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Enrichment experiments and primary production at Sagres (SW Portugal)

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ABSTRACT

Water was collected from the Sagres station (SW Portugal) in September 2002, at a site adjacent to the upwelling centre of Cabo São Vicente, during relaxation of upwelling conditions. Surface and depth samples were enriched with inorganic nutrients in order to evaluate their relative influence on the microalgal assemblage. Small-scale, short-term bioassays involved separate *in vitro* additions of nitrogen and phosphorus. Enrichments with nitrogen led to a general increase of primary production, suggesting nitrogen as the primary potential nutrient limiting microalgal growth during this period, as well as altering the relative microplanktonic composition in favour of diatoms.

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1. Introduction

Coastal upwelling areas are amongst the most productive habitats of marine ecosystems (Smith and Hollibaugh, 1993). Phytoplankton production is primarily enhanced by nitrate supply (new production, *sensu* Dugdale and Goering, 1967) to the euphotic zone during the active stages of upwelling (Barber and Smith, 1981) but, as this supply decreases during the relaxation of upwelling, this production becomes mainly supported by regenerated forms of nitrogen such as ammonium and urea (Codispoti, 1983; Bode et al., 1997). Phytoplankton must be able to adapt to the rapidly changing physical and chemical conditions in these areas (Hutchinson, 1961; Cloern and Dufford, 2005), which is achieved by storing nitrogen (N) compounds in intracellular pools during periods of N excess, when luxury uptake exceeds growth rates, allowing for the continuation of growth after depletion of external nutrients (Dortch et al., 1984). Consequently, primary production and biomass accumulation (as chlorophyll *a*) is often controlled by N availability in marine waters (Smith, 1984; Howarth, 1988), including upwelling areas (Packard et al., 1978; Kudela and Dugdale, 2000).

A variety of approaches have been used to assess which nutrient potentially regulates the algal community including: nutrient ratios (Redfield et al., 1963; Boynton et al., 1982), nutrient concentrations

and budgets (Smith, 1984; Fisher et al., 1988; Philippart et al., 2000; Prego, 2002), nutrient addition bioassays (Healey, 1979; Granéli, 1987; Schülter, 1998; Ault et al., 2000), and physiological indicators (Healey and Hendzel, 1980; Sala et al., 2001). However, the accurate determination of nutrient concentrations is not reliable, especially in oligotrophic environments where nutrients are near detection limits. Therefore, experimental studies have been used to observe the response of natural communities of phytoplankton to the addition of nutrients. There are limitations to these type of experiments caused by confinement in micro- or mesocosms, where both the organisms and their habitat are effectively separated from the nutrient supply of the open sea. Nonetheless, enrichment bioassays have been useful for estimating potential nutrient limitation and gaining insight to nutrient conditions (Sanders et al., 1987; Maestrini et al., 1984; Fong et al., 1993; Balode et al., 1998; Loureiro et al., 2005b).

The Sagres area, off the south west coast of the Iberian peninsular (Fig. 1) is affected by seasonal upwelling induced by northerly winds from May to September (Wooster et al., 1976; Fiúza, 1983; Sousa and Bricaud, 1992). The upwelled, nutrient-rich waters may flow counter-clockwise around the Cabo de São Vicente (Fig. 1), and flood the southern coastal shelf of Portugal (Fiúza et al., 1982; Relvas and Barton, 2002; Loureiro et al., 2005a). Furthermore, this shelf is additionally affected by local westerly winds, that may also induce upwelling episodes, and influenced by the presence of a warm coastal counter current (CCC) originating in the Gulf of Cádiz, that may progress from the southern to the western platform of the peninsular, depending on

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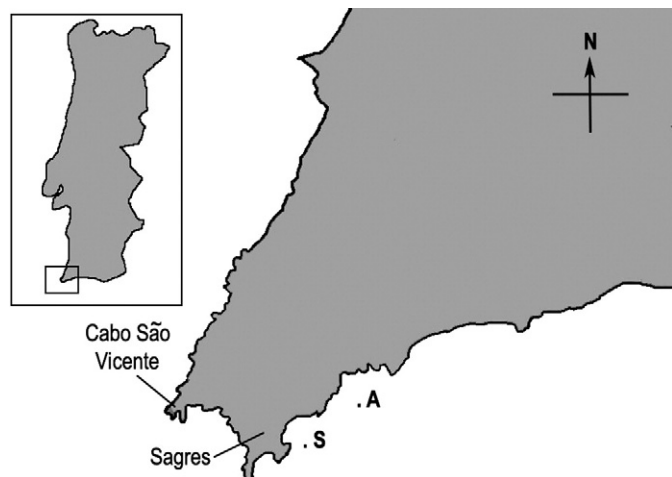


Fig. 1. Location of the sampling station (S), and the oyster aquaculture (A) at Sagres SW Portugal.

pressure gradients and southeasterly wind forcing (Relvas and Barton, 2002).

