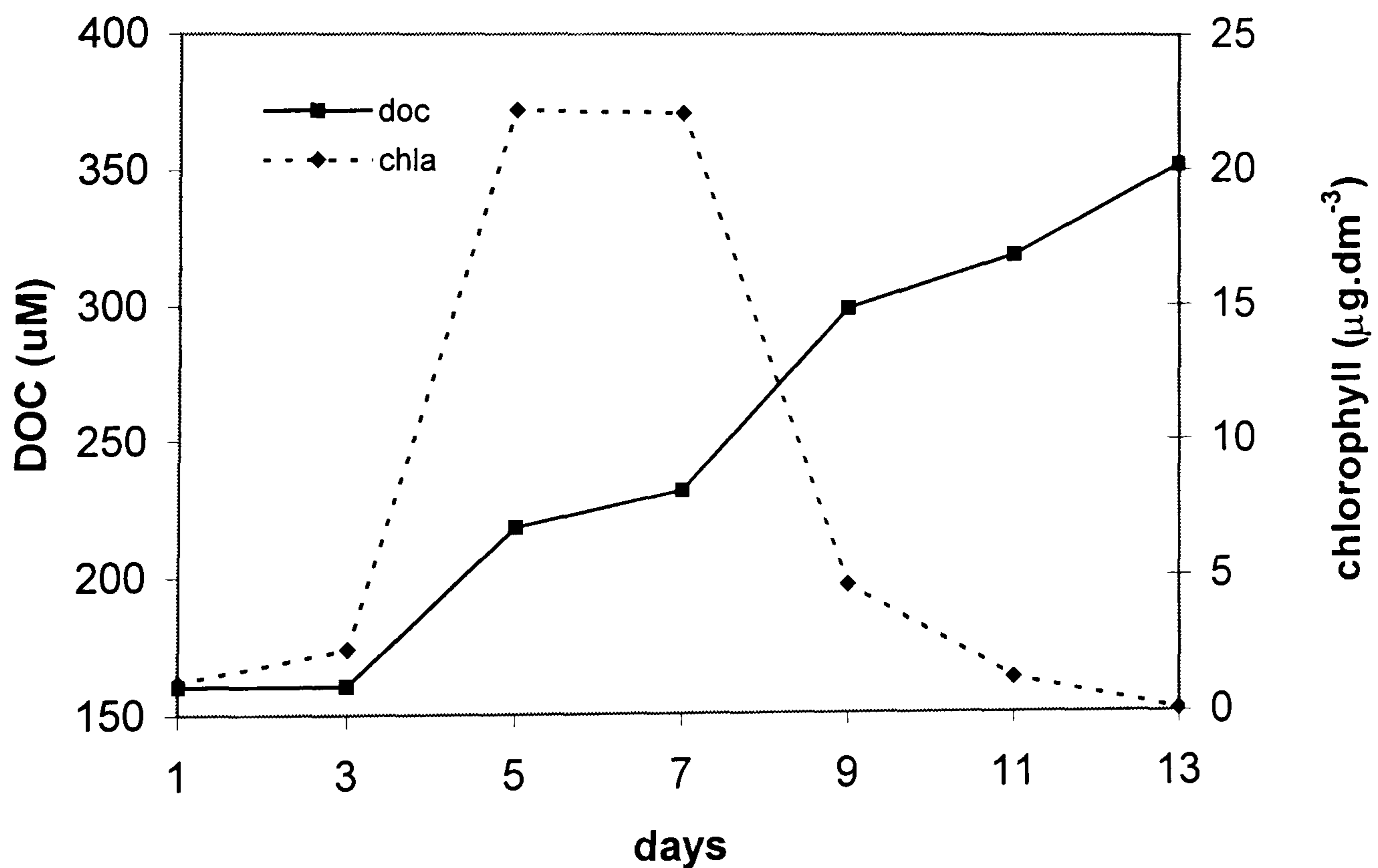
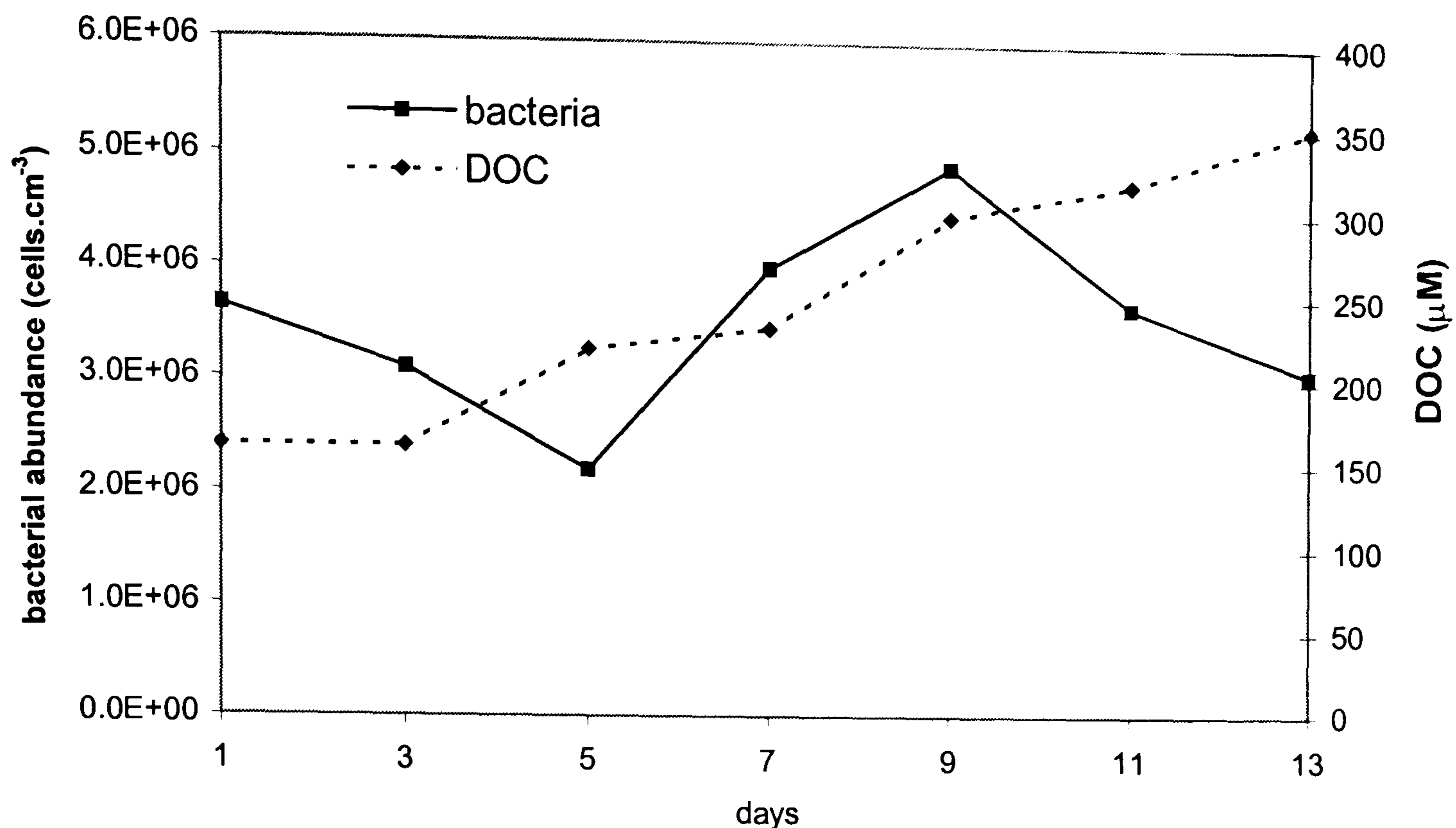


Figure 7.14. Changes in DOC in relation to chlorophyll *a* in *Skeletonema sp* culture showing the increase of DOC before the onset of chlorophyll *a*.

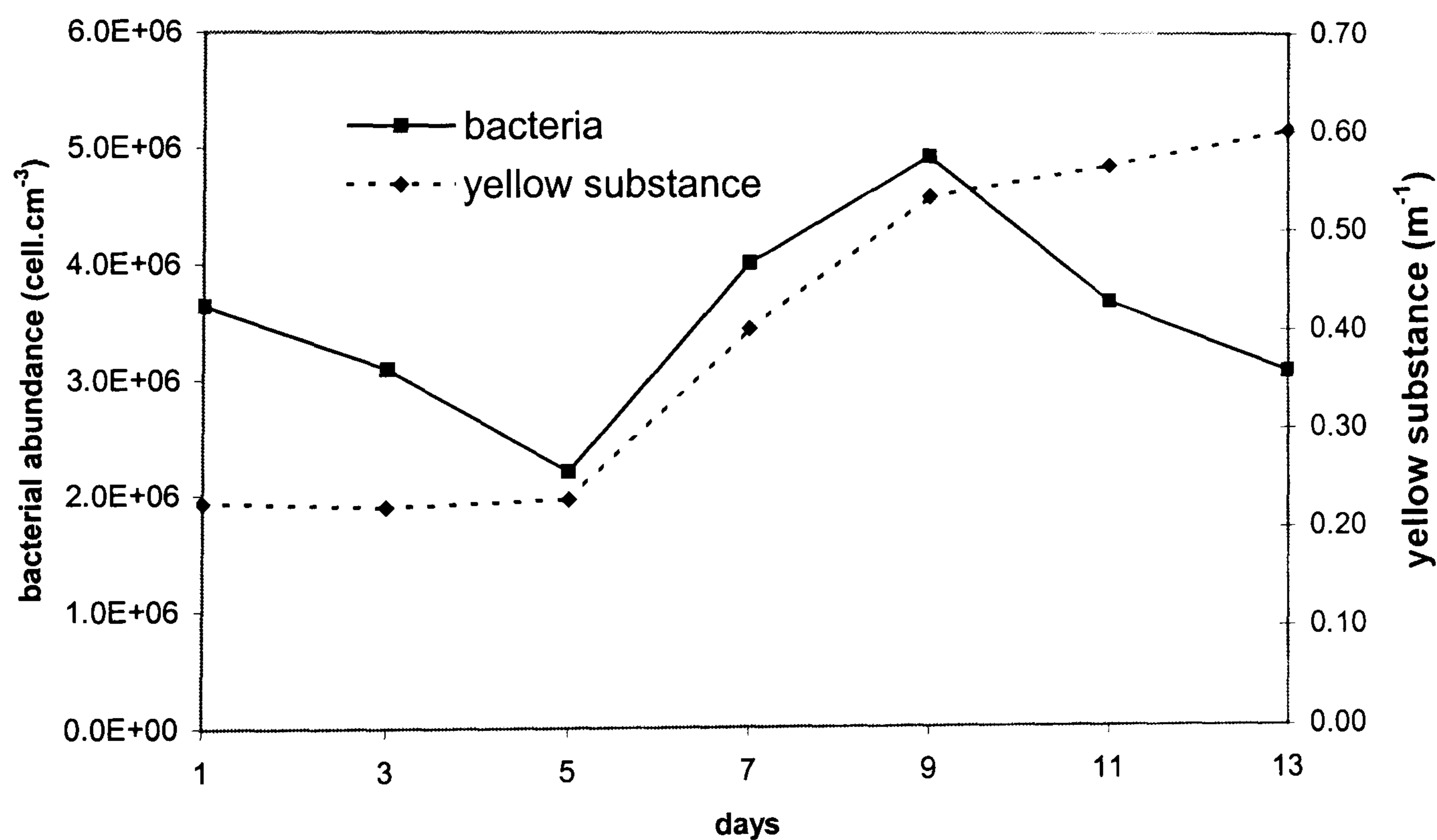


The composition of the freshly produced phytoplankton organic matter was not determined in the present study. However a prevailing accumulation of DOM in this study indicated that either the production of the organic matter exceeded consumption or the nature of the produced DOM was of less importance to the available consumer. From Figure (7.15) and Figure (7.16) it appears that bacterial abundance increased with DOC and yellow substance. No correlation was found between DOC and/or yellow substance and bacterial abundance. Significant correlation ( $p=0.001$ ) was found between DOC and yellow substances (Figure 7.17). Eberlein *et al.* (1985) reported that phytoplankton in cultures release large amount of carbohydrates in the form of exopolymers. Consequently, it was reported that carbohydrates constitute the major component of DOM released by phytoplankton along with amino acids (Morris & Foster, 1971). Other studies have suggested that dissolved carbohydrates may support a significant portion of bacterial carbon and energy demand (Amon&Benner 1996; Norrman *et al.* 1995). However this was not the case in this study since there was no correlation between DOC or YS with bacterial number.

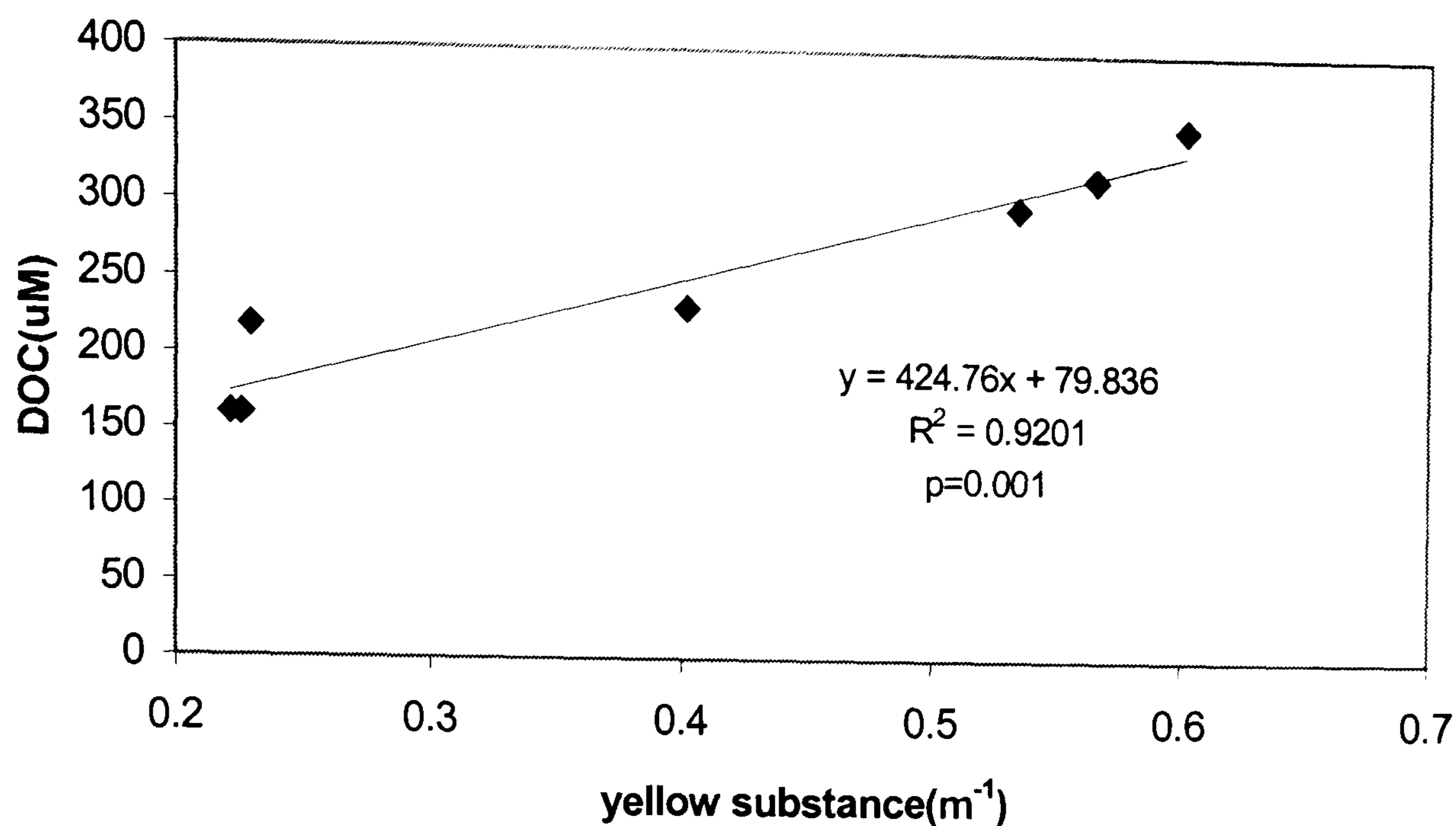
The highest accumulation of DOC and yellow substance occurred at the end of the cultures in all experiments in this study. This is in accordance with several studies (Biddanda & Benner 1997; Mykelstad 1974; Eberlein *et al.* 1985) which indicate that the accumulation of DOC and yellow substance is a combination of *in situ* production and degradation of DOM.



