

The yield of chlorophyll from nitrogen: a comparison between the shallow Ria Formosa lagoon and the deep oceanic conditions at Sagres along the southern coast of Portugal

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Abstract

The yield of chlorophyll from dissolved inorganic nitrogen (DIN) has been shown to be a potentially useful parameter for predicting eutrophication, particularly, in the northerly, coastal waters of the North East Atlantic (NEA). This study investigates whether this parameter might also be appropriate for the southerly, coastal waters of the NEA. Nitrogen enrichment experiments were carried out using microcosms to determine the microplanktonic yield of chlorophyll from DIN in waters from the Ria Formosa (April 2002) and from Sagres (September 2002) on the south coast of Portugal. Continuous culture techniques enabled experiments to be run for 7 days after enrichment so that changes in the cumulative yield over time could be calculated. Yields from the Sagres experiment were consistently higher than those from the Ria Formosa experiment, with respective maximum yields of 4.7 and 2.1 $\mu\text{g chl } (\mu\text{mol N})^{-1}$, and respective steady-state yields of 3.1 and 0.9 $\mu\text{g chl } (\mu\text{mol N})^{-1}$. In addition, regressions carried out on historical data sets from the two study sites showed poor correlation between chlorophyll and nitrate. Other differences between the microcosm experiments at the two sites, included: background concentrations of DIN, silicate and phosphate that were, respectively, 5.6 μM , 8.1 μM , and 0.3 μM higher in the Ria; chlorophyll concentrations at Sagres that were double those of the Ria; accumulation of particulate nitrogen that was both more rapid and more substantial at Sagres; a different community structure for the diatoms at the two sites; more numerous autotrophic dinoflagellates, flagellates and cyanobacteria, as well as more numerous protozoan grazers, at Sagres. These differences may explain why the yield of chlorophyll from DIN at Sagres is one of the highest reported in the literature. This yield parameter requires further study under a range of seasonal conditions and with a range of microplankton communities before it could be considered useful for predicting eutrophication throughout the coastal waters of the NEA.

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1. Introduction

Eutrophication of natural waters produced by an enhanced supply of nutrients, has been recognised in recent years as a serious environmental problem (Nixon, 1995), requiring the development of simple procedures and screening models for predicting this phenomenon (Tett et al., 2003). In freshwater situations, the relationship between phosphorus nutrient loading and mean

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phytoplankton chlorophyll has been demonstrated as a reliable parameter for predicting eutrophication (Dillon and Rigler, 1975; Schindler et al., 1978; Vollenweider, 1976). In coastal waters, nitrogen is considered to be the most likely nutrient limiting phytoplankton growth (Taylor et al., 1995; Tsirtsis, 1995). However, because coastal environments are more rapidly flushed than freshwater habitats of similar volume, a further understanding is needed of the dynamic relationship between nitrogen supply and increase of phytoplankton biomass (Gowen et al., 1992).

Procedures for estimating the yield parameter in the dynamic conditions of coastal waters have been developed by Gowen et al. (1992), using regression analysis on chlorophyll and nitrate data collected from a variety of Scottish sea-lochs. Further investigations of the yield of chlorophyll from dissolved inorganic nitrogen (DIN) have been carried out using microcosms and continuous culture techniques (Edwards, 2001; Edwards et al., 2003). The results from these studies confirm that, in Scottish coastal waters, the yield of chlorophyll from nutrient is appropriate for use in models, such as the Comprehensive Studies Task Team model (CSTT, 1994, 1997; Tett, 2002) for diagnosing and predicting eutrophication. The median yield of $1.1 \mu\text{g chl } (\mu\text{mol N})^{-1}$, obtained from the Gowen et al. (1992) regression analyses and the Edwards et al. (2003) microcosm experiments, has been used in the screening models for eutrophication developed within the context of the European Union (EU) funded OAERRE (Oceanographic Applications to Eutrophication in Regions of Restricted Exchange) project (Tett et al., 2003).

However, most of the studies on the yield parameter have been carried out in northern, coastal areas (Edwards et al., 2003; Tett et al., 2003), within the continental shelf of the North East Atlantic (NEA). This current study tests whether the yield of chlorophyll from nitrogen could be useful for predicting eutrophication along the southern coast of the NEA. All the coastal areas of the NEA belong to the same Geographical Intercalibration Group (GIG) for the Common Implementation Strategy (CIS) of the Water Framework Directive (WFD). The Ria Formosa and Sagres (Fig. 1) have been selected as intercalibration sites for the CIS of the WFD (C.E.C., 2000) in coastal waters along the southern coast of the NEA zone, because they belong to different typologies with respect to the degree of exposure, despite their geographical proximity to each other (100 km).

The Ria Formosa (Fig. 1) is a sheltered, shallow lagoon with a tidal range varying between 1.35 m and 3 m for neap and spring tides respectively, with an estimated exchange of 50–75% water mass during each tide (Sprung, 1994; Newton and Icely, in press). It has a volume of $31 \times 10^6 \text{ m}^3$, an average depth of 6 m, although most areas are less than 2 m deep (Newton and

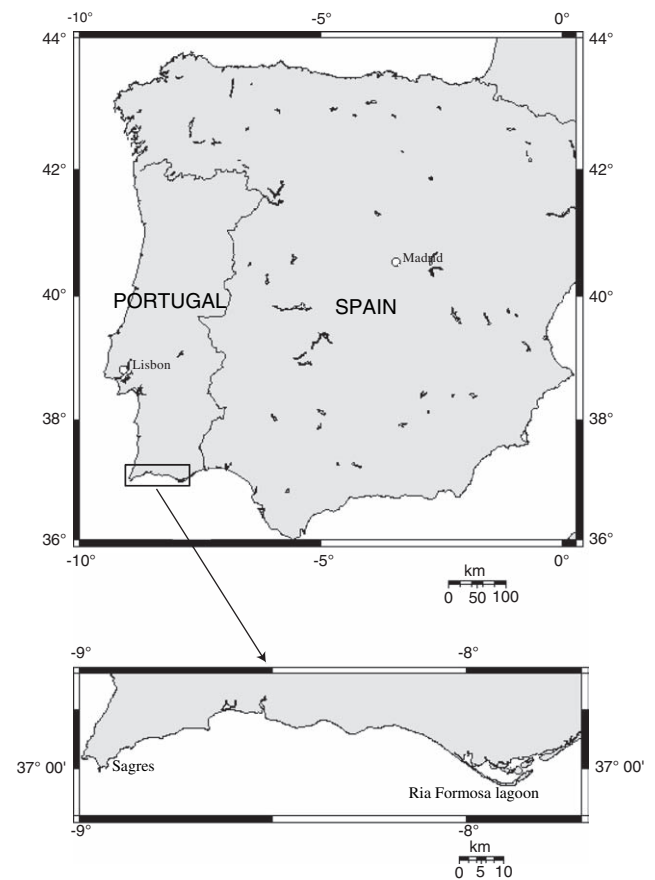


Fig. 1. A map showing the locations where the two microcosms were carried out: the Ria Formosa lagoon and Sagres.