Observations on the natural communities of phytoplankton at Sagres show that the microalgal assemblage peaks during active upwelling conditions and is dominated by fast growing diatoms, by then whereas during periods of relaxation flagellate forms are more prominent (Moita, 2001; Loureiro et al. 2005a). Production rates (max: $61 \mu\text{M O}_2 \text{ d}^{-1}$), chlorophyll *a* concentrations (max: 6 mg m^{-3}), and nitrate inputs (max: $19 \mu\text{M}$) are comparable to other productive coastal upwelling regions (Loureiro et al., 2005a). Relaxation of upwelling is associated with low nutrient conditions (Loureiro et al., 2005a, and references therein). In this study, we simulate an upwelling event by adding inorganic nutrients to water sampled from different depths of the euphotic zone during a relaxation period. This small-scale bioassay aims to observe short-term changes in the microalgal community and in the nutrient concentrations after enrichment with inorganic N and phosphorus (P).

2. Materials and methods

2.1. Study area

Under the terminology of the European Union (EU) Water Framework Directive (WFD), the Sagres area (SW Portugal) is classified as a mesotidal, moderately exposed Atlantic coastal type (Bettencourt et al., 2004), characterized by a narrow continental shelf. It is one of the intercalibration sites for the North East Atlantic used for the Common Implementation Strategy (CIS) of the WFD. The sampling station (Fig. 1) is located between the upwelling centre off Cabo S. Vicente and an offshore installation for bivalve aquaculture ($37^\circ 00' 63'' \text{ N}$, $8^\circ 55' 62'' \text{ W}$). The hinterland of Sagres has an area of 179.76 km^2 , of which 107.8 km^2 is part of the “Sudoeste Alentejano e Costa Vicentina” National Park. The small population of 7000 inhabitants produces low levels of sewage and agricultural run off, with minimal anthropogenic impact on the surrounding ocean, particularly as the fluvial input is irregular and restricted to small torrential streams (Peliz and Fiúza, 1999). However, natural eutrophication processes are induced by high concentrations of nutrients supplied by the seasonal upwelling of deep, cold oceanic water from spring to late summer. The resulting increase in biological productivity contributes to a commercially valuable bivalve aquaculture and fishery at Sagres. This productivity also induces nevertheless episodic toxic blooms that result in periodic restrictions on bivalve sales (Moita, 1993; Sampayo et al., 1997; Moita et al., 1998). Additional economic activities include harvesting of stalk barnacles and tourism.

2.2. Sampling and analysis

Water samples were collected during the morning of the 29th September 2002 with a Niskin flask, from the surface and from depths of 2.4 m, 4.8 m, and 8.1 m, representing light attenuation within the euphotic zone of 50%, 25% and 10%, respectively. The euphotic depth was estimated with a Secchi disc. The water was not sampled at the depth where light intensity falls to 1% of that at the surface, due to the shallowness of the sampling station ($20 \pm 3 \text{ m}$ due to tidal fluctuations). Samples were filtered through a $200 \mu\text{m}$ mesh size net to select for the microplankton community and to remove the larger grazing organisms and particles. Sea surface temperature (SST; $^\circ\text{C}$) was recorded with a Tinytalk PT 100 logger attached to a “long-line” for oyster culture. The temperature and salinity profiles were recorded with a Seacat SBE 19 CTD. Wind direction and magnitude were obtained from the National Meteorological Office station at Sagres. The Ekman transport of surface water was estimated according to Bakun's (1973) method, and used as a coastal upwelling index:

$$q_{x,y} = \frac{\tau_{x,y}}{f\rho_w} = \frac{\rho_a C_D |V| V_{x,y}}{f\rho_w}$$

where $\tau_{x,y}$ is the wind stress vector, ρ_a is the air density (1.22 kg m^{-3}), C_D is an empirical dimensionless drag coefficient (1.14×10^{-3} , see Large and Pond, 1982), $V_{x,y}$ is the wind speed vector on the sea surface, with magnitude $|V|$, f is the Coriolis parameter ($8.78 \times 10^{-5} \text{ s}^{-1}$ for Sagres), and ρ_w is the density of seawater ($\sim 1025 \text{ kg m}^{-3}$).