**Figure 7.15.** Variations of bacterial abundance in relation to dissolved organic carbon (y-axis represents bacterial abundance in cells.cm<sup>-3</sup>, 2<sup>nd</sup> y-axis represents DOC in μM).in *Skeletonema sp* culture.



**Figure 7.16.** Variations of bacterial abundance in relation to yellow substance in (y-axis represents bacterial abundance in cells.cm<sup>-3</sup>, 2<sup>nd</sup> y-axis represents yellow substance). *Skeletonema sp* culture.



**Figure 7.17. Correlation between bacterial abundance and yellow substance in *Skeletonema sp* culture.**

Although most of phytoplankton exudates are LMW DOM (Jensen 1983; Lancelot 1984), phytoplankton also produces a large amount HMW DOM (Amon & Benner 1996). The HMW DOM is carbon-rich and supports high rates of bacterial respiration, while the LMW DOM is nitrogen-rich and supports higher rates of bacterial growth (Amon & Benner 1996; Gardner *et al.* 1996). In this study, the lack of correlation between DOC and bacterial abundance, in addition to high accumulation of DOC and yellow substance before the onset of the phytoplankton growth, could be an indication that the DOM released was HMW DOM. This is in accordance with the study of Biddanda & Benner (1997) who reported a low rate (4.1) C:N of DOM in *Synechococcus* culture and a high (14.1) C:N of DOM produced in *Skeletonema* culture. This could be explained as a result of the composition of the DOM produced by cyanobacteria which comprises a large portion of amino sugars, amino acids and nucleic acids (Biddanda & Benner 1997), all of which are rich in nitrogen, thus reducing the C:N ratio. However the DOM produced by *Skeletonema* may be carbon-rich (Goldman *et al.* 1992; Williams 1995) thus producing high a C:N DOM.

DOC accumulation has been also linked to nutrient depletion (Ittekkot *et al.* 1981). The effect of light intensity and nutrients on DOC and yellow substances is shown in Figures (7.1&7.2). Both DOC and yellow substances exhibited similar patterns in different cultures. Culture work has demonstrated that large a production of extracellular DOC may result from nutrient depletion (Goldman *et al.* 1992). From experiment 1 high concentrations of DOC occurred in culture vessel without nutrient addition. It could be assumed that the addition of nutrient increased bacteria activity thus decreasing the concentration of DOC in the culture. By contrast in the culture without nutrient addition bacteria activity could have been reduced due to the lack of nutrients. In addition production could have exceeded consumption, allowing high accumulation of DOC.

In contrast to DOC, nutrients seemed to play a minor role in yellow substance accumulation. High values occurred in culture vessels with high light (experiment 1), with or without nutrient addition. This could be explained as a result of the nature of the dissolved organic matter produced. Unlike DOC, yellow substance represents mostly the refractory fraction of the DOM produced. In this case exposure to high light leads to increased primary production and DOM release. Bacterial activity is influenced by the addition of nutrient, thus removing the labile fraction of DOC and the rest accumulates as refractory DOC, which is mostly represented by yellow substance. It would have been interesting to have information on bacteria production, along with primary production in order to support this hypothesis.

DOM production and accumulation involve a number of parameters, however, at least in this study (experiments 1&2), light was shown to be one of the most important factors influencing the total organic production and consumption. The production rate in the dark culture did not show significant changes despite the nutrient input. Ignatiades & Fogg (1973) reported from their study that one of the factors affecting the release of organic matter in *Skeletonema costatum* culture, was the influence of light. All the cultures which were exposed to 10 klux (close to light intensity used in this study ~12klux) of illumination released 2.5 to 12.2% of organic

matter while in those which were exposed at 0.6 klux no excretion products were detected.

Hellebust (1965) discussed the influence of light as factor affecting the release of organic matter. From his study, he suggested that high light intensities might cause damage to the cell membranes and thus increase their permeability to organic matter. Although the results in the present study support the idea of light influence on the release of organic matter, more investigation is needed to look into different aspects related to the nature of light, i.e. whether the light used in the experiment resembles the natural light underwater, its intensity, and the response from different phytoplankton species to light exposure. The difference between the two sources of light (natural and *in vitro*) could influence the growth behaviour of different species.

The experiment with *Skeletonema costatum* showed that changes in the physiological state of the algae associated with nutrient deficiency and light intensity affected excretory behaviour. In addition DOC and yellow substance accumulated at the end of the phytoplankton bloom although sometimes this depends among other factors on the species composition of the bloom. However the explanation of the pattern of DOC and yellow substance in an environment such as the Menai Strait involves a lot of parameters that interact each other. For instance the time between chlorophyll *a* decrease and DOC increase argues against the decay of phytoplankton itself being the main source of DOM. The fate of planktonic algae in the sea is to be eaten as fast as they are produced and almost all of the phytoplankton cells produced enter the grazing food chain. In environments such as the Menai Strait phytoplankton development during spring and summer occurred gradually and this could be related to some extent to the zooplankton present to deal with the phytoplankton present at the time. Therefore it is probable that losses during grazing, death, decay of other organisms that developed at the expense of phytoplankton, and their excretory products, were responsible for the accumulation of DOC in the environment after a phytoplankton bloom. The consistency in the time lag between chlorophyll *a* and DOC peaks in experimental studies and in the Menai Strait, regardless of the time of the year or the concentration of chlorophyll *a*, adds strength to this conclusion.

### 7.3.5. Future research based on the experimental results:

Although the experimental investigations produced promising results, they can at best, only be considered to be preliminary experiments providing a basis for more extensive experimental investigations. The mechanisms controlling DOM dynamics in the Menai Strait will be many and varied, and for the rest of this chapter several proposals are put forward for the possible development of future experiments to investigate this.

Preliminary results have pointed towards the important role played by light and nutrients on the production and consumption of DOM. However, these results clearly indicate that a more comprehensive and detailed study is needed. This is because there are a number of questions on the role of other parameters and experimental techniques. For instance, the concentrations of nutrients used in the third experiment were close to the *in situ* nutrient concentration in the Menai Strait during early spring, but it seems the light intensity used in this study was high compared to Menai Strait PAR. In addition it is difficult to produce a general conclusion using results from a single species since there is more than one species dominating during spring. The role of protozoa was also not investigated in this study. However, it is difficult to investigate all these parameters in the limited time of this study. It is therefore necessary to design future experimental studies looking at the above-mentioned questions. Furthermore, these experiments should look at the physiological response of different phytoplankton species and heterotrophic microorganisms (bacteria & protozoa) under a wide range of irradiances, nutrient concentrations and temperature.

As a first step towards a comprehensive study of the production and consumption of DOM, three questions were formulated as testable hypotheses in order to understand some of the processes which were not clear in the preliminary experimental work.

These questions were as follows:

- What is the effect of nutrients and light intensity on the dynamics of dissolved organic matter? This was investigated as presented at the beginning of this chapter.

- Is the quantity and quality of dissolved organic matter released by phytoplankton, species related?
- What is the role of protozoa on dissolved organic matter production and consumption?

#### **7.4. Is the quantity and quality of dissolved organic matter released by phytoplankton, species related?**

The importance of dissolved organic matter (DOM) in the biogeochemical cycle of the ocean systems has been investigated and reported quite extensively by a number of scientists (Kirchman *et al.* 1991; Carlson & Ducklow 1995; Karl *et al.* 1998). Subsequently, the concentrations and the molecular weight composition of dissolved organic matter and their relation to bioreactivity have been reported (Amon & Benner 1994; Sharp *et al.* 1995; Guo *et al.* 1995). The excretion of photosynthetic extracellular release (PER) by phytoplankton is well documented (Myklestad & Haug 1972; Myklestad 1974; Lancelot 1983; Hong *et al.* 1997). It is now known that highest PER occurs during stationary phase of phytoplankton growth (Mague *et al.* 1980; Myklestad *et al.* 1989). In addition several studies (Wiebe & Smith 1977; Larsson & Hagstrom 1982; Wolter 1982; Sell & Overbeck 1992; Hong *et al.* 1997) have demonstrated the tight coupling between primary production and bacterial utilization. However part of the complexity of studying PER in relation to bacteria may arise because of the different physiological behaviors of the various taxonomic species which change with environmental changes.

It is known that different species have different PER (Myklestad 1974; Wolter 1982), however the quantity and quality of dissolved organic matter released by different phytoplankton taxonomical groups have not been taken into account when studying the coupling of phytoplankton production and bacterial consumption. During a phytoplankton bloom species succession and the accumulation of dissolved organic matter can be investigated, but it is difficult to determine the concentration and the molecular weight composition of DOM and their relationship to phytoplankton species composition. This is because in the natural environment, it is difficult to differentiate between the freshly produced DOM from phytoplankton and

that originating from other sources. In addition it is difficult to distinguish the amount and composition of DOM released by individual phytoplankton species in the natural environment. On the other hand, culture studies could help determine the composition of released DOM because organic compounds found in the culture media can be assumed to originate from phytoplankton photosynthesis.

It would therefore be the aim of this study to investigate the contribution of some of the dominant of phytoplankton species in the Menai Strait to the quantity and quality of DOM. It is anticipated that the outcome of this experiment will contribute to the understanding of the DOM dynamics. For instance, considering the type of DOM accumulating during phytoplankton bloom in the Menai Strait: Is it dominated by freshly produced DOM from phytoplankton? If so, how many different species are contributing, i.e. the concentration and bioreactivity of the DOM produced and their impact on other microorganisms. Is the phytoplankton contribution related to size or it is related to the response of different species to biological and physico-chemical parameters within the Menai Strait? In the field study of the Menai Strait I hypothesized about the type of organic matter passed from phytoplankton to bacterial in relation to species succession. This type of experiment will provide information to test the validity of the hypothesis.

#### **7.4.1. Objective 1:**

To study the effect of phytoplankton taxonomic composition on the nature of the dissolved organic matter released.

#### **7.4.2. Experimental design:**

Four species (*Skeletonema costatum*, *Cheateoceros affinis*, *Rhizosolenia* sp., and *Phaeocystis pouchetii*) will be isolated and grown to obtain unialgal cultures. These species are considered to be dominant during the spring bloom in the Menai Strait. Water will be collected from the Menai Strait and filtered through pre-combusted 0.45 $\mu$ m pore-size GF/F Whatman filter and through 0.2 $\mu$ m Nucleopore membranes by gentle vacuum filtration to remove phytoplankton and bacteria. Initial concentrations and composition of DOM of the water used for culturing will be recorded.



Two liters ( $2\text{dm}^3$ ) of filtered water will be transferred into each of culturing vessels (Pyrex Erlenmeyer flasks, acid washed and sterilized) and nutrient salt solution ( $2\text{cm}^3$  of F/2 medium (Guillard & Ryther 1962) added and mixed thoroughly. The algal inocula will be added to the culturing vessels so that the initial Chl *a* concentration in each culture is at least  $5\ \mu\text{g}\cdot\text{dm}^{-3}$  (average concentration during spring). The cultures bottles will be maintained at  $15^\circ\text{C}$  and under a changing daily irradiance equivalent to Menai Strait PAR (during spring at 1m deep) and grown in replicates of three for each species. Sterile air will be bubbled through the cultures for mixing and to supply  $\text{CO}_2$ . The culture will be enriched with  $\text{KNO}_3$  and  $\text{KH}_2\text{PO}_4$  to a final concentration of  $16\ \mu\text{M}$  and  $2\ \mu\text{M}$  respectively and grown for about 2 weeks. The filtered Menai Strait water will be used as control.

The cultures will be sampled daily for nitrate, phosphate, Chl *a*, cell number, particulate organic carbon (POC), dissolved organic (DOC) and yellow substance (YS). The methods and material used to analyze the above parameters are described in details in Chapter III. The biochemical compounds such as carbohydrates, lipids and amino acids will be analyzed following the techniques described by Smith & Underwood (2000) and Hamanaka *et al.* (2002).

### **7.4.3. Objective 2:**

The above experiment could be expanded to investigate the response of bacteria on the DOM produced by different phytoplankton species. This could be achieved by studying bacterial production under a wide range of temperature and light intensity.

### **7.4.4. Experimental design:**

Water will be collected from Menai Strait and treated as explained in the experiment looking at the impact of species composition on DOM production in this chapter. Seawater used for culturing will be checked for any contamination caused by filtration procedure by measuring inorganic nutrients and organic carbon before and after filtration. Species (*Skeletonema costatum*, *Cheatocecos affinis*, *Rhizosolenia sp.*, and *Phaeocystis pouchetii*) will be grown as unialgal batch cultures in autoclaved  $2\text{dm}^3$  in Pyrex Erlenmeyer flasks containing  $200\text{cm}^3$  of F/2 medium. The initial chl *a* concentration in batch culture will be at least  $5\ \mu\text{g}\cdot\text{dm}^{-3}$ . Water for

bacterial inoculation will be filtered through 0.45 $\mu$ m Whatman GF/F filters leaving about 50% of bacteria in the filtrate. About 10cm<sup>3</sup> of filtrate containing microorganisms will be transferred to each culture. The cultures will be exposed to different regimes of light and dark in a temperature-controlled incubator. At least three light intensities will be used corresponding to the lowest, medium and highest light intensity in the Menai Strait. Three incubation temperatures (5, 10 and 20°C) will be used. These cover the range of water temperature encountered in the Menai Strait. The culture will be grown for one week and sampled for chl *a*, phytoplankton cells, bacterial abundance, bacterial production, DOC, and yellow substance. Except for bacterial production, other parameters will be analyzed as explained earlier. Bacterial production will be measured using the [<sup>3</sup>H] thymidine incorporation method (Fuhrman & Azam 1982).

#### **7.4.5. Statistical analysis of data:**

Replicate measurements will be performed by taking subsamples from a single culture. This will help to overcome the problem of discriminating between variation caused by changes in specific parameters and the variation caused by the culture itself. Data will be checked for normal distribution and all parameters will be compared statistically for significant differences between culture types with multi-way (Factorial) ANOVA. Positive or negative relationships will be checked for specific parameters in all treatments using linear regression analysis.

In this experiment the quantity and quality of DOM produced by different phytoplankton species will be studied. This will be achieved through comparing the total DOM concentration in cultures followed by fractionation to find out which species produce what fraction of DOM and in what quantity. Then this will be related to change in irradiance and nutrient availability. The response of bacteria to the DOM produced and its production will be investigated by correlating its production with different variable in the experiment. These experiments will indicate the significance contribution of different phytoplankton species as a source of DOM and the response of bacteria to the produced DOM from different phytoplankton species.

## 7.5. What is the role of protozoan in dissolved organic matter production and consumption?

There has been much controversy over the roles that bacteria and protozoa play in the marine food web (Ammerman & Azam 1985). This controversy is based on the argument as to which group of microorganism plays the major role in regenerating nutrients. Both protozoa and bacterial were reported to play an important role in nutrient recycling (Andersen *et al.* 1986). A number of studies have reported the role of protozoa as a major factor in nutrient recycling, especially ammonia (Anderson *et al.* 1985; Goldman & Caron 1985; VanWambeke & Bianchi 1985). In addition protozoa are capable of preying on both phytoplankton and bacteria, and nitrogen is regenerated from all grazed microbes (Goldman & Caron 1985).