Mudge, 2003), and is generally well mixed vertically. However, the inner parts of the lagoon, which are less well mixed, may be susceptible to water quality problems, especially eutrophication. Freshwater inputs are localised or are temporarily created by torrential downpours. The concentration of nutrients is variable, particularly in winter, with nitrates varying between 0.2 and $10 \mu\text{M}$ (Falcão and Vale, 2003), but with values as high as $40 \mu\text{M}$ occurring after periods of intense rainfall (Newton and Mudge, 2005). Phosphate varies between 0.05 and $3 \mu\text{M}$ (Falcão and Vale, 2003; Newton and Mudge, 2005). The chlorophyll concentration in the water column is low with ranges of between 1 and $3 \mu\text{g l}^{-1}$ (Newton et al., 2003). The sediment is covered by green macro-algae, *Ulva* sp. and filamentous algae, which compete with the phytoplankton for nutrients and may partly explain the low chlorophyll concentrations in the water column. The lagoon is in an urbanised area surrounded by agricultural land and is, therefore, vulnerable to anthropogenic eutrophication (Newton et al., 2003).

Sagres (Fig. 1) is located on an exposed coast characterised by steep, limestone cliffs of 30–40 m, and a narrow continental shelf that slopes steeply to deep

Atlantic water (4000 m). There are no permanent rivers in the area but only torrential streams that flow in winter and are fed by the runoff from Atlantic storms and strong south-west winds (Peliz and Fiuza, 1999). In the summer a dominant, northerly wind drives a seasonal, coastal upwelling (Wooster et al., 1976; Fiúza et al., 1982; Frouin et al., 1990; Sousa and Bricaud, 1992; Relvas and Barton, 2002) that supplies nutrients to the euphotic zone. The concentrations of nitrates vary between 4 and 20 μM , depending on the upwelling conditions, whilst the phosphate concentrations are more constant, varying between 0.2 and 0.5 μM (Loureiro et al., in press). Chlorophyll *a* is relatively low at 2 $\mu\text{g l}^{-1}$ (Villa et al., 1997) increasing up to 6 $\mu\text{g l}^{-1}$ during periods of upwelling (Loureiro et al., in press). This area supports a rich fishing industry and, in recent years, a successful offshore aquaculture industry for bivalves. The Portuguese fisheries authorities regularly inspect these bivalves for toxins from blooms of harmful algae, which are sometimes detected, especially during the autumn. Although summer upwelling conditions may produce natural eutrophication at Sagres, the low resident population and limited intensive agriculture will minimize anthropogenic eutrophication.

This paper shows the results of microcosm experiments undertaken using the lagoon water of the Ria Formosa in April 2002, and the oceanic water of Sagres in September, 2002. The dates were selected as periods when productivity is generally high at the respective sites. The results are also presented of regression analysis carried out on data sets of chlorophyll and dissolved inorganic nitrogen (DIN) collected from both of these sites.

2. Materials and methods

2.1. Water collection

Approximately 100 l of seawater was collected at low water on the 11th April, 2002 from a depth of 0.5 m at the Ponte site ($37^{\circ} 00' 32'' \text{ N}$ and $07^{\circ} 59' 37'' \text{ W}$ in Fig. 1) in the Ria Formosa, which had been used for routine sampling over the OAERRE project. There was substantial rainfall during the previous week, but the temperature and salinity in the Ria, at 16.7°C and 36, were similar to oceanic conditions due to the high rate of exchange between the lagoon and the ocean. After collection, the water was quickly transported to the microcosms that had been set up in the University of Algarve.

A similar volume of water was collected on 18th September, 2002 from a depth of 0.5 m at the Sagres site ($37^{\circ} 00' 40'' \text{ N}$ and $8^{\circ} 55' 30'' \text{ W}$ in Fig. 1), which was adjacent to a mooring in 35 m depth at an offshore aquaculture structure on the edge of the continental slope. On the 7th September, the north wind changed

from north-west to south-west culminating in south-east winds on the 18th. There was substantial rainfall during the south-westerly conditions, with a steady increase in temperature from 15.0°C to 18.1°C on the sampling day, whilst the salinity remained constant at 36. Essentially, the oceanic water was collected after a period of summer upwelling when the wind changed from the northerly direction on the 7th September (Loureiro et al., in press). After collection, the water was quickly transported to the microcosms that had been set up in the cold room belonging to Sagremarisco L^{da}.

2.2. Experimental design

A diagram of a microcosm is shown in Fig. 2. It comprises a reservoir containing nutrients, a reactor containing the study population, and a sump for receiving overflow from the reactor. The reservoirs and reactors used for the study were transparent 10 l polycarbonate carboys. Filtered air was blown into the reactors to supply carbon dioxide, and mixing was aided by the use of magnetic stirrer bars. The same configuration was used for both the experiment at the University of Algarve in April, 2002 and the experiment at Sagremarisco L^{da} in September, 2002.

Diluent for the reservoirs was filtered through a 0.5 μm heavy-duty filtration unit, 3 l was added to the reservoirs, and the rest was stored in a cold room until needed. 10 l of the collected seawater was added to each reactor after filtration through a 200 μm mesh, which was used to restrict the filtrate to microplankton, defined for our purposes as organisms less than 200 μm in size. A peristaltic pump was used to create a dilution rate of 0.2 d^{-1} . The microcosm apparatus and the conditions for continuous culture were operational within 4 h of the initial water collection. The temperature, irradiance, and day length used for each experiment are shown in Table 1.

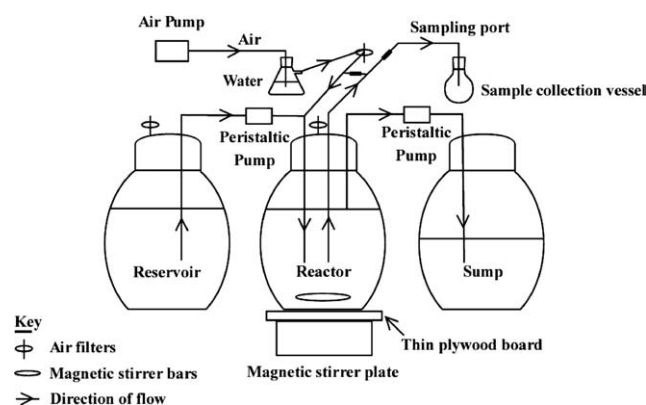


Fig. 2. A diagram showing the main components of the microcosm set-up (adapted from Fig. 1 in Edwards et al., 2003).