Samples for nutrient determination were frozen at -20°C . Ammonium, nitrate and phosphate were determined by the methods described in Grasshoff et al. (1983) with modifications described in Newton (1995). Seawater was filtered through Whatman GF/F filters for the determination of chlorophyll *a* (chl *a*) concentration. The filters were stored at -20°C and chlorophyll was subsequently extracted in acetone, following the JGOFS (1994) protocol. Concentrations were measured with a calibrated Jasco FP-777 spectrofluorometer (Strickland and Parsons, 1972). Dissolved oxygen concentration was estimated by the Winkler method, following modifications by Strickland and Parsons (1972). Concentrations are expressed as $\mu\text{M O}_2$. Oxygen saturation (%) was calculated using the standard equations for the solubility of oxygen in seawater (Aminot and Chaussepied, 1983).

2.3. Microscopic identification

Samples for microscopy were preserved with acidified Lugol's solution and subsequently settled in sediment chambers for identification (Tomas, 1997) and enumeration (Utermöhl, 1958), using a Zeiss Axiovert 25 inverted microscope. Smaller cells were observed at $400\times$ magnification up to a total of 100 optical fields, whereas less abundant and larger organisms were observed at $100\times$ magnification over the entire chamber. Identification was generally performed to genus level. The assemblage was divided into four major taxonomic groups: diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae), ciliates (Ciliata) and nanoflagellates. Wherever possible the nanoflagellates

Table 1

Initial parameters for the nutrient enrichment bioassays performed at Sagres. Chl *a* = chlorophyll *a*; Diat. = diatoms; Dino. = dinoflagellates; Nano. = nanoflagellates; Cilia. = ciliates; – data not available

Sample depth (m)	Temperature ($^\circ\text{C}$)	Salinity	Chl <i>a</i> (mg m^{-3})	%O ₂	Diat. Dino. Nano. Cilia.			
					($\times 10^3 \text{ cell l}^{-1}$)			
Surface	18.8	36.3	2.53	103	139	216	98	10
2.4	18.8	36.5	2.88	102	203	152	61	9
4.8	19.0	–	2.93	102	142	163	58	7
8.1	19.8	–	1.23	103	110	153	82	4

Table 2
Initial nutrient concentrations for the nutrient enrichment bioassays performed at Sagres

Treatment	Sample depth (m)	NH ₄ ⁺	NO ₃ ⁻	PO ₄ ³⁻	N:P
		μM			
Control	Surface	0.29	2.26	0.28	9.3
	2.4	0.31	0.11	0.29	1.7
	4.8	0.08	*	0.29	0.5
	8.1	0.43	*	0.33	1.5
+N	Surface	0.29	7.35	0.28	27.3
	2.4	0.31	4.46	0.29	16.8
	4.8	0.08	4.36	0.29	15.4
	8.1	0.43	4.29	0.33	14.3
+P	Surface	0.29	2.26	2.05	1.63
	2.4	0.31	0.11	1.87	0.26
	4.8	0.08	*	1.89	0.10
	8.1	0.43	*	1.94	0.26

* Below detection limits; N:P represents the Redfield Ratio where N=Nitrate + Nitrite (data not shown)+Ammonium.

were separated into two classes: Cryptophyceae and Dictyochophyceae; where this was not possible, they were included in the group of unidentified nanoflagellates.

2.4. Net production

Water samples for net primary production determination were siphoned into ~300 cm³ Winkler bottles with silicon tubing. Five replicates were fixed immediately for the measurement of initial dissolved oxygen concentrations. Five additional bottles were clamped onto white circular fibreglass disks and suspended *in situ* along a “long-line” over 24 h, at the depth from which they were originally sampled. Triplicates were then fixed and analysed for dissolved oxygen concentration. Net community production (NCP) was calculated as the difference in oxygen concentration between the incubated and the initial samples. The samples from the two remaining bottles were used for nutrient determination and microscopic identification.