In order to understand the dynamics and control of bacterial consumption of organic matter in the natural environment we need to understand the factors controlling bacterial growth and abundance. These factors could be controlled by physiological properties of bacteria and interaction between bacteria and other microorganisms (Thingstad & Lignell 1997). These factors include temperature, viral infection, resource limitation, and grazing (del Giorgio & Scarborough 1995). Grazing of bacteria is one of the major factors influencing DOC accumulation. The existence of active and inactive bacteria is ecologically significant since many flagellates are selective feeders. There are reports suggesting that larger bacterial size-fractions have high specific growth and production rates (Bird & Kalff 1993) and that predation on bacteria by nanoflagellates and other planktonic grazers, involves selection of large active bacteria (Jurgens & Gude 1994).

Therefore, it may be hypothesized that protozoa could control the accumulation of DOC by limiting the number of active bacteria in the water system during the productive season, thereby limiting the ability of bacteria to degrade the labile DOC and regenerate nutrients. The biomass and seasonality of protozoa were not investigated in the Menai Strait in the current study. This raises a number of questions about their roles. For example: what was the impact of protozoa on both phytoplankton and bacteria, and what was their possible role in nutrient regeneration

during the summer period in the Menai Strait? Was their role in DOM dynamics more efficient during spring or summer? It has been reported that protozoa regenerate large quantities of ammonia while grazing; sometimes the regeneration exceeds that for most macrozooplankton (Gast & Horstmann 1983; Sherr *et al.* 1983; Andersson *et al.* 1985; Goldman *et al.* 1985).

It is therefore assumed that the outcome of this experiment will enhance the understanding of the dynamics of DOM in the Menai Strait. For example: was the variation in phytoplankton and bacterial abundance in the Menai Strait during spring and summer caused by protozoa grazing. What was their role in the rapid regeneration of ammonia and phosphate during summer? Therefore information from this experiment will provide more insights on the dynamics of DOM in the Menai Strait especially, during spring and summer.

### **7.5.1. Objective:**

To study the role of protozoa on dissolved organic matter production and consumption.

### **7.5.2. Experimental design:**

Water will be collected in the Menai Strait and filtered through 200 and 40 $\mu$ m pore-size screens, and through 0.8 $\mu$ m Nucleopore membranes, by gentle vacuum filtration. Three replicate culture vessels will be filled with 2dm<sup>3</sup> water containing 4 different size spectrum of particles: A (unfiltered water), B (water filtered through 200 $\mu$ m), C (water filtered through 40 $\mu$ m), D (water filtered through 0.8 $\mu$ m) and E (water filtered through 0.2 $\mu$ m) as a control. The cultures bottles will be maintained at 15°C and at constant light intensity close to the PAR characteristic of the Menai Strait during spring. Sterile air will be bubbled through the cultures for mixing and to supply CO<sub>2</sub>. The culture will be grown for 10 days and sampled daily for chl *a*, phytoplankton abundance, bacterial abundance, protozoa abundance, DOC and yellow substance.

To determine the heterotrophic nanoflagellates in all samples, 15-20ml of samples will be preserved in gluteraldehyde, stained with DAPI (5 $\mu$ gml<sup>-1</sup>) and filtered

through black 0.8 $\mu$ m Nuclepore filters. Protozoa abundance will be assessed using epifluorescence microscopy (Olympus 2HB RFL Epifluorescence Microscopy) under 1000X magnification. Bacterial net growth rates in the culture will be estimated from difference in abundance from day 0 to day 10, assuming exponential growth (Ducklow *et al.* 1985).

### 7.5.3. Statistical analysis of data:

Replicate measurements will be performed by taking subsamples from a single culture. This will help to overcome the problem of discriminating between variation caused by changes in specific parameters and the variation caused by the culture itself. Data will be checked for normal distribution and all parameters will be compared statistically for significant differences between culture types using multi-way (Factorial) ANOVA. Positive or negative relationships will be checked for specific parameters in all treatments using linear regression analysis.

In this experiment the role of protozoa on DOC production and consumption will be studied. This will be achieved through investigating the DOM produced in different cultures and correlate to chl *a*, bacteria and protozoan abundance. The fractionation of cultures will help understanding the role of bacteria and protozoa in DOM production and consumption. For example in the culture A (unfiltered) the DOM produced is expected to be influenced directly or indirectly by both bacteria and protozoa but other large heterotrophs are also expected to play a major role in the production and consumption of DOC.

In culture B (<200 $\mu$ m) the DOC release will be attributed to process by microzooplankton on phytoplankton or bacteria. It is assumed that protozoa will be dominating the heterotrophs and the DOC will be produced through sloppy feeding. If this is the case, it is expected that the most of the DOC produced will be in the form of high molecular weight (HMW).

In culture C (<40 $\mu$ m), this fraction was designed to isolate bacteria at least from the larger protozoa and reducing protozoan predation pressure on bacterial community enhancing bacterial growth and DOC uptake. It is assumed that the reduction of

protozoan predation on bacteria will have influence on the type of DOC released. For instance is hypothesized that the type of DOC released will be more likely due to photosynthetic autotrophs, thus the most of the DOC produced will be in the form of low molecular weight (LMW). While in culture D ( $<0.2\mu\text{m}$ ) it is assumed that all phytoplankton and heterotrophs are excluded and DOC will not be influenced. Therefore this culture will be used as control.

The fractionation of plankton communities in this experiment will help to create different plankton fractions which will have influence on the DOC variability. This fractionation of plankton communities may cause difficulties in the interpretation of some of the experimental results but it will help understanding the alternative roles of both bacteria and protozoa in term of DOC production and consumption in the marine environment. For instance the high abundance of protozoa will increase predation pressure on bacteria and increases the accumulation of DOC, while reduction of predation pressure on bacteria might increase the uptake of DOC by bacteria and reducing the total DOC concentration.

The results of the experimental work presented in this chapter lead to the conclusion that the phytoplankton is the major source of DOM in the Menai Strait, and that nutrients and irradiance play a major role in DOM production. In addition, the experimental work has shown a significant correlation between DOC and yellow substance. However, future work need to address the nature of the relationships among different parameters controlling the mechanisms of the production and consumption of DOM.

# CHAPTER VIII.

## 8. GENERAL DISCUSSION AND CONCLUSION.

The study of the seasonal variation of dissolved organic matter in the coastal water such as the Menai Strait is of interest for various reasons: this region is characterized with high primary production. The effect of this high primary production exhibits phenomena, which may not be seen easily in other regions. Several studies have been conducted in the Menai Strait covering different aspects (chapter II), this has provided a good picture of the dynamics of the chemical, biological and physical parameters. However aspects related to dissolved organic matter have been poorly investigated in this region. Therefore this study has brought together all the information collected in the field and in the laboratory during the study period to understand the dynamics of the dissolved organic matter in relation to other parameters.

Blight et al (1995) hypothesized the flow of organic matter from primary producer to bacteria in the Menai Strait but didn't provide information on the magnitude of the dissolved organic matter and their relationship among each other. Therefore the work reported in this chapter attempts to bring together all the information available trying to build a picture of the dynamics of the dissolved organic matter in the Menai Strait. This discussion is structured to answer the following questions as stated in the objectives:

- 1) What is the role of phytoplankton and bacteria on the seasonal variation of dissolved organic carbon (DOC), nitrogen (DON) and yellow substance ( $g_{440}$ )?
- 2) Does yellow substance influence the changes in the DOM?

## **8.1. Role of phytoplankton and bacteria in the seasonal variation of DOC, DON and yellow substance.**

During the present study DOC exhibited a seasonal variation characterized by high accumulation towards the end of spring followed by high values during summer. Low concentrations occurred during winter when phytoplankton production was low. A time lag between maximum primary production, bacterial abundance and DOC maximum was observed. Thus, accumulation of DOC depends at least in part on the decoupling of microbial consumption from DOC production. This decoupling depends on the rate of DOC production, the type of material produced, and the microbial activity. Several studies have reported the lack of correlation between bacterial and phytoplankton parameters, however in this study significant correlation was found. This is in agreement with Bird & Kalff (1984) and Cole *et al.* (1988) who also reported a strong positive correlation between phytoplankton and bacterial abundance. Despite the significant correlation between phytoplankton and bacterial parameters, most of the time there is a time lag between the maximum phytoplankton production and bacterial abundance. This has been hypothesized that is due to either the low temperature (Pomeroy & Diebel 1986) or the high molecular weight of the organic matter produced (Billen & Fontigny 1987), or nutrient depletion (Thingstad & Billen 1994).

In the current study DOC accumulation during the period dominated by diatoms was low and this could be due to either the labile nature of organic matter released and assimilated by bacteria or a low quantity of organic matter released. Tupas *et al.* (1994) also reported low accumulation of DOC during diatom blooms. The maximum accumulation of DOC occurred during and after the *Phaeocystis* bloom, which coincided with the depletion of inorganic nitrogen. Other studies have demonstrated the accumulation of DOC that coincides with increases in *Phaeocystis* primary production (Billen & Fontigny 1987) and biomass (Eberlein *et al.* 1985), indicating that the accumulation of DOC could have happened at earlier stages of a bloom when nutrients were not limiting. This may give an indication that nutrient depletion could offer some explanation but it is not likely to be the only factor controlling the accumulation of DOC.



As stated earlier the high accumulation of DOC coincided with the depletion of nutrient (6.4). It could be assumed that during this period, the dissolved organic matter released by algae will be rich in carbon relative to nitrogen (Lancelot 1983; Williams 1995). This was evident in this study from the low concentration of DON relative to DOC from day 90 to 150 (Figure 6.4). If we are to consider bacteria as major consumers of DOC, they require inorganic nitrogen to assimilate the nitrogen-poor DOM (Lancelot & Billen 1985). Therefore the depletion of inorganic nitrogen during the *Phaeocystis* bloom and a poor competition of bacteria with algae for inorganic nitrogen during summer could be considered to be at least one of the factors allowing DOC accumulation during spring and summer.

The composition of the plankton community and their size structure could play a vital role in controlling the DOM production (Carlson *et al.* 1998). For example the quantity and quality of the dissolved organic matter may differ from genera to genera and from class to class of algae. The role of pico- and nanophytoplankton was discussed earlier (Chapter VI), in addition these organisms contribute to the cycling of organic matter through the microbial food web (Michaels & Silver 1988). Unfortunately the pico and nanophytoplankton were not enumerated in this study along with other microheterotrophic organisms. Carlson *et al.* (1998) reported that 11% of the carbon flows from phytoplankton production to bacterioplankton in a system with less pico and nanophytoplankton, whilst in a system with high pico- and nanophytoplankton abundance the carbon flow to bacterioplankton increased to 31%. Carlson *et al.* (1998) reported differences in the lability of the dissolved organic matter produced by a bloom dominated by *Phaeocystis* in the Ross Sea and Sargasso Sea. In the Ross Sea, 72% of the DOC produced was labile and utilized by bacteria, whilst in the Sargasso Sea half of the newly produced DOC was refractory and escaped bacteria degradation. This indicates that despite the dominance of the same species in both regions, the mechanisms through which DOC was released influenced the type of dissolved organic matter available to heterotrophic organisms.

The fate of DON and yellow substance is discussed in chapter VI. Phytoplankton seems to influence both fractions. However the author doesn't rule out the possibility of other factors since the present study was limited to the measurement of

total DOC, DON, and yellow substance and no size fractionation of DOM was investigated.

Further investigation is needed to qualify and quantify the exact mechanisms responsible for the observed accumulation of DOC, DON and yellow substance in spring and summer and decline during autumn and winter. However from the available data collected in the present study, the fact remained that DOC in the Menai Strait is produced by phytoplankton during spring and accumulate during summer, however the contribution of other sources such as macroalgae and the mechanisms involved in the release of DOC remains to be determined.

## **8.2. Does yellow substance influence the changes in the DOM concentrations or Vice Versa?**

Until recently, primary production was considered as a major source of labile DOC which is assimilated by bacteria. However several authors (Tranvik 1988; Moran & Hodson 1990; Bano *et al.* 1997) have reported on the possibility of changes of allochthonous fraction from refractory to labile DOC indicating the influence of DOC from algal production to yellow substances and vice versa. The relationship between these two fractions is still complex to understand since there are several factors involved.

The variation of the DOC and yellow substance in both field and experimental work, exhibited trends which are more or less the same. Both fractions increased with the increase in phytoplankton biomass. However they exhibited a poor significant correlation in the field, while a positive significant correlation was observed in the experimental work. This could be due to the interaction of different factors in the field. However in the experimental work DOC seemed to increase earlier followed by yellow substance. This feature was also observed in the field. This may indicate that the increase of yellow substance may have been influenced by the process by which DOM from phytoplankton was released. However the experiments in this study were not designed to investigate details of this processes such as the effect of photobleaching of refractory material to labile material, rather they were designed

to show general impact of light and nutrient on DOM release. One would expect a decline of yellow substance followed by an increase of DOC if yellow substance influences DOC. In the present study the relationship between DOC and yellow substance or vice versa was proven through the significant correlation between the two parameters, however the nature of this relationship is somehow beyond the scope of this study. DOC concentration remained high during summer period in the Menai Strait and this was explained as coupling between production and consumption of dissolved organic matter. While yellow substance exhibited a clear decline during summer which could be as a result of photobleaching thus contributing to accumulation of DOC during this period, in addition to other factors as discussed in chapter VI. More investigations are required to prove this hypothesis.

### **8.3. Conclusions**

The present study has shown also that the seasonal variation of DOM is mainly controlled by primary production and consumption of organic matter. In addition, the accumulation of DOC, DON and yellow substance during summer is associated with the rate of supply of algae production and degradation by heterotrophic organisms. However there also a significant contribution of DOM from other sources such as macroalgae and river input as this may yet need to be determined since this contribution could be from the Irish Sea and Liverpool Bay. The relative importance of sediments and bacteria as a source of DOC and yellow substance is difficult to separate in this study. The relationship between yellow substance and DOC was proven in this study in the culture and field, however more investigations are required to find out the nature of this relationship.

### **8.4. Future studies:**

Despite the effort put in the present study to cover different aspects in order to fully understand the dynamics of the DOM in the Menai Strait, there is still a need to do

more investigation looking in more detail at some of the parameters as described below:

#### **8.4.1.Importance of the microbial loop in the Menai Strait**

The role of bacteria in the distribution of DOM organic matter was discussed in this study and proved to play a vital role, however there is a need to investigation the importance of the microbial loop. The organic matter produced by phytoplankton is utilized by bacteria and the latter is grazed by protozoa (ciliates and flagellates) which contribute to the energy flow through the microbial loop (DOC-bacteria-protozoa-zooplankton). Although there may be studies on the abundance of bacteria, protozoa and zooplankton abundance in the Menai Strait, these studies are not linked to the dynamics of DOM in the Menai Strait. Therefore more studies on the role of the microbial loop are needed in the Menai Strait covering aspects such as the their role in DOM transformation and size, and grazing pressure of zooplankton on phytoplankton.

#### **8.4.2.The role of DON as a source for primary production**

As it is the case with the dissolved organic carbon, it would be interesting to investigate the fractionation of the dissolved organic nitrogen (DON) in order to understand the role of each fraction to the primary production. For example it is well known that urea can be used as a nitrogen source especially in the coastal area (Remsen 1971; Kaufman et al. 1983; Kristiansen 1983). It would be interesting to investigate the seasonal variations of different DON fractions. Whether these fractions are occurring on the seasonal basis or are only linked to small-scale excretion and what is their role in the seasonal variation of the primary production.