Table 1
Temperature and light regimes used during the Ria Formosa and Sagres microcosm experiments

Location	Mean light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Daylength (hours L:D)	Temperature ($^{\circ}\text{C}$)
Ria Formosa	126	15:9	22.90 ± 2.1
Sagres	103	13:11	19.22 ± 3.4

Errors are SE of the mean.

After 24 h, nitrate was added to the reservoirs and reactors. Nitrate additions were intended to increase the ambient concentration by approximately 12 μM , with actual concentrations determined by analysis (Table 2). Since the aim of the experiment was to determine the yield of chlorophyll from DIN under nitrogen limiting conditions, other nutrients, vitamins and trace elements were added to the reactors and reservoirs in concentrations that were present in excess to the known requirements for phytoplankton growth (Brzezinski, 1985). The proportions of phosphate, silicate, vitamins and trace elements were the same as those in Guillard's medium with approximately 18 μM of silicate and 1.8 μM of phosphate added to each reservoir and reactor (Table 2). The resulting molar ratios in diluent after enrichment with nutrients were (Si:N:P) \sim 10:7:1. The N:P was substantially less than the Redfield 16:1 ensuring that phosphate would not limit phytoplankton growth, whilst the Si:N of 1.43 was almost twice the 0.75 the ratio that Brzezinski (1985) estimated was necessary to provide adequate silicate for diatom growth. After enrichment, the experiment was run for 7 days.

2.3. Sampling strategy and analytical methods

Samples were collected daily from the reactors for the analysis of chlorophyll, dissolved inorganic nutrients,

Table 2
DIN, silicate and phosphate concentrations measured at the sampling site and in the diluent before and after enrichment

Location	Average concentration (μM)		
	Diluent		
	DIN	SiO_2	PO_4^{-3}
	At collection site		
Ria Formosa	7.16	9.45	0.53
Sagres	1.52	1.34	0.22
	Pre-enrichment ^a		
Ria Formosa	3.59	9.35	0.46
Sagres	0.57	1.04	0.20
	Post-enrichment (30 min)		
Ria Formosa	16.50	30.67	4.30
Sagres	12.98	16.95	2.09

^a Pre-enrichment nutrient values were measured in the reactors just prior to enrichment as no samples were taken from the reservoirs prior to enrichment. There is an assumption that at the start of the experiment nutrient levels in the reservoir and reactor were similar.

particulate nitrogen and carbon, and microplankton, including bacteria. Concentrations of nutrients, particulates and pigments were determined by comparison with standards. Chlorophyll values were estimated using the spectrophotometric techniques and equations of Lorenzen (1967) which distinguishes pheopigments from chlorophyll.

A Skalar autoanalyser was used to measure dissolved inorganic nutrients using standard chemical methods: nitrate and nitrite (Navone, 1964; Walinga et al., 1989); ammonium (Krom, 1980; Searle, 1984); phosphate (Boltz and Mellon, 1948; Walinga et al., 1989); silicate (Babulak and Gildenberg, 1973).

A Carlo Erba NA 2500 CHN analyser was used to analyse particulate nitrogen (PON) and particulate organic carbon (POC) according to the procedures of Verardo et al. (1990).

A Wild Heerbrugg inverted light microscope was used to identify and count diatoms, dinoflagellates and larger protozoa according to the procedures of Tett (1987). Fluorescence techniques were used to enumerate heterotrophic and autotrophic nanoflagellates (< 10 μm in size) and bacteria using a Leica epifluorescent microscope according to the procedures of Porter and Feig (1980). The microscope was fitted with an Attoarc adjustable light source, 02 UV excitation G-365 filter set and 09 blue excitation 450/490 filter set. For the present purpose, eukaryotic cells were categorized as either autotrophs or heterotrophs depending on whether pigments were present, and the latter were further divided into organisms greater or less than 20 μm in size. The most abundant phytoplankters, excluding nanoflagellates, were identified down to genus.

2.4. Methods used for the determination of the yield of chlorophyll from DIN

The yield of chlorophyll from DIN obtained from the microcosm studies was calculated as the ratio of cumulative increase in chlorophyll to cumulative decrease in DIN at each sampling period. The rationale for this method of yield estimation, and its appropriateness for dynamic coastal waters is fully explained in Edwards et al. (2003). The calculations take into account the daily input of nutrients into each reactor from the reservoir, and the daily loss of microplankters and nutrients from the reactor to the sump.

Regression analysis was carried out on chlorophyll and nitrate data obtained from samples taken from the Ria Formosa lagoon and Sagres. Data used for each analysis were collected during one sampling trip (Ria Formosa) or over a season (Sagres), and the synoptic data were used to represent temporal variations in chlorophyll and nitrate, with chlorophyll increasing as nitrate decreases. This method utilizes the fact that, when no other resources are limiting, there will be an

inverse relationship between chlorophyll and nitrate. Chlorophyll was regressed against nitrate to produce a measure of the yield of chlorophyll from nitrate from the negative slope of the regression.

2.5. Statistical analysis

Enrichment of the microcosms stimulated blooms during both experiments. After reaching a maximal value, chlorophyll decreased until the yield had reached a quasi-steady-state by day 6. One-way ANOVA analysis was carried out on both the data for maximal and day 6 yields to determine whether there was a significant difference in the yield of chlorophyll from DIN between the lagoon water of the Ria Formosa and oceanic water of Sagres under bloom and steady-state conditions.

3. Results

Time series diagrams of the results from the microcosm experiments are presented in Figs. 3–8. In these Figures, day 0 is the day on which water was collected and the microcosms were initiated, and day 1 is when nutrient enrichment took place. Each variable is shown as the mean value of the data from the replicated

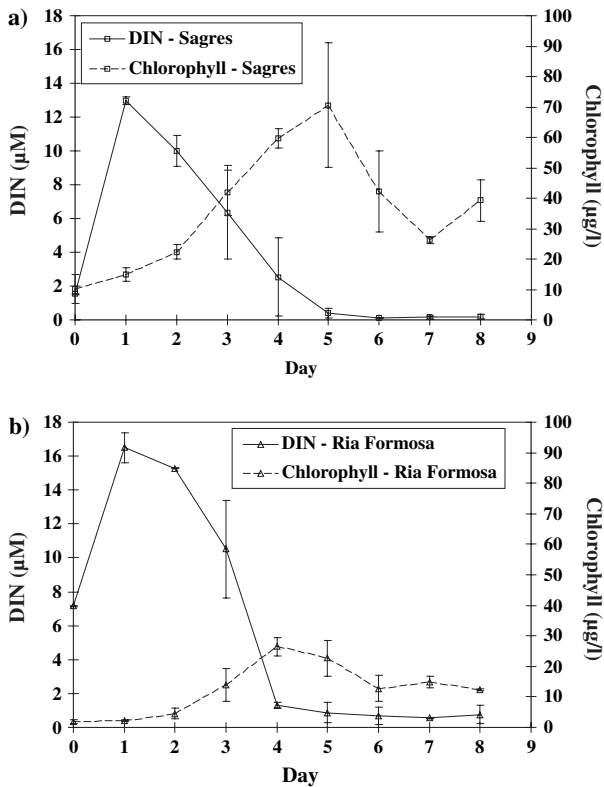


Fig. 3. A time series of the change in dissolved inorganic nitrogen (DIN) and Chlorophyll during: (a) the Sagres microcosm experiment; and (b) the Ria Formosa microcosm experiment.