2.5. Enrichment experiment

The initial chemical and biological parameters were obtained by sub-sampling each control bottle at the respective sampling depths, assuming that these data were representative of the initial conditions in the remaining bottles (Tables 1 and 2). The oxygen bottles (~300 cm³) were filled with pre-filtered water samples from the surface, 2.4 m,

4.8 m, and 8.1 m (euphotic zone) and were enriched with a single-pulse of nitrogen (as NaNO₃) or phosphorus (as Na₂HPO₄). Nitrate was selected as the inorganic nitrogen form for these experiments, as it is the dominant form of nitrogen under upwelling conditions (Loureiro et al., 2005a), thereby simulating experimentally the effect of upwelling on phytoplankton (Granéli, 1987). The concentrations of added nutrients for enrichment were based upon the mean values found at the sampling location during previous years (Loureiro et al., 2005a), to provide final concentrations for a Redfield ratio of N:P > 16 for N additions, and N:P < 16 for P additions. Water samples without additions of nutrient were used as controls. One disc for each of these three conditions was incubated *in situ* for 24 h at each depth sampled from the euphotic zone. The technique for estimating the net production from these 12 discs is described above.

2.6. Statistics

No true replication of this experiment was performed. Nevertheless, similar bioassays conducted further along the coast from Sagres, in the Ria Formosa coastal lagoon, had a high degree of reproducibility (Loureiro et al., 2005b). For statistical purposes, each treated Winkler bottle was considered as an enrichment replicate. Each sample was assumed to be representative of its sampling location and depth (Clarke and Warwick, 2001). STATISTICA© software was used for non-parametric tests. Kruskal–Wallis was used to evaluate the statistical differences between the control samples from different depths, whereas Mann–Whitney *U* test evaluated the results from the enrichment experiments.

3. Results

3.1. Wind and hydrographic conditions

Fig. 2 shows the fluctuations in upwelling conditions along both the south and west coasts of Portugal during September 2002. At the beginning of the month, the wind conditions were favourable for upwelling on both coasts ($q_x > 0$ and $q_y < 0$ in Fig. 2a), but as the northerly winds decreased towards the middle of the month, a period of downwelling was observed along the west coast ($q_y > 0$). Favourable conditions for upwelling continued along the south coast, with positive eastward Ekman transport (q_x), associated with high wind-speed values (Fig. 2b). The relatively low sea surface temperature (SST) recorded between the 14th and the 24th September (15 °C–16 °C in Fig. 2c) probably reflected these upwelling conditions. However, there

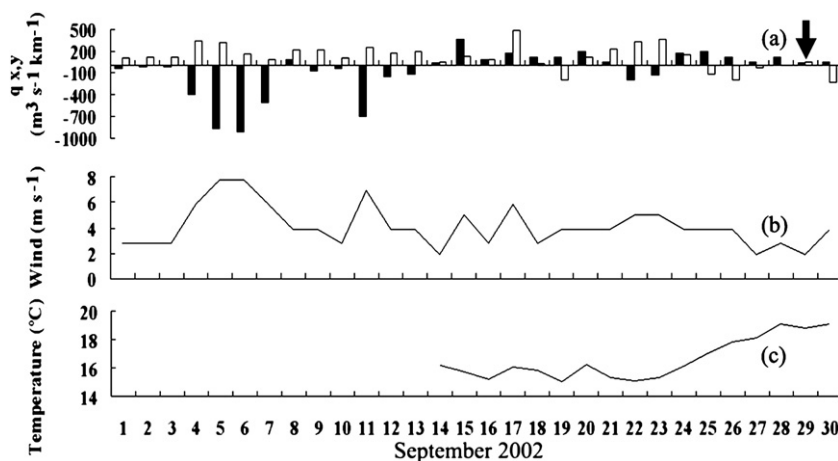


Fig. 2. Eastward (q_x , white bar) and northward (q_y , black bar) Ekman transport (a) during September 2002 at Sagres; positive q_x values indicates favourable upwelling conditions for the south coast, whereas negative q_y represents favourable upwelling conditions for the west coast; black arrow indicates the sampling date. Temporal evolution of wind speed (b) and temperature (c).