#### **8.4.3.The role of picoplankton and microzooplankton**

Studies of primary production and nutrient availability at small time scales (days), and experiments examining the effect of grazing by microzooplankton and zooplankton , are required to understand mechanisms causing the unusual duration of

the period of dominance by picoplankton cells. These organisms are known to contribute significantly to primary production. It could be interesting to investigate their role especially during summer.

#### **8.4.4. Size fractionation of DOM and the role of photobleaching of DOM on the seasonal variation of DOC in the Menai Strait.**

The dynamics of the DOM was discussed in this study. However information on the size fractionation of DOM could be useful in order to understand aspects related to carbon transfer and the reaction of heterotrophic microorganisms. The decline of yellow substance during summer was hypothesized to be due to sediment adsorption or photobleaching. More detailed study combining the experimental and field work on the role of photobleaching of DOM in the Menai Strait is necessary to fully understand the relationship between DOC and yellow substance as well as bacteria.

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# Appendix 1. All data collected in the Menai Strait in 1998 and 1999.

| Date      | Day | DOC | DON | C:N | g440            | POC | PON | C:N | chl a               | bacteria               | phytopl.               | GCP                                | NCP                                | DCR                                | nitrate | nitrite | ammonia | Phosphate | salinity | W temp | sec |  |
|-----------|-----|-----|-----|-----|-----------------|-----|-----|-----|---------------------|------------------------|------------------------|------------------------------------|------------------------------------|------------------------------------|---------|---------|---------|-----------|----------|--------|-----|--|
|           |     | μM  | μM  |     | m <sup>-1</sup> | μM  | μM  |     | μg.dm <sup>-3</sup> | cells.cm <sup>-3</sup> | cells.dm <sup>-3</sup> | μM.O <sub>2</sub> .d <sup>-1</sup> | μM.O <sub>2</sub> .d <sup>-1</sup> | μM.O <sub>2</sub> .d <sup>-1</sup> | μM      | μM      | μM      | μM        | psu      | °C     | m   |  |
| 3-Jun-98  | 154 | 190 | 5.4 | 35  |                 | 77  | 8   | 9   | 2.3                 | 6.0E+06                | 9.9E+04                | 3                                  | -4                                 | 7                                  | 0.39    | 0.04    | 0.94    | 0.51      | 32.6     | 14.2   | 1.7 |  |
| 12-Jun-98 | 163 | 205 | 8.7 | 23  |                 | 107 | 12  | 9   | 6.6                 | 5.6E+06                | 4.4E+05                | 4                                  | -1                                 | 5                                  | 0.69    | 0.06    | 3.02    | 0.64      | 32.9     | 13.8   | 1.2 |  |
| 17-Jun-98 | 168 | 171 | 7.1 | 24  |                 | 79  | 10  | 8   | 6.3                 | 3.6E+06                | 8.2E+05                | 17                                 | 14                                 | 3                                  | 1.18    | 0.10    | 2.74    | 0.66      | 32.9     | 11.6   | 1.8 |  |
| 25-Jun-98 | 176 | 150 | 5.7 | 26  |                 | 102 | 10  | 10  | 8.7                 | 6.4E+06                | 7.9E+05                | 9                                  | 5                                  | 4                                  | 0.03    | 0.04    | 0.30    | 0.38      | 33.4     | 14.9   | 1.5 |  |
| 1-Jul-98  | 182 | 149 | 8.6 | 17  | 0.19            | 51  | 5   | 10  | 2.3                 | 4.1E+06                | 7.8E+04                | 4                                  | 1                                  | 3                                  | 0.39    | 0.06    | 2.30    | 0.53      | 33.4     | 16.3   | 2.5 |  |
| 8-Jul-98  | 189 | 149 | 9.7 | 15  | 0.19            | 49  | 6   | 8   | 2.9                 | 3.7E+06                | 1.1E+05                | 1                                  | -2                                 | 4                                  | 0.16    | 0.06    | 2.79    | 0.44      | 33.8     | 15.4   | 2.5 |  |
| 17-Jul-98 | 198 | 147 | 5.8 | 25  | 0.10            | 43  | 4   | 12  | 3.3                 | 4.6E+06                | 6.0E+04                | 5                                  | 3                                  | 3                                  | 0.57    | 0.10    | 1.83    | 0.65      | 33.8     | 16.0   | 2.7 |  |
| 23-Jul-98 | 204 | 161 | 5.5 | 29  | 0.23            | 71  | 7   | 10  | 4.9                 | 3.6E+06                | 6.8E+05                | 3                                  | -1                                 | 5                                  | 0.31    | 0.06    | 0.24    | 0.27      | 33.6     | 15.8   | 3.0 |  |
| 30-Jul-98 | 211 | 155 | 5.9 | 26  | 0.16            | 24  | 3   | 8   | 2.3                 | 3.5E+06                | 1.1E+05                | 3                                  | -2                                 | 5                                  | 0.31    | 0.08    | 1.33    | 0.63      | 33.6     | 15.7   | 4.5 |  |
| 6-Aug-98  | 218 | 155 | 6.0 | 26  | 0.25            | 18  | 3   | 7   | 2.0                 | 4.7E+06                |                        | 4                                  | 2                                  | 1                                  | 0.28    | 0.08    | 0.86    | 0.69      | 33.6     | 16.4   | 3.5 |  |
| 13-Aug-98 | 225 | 150 | 5.9 | 25  | 0.15            | 19  | 2   | 10  | 1.4                 | 2.1E+06                | 2.9E+05                | 7                                  | 3                                  | 4                                  | 0.51    | 0.10    | 1.40    | 0.71      | 33.9     | 16.4   | 3.0 |  |
| 22-Aug-98 | 234 | 143 | 3.8 | 37  | 0.17            | 15  |     |     | 1.6                 | 2.4E+06                | 5.6E+05                | 2                                  | 0                                  | 2                                  | 0.39    | 0.08    | 1.55    | 0.63      | 34.0     | 16.3   | 3.0 |  |
| 27-Aug-98 | 239 | 132 | 5.1 | 26  | 0.16            | 19  | 2   | 8   | 2.7                 | 4.2E+06                | 2.9E+05                | 22                                 | 9                                  |                                    | 0.36    | 0.10    | 0.67    | 0.63      | 34.0     | 16.2   | 3.0 |  |
| 3-Sep-98  | 246 | 148 | 4.8 | 31  | 0.16            | 21  | 3   | 8   | 3.5                 | 4.2E+06                | 5.6E+05                | 7                                  | 6                                  | 1                                  | 0.05    | 0.04    | 0.27    | 0.59      | 33.8     | 17.5   | 4.0 |  |
| 10-Sep-98 | 253 | 143 | 5.8 | 24  | 0.18            | 30  | 3   | 9   | 3.8                 | 6.6E+06                | 1.2E+05                | 12                                 | 9                                  | 3                                  | 0.13    | 0.06    | 0.66    | 0.60      | 33.4     | 16.2   | 2.0 |  |
| 16-Sep-98 | 259 | 138 | 2.5 | 56  | 0.20            | 22  | 3   | 9   | 1.6                 | 5.4E+06                | 3.9E+04                | 3                                  | 1                                  | 2                                  | 1.43    | 0.22    | 2.05    | 0.65      | 33.2     | 14.7   | 1.8 |  |
| 24-Sep-98 | 267 | 135 | 2.1 | 64  | 0.18            | 36  | 4   | 9   | 2.3                 | 4.6E+06                | 3.3E+04                | 6                                  | 4                                  | 2                                  | 1.51    | 0.22    | 2.39    | 0.80      | 33.4     | 16.5   | 1.5 |  |
| 1-Oct-98  | 274 | 156 | 3.9 | 40  | 0.28            | 22  |     |     | 2.3                 | 6.7E+06                | 3.4E+04                | 11                                 | 9                                  | 2                                  | 3.61    | 0.27    | 1.38    | 0.95      | 32.6     | 15.4   | 1.5 |  |
| 6-Oct-98  | 279 | 152 | 3.2 | 48  | 0.24            | 31  | 3   | 9   | 2.3                 | 4.3E+06                | 2.4E+04                | 10                                 | 9                                  | 1                                  | 4.32    | 0.49    | 2.99    | 0.98      | 32.9     | 13.8   | 1.0 |  |
| 14-Oct-98 | 287 | 155 | 3.2 | 48  | 0.29            | 22  | 3   | 8   | 1.2                 | 5.5E+06                | 1.4E+04                | 4                                  | 2                                  | 2                                  | 6.27    | 0.63    | 3.96    | 1.29      | 32.3     | 15.0   | 1.5 |  |
| 20-Oct-98 | 293 | 155 | 0.6 |     | 0.23            | 26  | 3   | 9   | 1.1                 | 3.4E+06                | 3.2E+04                | 2                                  | 2                                  | 0                                  | 7.65    | 0.41    | 1.95    | 1.06      | 32.5     | 12.1   | 1.5 |  |
| 4-Nov-98  | 308 | 123 | 0.5 |     | 0.16            | 47  | 5   | 10  | 1.6                 | 3.5E+06                | 4.0E+04                | 4                                  | 3                                  | 1                                  | 9.13    | 0.22    | 1.11    | 0.88      | 32.4     | 10.2   | 0.5 |  |
| 10-Nov-98 | 314 | 130 | 1.1 |     | 0.18            | 17  |     |     | 1.5                 | 3.2E+06                | 3.2E+04                | 3                                  | 2                                  | 0                                  | 8.21    | 0.29    | 0.90    | 0.79      | 33.4     | 10.7   | 1.5 |  |
| 18-Nov-98 | 322 | 113 |     |     | 0.13            | 17  |     |     | 0.6                 | 3.2E+06                | 1.5E+04                | 2                                  | 2                                  | 0                                  | 9.70    | 0.43    | 1.51    | 0.87      | 33.2     | 8.7    | 1.5 |  |
| 25-Nov-98 | 329 | 137 |     |     | 0.26            | 16  | 2   | 9   | 0.6                 | 3.1E+06                | 3.8E+04                | 1                                  | 0                                  | 0                                  | 10.21   | 0.65    | 2.16    | 0.97      | 32.7     | 8.5    | 2.0 |  |
| 5-Dec-98  | 339 | 105 |     |     | 0.19            | 47  | 5   | 9   | 0.9                 | 3.7E+06                | 1.9E+04                | 2                                  | 1                                  | 1                                  | 11.49   | 0.41    | 1.04    | 1.11      | 33.0     | 7.9    | 1.5 |  |
| 10-Dec-98 | 344 | 97  | 0.6 |     | 0.16            | 18  | 2   | 9   | 0.4                 | 3.5E+06                | 1.4E+04                | 1                                  | 0                                  | 1                                  | 13.35   | 0.45    | 1.78    | 1.09      | 32.7     | 8.3    | 1.5 |  |
| 26-Dec-98 | 360 |     |     |     |                 |     |     |     |                     |                        |                        |                                    |                                    |                                    |         |         |         |           |          |        |     |  |
| 29-Jan-99 | 29  | 83  | 0.0 |     | 0.07            | 19  | 2   | 10  | 0.4                 | 2.1E+06                | 6.9E+03                |                                    | -1                                 | 2                                  | 11.33   | 0.20    | 1.21    | 0.78      | 33.0     | 7.6    | 1.5 |  |
| 4-Feb-99  | 35  | 93  | 1.1 |     | 0.04            | 16  | 1   | 12  | 0.6                 | 2.0E+06                | 2.1E+04                | 1                                  | 1                                  |                                    | 9.49    | 0.18    | 1.10    | 0.83      | 33.7     | 8.1    | 1.5 |  |
| 12-Feb-99 | 43  | 85  | 0.0 |     | 0.05            | 5   |     |     | 0.4                 | 4.2E+06                | 2.5E+04                | 2                                  | 1                                  | 1                                  | 12.15   | 0.20    | 1.94    | 0.85      | 33.5     | 6.2    | 1.8 |  |
| 17-Feb-99 | 48  | 87  | 0.0 |     | 0.05            | 10  | 1   | 7   | 0.7                 | 3.0E+06                | 5.6E+04                | 2                                  | -1                                 | 4                                  | 12.07   | 0.22    | 1.10    | 0.86      | 33.5     | 7.0    | 1.1 |  |
| 25-Feb-99 | 56  | 94  | 0.0 |     | 0.10            | 9   | 1   | 6   | 0.6                 | 2.8E+06                | 1.1E+04                | 2                                  | 1                                  | 0                                  | 15.81   | 0.27    | 1.48    | 0.99      | 32.6     | 6.1    | 1.3 |  |