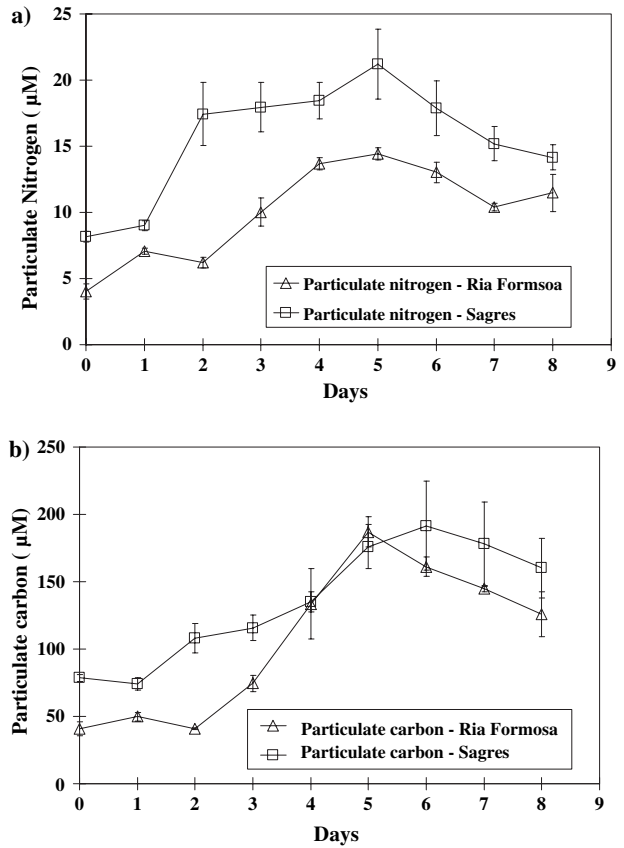


Fig. 4. A time series showing the change in particulate concentrations during the course of the microcosm experiments: (a) particulate nitrogen; and (b) particulate carbon.

microcosms with bars giving ± one standard error of the mean ($n=2$). Some of the data have been logarithmically transformed, for two reasons: firstly, growth of micro-organisms is exponential; secondly, because some of the variables are ratios, they have been calculated, and are plotted, logarithmically in order that error bars

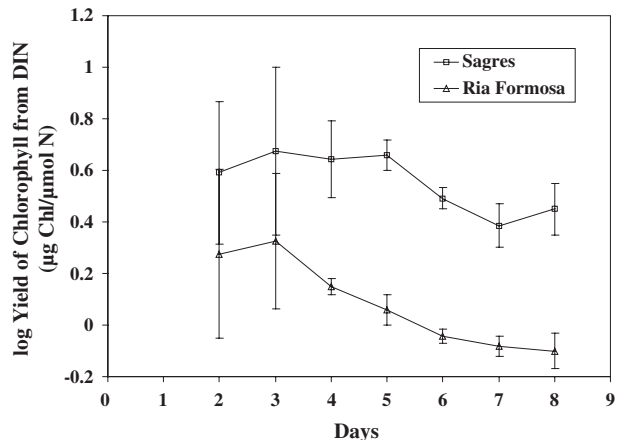


Fig. 5. A time series of the cumulative yield of chlorophyll from DIN.

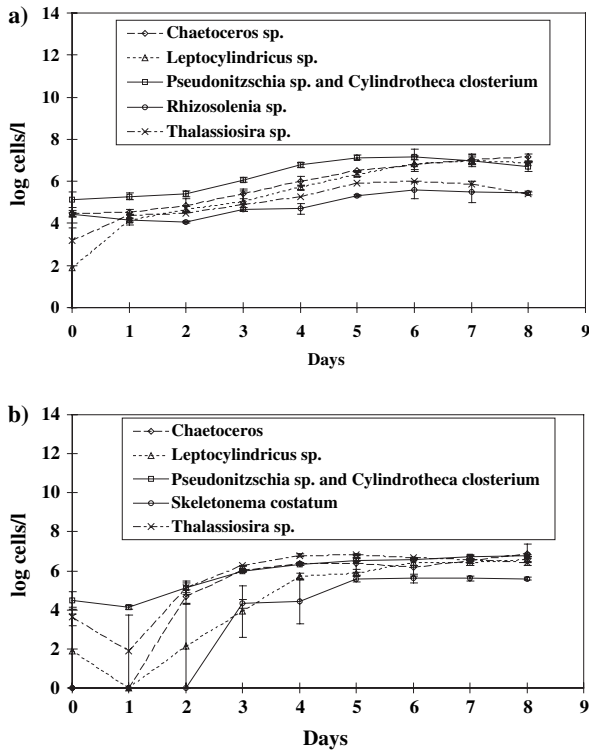


Fig. 6. A time series showing the change in diatom abundance during: (a) the Sagres microcosm experiment; and (b) the Ria Formosa microcosm experiment.

can be calculated by summing variances due to each component.

Figs. 3a,b show changes in the concentration of DIN and chlorophyll during the course of each microcosm experiment. Background levels of DIN were much higher in the Ria Formosa lagoon, $7.2 \mu\text{M}$ compared to $1.5 \mu\text{M}$ in Sagres waters, causing differences in the concentration of nitrogen available for uptake by phytoplankton at the start of each experiment. There was a decrease of DIN after nutrient enrichment in both experiments, but the initial decline, between days 1–2, was slower during the Ria Formosa experiment, compared to the Sagres experiment. After day 2, DIN decreased faster in the Ria Formosa microcosms with concentrations as low as $1.3 \mu\text{M}$ by day 4. In the Sagres experiment, the decline in DIN was more constant, and low levels of $0.4 \mu\text{M}$ did not occur until day 5.

Chlorophyll concentrations began to rise after enrichment on day 1, reaching a maximum of $26.5 \mu\text{g l}^{-1}$ on day 4 of the Ria Formosa experiment, and $70.6 \mu\text{g l}^{-1}$ on day 5 of the Sagres experiment. Both chlorophyll maximas corresponded to low levels of DIN in the microcosms. Thereafter, chlorophyll proceeded to decline until day 6 when it subsequently fluctuated between 12.4 and $14.9 \mu\text{g l}^{-1}$ in the Ria Formosa experiment, and 26.3 – $42.2 \mu\text{g l}^{-1}$ in the Sagres experiment. Throughout the time series, concentrations of chlorophyll measured in the Ria Formosa experiment

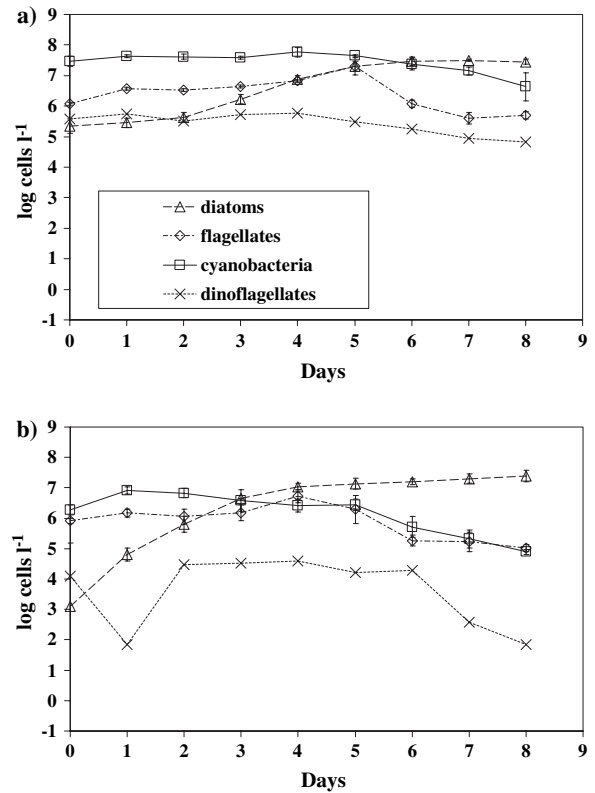


Fig. 7. A time series showing the change in abundance of the four main groups of phytoplankters present during: (a) the Sagres microcosm experiment; and (b) the Ria Formosa microcosm experiment.

were always significantly lower than in the Sagres experiment.

Fig. 4a shows changes in particulate nitrogen (PN) during the course of the two microcosm experiments. Background concentrations of PN were half as high in the Ria Formosa lagoon compared to those measured in the Sagres waters. PN increased after enrichment during both microcosm experiments. PN was slower to increase in the Ria Formosa experiment, and levels actually fell between days 1–2. The increase in PN after day 2 was relatively constant compared to the Sagres experiment, and a maximum PN of $14.4 \mu\text{M}$ was attained on day 5, after which levels fell. There was a large gain of $8.4 \mu\text{M}$ of PN between days 1–2 during the Sagres experiment when concentrations then remained relatively constant until reaching a peak of $21.2 \mu\text{M}$ on day 5, after which levels declined throughout the time series. PN levels in the Ria Formosa experiment were always significantly lower than during the Sagres experiment.

Fig. 4b shows the change in particulate carbon (PC) over time. Background concentrations of PC were half as high in the Ria Formosa lagoon compared to the Sagres waters. PC increased after enrichment during both microcosm experiments. PC measured during days 0–3 was significantly lower in the Ria Formosa compared to the Sagres experiment. Concentrations of

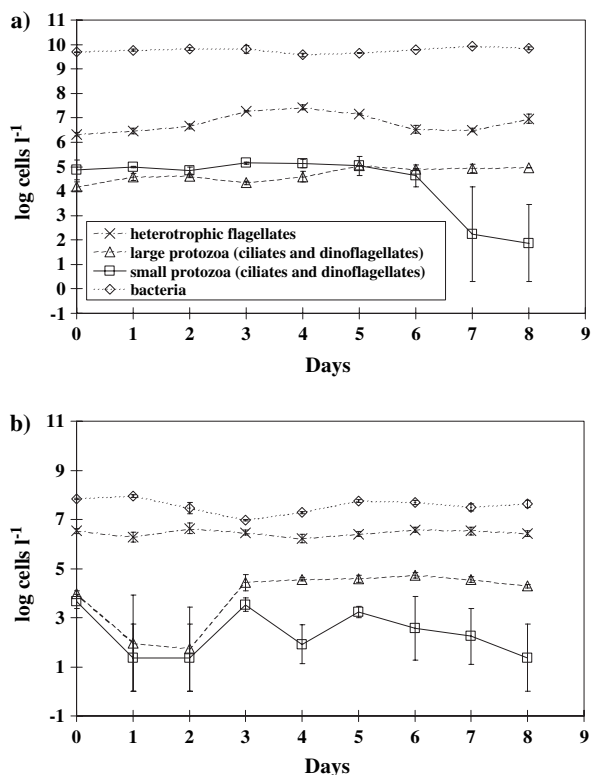


Fig. 8. A time series showing the change in abundance of protozoa present during: (a) the Sagres microcosm experiment; and (b) the Ria Formosa microcosm experiment.

PC actually declined between days 1–2 in the Ria Formosa experiment, after which levels rose quickly reaching a maximum of 187.0 μM by day 5, thereafter PC decreased until reaching 125.8 μM by day 8. PC levels rose after day 1 in the Sagres experiment until reaching a maximum of 191.6 μM by day 6, after which PC declined slightly, reaching 160.1 μM by day 8.

Fig. 5 shows the time series of cumulative yield of chlorophyll from DIN for the Ria Formosa and the Sagres microcosm experiments. The error bars for days 2 and 3 in both experiments are due to differences in the timing of DIN uptake and chlorophyll synthesis between replicated microcosms. The highest yield was obtained on day 3 during both experiments with a Ria Formosa and Sagres maximum yield of 2.1 $\mu\text{g chl} (\mu\text{mol N})^{-1}$ and 4.7 $\mu\text{g chl} (\mu\text{mol N})^{-1}$, respectively. After day 3, there was a steady decline in yield until day 6 in the Ria Formosa experiment. Thereafter there was no significant difference in yield, and the microcosms were assumed to have reached a quasi-steady state with a yield of 0.9 $\mu\text{g chl} (\mu\text{mol N})^{-1}$ by day 6. There was no significant difference in yield from days 2–5 during the Sagres experiment. There was a decrease between days 5–6, after which there was no significant difference in yield, and the microcosms were assumed to have reached a quasi-steady-state with a yield of 3.1 $\mu\text{g chl} (\mu\text{mol N})^{-1}$ by day 6. The yield was always higher during the

Sagres experiment, although this was only significant from day 4 onwards.