| Date      | Day | DOC           | DON           | C:N | g440            | POC           | PON           | C:N | chl a                 | bacteria               | phytopl.               | GCP                            | NCP                            | DCR                            | nitrate       | nitrite       | ammonia       | Phosphate     | salinity | W.temp             | secch |
|-----------|-----|---------------|---------------|-----|-----------------|---------------|---------------|-----|-----------------------|------------------------|------------------------|--------------------------------|--------------------------------|--------------------------------|---------------|---------------|---------------|---------------|----------|--------------------|-------|
|           |     | $\mu\text{M}$ | $\mu\text{M}$ |     | $\text{m}^{-1}$ | $\mu\text{M}$ | $\mu\text{M}$ |     | $\mu\text{g.dm}^{-3}$ | $\text{cells.cm}^{-3}$ | $\text{cells.dm}^{-3}$ | $\mu\text{M.O}_2\text{d}^{-1}$ | $\mu\text{M.O}_2\text{d}^{-1}$ | $\mu\text{M.O}_2\text{d}^{-1}$ | $\mu\text{M}$ | $\mu\text{M}$ | $\mu\text{M}$ | $\mu\text{M}$ | psu      | $^{\circ}\text{C}$ | m     |
| 4-Mar-99  | 63  | 134           | 2.2           | 61  | 0.14            | 29            | 3             | 9   | 0.3                   | 3.0E+06                | 2.2E+04                | 2                              | 1                              | 1                              | 16.60         | 0.20          | 1.80          | 1.07          | 31.9     | 6.7                | 1.2   |
| 11-Mar-99 | 70  | 126           | 2.4           | 54  | 0.18            | 19            |               |     | 0.4                   | 4.8E+06                | 3.7E+04                | 1                              | 0                              | 1                              | 16.70         | 0.20          | 2.28          | 1.07          | 32.4     | 5.7                | 1.6   |
| 18-Mar-99 | 77  | 146           | 2.0           |     | 0.15            | 35            | 4             | 9   | 1.6                   | 3.3E+06                | 9.6E+04                | 5                              | 3                              | 2                              | 16.60         | 0.27          | 1.26          | 1.18          | 32.1     | 7.3                | 1.5   |
| 22-Mar-99 | 81  | 128           | 2.0           | 63  | 0.12            | 42            | 5             | 9   | 1.4                   | 3.6E+06                | 6.4E+04                | 9                              | 9                              | 0                              | 16.10         | 0.24          | 1.14          | 1.15          | 32.1     | 6.7                | 1.0   |
| 23-Mar-99 | 82  | 128           |               |     | 0.07            | 48            | 5             | 9   | 1.1                   |                        |                        |                                |                                |                                | 15.90         | 0.18          |               | 1.16          | 32.2     | 7.0                | 1.0   |
| 24-Mar-99 | 83  | 127           |               |     | 0.05            | 39            | 4             | 11  | 1.2                   |                        |                        |                                |                                |                                | 15.80         | 0.22          |               | 1.18          | 32.2     | 7.2                | 1.0   |
| 25-Mar-99 | 84  | 138           | 0.3           |     | 0.16            | 24            |               |     | 1.0                   | 5.8E+06                | 5.3E+04                | 1                              | 1                              | 0                              | 16.10         | 0.20          | 1.04          | 1.19          | 32.2     | 8.2                | 1.1   |
| 29-Mar-99 | 88  | 157           |               |     | 0.13            | 38            | 4             | 10  | 1.4                   |                        |                        |                                |                                |                                | 15.60         | 0.24          |               | 1.15          | 32.3     | 7.6                | 1.0   |
| 30-Mar-99 | 89  | 157           |               |     | 0.17            | 29            | 3             | 11  | 1.3                   |                        |                        |                                |                                |                                | 15.10         | 0.22          |               | 1.14          | 32.2     | 8.0                | 1.0   |
| 1-Apr-99  | 91  | 131           | 0.5           |     | 0.15            | 31            | 3             | 10  | 1.7                   | 5.2E+06                | 2.4E+05                | 9                              | 7                              | 1                              | 16.60         | 0.18          | 0.55          | 0.85          | 32.5     | 8.6                | 1.2   |
| 3-Apr-99  | 93  | 138           |               |     | 0.13            | 29            | 3             | 10  | 1.6                   |                        |                        |                                |                                |                                | 11.43         | 0.18          |               | 0.88          |          | 8.7                | 1.4   |
| 5-Apr-99  | 95  | 143           |               |     | 0.13            | 32            | 3             | 10  | 1.8                   |                        |                        | data                           |                                |                                | 9.44          | 0.16          |               | 1.14          |          | 9.2                | 1.3   |
| 7-Apr-99  | 97  | 137           | 1.7           |     | 0.14            | 30            | 3             | 9   | 1.9                   | 4.8E+06                | 4.4E+05                | 20                             | 18                             | 2                              | 11.54         | 0.16          | 1.12          | 1.12          | 32.3     | 9.2                | 1.3   |
| 10-Apr-99 | 100 | 140           |               |     | 0.15            | 21            | 2             | 9   | 2.3                   |                        |                        |                                |                                |                                | 16.47         | 0.24          |               | 1.02          |          | 9.5                | 1.2   |
| 12-Apr-99 | 102 | 143           |               |     | 0.12            | 26            | 3             | 8   | 3.0                   | 9.3E+06                |                        |                                |                                |                                | 15.40         | 0.24          |               | 1.06          |          | 8.9                | 1.6   |
| 13-Apr-99 | 103 | 142           |               |     | 0.15            | 33            | 4             | 9   | 2.7                   |                        |                        |                                |                                |                                | 15.65         | 0.27          |               | 0.93          |          | 8.2                | 1.6   |
| 14-Apr-99 | 104 | 140           | 1.6           |     | 0.12            | 50            | 6             | 9   | 2.6                   | 7.0E+06                | 3.1E+05                | 16                             | 14                             | 2                              | 13.40         | 0.22          | 0.74          | 0.91          | 32.5     | 7.9                | 1.0   |
| 15-Apr-99 | 105 | 141           |               |     | 0.14            | 47            | 5             | 9   | 2.3                   |                        |                        |                                |                                |                                | 12.79         | 0.18          |               | 0.92          |          | 8.0                | 0.8   |
| 16-Apr-99 | 106 | 136           |               |     | 0.12            | 55            | 6             | 9   | 1.9                   |                        |                        |                                |                                |                                | 12.89         | 0.18          |               | 0.89          |          | 8.1                | 0.8   |
| 17-Apr-99 | 107 | 150           |               |     | 0.08            | 53            | 6             | 9   | 2.6                   |                        |                        |                                |                                |                                | 12.18         | 0.18          |               | 0.87          |          | 8.6                | 0.9   |
| 18-Apr-99 | 108 | 140           |               |     | 0.14            | 52            | 7             | 8   | 2.9                   |                        | 5.2E+05                |                                |                                |                                | 12.07         | 0.16          |               | 0.83          |          | 8.6                | 1.0   |
| 19-Apr-99 | 109 | 142           |               |     | 0.15            | 30            |               |     | 2.7                   |                        |                        |                                |                                |                                | 12.92         | 0.18          |               | 0.81          |          | 8.7                | 1.0   |
| 20-Apr-99 | 110 | 135           | 0.6           |     | 0.13            | 40            | 5             | 8   | 4.0                   | 5.4E+06                | 1.2E+06                | 32                             | 30                             | 2                              | 11.72         | 0.18          | 0.37          | 0.77          | 32.4     | 8.7                | 1.0   |
| 21-Apr-99 | 111 | 170           |               |     | 0.13            | 44            | 5             | 10  | 4.5                   |                        |                        |                                |                                |                                | 13.46         | 0.18          |               | 0.74          |          | 9.0                | 1.0   |
| 22-Apr-99 | 112 | 151           |               |     | 0.15            | 43            | 5             | 8   | 4.0                   |                        |                        |                                |                                |                                | 12.02         | 0.20          |               | 0.70          |          | 9.3                | 1.0   |
| 23-Apr-99 | 113 | 148           |               |     | 0.19            | 29            | 4             | 8   | 4.0                   |                        |                        |                                |                                |                                | 11.74         | 0.18          |               | 0.66          |          | 9.4                | 1.5   |
| 24-Apr-99 | 114 | 136           |               |     | 0.15            | 37            | 4             | 8   | 3.7                   |                        |                        |                                |                                |                                | 10.82         | 0.20          |               | 0.63          |          | 9.8                | 1.6   |
| 25-Apr-99 | 115 | 140           |               |     | 0.17            | 28            | 4             | 6   | 3.7                   |                        |                        |                                |                                |                                | 9.72          | 0.20          |               | 0.57          |          | 9.3                | 2.0   |
| 26-Apr-99 | 116 | 140           |               |     | 0.15            | 33            | 5             | 7   | 5.0                   |                        |                        |                                |                                |                                | 8.95          | 0.20          |               | 0.57          |          | 9.5                | 2.0   |
| 27-Apr-99 | 117 | 150           | 1.7           |     | 0.17            | 70            | 8             | 9   | 6.9                   | 9.4E+06                | 1.0E+06                | 42                             | 39                             | 3                              | 7.60          | 0.20          | 0.84          | 0.54          | 32.3     | 10.0               | 1.6   |
| 28-Apr-99 | 118 | 153           |               |     | 0.17            | 56            | 7             | 8   | 5.8                   |                        |                        |                                |                                |                                | 6.52          | 0.20          |               | 0.47          |          | 10.5               | 1.5   |
| 29-Apr-99 | 119 | 191           |               |     | 0.16            | 52            | 7             | 7   | 8.6                   |                        |                        |                                |                                |                                | 5.58          | 0.20          |               | 0.39          |          | 10.7               | 1.3   |
| 30-Apr-99 | 120 | 143           |               |     | 0.17            | 61            | 8             | 8   | 8.9                   |                        |                        |                                |                                |                                | 4.66          | 0.20          |               | 0.38          |          | 10.6               | 1.3   |

| Date      | Day | DOC | DON | C:N | g440            | POC | PON | C:N | chl a               | bacteria               | phytopl.               | GCP                               | NCP                               | DCR                               | nitrate | nitrite | ammonia | Phosphate | salinity | W.temp. | secchi |
|-----------|-----|-----|-----|-----|-----------------|-----|-----|-----|---------------------|------------------------|------------------------|-----------------------------------|-----------------------------------|-----------------------------------|---------|---------|---------|-----------|----------|---------|--------|
|           |     | μM  | μM  |     | m <sup>-1</sup> | μM  | μM  |     | μg.dm <sup>-3</sup> | cells.cm <sup>-3</sup> | cells.dm <sup>-3</sup> | μM.O <sub>2</sub> d <sup>-1</sup> | μM.O <sub>2</sub> d <sup>-1</sup> | μM.O <sub>2</sub> d <sup>-1</sup> | μM      | μM      | μM      | μM        | psu      | °C      | m      |
| 1-May-99  | 121 | 139 |     |     | 0.12            | 51  | 7   | 7   | 8.9                 |                        |                        |                                   |                                   |                                   | 4.20    | 0.18    |         | 0.36      |          | 10.6    | 1.6    |
| 2-May-99  | 122 | 142 |     |     | 0.15            | 52  | 7   | 7   | 10.9                |                        |                        |                                   |                                   |                                   | 3.69    | 0.18    |         | 0.34      |          | 10.8    | 1.5    |
| 3-May-99  | 123 | 132 |     |     | 0.16            | 42  | 6   | 7   | 8.1                 | 8.1E+06                |                        |                                   |                                   |                                   | 3.40    | 0.18    |         | 0.32      |          | 11.4    | 1.6    |
| 4-May-99  | 124 | 135 | 3.6 | 38  | 0.15            | 47  | 7   | 7   | 7.8                 | 8.9E+06                | 1.3E+06                | 46                                | 38                                | 8                                 | 2.48    | 0.16    | 0.36    | 0.27      | 32.7     | 11.8    | 1.7    |
| 5-May-99  | 125 | 150 |     |     | 0.16            | 55  | 7   | 8   | 7.2                 |                        |                        |                                   |                                   |                                   | 1.23    | 0.16    |         | 0.27      |          | 11.6    | 1.7    |
| 6-May-99  | 126 | 144 |     |     | 0.25            | 71  | 9   | 8   | 9.8                 |                        |                        |                                   |                                   |                                   | 0.90    | 0.14    |         | 0.24      |          | 11.5    | 1.5    |
| 7-May-99  | 127 | 142 |     |     | 0.17            | 55  | 7   | 8   | 9.8                 |                        |                        |                                   |                                   |                                   | 0.90    | 0.10    |         | 0.24      |          | 11.0    | 1.6    |
| 8-May-99  | 128 | 146 |     |     | 0.15            | 61  | 8   | 8   | 6.9                 |                        |                        |                                   |                                   |                                   | 0.77    | 0.10    |         | 0.23      |          | 11.6    | 2.0    |
| 9-May-99  | 129 | 146 |     |     | 0.05            | 49  | 7   | 7   | 10.1                |                        |                        |                                   |                                   |                                   | 0.77    | 0.10    |         | 0.23      |          | 11.6    | 1.5    |
| 10-May-99 | 130 | 143 |     |     | 0.20            | 51  | 6   | 9   | 9.8                 |                        |                        |                                   |                                   |                                   | 0.54    | 0.10    |         | 0.18      |          | 11.7    | 2.0    |
| 11-May-99 | 131 | 151 | 2.8 | 53  | 0.15            | 52  | 7   | 7   | 8.6                 | 1.3E+07                | 2.3E+06                | 56                                | 53                                | 4                                 | 0.16    | 0.08    | 0.33    | 0.18      | 32.3     | 11.6    | 2.0    |
| 14-May-99 | 134 | 137 |     |     | 0.09            | 59  | 7   | 9   | 10.4                |                        |                        |                                   |                                   |                                   | 0.00    | 0.02    |         | 0.14      |          | 11.9    | 1.6    |
| 15-May-99 | 135 | 141 |     |     | 0.19            | 55  | 7   | 8   | 10.9                |                        |                        |                                   |                                   |                                   | 0.00    | 0.02    |         | 0.14      |          | 12.1    | 1.8    |
| 16-May-99 | 136 | 136 |     |     | 0.19            | 58  | 7   | 8   | 5.2                 |                        |                        |                                   |                                   |                                   | 0.00    | 0.02    |         | 0.10      |          | 12.0    | 1.6    |
| 17-May-99 | 137 | 146 |     |     | 0.16            | 78  | 7   | 11  | 8.4                 |                        |                        |                                   |                                   |                                   | 0.00    | 0.00    |         | 0.10      |          | 11.6    | 1.4    |
| 18-May-99 | 138 | 166 | 4.5 | 37  | 0.16            | 89  | 8   | 11  | 10.9                | 7.6E+06                | 2.8E+05                | 40                                | 33                                | 7                                 | 0.00    | 0.00    | 0.09    | 0.14      | 33.0     | 11.7    | 1.5    |
| 19-May-99 | 139 | 190 |     |     | 0.22            | 74  | 8   | 9   | 6.3                 |                        |                        |                                   |                                   |                                   | 0.00    | 0.02    |         | 0.14      |          | 12.4    | 1.5    |
| 20-May-99 | 140 | 169 |     |     | 0.23            | 93  | 7   | 13  | 9.2                 |                        |                        |                                   |                                   |                                   | 0.00    | 0.02    |         | 0.17      |          | 12.5    | 1.6    |
| 21-May-99 | 141 | 167 |     |     | 0.22            | 102 | 8   | 13  | 9.5                 |                        |                        |                                   |                                   |                                   | 0.00    | 0.02    |         | 0.12      |          | 12.1    | 1.5    |
| 23-May-99 | 143 | 149 |     |     | 0.19            | 106 | 7   | 15  | 10.4                |                        |                        |                                   |                                   |                                   | 0.00    | 0.02    |         | 0.11      |          | 12.0    | 1.7    |
| 25-May-99 | 145 | 184 | 4.2 | 44  | 0.19            | 115 | 9   | 13  | 11.5                | 1.0E+07                | 7.7E+06                | 21                                |                                   |                                   | 0.00    | 0.00    | 0.11    | 0.02      | 33.3     | 12.8    | 2.0    |
| 28-May-99 | 148 | 148 |     |     | 0.20            | 60  | 6   | 10  | 8.6                 |                        |                        |                                   |                                   |                                   | 0.00    | 0.00    |         | 0.02      |          | 12.0    | 1.8    |
| 1-Jun-99  | 152 | 144 | 5.9 | 24  | 0.19            | 84  | 6   | 14  | 6.0                 | 7.3E+06                | 1.9E+06                | 12                                | 5                                 | 7                                 | 0.00    | 0.02    | 0.18    | 0.12      | 33.3     | 12.2    | 1.6    |
| 6-Jun-99  | 157 | 155 | 3.9 | 39  | 0.18            | 121 | 8   | 15  | 5.2                 | 6.4E+06                | 1.5E+06                | 5                                 | -1                                | 7                                 | 0.00    | 0.12    | 0.20    | 0.12      | 33.1     | 13.2    | 1.5    |
| 17-Jun-99 | 168 | 198 | 3.6 | 55  | 0.30            | 58  | 7   | 9   | 2.2                 | 6.5E+06                | 5.6E+04                |                                   |                                   |                                   | 0.00    | 0.18    | 0.23    | 0.18      | 33.5     | 14.7    | 1.5    |
| 22-Jun-99 | 173 | 159 | 5.2 | 30  | 0.31            | 42  | 5   | 9   | 2.2                 | 9.3E+06                | 1.3E+05                |                                   |                                   |                                   | 0.10    | 0.15    | 1.61    | 0.44      | 33.1     | 14.6    | 2.5    |
| 6-Jul-99  | 187 | 150 | 6.1 | 24  | 0.10            | 34  | 4   | 9   | 2.2                 | 1.2E+06                | 1.1E+06                | 7                                 | 1                                 | 6                                 | 0.03    | 0.13    | 1.04    | 0.43      | 33.5     | 16.7    | 2.5    |
| 14-Jul-99 | 195 | 149 | 4.7 | 32  | 0.15            | 22  | 3   | 7   | 3.0                 | 2.9E+06                | 2.8E+05                | 6                                 | 2                                 | 4                                 | 1.20    | 0.14    | 0.99    | 0.48      | 33.5     | 16.5    | 2.5    |
| 20-Jul-99 | 201 | 143 | 5.3 | 27  | 0.17            | 28  | 4   | 7   | 3.6                 | 4.1E+06                | 1.7E+05                | 7                                 | 4                                 | 3                                 | 0.13    | 0.06    | 1.16    | 0.41      | 33.5     | 17.5    | 2.5    |
| 28-Jul-99 | 209 | 139 | 5.0 | 28  | 0.17            | 29  | 4   | 7   | 2.0                 | 3.9E+06                | 7.6E+04                | 8                                 | 4                                 | 4                                 | 0.05    | 0.04    | 1.17    | 0.48      | 33.7     | 15.3    | 2.5    |
| 13-Aug-99 | 225 | 172 | 3.2 | 54  | 0.1             | 42  | 6   | 7   | 3.7                 | 4.6E+06                | 1.5E+05                | 24                                | 20                                | 4                                 | 0.92    | 0.16    | 1.51    | 0.75      | 33.3     | 13.0    | 1.5    |
| 25-Aug-99 | 237 | 149 | 6.4 | 23  | 0.2             | 27  | 4   | 7   | 2.6                 | 5.7E+06                | 9.7E+04                | 13                                | 8                                 | 6                                 | 0.28    | 0.08    | 0.90    | 0.64      | 33.0     | 16.0    | 1.5    |
| 14-Sep-99 | 257 | 171 | 3.0 | 57  | 0.1             | 28  | 4   | 7   | 2.0                 | 3.4E+06                | 9.2E+04                | 10                                | 6                                 | 3                                 | 0.28    | 0.10    | 0.75    | 0.63      | 33.4     | 13.0    | 2.7    |
| 27-Sep-99 | 270 | 152 | 2.3 | 65  | 0.2             | 34  | 4   | 8   | 1.8                 | 1.0E+07                | 1.1E+05                | 8                                 | 6                                 | 2                                 | 1.36    | 0.14    | 1.35    | 0.66      | 32.8     | 14.6    | 1.7    |