Figs. 6a,b show time series of the dominant diatom species present during the two microcosm experiments, including *Pseudonitzschia* sp., *Cylindrotheca closterium*, *Thalassiosira* sp., *Chaetoceros* sp. and *Leptocylindricus* sp. The centric diatom *Thalassiosira* sp. increased to a maximum of 6.64×10^6 cells l^{-1} by day 5 in the Ria Formosa experiment (Fig. 6b), and they were the most abundant species until day 6 when pennate diatoms numbers rose to similar levels. The most numerous diatoms observed during the Sagres experiment were the pennates *Pseudonitzschia* sp. and *Cylindrotheca closterium* which reached a maximum of 1.49×10^7 cells l^{-1} by day 6. During the Ria Formosa experiment *Skeletonema costatum* was an important component of the phytoplankton after day 3 (Fig. 6b), but was only present in low numbers during the Sagres experiment. *Rhizosolenia* sp. was abundant during the Sagres experiment (Fig. 6a), but was only observed in low numbers during the Ria Formosa experiment.

In Figs. 7a,b, phytoplankton have been grouped into diatoms, dinoflagellates, flagellates and cyanobacteria, to show changes in the abundance of these groups of phytoplankton during the microcosm experiments. There is no significant difference in diatom numbers between the Ria Formosa and Sagres experiments, except between days 0–1 when numbers were lower in the Ria Formosa experiment. Diatom abundance increased throughout both experiments reaching a maximum by day 8, although after day 5 there was no significant difference in abundance in the microcosms, and it appeared that the cells were in a stationary phase of growth. The initial numbers of total autotrophic dinoflagellates were much lower in the lagoon water of the Ria Formosa compared to the oceanic water of Sagres on day 0, with respective counts of 1.26×10^4 cells l^{-1} and 3.85×10^5 cells l^{-1} . Autotrophic dinoflagellate numbers initially fell between days 0 and 1 in the Ria Formosa experiment, but numbers then rose to 2.98×10^4 cells l^{-1} by day 2 and remained relatively constant until day 6 after which cells declined to very low numbers. In the Sagres experiment, autotrophic dinoflagellate abundance was significantly higher than in the Ria Formosa experiment, with numbers reaching a maximum of 5.91×10^5 cells l^{-1} by day 4. Autotrophic flagellate numbers rose to a maximum of 5.25×10^6 cells l^{-1} by day 4 during the Ria Formosa experiment and then declined until day 6, after which abundance did not significantly change, fluctuating between 1.02×10^5 and 1.82×10^5 cells l^{-1} . Autotrophic flagellates increased to a maximum of 2.00×10^7 cells l^{-1} on day 5 in the Sagres microcosms and then decreased to a minimum of 4.90×10^5 cells l^{-1} by day 8. Cyanobacterial abundance was always much lower throughout the Ria Formosa experiment compared to the Sagres experiment, with maximum counts of 5.89×10^7 cells l^{-1}

and 8.13×10^6 cells l^{-1} , respectively. Overall, autotrophic dinoflagellates, autotrophic flagellates and cyanobacteria were always present in greater numbers throughout the Sagres experiment.

Figs. 8a,b show time series for the abundance of bacteria, heterotrophic nanoflagellates, small protozoa and large protozoa, with small protozoa being defined as those less than 20 μm in size. Data has been pooled for ciliates and dinoflagellates. Bacterial numbers were two magnitudes lower in the Ria Formosa experiment compared to the Sagres experiment, with respective maxima of 9.16×10^7 cells l^{-1} and 7.00×10^9 cells l^{-1} . Heterotrophic flagellates did not vary much during the Ria Formosa experiment fluctuating between 1.66×10^6 and 4.37×10^6 cells l^{-1} . In the Sagres experiment, there was an increase in numbers during the middle and at the end of the time series, and a maximal abundance of 2.63×10^7 cells l^{-1} on day 4. Small heterotrophic protozoa were fewer throughout the Ria Formosa experiment compared to the Sagres experiment, with respective maximal abundances of 4.62×10^3 cells l^{-1} and 1.42×10^5 cells l^{-1} , until days 7–8 when numbers were similar. The numbers of large heterotrophic protozoa were not significantly different between the Ria Formosa and Sagres experiments, except at the beginning of the time series between days 1–2 when numbers declined and were lower in the Ria Formosa microcosms, and at the end of the experiment on days 7 and 8. Maximal numbers of large heterotrophic protozoa in the Ria Formosa and Sagres experiments were 5.25×10^4 and 1.13×10^5 cells l^{-1} , respectively. Overall there were lower numbers of protozoan grazers estimated during the Ria Formosa experiment compared to those counted during the Sagres experiment.

A one-way analysis of variance was carried out comparing yields obtained from the Ria Formosa microcosm and Sagres experiments on days 3 and 6, with day 3 yield corresponding to the maximum yield obtained during the phytoplankton bloom and day 6 yield corresponding to quasi-steady-state conditions. The results showed that there was no significant difference between day 3 yields. This was not surprising as the initial values have large error bars caused by differences in the timing of the phytoplankton response to enrichment between replicated microcosms. However, analysis of variance showed that there was a significant difference between Ria Formosa and Sagres for day 6 yields ($P=0.01$).

Regression of chlorophyll against nitrate for both the Ria Formosa lagoon and the Sagres waters showed a poor correlation. Only 1 out of 8 regressions carried out for the Ria Formosa gave a significant relationship between chlorophyll and DIN (Fig. 9), with approximately 37% of the spread of data accounted for by the trend line, and that was during the spring, with high background nitrate and low chlorophyll levels. No

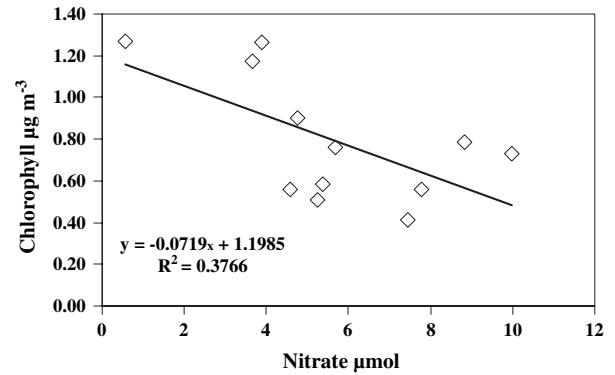


Fig. 9. The regression of chlorophyll against nitrate using data obtained from water samples collected from the Ria Formosa lagoon during March 2001.

significant correlations were found between chlorophyll and nitrate for any of the Sagres dataset.

4. Discussion

4.1. A summary of the differences between the microcosm experiments at the Ria Formosa and Sagres

The main differences observed between the microcosm experiments at the Ria Formosa and Sagres are summarised below:

1. before nutrient enrichment, the background concentration of dissolved inorganic nitrogen (DIN) is 5 μM higher in the lagoon compared to the ocean;
2. after nutrient enrichment, a shorter lag time at Sagres before the decline of dissolved inorganic nitrogen;
3. chlorophyll concentrations at Sagres that are generally double those of the Ria Formosa;
4. accumulation of particulate nitrogen (PN) is more rapid and significantly more substantial at Sagres;
5. accumulation of particulate carbon (PC) is greater at Sagres, although the rate of accumulation is more rapid in the Ria Formosa peaking at maximal value of around 190 μM , which is similar for both sites;
6. the dominant members of the diatom community are the pennate diatoms *Pseudonitzschia* sp and *Cylindrotheca closterium* at Sagres and the centric diatom *Thalassiosira* sp at the Ria Formosa, although there is little difference in overall diatom abundance at both sites;
7. autotrophic dinoflagellates, autotrophic flagellates, and cyanobacteria are all more abundant at Sagres;
8. protozoan grazers are more abundant at Sagres.

These differences are reflected in the estimates obtained for the yield of chlorophyll from DIN, where

the maximum yield and the steady-state yield at Sagres are double those for the Ria Formosa.

4.2. Comparison between the yield of chlorophyll from DIN from both the northerly and southerly coastal regions of the North East Atlantic

Table 3 compares the differences in the yield of chlorophyll from DIN estimated from microcosm experiments at the Portuguese sites with those from Scottish waters. Essentially, the mean values for both the maximum and steady-state yield at the Ria Formosa are similar to those obtained for the Lynn of Lorne during the spring. In addition, the steady-state yield of $0.9 \mu\text{g chl } (\mu\text{mol N})^{-1}$ is similar to the median value of $1.1 \mu\text{g chl } (\mu\text{mol N})^{-1}$ obtained from regression analysis of 38 data sets in north-west Scotland by Gowen et al. (1992) and of $0.8 \mu\text{g chl } (\mu\text{mol N})^{-1}$ calculated by Larsson and Kratzer (in Tett et al., 2003) from a large data set for the Himmer fjord in Sweden. In contrast, the late summer values in the Scottish waters are higher than those in the lagoon, but not as high as in the oceanic waters of Sagres.

Edwards et al. (2003) discuss the significance of yield values for chlorophyll from DIN and suggest that the steady-state yield is the most appropriate parameter for assessing the general potential for eutrophication at sites exposed to continuous nutrient enrichment. Maximum yield is more appropriate for sporadic nutrient enrichment where a rapid period of unrestricted growth may be detrimental to a sensitive site such as an aquacultural installation. On the basis of these various studies, Tett et al. (2003) have suggested a single value of $1.1 \mu\text{g chl } (\mu\text{mol N})^{-1}$ as a good approximation for use in screening models for predicting eutrophication throughout the NEA coastal waters. However, in light of microcosm studies in Portugal, this assumption must be examined more critically as the steady-state yield of $3.1 \mu\text{g chl } (\mu\text{mol N})^{-1}$ at Sagres is substantially higher than the $1.1 \mu\text{g chl } (\mu\text{mol N})^{-1}$. Indeed, taking the maximum yield into account, a value $4.7 \mu\text{g chl } (\mu\text{mol N})^{-1}$ for Sagres is still higher than the upper 95%

percentile of $4.4 \mu\text{g chl } (\mu\text{mol})^{-1}$ obtained from Scottish waters (Gowen et al., 1992). The poor correlation between chlorophyll and nitrate at Sagres also devalues the potential of this parameter for predicting eutrophication. Nonetheless, the microcosm values for the maximum and the steady-state yield at the Ria Formosa conform to those for the more northerly waters of the NEA, but again there is poor correlation between chlorophyll and nitrate data sets for the lagoon.

4.3. Possible explanations for variations in yield values between the Ria Formosa and Sagres

It is evident that further study is necessary to fully understand the large differences in yield observed between the Ria Formosa and Sagres, and it is worth exploring possible explanations for this variation for effective design of future microcosm experiments.

4.3.1. Contrasting site characteristics

Many of the differences are attributable to the very different characteristics at these two sites (see Section 1).

The pelagic microplankton in the sheltered, shallow conditions of the Ria Formosa will be affected by three principle factors:

- sediment re-suspension produced by the influence of tides, freshwater inputs and wind action (Lopes et al., 2001) can reduce light availability for phytoplankton photosynthesis (Tett et al., 1993);
- the macroalgae and microphytobenthos inhabiting the extensive areas of lagoonal sediment will compete for nutrients thereby affecting the concentrations available for the pelagic microplankton;
- the 50–70% exchange rate between the ocean and the lagoon (Newton and Icely, in press) would remove substantial numbers pelagic phytoplankton from the lagoon before they could utilize the nutrient in the lagoon.

The pelagic microplankton in the exposed oceanic conditions of Sagres will be affected more by how the general oceanographic processes that occur around Cape S. Vincente peninsular (Fiúza et al., 1982; Relvas and Barton, 2002) will influence balance of between the autotrophic and heterotrophic systems (Williams, 1998; Gasol and Duarte, 2000).

4.3.2. Effect of seasonal differences

Seasonal effects have been demonstrated in Scottish waters (Edwards, 2001) where both the maximum and the steady-state yield are higher in late summer than in spring (Table 3). These differences are due to annual cycles in daylength, light intensity, temperature, nutrient cycles, etc. However, there are also more subtle factors which could account for difference in the uptake

Table 3

Mean values of the yield of chlorophyll from DIN determined from microcosm time-series studies using microplankton collected from the Ria Formosa lagoon in April 2002, Sagres oceanic water in September 2002, and the Lynn of Lorne water in March/April 1999 and August 1999

	$\mu\text{g chl } (\mu\text{mol N})^{-1}$			
	Spring		Late Summer	
	Maximum	Steady-state	Maximum	Steady-state
Ria Formosa	2.12	0.91		
Sagres			4.72	3.10
Lynn of Lorne	1.80	0.70	3.01	1.50

The values are means obtained from the studies.

patterns of DIN observed in the microcosms at the Ria Formosa and Sagres.