## Appendix 2. Phytoplankton abundance in the Menai Strait June 1998-September 1999.

| Data are cells dm <sup>-3</sup> |                                    | cells number |                  | cells number                      |        |
|---------------------------------|------------------------------------|--------------|------------------|-----------------------------------|--------|
| <b>3-Jun-98</b>                 | <i>Leptocylindrus danicus</i>      | 32360        | <b>17-Jun-98</b> | <i>Rhizosolenia delicatula</i>    | 404150 |
|                                 | <i>Nitzschia</i> sp.               | 15836        |                  | <i>Nitzschia closterium</i>       | 2066   |
|                                 | <i>Navicula</i> sp.1               | 4820         |                  | <i>Melosira sulcata</i>           | 13770  |
|                                 | <i>Nitzschia</i> sp.2              | 12393        |                  | <i>Coscinodiscus</i> sp.          | 689    |
|                                 | <i>Nitzschia</i> sp.3              | 2066         |                  | <i>Eucampia zoodiacus</i>         | 128750 |
|                                 | <i>Pleurosigma</i> sp.             | 1377         |                  | <i>Phaeocystis</i> sp.            | 64031  |
|                                 | <i>Navicula</i> sp.2               | 1377         |                  | <i>Leptocylindrus danicus</i>     | 166617 |
|                                 | <i>Paralia sulcata</i>             | 25475        |                  | <i>Navicula</i> sp.               | 3443   |
|                                 | <i>Coscinodiscus</i> sp.           | 2066         |                  | <i>Nitzschia</i> sp.              | 19967  |
|                                 | <i>Rhizosolenia</i> sp.            | 689          |                  | <i>Rhizosolenia</i> sp.           | 4820   |
|                                 | <i>Ceratium fusus</i>              | 689          |                  | <i>Melosira</i> sp.               | 1377   |
|                                 |                                    |              |                  | <i>Ditylum brightwellii</i>       | 689    |
|                                 |                                    |              |                  | <i>Guinardia</i> sp.              | 4820   |
|                                 |                                    |              |                  | <i>Thalassiosira</i> sp.          | 1377   |
| <b>12-Jun-98</b>                | <i>Chaetoceros</i> sp.             | 18278        |                  |                                   |        |
|                                 | <i>Nitzschia longissima</i>        | 60588        | <b>25-Jun-98</b> | <i>Leptocylindrus danicus</i>     | 722925 |
|                                 | <i>Navicula</i> sp.                | 6885         |                  | <i>Melosira</i> sp.               | 11016  |
|                                 | <i>Nitzschia closterium</i>        | 18590        |                  | <i>Nitzschia closterium</i>       | 15836  |
|                                 | <i>Eucampia zoodiacus</i>          | 98456        |                  | <i>Rhizosolenia</i> sp.           | 4131   |
|                                 | <i>Rhizosolenia</i> sp.            | 1377         |                  | <i>Rhizosolenia stouterfothii</i> | 2754   |
|                                 | <i>Leptocylindrus danicus</i>      | 194846       |                  | <i>Gyrodinium</i> sp.             | 4131   |
|                                 | <i>Coscinodiscus</i> sp.           | 10328        |                  | <i>Coscinodiscus</i> sp.          | 689    |
|                                 | <i>Rhizosolenia pungens</i>        | 689          |                  | <i>Rhizosolenia delicatula</i>    | 1377   |
|                                 | <i>Nitzschia delicatissima</i>     | 4131         |                  | <i>Bacillaria paradoxa</i>        | 11705  |
|                                 | <i>Bacillaria paradoxa</i>         | 7574         |                  | <i>U-ID</i> sp.                   | 2754   |
|                                 | <i>Rhizosolenia</i> sp.            | 2066         |                  | <i>Ceratium fusus</i>             | 689    |
|                                 | <i>Ditylum brightwellii</i>        | 689          |                  | <i>Rhizosolenia</i> sp.           | 2754   |
|                                 | <i>Melosira</i> sp.                | 1377         |                  | <i>U-ID</i> sp.                   | 2754   |
|                                 | <i>Guinardia flaccida</i>          | 4131         |                  | <i>Navicula</i> sp.               | 1377   |
|                                 | <i>Biddulphia sinensis</i>         | 689          |                  | <i>Biddulphia sinensis</i>        | 689    |
|                                 | <i>Rhizosolenia delicatula</i>     | 2754         |                  |                                   |        |
|                                 | <i>Melosira sulcata</i>            | 8262         |                  |                                   |        |
|                                 | <i>Thalassionema nitzschioides</i> | 2066         |                  |                                   |        |

|                 |                                |       |                  |                                   |        |
|-----------------|--------------------------------|-------|------------------|-----------------------------------|--------|
| <b>1-Jul-98</b> | <i>Leptocylindrus</i> sp.      | 31671 | <b>17-Jul-98</b> | <i>Chaetoceros</i> sp.            | 3443   |
|                 | <i>Navicula</i> sp.            | 1377  |                  | <i>Guinardia</i> sp.              | 6885   |
|                 | <i>Ceratium furca</i>          | 689   |                  | <i>Rhizosolenia</i> sp.           | 15836  |
|                 | U-ID dinoflagellate            | 13770 |                  | <i>Rhizosolenia delicatula</i>    | 16524  |
|                 | <i>Nitzschia longissima</i>    | 689   |                  | <i>Navicula</i> sp.               | 1377   |
|                 | <i>Nitzschia closterium</i>    | 5508  |                  | <i>Nitzschia</i> sp.              | 2066   |
|                 | <i>Rhizosolenia</i> sp.        | 8951  |                  | U-ID sp. ( <i>Navicula</i> sp.??) | 6885   |
|                 | <i>Rhizosolenia delicatula</i> | 13082 |                  | <i>Melosira</i> sp.               | 4820   |
|                 | <i>Navicula</i> sp.            | 2066  |                  | <i>Coscinodiscus</i> sp.          | 689    |
|                 | <i>Guinardia flaccida</i>      | 689   |                  | <i>Pleurosigma</i> sp.            | 1377   |
| <b>8-Jul-98</b> | <i>Noctiluca</i> sp.           | 1377  | <b>23-Jul-98</b> | <i>Rhizosolenia</i> sp.           | 80555  |
|                 | U-ID sp.(dinos)                | 17901 |                  | <i>Guinardia flaccida</i>         | 36491  |
|                 | <i>Leptocylindrus danicus</i>  | 11016 |                  | <i>Navicula</i> sp.               | 2754   |
|                 | <i>Rhizosolenia</i> sp.        | 22721 |                  | <i>Rhizosolenia delicatula</i>    | 526703 |
|                 | <i>Nitzschia closterium</i>    | 6885  |                  | <i>Leptocylindrus danicus</i>     | 19967  |
|                 | <i>Rhizosolenia delicatula</i> | 19967 |                  | <i>Nitzschia</i> sp.              | 1377   |
|                 | <i>Navicula</i> sp.            | 4820  |                  | UN-ID dinoflagellate              | 2066   |
|                 | <i>Nitzschia delicatissima</i> | 15836 |                  | <i>Prorocentrum</i> sp.           | 1377   |
|                 | U-ID sp.                       | 689   |                  | UN-ID dinoflagellate1             | 689    |
|                 | <i>Coscinodiscus</i> sp.       | 2066  |                  | UN-ID diatom                      | 1377   |
|                 | <i>Guinardia flaccida</i>      | 3443  |                  | <i>Nitzschia</i> sp.              | 9639   |
|                 | <i>Prorocentrum</i> sp.        | 3443  | <b>30-Jul-98</b> | <i>Navicula</i> sp.               | 689    |
|                 | <i>Peridinium</i> sp.          | 4131  |                  | <i>Guinardia flaccida</i>         | 33737  |
|                 | <i>Pleurosigma</i> sp.         | 689   |                  | <i>Rhizosolenia</i> sp.           | 64031  |
|                 |                                |       |                  | UN-ID diatom                      | 2754   |
|                 |                                |       |                  | <i>Navicula</i> sp.1              | 689    |
|                 |                                |       |                  | <i>Leptocylindrus danicus</i>     | 11705  |
|                 |                                |       |                  | <i>Nitzschia</i> sp 1             | 689    |

|                  |                            |               |                  |                                     |              |
|------------------|----------------------------|---------------|------------------|-------------------------------------|--------------|
| <b>27-Aug-98</b> | <b>Rhizosolenia sp.</b>    | <b>26163</b>  | <b>10-Sep-98</b> | <b>Melosira sp.</b>                 | <b>13770</b> |
|                  | Rhizosolenia delicatula    | 37179         |                  | Leptocylindrus sp                   | 79178        |
|                  | Leptocylindrus danicus     | 188649        |                  | Ceratium sp.                        | 1377         |
|                  | Nitzschia sp               | 14459         |                  | Coscinodiscus sp.                   | 2066         |
|                  | UN-ID dinoflagellate       | 1377          |                  | Pleurosigma sp.                     | 2066         |
|                  | Coscinodiscus sp.          | 2754          |                  | Nitzschia sp.                       | 10328        |
|                  | Ceratium sp.               | 3443          |                  | Navicula sp.                        | 1377         |
|                  | Navicula sp.               | 7574          |                  | Rhizosolenia sp.                    | 689          |
|                  | Prorocentrum sp.           | 1377          |                  | Navicula sp 1                       | 1377         |
|                  | Pyrocystis sp.             | 689           |                  | Thalassionema sp.                   | 2754         |
|                  | Gyrodinium sp.             | 689           |                  | UN-ID dinoflagellate(protoperidiniu | 3443         |
|                  | Pleurosigma sp.            | 689           |                  | Biddulphia sp.                      | 689          |
|                  | Chaetoceros sp.            | 1377          |                  |                                     |              |
|                  |                            |               | <b>16-Sep-98</b> | <b>Biddulphia sp.</b>               | <b>3443</b>  |
| <b>3-Sep-98</b>  | <b>Leptocylindrus sp.</b>  | <b>488147</b> |                  | Ceratium sp.                        | 3443         |
|                  | UN-ID dinoflagellate       | 689           |                  | Peridinium sp.                      | 9639         |
|                  | Navicula sp.               | 1377          |                  | Melosira sp                         | 7574         |
|                  | Eucampia zoodiacus         | 45441         |                  | Prorocentrum sp.                    | 3443         |
|                  | Melosira sp.               | 2754          |                  | Navicula sp                         | 689          |
|                  | Rhizosolenia stolterfothii | 3443          |                  | Eucampia sp.                        | 11016        |
|                  | Dictyocha sp.              | 689           |                  |                                     |              |
|                  | Ceratium sp.               | 1377          | <b>24-Sep-98</b> | <b>Ceratium sp.</b>                 | <b>3443</b>  |
|                  | Rhizosolenia sp.           | 689           |                  | Melosira sp.                        | 8951         |
|                  | UN-ID diatom               | 17901         |                  | Pendinium sp.                       | 2066         |
|                  | Biddulphia sinensis        | 1377          |                  | Thalassionema sp.                   | 3443         |
|                  |                            |               |                  | Biddulphia sp.                      | 3443         |
|                  |                            |               |                  | UN-ID diatom                        | 689          |
|                  |                            |               |                  | Prorocentrum sp.                    | 2754         |
|                  |                            |               |                  | UN-ID dinoflagellates               | 689          |
|                  |                            |               |                  | Pyrocystis sp                       | 1377         |
|                  |                            |               |                  | Rhizosolenia sp.                    | 5508         |
|                  |                            |               |                  | UN-ID diatom1                       | 689          |

|                  |                    |       |                  |                   |       |
|------------------|--------------------|-------|------------------|-------------------|-------|
| <b>1-Oct-98</b>  | Ceratium sp        | 4131  | <b>20-Oct-98</b> | Rhizosolenia sp   | 589   |
|                  | Biddulphia sp.     | 689   |                  | Leptocyindrus sp  | 13082 |
|                  | Peridinium sp.     | 4131  |                  | Guinardia sp      | 3443  |
|                  | UN-ID diatom       | 4131  |                  | Pleurosigma sp    | 689   |
|                  | Melosira sp        | 16524 |                  | Coscinodiscuss sp | 1377  |
|                  | Pyrocystis sp.     | 689   |                  | Melosira sp       | 9639  |
|                  | Coscinodiscus sp.  | 689   |                  | Ceratium sp       | 689   |
|                  | Prorocentrum sp.   | 2066  |                  | UN-ID sp          | 689   |
|                  | Rhizosolenia sp.   | 689   |                  | Biddulphia sp     | 1377  |
|                  |                    |       |                  | UN-ID diatom      | 689   |
| <b>6-Oct-98</b>  | Biddulphia sp.     | 10328 |                  |                   |       |
|                  | Melosira sp.       | 11016 |                  |                   |       |
|                  | Rhizosolenia sp.   | 689   |                  |                   |       |
|                  | Navicula sp.       | 689   |                  |                   |       |
|                  | Coscinodiscuss sp. | 1377  |                  |                   |       |
| <b>14-Oct-98</b> | UN-ID diatom       | 689   |                  |                   |       |
|                  | Biddulphia sp.     | 1377  |                  |                   |       |
|                  | UN-ID diatom 2     | 4820  |                  |                   |       |
|                  | Protoperdinium sp. | 689   |                  |                   |       |
|                  | Rhizosolenia sp.   | 689   |                  |                   |       |
|                  | UN-ID diatom 3     | 689   |                  |                   |       |
|                  | Melosira sp.       | 4820  |                  |                   |       |

|                                    |       |                                    |       |
|------------------------------------|-------|------------------------------------|-------|
| <b>4-Nov-98</b> Biddulphia sp.     | 2066  | <b>25-Nov-98</b> Coscinodiscus sp. | 2066  |
| Coscinodiscus sp.                  | 4131  | UN-ID diatom                       | 3443  |
| Bacillaria sp.                     | 8282  | UN-ID diatom                       | 689   |
| Pleurosigma                        | 689   | Leptocyindrus sp.                  | 4131  |
| Melosira sp.                       | 15836 | Ceratium sp.                       | 689   |
| UN-ID diatom                       | 3443  | Rhizosolenia sp.                   | 689   |
| Thalassiosira sp.                  | 5508  | Melosira sp.                       | 26163 |
| <br>                               |       |                                    |       |
| <b>10-Nov-98</b> Melosira sp.      | 16524 | <b>5-Dec-98</b> Melosira sp.       | 12393 |
| Thalassiosira sp.                  | 4131  | Coscinodiscus sp.                  | 1377  |
| Biddulphia sp.                     | 4131  | UN-ID diatom                       | 2066  |
| UN-ID diatom                       | 2066  | Pleurosigma sp.                    | 2066  |
| Coscinodiscus sp.                  | 1377  | Chaetoceros sp.                    | 689   |
| Ceratium sp.                       | 689   | Ceratium sp.                       | 689   |
| Thalassionema sp.                  | 2754  |                                    |       |
| <br>                               |       |                                    |       |
| <b>18-Nov-98</b> Coscinodiscus sp. | 4131  | <b>10-Dec-98</b> Pleurosigma sp.   | 689   |
| Nitzschia sp.                      | 2754  | Melosira sp.                       | 7574  |
| Navicula sp.                       | 1377  | UN-ID diatom                       | 3443  |
| Chaetoceros sp.                    | 689   | Coscinodiscus sp.                  | 689   |
| Biddulphia sp.                     | 689   | Thalassiosira sp.                  | 2066  |
| Rhizosolenia sp.                   | 689   |                                    |       |
| Melosira sp.                       | 4820  |                                    |       |

|                  |                      |       |                  |                   |      |
|------------------|----------------------|-------|------------------|-------------------|------|
| <b>29-Jan-99</b> | Melosira sp.         | 5508  | <b>25-Feb-99</b> | Chaetoceros sp.   | 6197 |
|                  | Thalassiosira sp.    | 1377  |                  | Melosira sp.      | 3443 |
|                  |                      |       |                  | Coscinodiscus sp. | 1377 |
| <b>4-Feb-99</b>  | Melosira sp.         | 2066  |                  |                   |      |
|                  | coscinodiscus sp.    | 689   |                  |                   |      |
|                  | Ditylum brightwellii | 689   |                  |                   |      |
|                  | U-ID diatom          | 689   |                  |                   |      |
|                  | Rhizosolenia sp.     | 689   |                  |                   |      |
|                  | Bacillaria sp.       | 11016 |                  |                   |      |
|                  | Thalassionema sp.    | 5508  |                  |                   |      |
| <b>12-Feb-99</b> | Melosira sp.         | 2066  |                  |                   |      |
|                  | Bacillaria sp.       | 11705 |                  |                   |      |
|                  | Chaetoceros sp.      | 8262  |                  |                   |      |
|                  | Thalassionema sp.    | 3443  |                  |                   |      |
| <b>17-Feb-99</b> | Thalassiosira sp.    | 9639  |                  |                   |      |
|                  | Ditylum brightwellii | 4131  |                  |                   |      |
|                  | Lauderia borealis    | 2754  |                  |                   |      |
|                  | Bacillaria sp.       | 24098 |                  |                   |      |
|                  | Coscinodiscus sp.    | 689   |                  |                   |      |
|                  | Rhizosolenia sp.     | 689   |                  |                   |      |
|                  | UN-ID diatom         | 689   |                  |                   |      |
|                  | Melosira sp.         | 11705 |                  |                   |      |
|                  | Pleurosigma sp.      | 689   |                  |                   |      |
|                  | Biddulphia sp.       | 689   |                  |                   |      |

|                  |                   |       |                  |                   |       |
|------------------|-------------------|-------|------------------|-------------------|-------|
| <b>4-Mar-99</b>  | Chaetoceros sp.   | 6885  | <b>24-Mar-99</b> | Navicula sp.      | 1377  |
|                  | Pleurosigma sp.   | 1377  |                  | Coscinodiscus sp. | 689   |
|                  | Melosira sp.      | 6885  |                  | Chaetoceros sp.   | 28917 |
|                  | Biddulphia sp.    | 1377  |                  | Bacillaria sp.    | 7574  |
|                  | Coscinodiscus sp. | 1377  |                  | Pleurosigma sp.   | 689   |
|                  | Ditylum sp.       | 1377  |                  | Melosira sp.      | 2754  |
|                  | Ceratium sp.      | 689   |                  |                   |       |
|                  | Navicula sp.      | 1377  |                  |                   |       |
|                  | Rhizosolenia sp.  | 689   |                  |                   |       |
| <b>11-Mar-99</b> | Nitzschia sp.     | 3443  | <b>25-Mar-99</b> | Chaetoceros sp.   | 4131  |
|                  | Navicula sp.      | 2066  |                  | Skeletonema sp.   | 19967 |
|                  | Coscinodiscus sp. | 689   |                  | Navicula sp.      | 2754  |
|                  | Melosira sp.      | 8951  |                  | Nitzschia sp.     | 689   |
|                  | Chaetoceros sp.   | 15147 |                  | Asterionella sp.  | 8262  |
|                  | Thalassionema sp. | 6885  |                  | Melosira sp.      | 14459 |
|                  |                   |       |                  | Coscinodiscus sp. | 1377  |
| <b>18-Mar-99</b> | Bacillaria sp.    | 61965 |                  | Pleurosigma sp.   | 689   |
|                  | Nitzschia sp.     | 5508  |                  | Biddulphia sp.    | 689   |
|                  | Thalassionema sp. | 1377  |                  |                   |       |
|                  | Skeletonema sp.   | 15836 | <b>30-Mar-99</b> | Nitzschia sp.     | 1377  |
|                  | Pleurosigma sp.   | 689   |                  | Chaetoceros sp.   | 12393 |
|                  | Coscinodiscus sp. | 1377  |                  | Bacillaria sp.    | 3443  |
|                  | Asterionella sp.  | 8951  |                  | Melosira sp.      | 13770 |
|                  | Biddulphia sp.    | 689   |                  | Ditylum sp.       | 2066  |
|                  |                   |       |                  | Coscinodiscus sp. | 689   |
| <b>22-Mar-99</b> | Coscinodiscus sp. | 3443  |                  | Rhizosolenia sp.  | 2066  |
|                  | Pleurosigma sp.   | 2754  |                  | Navicula sp.      | 3443  |
|                  | Rhizosolenia sp.  | 689   |                  | Skeletonema sp.   | 30294 |
|                  | Melosira sp.      | 9639  |                  |                   |       |
|                  | Chaetoceros sp.   | 40622 |                  |                   |       |
|                  | Biddulphia sp.    | 1377  |                  |                   |       |
|                  | Asterionella sp.  | 2754  |                  |                   |       |
|                  | Thalassiosira sp. | 2754  |                  |                   |       |

|                  |                   |       |                  |                   |       |
|------------------|-------------------|-------|------------------|-------------------|-------|
| <b>4-Mar-99</b>  | Chaetoceros sp.   | 6885  | <b>24-Mar-99</b> | Navicula sp.      | 1377  |
|                  | Pleurosigma sp.   | 1377  |                  | Coscinodiscus sp. | 689   |
|                  | Melosira sp.      | 6885  |                  | Chaetoceros sp.   | 28917 |
|                  | Biddulphia sp.    | 1377  |                  | Bacillaria sp.    | 7574  |
|                  | Coscinodiscus sp. | 1377  |                  | Pleurosigma sp.   | 689   |
|                  | Ditylum sp.       | 1377  |                  | Melosira sp.      | 2754  |
|                  | Ceratium sp.      | 689   |                  |                   |       |
|                  | Navicula sp.      | 1377  |                  |                   |       |
|                  | Rhizosolenia sp.  | 689   |                  |                   |       |
| <b>11-Mar-99</b> | Nitzschia sp.     | 3443  | <b>25-Mar-99</b> | Chaetoceros sp.   | 4131  |
|                  | Navicula sp.      | 2066  |                  | Skeletonema sp.   | 19967 |
|                  | Coscinodiscus sp. | 689   |                  | Navicula sp.      | 2754  |
|                  | Melosira sp.      | 8951  |                  | Nitzschia sp.     | 689   |
|                  | Chaetoceros sp.   | 15147 |                  | Asterionella sp.  | 8262  |
|                  | Thalassionema sp. | 6885  |                  | Melosira sp.      | 14459 |
|                  |                   |       |                  | Coscinodiscus sp. | 1377  |
| <b>18-Mar-99</b> | Bacillaria sp.    | 61965 |                  | Pleurosigma sp.   | 689   |
|                  | Nitzschia sp.     | 5508  |                  | Biddulphia sp.    | 689   |
|                  | Thalassionema sp. | 1377  |                  |                   |       |
|                  | Skeletonema sp.   | 15836 | <b>30-Mar-99</b> | Nitzschia sp.     | 1377  |
|                  | Pleurosigma sp.   | 689   |                  | Chaetoceros sp.   | 12393 |
|                  | Coscinodiscus sp. | 1377  |                  | Bacillaria sp.    | 3443  |
|                  | Asterionella sp.  | 8951  |                  | Melosira sp.      | 13770 |
|                  | Biddulphia sp.    | 689   |                  | Ditylum sp.       | 2066  |
|                  |                   |       |                  | Coscinodiscus sp. | 689   |
| <b>22-Mar-99</b> | Coscinodiscus sp. | 3443  |                  | Rhizosolenia sp.  | 2066  |
|                  | Pleurosigma sp.   | 2754  |                  | Navicula sp.      | 3443  |
|                  | Rhizosolenia sp.  | 689   |                  | Skeletonema sp.   | 30294 |
|                  | Melosira sp.      | 9639  |                  |                   |       |
|                  | Chaetoceros sp.   | 40622 |                  |                   |       |
|                  | Biddulphia sp.    | 1377  |                  |                   |       |
|                  | Asterionella sp.  | 2754  |                  |                   |       |
|                  | Thalassiosira sp. | 2754  |                  |                   |       |



|                 |                          |              |                  |                        |               |
|-----------------|--------------------------|--------------|------------------|------------------------|---------------|
| <b>1-Apr-99</b> | <b>Coscinodiscus sp.</b> | <b>2754</b>  | <b>7-Apr-99</b>  | <b>Skeletonema sp.</b> | <b>256811</b> |
|                 | Navicula sp.             | 6885         |                  | Navicula sp.           | 2066          |
|                 | Asterionella sp.         | 10328        |                  | Coscinodiscus sp.      | 3443          |
|                 | Skeletonema sp.          | 105341       |                  | Chaetoceros sp.        | 112914        |
|                 | Nitzschia sp.            | 12393        |                  | Melosira sp.           | 2754          |
|                 | Chaetoceros sp.          | 53015        |                  | Nitzschia sp.          | 14459         |
|                 | Melosira sp.             | 26163        |                  | Thalassionema sp.      | 8262          |
|                 | Bacillaria sp.           | 8262         |                  | Biddulphia sp.         | 1377          |
|                 | Pleurosigma sp.          | 1377         |                  | Rhizosolenia sp.       | 689           |
|                 | Ditylum sp.              | 689          |                  | Pleurosigma sp.        | 689           |
|                 | Thalassionema sp.        | 5508         |                  | Asterionella sp.       | 33048         |
|                 | Rhizosolenia sp.         | 1377         |                  | Ditylum sp.            | 1377          |
|                 | Biddulphia sp.           | 1377         |                  | Peridinium sp.         | 689           |
|                 |                          |              |                  | Bacillaria sp.         | 3443          |
| <b>5-Apr-99</b> | <b>Melosira sp.</b>      | <b>21344</b> | <b>12-Apr-99</b> | <b>Skeletonema sp.</b> | <b>881969</b> |
|                 | Skeletonema sp.          | 305694       |                  | Thalassiosira sp.      | 42687         |
|                 | Rhizosolenia sp.         | 2754         |                  | Asterionella sp.       | 43376         |
|                 | Asterionella sp.         | 42687        |                  | Chaetoceros sp.        | 46864         |
|                 | Navicula sp.             | 3443         |                  | Nitzschia sp.          | 26229         |
|                 | Chaetoceros sp.          | 40622        |                  | Navicula sp.           | 9639          |
|                 | Ditylum sp.              | 689          |                  | UN-ID dp.              | 9639          |
|                 | Pleurosigma sp.          | 689          |                  | Thalassionema sp.      | 11706         |
|                 | Coscinodiscus sp.        | 1377         |                  | Coscinodiscus sp.      | 1377          |
|                 | Biddulphia sp.           | 1377         |                  | Ditylum sp.            | 689           |
|                 | Nitzschia sp.            | 1377         |                  | Bacillaria sp.         | 6197          |
|                 | Thalassiosira sp.        | 6885         |                  |                        |               |

|                  |                      |         |                  |                   |         |
|------------------|----------------------|---------|------------------|-------------------|---------|
| <b>14-Apr-99</b> | Navicula sp.         | 5508    | <b>25-Apr-99</b> | Skeletonema sp.   | 1173883 |
|                  | Skeletonema sp.      | 216878  |                  | Asterionella sp.  | 98456   |
|                  | Chaetoceros sp.      | 27540   |                  | Navicula sp.      | 6197    |
|                  | Coscinodiscus sp.    | 1377    |                  | Ditylum sp.       | 25475   |
|                  | Asterionella sp.     | 24786   |                  | Thalassionema sp. | 48195   |
|                  | Bacillaria sp.       | 13770   |                  | Chaetoceros sp.   | 58523   |
|                  | Nitzschia sp.        | 2066    |                  | Rhizosolenia sp.  | 17901   |
|                  | Melosira sp.         | 15836   |                  | Nitzschia sp.     | 39245   |
|                  | Pleurosigma sp.      | 689     |                  | Thalassiosira sp. | 9639    |
|                  |                      |         |                  | Melosira sp.      | 8951    |
|                  |                      |         |                  | Coscinodiscus sp. | 3443    |
| <b>18-Apr-99</b> | Melosira sp.         | 21344   |                  | Guinardia sp.     | 689     |
|                  | Coscinodiscus sp.    | 2066    |                  | UN-ID sp.         | 2754    |
|                  | Skeletonema sp.      | 438575  |                  | Biddulphia sp.    | 2066    |
|                  | Rhizosolenia sp.     | 4131    |                  |                   |         |
|                  | Chaetoceros sp.      | 16524   | <b>27-Apr-99</b> | Melosira sp.      | 2754    |
|                  | Stephanopyxis sp.    | 5508    |                  | Lauderia sp.      | 15147   |
|                  | Asterionella sp.     | 4820    |                  | Rhizosolenia sp.  | 20655   |
|                  | Navicula sp.         | 1377    |                  | Skeletonema sp.   | 417231  |
|                  | Thalassionema sp.    | 21344   |                  | Asterionella sp.  | 290547  |
|                  | Ditylum brightwellii | 689     |                  | Chaetoceros sp.   | 55080   |
|                  |                      |         |                  | Bacillaria sp.    | 39245   |
| <b>20-Apr-99</b> | Biddulphia sp.       | 1377    |                  | Biddulphia sp.    | 6885    |
|                  | Nitzschia sp.        | 11016   |                  | Thalassionema sp. | 94325   |
|                  | Skeletonema sp.      | 1032062 |                  | Nitzschia sp.     | 37868   |
|                  | Coscinodiscus sp.    | 689     |                  | Navicula sp.      | 2066    |
|                  | Asterionella sp.     | 39245   |                  | Ditylum sp.       | 27540   |
|                  | Thalassionema sp.    | 25475   |                  | Thalassiosira sp. | 3443    |
|                  | Navicula sp.         | 1377    |                  | Coscinodiscus sp. | 2066    |
|                  | Chaetoceros sp.      | 55080   |                  | Stephanopyxis sp. | 1377    |
|                  | Ditylum sp.          | 2754    |                  |                   |         |
|                  | Rhizosolenia sp.     | 2754    |                  |                   |         |
|                  | Pleurosigma sp.      | 2066    |                  |                   |         |