For example, in April in the Ria Formosa, the background levels of nitrate are not limiting to phytoplankton growth, but due to the overcast weather conditions in combination with high water turbulence and sediment disturbance, photosynthesis could have been limited by low light. Monoculture studies on phytoplankton show that uptake sites for nitrogen are reduced under conditions of nitrate sufficiency (Riegman et al., 1990; Everest et al., 1986; Dortch et al., 1984), whilst there is an increase in the synthesis of protein-pigment complexes for improving photosynthesis under conditions of light limitation (Coleman et al., 1988; Riemann et al., 1989; Sakshaug et al., 1989). However, in the case of Sagres during September, the contrasting situation occurs where the background level of nitrogen is low, but the light levels for photosynthesis are good. Under these conditions, the phytoplankton would be adapted to utilise all available nitrogen (Riegman et al., 1990; Everest et al., 1986; Dortch et al., 1984), including recycled nitrogen in the form of ammonium. It is notable that other components of the microplankton, such as cyanobacteria can also adapt their physiology to the background levels of nutrients. Under conditions of nitrate excess, cyanobacteria will select nitrate in preference to the energetically costly process of nitrogen fixation (Zehr et al., 2000; Flores and Herrero, 1994), but under conditions of nitrate scarcity these organisms will fix nitrogen as a source for growth (Zehr et al., 2000; Sellner, 1997) and this fixation can occur at very high rates (Wasmund et al., 2001).

One other important seasonal occurrence is the coastal upwelling conditions observed around Cape S. Vicente (see Sagres in Fig. 1) between May and September, depending on the strength of the northerly and westerly winds (see references in Introduction). At the time of the Sagres microcosm experiment, the upwelling season is essentially over (Loureiro et al., in press) with low concentrations of DIN at 1.5 μM , typical of oligotrophic oceanic waters. However, during a period of upwelling in July of the same year, the DIN concentration is up to 20 μM (Loureiro et al., in press).

4.3.3. Variation in the yield of chlorophyll from DIN between different microplankton groups

The microplankton community in the Sagres experiment are very different from those observed in other microcosm experiments, including the Ria Formosa. There is an abundance of *Prorocentrum* sp., especially *P. micans* and *P. triestimum* and small autotrophic dinoflagellates that remain relatively stable for the duration of the experiment, as well as large numbers of autotrophic nanoflagellates. Yield values may differ between phytoplankton groups. Data obtained from monoculture studies have been used to determine the

yield of chlorophyll from PN for flagellates, dinoflagellates and diatoms using regression analysis (Edwards, 2001). The results indicate that autotrophic flagellates, in particular, appear to synthesise more chlorophyll from their internal PN compared to the other phytoplankton groups, with a maximum of 9 $\mu\text{g chl } (\mu\text{mol N})^{-1}$ compared to 0.90–2.25 $\mu\text{g chl } (\mu\text{mol N})^{-1}$ for diatoms. Only one of the data sets for dinoflagellates gives a significant correlation with a value of 2.00 $\mu\text{g chl } (\mu\text{mol N})^{-1}$.

In contrast to the Scottish microcosm experiments (Edwards et al., 2003), cyanobacteria are observed in large numbers at both Sagres and the Ria Formosa. The cyanobacteria at both sites have the characteristics of *Synechococcus* spp, with unicellular coccoidal cells, sometimes grouped into colonies, sometimes present as single scattered cells, with a diameter of 1–1.5 μm along the longest axis. Early studies on cyanobacteria imply that only the heterocystous forms are capable of aerobic nitrogen fixation (Bergman et al., 1997), but recent studies have shown that non-heterocystous, coccoid cyanobacteria, including *Synechococcus* spp, are also capable of aerobic nitrogen fixation (León et al., 1986; Roger, 1985; Spiller and Shanmugam, 1987; Huang and Chow, 1986; Kostyaev, 1990) There are no data sets available to calculate whether there is a significant correlation between chlorophyll and nitrogen in this group of organisms.

4.3.4. Possible influence of recycled nitrogen on the yield value

When new nitrogen in the form of nitrate is unavailable, phytoplankton depend on remineralised nitrogen in the form of ammonium. Smaller phytoplankton, such as nanoflagellates tend to fare better under these oligotrophic conditions due to their greater surface to volume ratios (Stolte et al., 1994; Lazier and Mann, 1989). Regenerated nitrogen is significantly more available throughout the Sagres experiment and this is reflected by the greater abundance of nanoflagellates compared to the Ria Formosa. Recent research into the interactions between cyanobacteria and picoplankton in the Baltic Sea, using $^{15}\text{N}_2$ as a tracer by Ohlendieck et al. (2000), show that newly fixed N_2 is transferred from cyanobacteria to picoplankton. Other studies on tropical cyanobacteria by Glibert and Bronk (1994) observe that up to 50% of newly fixed N_2 is released as dissolved organic nitrogen (DON) during active growth. DON is used by bacteria, protozoa and some phytoplankton, thus helping to sustain the microbial food web.

On the basis of the above discussion, it would appear that the microplankton from waters with low nutrients would be physiologically prepared to respond rapidly to nutrient enrichment and this is supported by the results obtained from the Sagres microcosms with the time series shown in Figs. 3a for chlorophyll, 4a for PN, and 4b for PC – all showing a rapid response after nutrient

enrichment on day 1. In contrast, the microplankton from the waters of the Ria Formosa, with much higher concentrations of nutrients, responded slowly after nutrient enrichment of the microcosms, with both PN (Fig. 4a) and PC (Fig. 4b) showing an initial decline after day 1. In comparison with the Ria Formosa, the Sagres microcosms have higher numbers of microplanktons that can either fix nitrogen (e.g. cyanobacteria in Fig. 7), or utilise recycled nitrogen (e.g. bacteria, protozoa and small autotrophic phytoplankton Figs. 7 and 8) when ambient concentrations of nutrients are low.

5. Conclusions

The yield of chlorophyll from DIN could be an important parameter for use in screening models to predict eutrophication in coastal waters (Tett et al., 2003). However, both the maximum and steady-state yield values estimated from microcosm experiments are much higher in Sagres than in the Ria Formosa. Furthermore, there is poor correlation between chlorophyll and nitrate measurements at both these sites. It is important to understand why these southerly, coastal waters appear to be different from the northerly waters as both the sheltered Ria Formosa and the exposed Sagres sites are intercalibration sites in the North East Atlantic for the CIS of the EU-WFD (see Section 1). A standard parameter for the prediction of eutrophication would be most desirable for the implementation of this directive.

One striking contrast between the Portuguese and Scottish sites is the difference between microplankton communities and, in particular, the large numbers of cyanobacteria in the Portuguese waters. Future studies could establish whether increased yields in waters with low DIN, such as Sagres, are due to selection pressures for both nitrogen fixing cyanobacteria and a microplankton community that utilises DIN efficiently. It would also be interesting to obtain yield values from microplankton communities that have developed during periods of seasonal upwelling when there is a surplus of DIN. Future microcosm studies on the Ria Formosa, should take into account the rapid exchange of pelagic communities between the lagoon and the ocean also examine, the competition for DIN from macroalgae and microphytobenthos.

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