|                                   |         |                                   |         |
|-----------------------------------|---------|-----------------------------------|---------|
| <b>30-Apr-99</b> Biddulphia sp.   | 8262    | <b>5-May-99</b> Thalassionema sp. | 62654   |
| Ditylum sp.                       | 19278   | Lauderia sp.                      | 12393   |
| Nitzschia sp.                     | 34425   | Chaetoceros sp.                   | 23409   |
| Thalassiosira sp.                 | 8951    | Asterionella sp.                  | 501917  |
| Asterionella sp.                  | 628601  | Rhizosolenia sp.                  | 17213   |
| Rhizosolenia sp.                  | 21344   | Nitzschia sp.                     | 24786   |
| Pleurosigma sp.                   | 2066    | Navicula sp.                      | 4131    |
| Thalassionema sp.                 | 66096   | Skeletonema sp.                   | 5508    |
| Chaetoceros sp.                   | 61277   | Leptocylindrus sp.                | 5508    |
| Navicula sp.                      | 1377    | Guinardia sp.                     | 4131    |
| Coscinodiscus sp.                 | 2066    | UN-ID sp.                         | 5508    |
| Skeletonema sp.                   | 23409   | Bacillaria sp.                    | 22721   |
| Bacillaria sp.                    | 65408   | Ditylum sp.                       | 2066    |
| Pheocystis sp.                    | 29606   | Melosira sp.                      | 5508    |
| Stephanopyxis sp.                 | 6885    |                                   |         |
|                                   |         |                                   |         |
| <b>4-May-99</b> Thalassionema sp. | 77112   | <b>11-May-99</b> Biddulphia sp.   | 1377    |
| Asterionella sp.                  | 1067864 | Stephanopyxis sp.                 | 10328   |
| Rhizosolenia sp.                  | 44064   | Rhizosolenia sp.                  | 10328   |
| Chaetoceros sp.                   | 46130   | Nitzschia sp.                     | 51638   |
| Ditylum sp.                       | 2066    | Asterionella sp.                  | 442017  |
| Skeletonema sp.                   | 6197    | Leptocylindrus sp.                | 31671   |
| Nitzschia sp.                     | 30294   | Chaetoceros sp.                   | 38556   |
| Biddulphia sp.                    | 8951    | Phaeocystis sp.                   | 1699218 |
| Coscinodiscus sp.                 | 2066    | Bacillaria sp.                    | 29806   |
| Bacillaria sp.                    | 10328   | Coscinodiscus sp.                 | 689     |
| Stephanopyxis sp.                 | 6197    | Guinardia sp.                     | 1377    |
| Lauderia sp.                      | 11705   | Thalassionema sp.                 | 7574    |
| Navicula sp.                      | 689     | Melosira sp.                      | 3443    |
| Thalassiosira sp.                 | 2754    | Lauderia sp.                      | 4131    |
| Guinardia sp.                     | 689     | Thalassiosira sp.                 | 4131    |

|                  |                    |         |                  |                    |         |
|------------------|--------------------|---------|------------------|--------------------|---------|
| <b>18-May-99</b> | Nitzschia sp.      | 159732  | <b>25-May-99</b> | Phaeocystis sp.    | 7270560 |
|                  | Navicula sp.       | 4131    |                  | Rhizosolenia sp.   | 329792  |
|                  | Rhizosolenia sp.   | 48195   |                  | Lauderia sp.       | 1377    |
|                  | UN-ID species      | 2754    |                  | Nitzschia sp.      | 90194   |
|                  | Guinardia sp.      | 6885    |                  | Coscinodiscus sp.  | 4820    |
|                  | Leptocylindrus sp. | 7574    |                  | Guinardia sp.      | 11016   |
|                  | Thalassionema sp.  | 13770   |                  | Biddulphia sp.     | 689     |
|                  | Lauderia sp.       | 3443    |                  | Stephanopyxis sp.  | 9639    |
|                  | Biddulphia sp.     | 4820    |                  | Eucampia sp.       | 1377    |
|                  | Bacillaria sp.     | 8262    |                  | Thalassionema sp.  | 2754    |
|                  | Stephanopyxis sp.  | 7574    |                  | Ceratium sp.       | 1377    |
|                  | Pleurosigma sp.    | 689     |                  | Navicula sp.       | 4131    |
|                  | Chaetoceros sp.    | 15147   |                  |                    |         |
|                  | UN-ID diatom       | 1377    |                  |                    |         |
| <b>23-May-99</b> | Phaeocystis sp.    | 7123221 | <b>28-May-99</b> | Phaeocystis sp.    | 6428525 |
|                  | Rhizosolenia sp.   | 300875  |                  | Rhizosolenia sp.   | 273335  |
|                  | UN-ID sp.          | 3443    |                  | Lauderia sp.       | 4820    |
|                  | Guinardia sp.      | 8951    |                  | Leptocylindrus sp. | 16524   |
|                  | Melosira sp.       | 11016   |                  | Nitzschia sp.      | 65408   |
|                  | Nitzschia sp.      | 154224  |                  | Bacillaria sp.     | 11705   |
|                  | Ditylum sp.        | 689     |                  | Biddulphia sp.     | 2754    |
|                  | Coscinodiscus sp.  | 4131    |                  | Guinardia sp.      | 4131    |
|                  | Navicula sp.       | 2066    |                  | UN-ID sp.          | 2754    |
|                  | Leptocylindrus sp. | 50261   |                  | Stephanopyxis sp.  | 2754    |
|                  | Lauderia sp.       | 1377    |                  |                    |         |
|                  | Stephanopyxis sp.  | 2754    |                  |                    |         |

|                 |                       |              |                  |                         |              |
|-----------------|-----------------------|--------------|------------------|-------------------------|--------------|
| <b>1-Jun-99</b> | <b>UN -ID sp.</b>     | <b>1377</b>  | <b>17-Jun-99</b> | <b>Rhizosolenia sp.</b> | <b>18590</b> |
|                 | Bacillaria sp.        | 19278        |                  | Melosira sp.            | 17901        |
|                 | Melosira sp.          | 3443         |                  | Prorocentrum sp.        | 2754         |
|                 | Rhizosolenia sp.      | 471623       |                  | Navicula sp.            | 2066         |
|                 | Guinardia sp.         | 5508         |                  | Nitzschia sp.           | 9639         |
|                 | Nitzschia sp.         | 39245        |                  | Coscinodiscus sp.       | 689          |
|                 | Ceratium sp.          | 689          |                  | Peridinium sp.          | 4131         |
|                 | Peridinium sp.        | 1377         |                  | UN-ID dino              | 689          |
|                 | Prorocentrum sp.      | 2754         |                  |                         |              |
|                 | Coscinodiscus sp.     | 2754         |                  |                         |              |
|                 | Pleurosigma sp.       | 2066         |                  |                         |              |
|                 | Leptocyindrus sp.     | 22721        |                  |                         |              |
|                 | Ditylum sp.           | 1377         |                  |                         |              |
|                 | Navicula sp.          | 689          |                  |                         |              |
| <b>6-Jun-99</b> | <b>Bacillaria sp.</b> | <b>17213</b> | <b>22-Jun-99</b> | <b>Rhizosolenia sp.</b> | <b>53015</b> |
|                 | Rhizosolenia sp.      | 142520       |                  | Leptocyindrus sp.       | 39245        |
|                 | Guinardia sp.         | 2066         |                  | Nitzschia sp.           | 17213        |
|                 | Prorocentrum sp.      | 5508         |                  | Peridinium sp.          | 16524        |
|                 | UN-ID sp.             | 8951         |                  | Navicula sp.            | 3443         |
|                 | Leptocyindrus sp.     | 33737        |                  | Noctiluca sp.           | 2066         |
|                 | Nitzschia sp.         | 19278        |                  | UN-ID sp.               | 2066         |
|                 | Navicula sp.          | 1377         |                  | Ceratium sp.            | 689          |
|                 | Peridinium sp.        | 1377         |                  |                         |              |
|                 | Ceratium sp.          | 1377         |                  |                         |              |

|                  |                           |               |                  |                      |              |
|------------------|---------------------------|---------------|------------------|----------------------|--------------|
| <b>6-Jul-99</b>  | <b>Leptocylindrus sp.</b> | <b>760793</b> | <b>20-Jul-99</b> | <b>Guinardia sp.</b> | <b>30294</b> |
|                  | Nitzschia sp.             | 177633        |                  | UN-ID sp.            | 4820         |
|                  | Peridinium sp.            | 21344         |                  | Leptocylindrus sp.   | 121885       |
|                  | UN-ID sp (dino)           | 77801         |                  | Rhizosolenia sp.     | 13082        |
|                  | Navicula sp.              | 1377          |                  | Navicula sp.         | 689          |
|                  | Guinardia sp.             | 4131          |                  |                      |              |
|                  | Rhizosolenia sp.          | 10328         |                  |                      |              |
|                  | Ceratium sp.              | 2754          |                  |                      |              |
|                  | Coscinodiscus sp.         | 689           |                  |                      |              |
|                  | Pleurosigma sp.           | 689           |                  |                      |              |
|                  | Chaetoceros sp.           | 689           |                  |                      |              |
|                  | Prorocentrum sp.          | 1377          |                  |                      |              |
| <b>14-Jul-99</b> | <b>Prorocentrum sp.</b>   | <b>2754</b>   | <b>28-Jul-99</b> | <b>Navicula sp.</b>  | <b>6197</b>  |
|                  | Leptocylindrus sp.        | 103275        |                  | Rhizosolenia sp.     | 13770        |
|                  | Guinardia sp.             | 30294         |                  | UN-ID sp.            | 2086         |
|                  | Navicula sp.              | 4131          |                  | Guinardia sp.        | 10328        |
|                  | Coscinodiscus sp.         | 689           |                  | Leptocylindrus sp.   | 15147        |
|                  | Protoperidinium sp.       | 2086          |                  | Prorocentrum sp.     | 1377         |
|                  | Rhizosolenia sp.          | 2086          |                  | Peridinium sp.       | 3443         |
|                  | Nitzschia sp.             | 99144         |                  | Ceratium sp.         | 1377         |
|                  | UN-ID sp.(dino)           | 13770         |                  | Pleurosigma sp.      | 1377         |
|                  | Chaetoceros sp.           | 1377          |                  | Bacillaria sp.       | 6197         |
|                  | Peridinium sp.            | 4820          |                  | Chaetoceros sp.      | 1377         |
|                  | UN-ID sp.                 | 9639          |                  | Nitzschia sp.        | 5608         |
|                  | Noctiluca sp.             | 689           |                  | Meiosira sp.         | 2754         |
|                  | Ceratium sp.              | 1377          |                  | Skeletonema sp.      | 4131         |
|                  | Pleurosigma sp.           | 689           |                  | Noctiluca sp.        | 689          |

|                  |                    |       |                  |                   |       |
|------------------|--------------------|-------|------------------|-------------------|-------|
| <b>13-Aug-99</b> | Navicula sp.       | 9639  | <b>14-Sep-99</b> | Stauroneis sp.    | 12393 |
|                  | Pleurosigma sp.    | 1377  |                  | Rhizosolenia sp.  | 35114 |
|                  | Rhizosolenia sp.   | 33737 |                  | Chaetoceros sp.   | 8861  |
|                  | Prorocentrum sp.   | 3443  |                  | Nitzschia sp.     | 14459 |
|                  | Eucampia sp.       | 65408 |                  | Stephanopyxis sp. | 2754  |
|                  | Leptocylindrus sp. | 15147 |                  | Pleurosigma sp.   | 1377  |
|                  | Nitzschia sp.      | 8262  |                  | Navicula sp.      | 689   |
|                  | Thalassiosira sp.  | 4820  |                  | Coscinodiscus sp. | 4131  |
|                  | Ceratium sp.       | 689   |                  | Guinardia sp.     | 689   |
|                  | Melosira sp.       | 6197  |                  | Ceratium sp.      | 1377  |
|                  | Guinardia sp.      | 689   |                  | Eucampia sp.      | 2066  |
|                  | Coscinodiscus sp.  | 2754  |                  | Lauderia sp.      | 6885  |
|                  |                    |       |                  | Prorocentrum sp.  | 689   |
| <b>25-Aug-99</b> | Leptocylindrus sp. | 7574  | <b>27-Sep-99</b> | Rhizosolenia sp.  | 75047 |
|                  | Eucampia sp.       | 59900 |                  | Thalassiosira sp. | 2066  |
|                  | Melosira sp.       | 10328 |                  | Melosira sp.      | 12393 |
|                  | Navicula sp.       | 6885  |                  | Navicula sp.      | 2754  |
|                  | Ceratium sp.       | 1377  |                  | Prorocentrum sp.  | 689   |
|                  | Peridinium sp.     | 2754  |                  | Chaetoceros sp.   | 5508  |
|                  | Rhizosolenia sp.   | 4820  |                  | Ceratium sp.      | 689   |
|                  | Pleurosigma sp.    | 1377  |                  | Pleurosigma sp.   | 1377  |
|                  | Prorocentrum sp.   | 2066  |                  | Lauderia sp.      | 5508  |
|                  |                    |       |                  | Peridinium sp.    | 689   |

## Appendix 3

### COMPARISON BETWEEN WHATMAN GF/F FILTERS AND POLYCARBONATE(O.2um)

#### Experiment # 3

DOC concentration

| GF/F | Polycarbonate |
|------|---------------|
| 120  | 208           |
| 142  | 225           |
| 138  | 200           |
| 136  | 197           |
| 129  | 119           |
| 137  | 239           |
| 152  | 233           |
| 146  | 211           |
| 131  | 238           |
| 132  | 244           |

#### T-test to check the mean difference between GF/F and Polycarbonate filters

t-Test: Paired Two Sample for Means

|                              | Variable 1 | Variable 2 |
|------------------------------|------------|------------|
| Mean                         | 136.3323   | 211.3493   |
| Variance                     | 86.42066   | 1352.952   |
| Observations                 | 10         | 10         |
| Pearson Correlation          | 0.287207   |            |
| Hypothesized Mean Difference | 0          |            |
| df                           | 9          |            |
| t Stat                       | -6.7287    |            |
| P(T<=t) one-tail             | 4.28E-05   |            |
| t Critical one-tail          | 1.833114   |            |
| P(T<=t) two-tail             | 8.57E-05   |            |
| t Critical two-tail          | 2.262159   |            |

variable 1 = GF/F filters  
variable 2 = Polycarbonate filters



## Appendix 4

### Estimation of phytoplankton cell volume

Cells volumes were determined from linear dimensions assuming the cells to be spherical, cylindrical, or ellipsoidal. A number of species were included in the genetic groups designated as *Rhizosolenia sp.* or *Chaetoceros sp.* Therefore the volume of the genetic group represent the mean of different species in a particular group and the total cell volumes of different genetic groups were estimated by multiplying by the total cell numbers.

| Species                    | Volume ( $\mu\text{m}^3$ ) |
|----------------------------|----------------------------|
| <i>Asterionella sp.</i>    | 2500                       |
| <i>Baccillaria sp.</i>     | 4400                       |
| <i>Biddulphia sp.</i>      | 241000                     |
| <i>Ceratium sp.</i>        | 10000                      |
| <i>Chaetoceros sp.</i>     | 6500                       |
| <i>Coscinodiscus sp.</i>   | 350000                     |
| <i>Dictyocha sp.</i>       | 500                        |
| <i>Ditylum sp.</i>         | 27000                      |
| <i>Eucampia sp.</i>        | 4600                       |
| <i>Guinardia sp.</i>       | 300000                     |
| <i>Gyrodinium sp.</i>      | 39750                      |
| <i>Lauderia sp.</i>        | 21000                      |
| <i>Leptocylindrus sp.</i>  | 2000                       |
| <i>Melosira sp.</i>        | 3100                       |
| <i>Navicula sp.</i>        | 2200                       |
| <i>Nitzschia sp.</i>       | 220                        |
| <i>Noctiluca sp.</i>       | 100000                     |
| <i>Paralia sp.</i>         | 3100                       |
| <i>Peridinium sp.</i>      | 30000                      |
| <i>Phaecosystis sp.</i>    | 60                         |
| <i>Pleurosigma</i>         | 11000                      |
| <i>Prorocentrum sp.</i>    | 30000                      |
| <i>Protoperidinium sp.</i> | 30000                      |
| <i>Pyrocystis sp.</i>      | 1000                       |
| <i>Rhizosolenia sp.</i>    | 39750                      |
| <i>Skeletonema sp.</i>     | 180                        |
| <i>Stauroneis sp.</i>      | 10000                      |
| <i>Stephanopyxis sp.</i>   | 100000                     |
| <i>Thalassionema sp.</i>   | 1200                       |
| <i>Thalassiosira sp.</i>   | 2600                       |