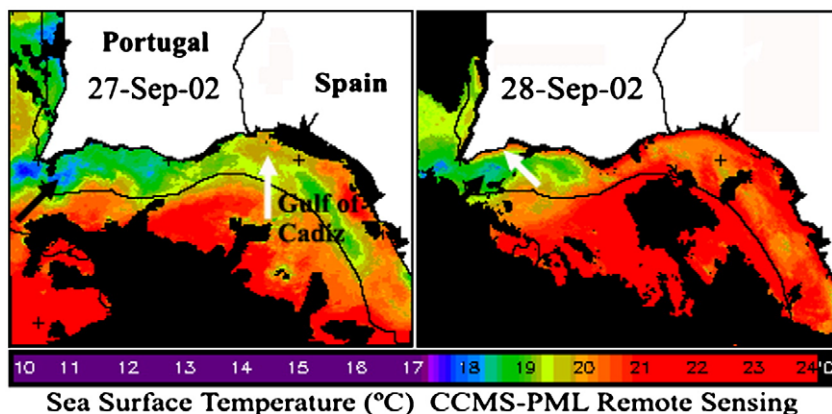


Fig. 3. Sea Surface Temperature (SST) satellite images (NOAA/AVHRR) processed at the Plymouth Marine Lab, UK. Dates are indicated in the images. Black arrows show colder waters, whereas white arrows indicate the influence of the warm water band coming from the Gulf of Cádiz, to the south continental shelf of Portugal.

was a steady rise in temperature up to 19 °C for the rest of the month. At the time of sampling, on the 29th September, the temperature was 18.8 °C, with a low wind speed $\approx 2 \text{ m s}^{-1}$, and $q_x, q_y \approx 0$, all features of a period of relaxation in upwelling conditions. Indeed, satellite images (Fig. 3) from the two days previous to the sampling date (quality of image poor for the 29th) show the retraction of colder waters from the south coast, and the progression of the warm coastal counter current (CCC) from the Gulf of Cádiz to the Portuguese south continental shelf. The higher salinities which tend to be associated with the warm CCC (Sánchez and Relvas, 2003) were recorded at the sampling station with values > 36.2 (Table 1).

3.2. Initial chemical and biological parameters

Table 1 shows the range of the physical, chemical and biological variables of the initial water samples used for the nutrient bioassays.

Table 3
List of identified taxa from initial samples

Code	Taxa	Abundance $10^3 \text{ cell. l}^{-1}$	Code	Taxa	Abundance $10^3 \text{ cell. l}^{-1}$
	Bacillariophyceae (Diatoms)		Din	<i>Dinophysis</i> spp.	0.4 ± 0.6
	Centrales		Gon	<i>Gonyaulax</i> spp.	12.2 ± 19.4
Cha	<i>Chaetoceros</i> spp.	4.1 ± 3.2	Gym	<i>Gymnodinium</i> spp.	16.4 ± 13.6
Dac	<i>Dactyliosolen</i> spp.	2.9 ± 4.1	GmGr	<i>Gymnodinium</i> + <i>Gyrodinium</i> spp.	92.7 ± 19.2
Gui	<i>Guinardia</i> spp.	0.9 ± 0.4	Gyr	<i>Gyrodinium</i> spp.	6.8 ± 4.6
GuiS	<i>Guinardia striata</i>	1.0 ± 1.3	Kat	<i>Katodinium</i> spp.	6.3 ± 8.3
Hem	<i>Hemiaulus</i> spp.	0.4 ± 0.3	ProC	<i>Prorocentrum</i> spp.	29.6 ± 16.9
Lep	<i>Leptocylindrus</i> spp.	20.1 ± 15.6	ProP	<i>Protoperidinium</i> spp.	1.6 ± 1.0
Rhi	<i>Rhizosolenia</i> spp.	3.5 ± 3.0	Scr	<i>Scrippsiella</i> spp.	9.2 ± 3.3
Ske	<i>Skeletonema</i> spp.	6.5 ± 0.9	DNs	Small $< 20 \mu\text{m}$ Unidentified	2.5 ± 1.1
DCs	Small $< 20 \mu\text{m}$ Unidentified	3.1 ± 2.6	Hap	Haptorida	1.0 ± 0.8
Nav	<i>Navicula</i> spp.	1.5 ± 0.9	Oli	Oligotrichida	4.7 ± 2.0
Nit	<i>Nitzschia</i> spp.	91.7 ± 45.0	Tin	Tintinnina	0.9 ± 0.3
PSN	<i>Pseudo-nitzschia</i> spp.	9.9 ± 3.8	Cil	Unidentified	1.0 ± 0.6
DPb	Big ($> 20 \mu\text{m}$) Unidentified	8.5 ± 1.0	Cry	Cryptomonadales	41.2 ± 6.5
Amp	<i>Amphidinium</i> spp.	1.8 ± 0.7		Dictyochophyceae	
Cer	<i>Ceratium</i> spp.	0.4 ± 0.1	Dic	Dictyochaceae (Silicoflagellates)	2.1 ± 0.9
			Nan	Nanoflagellates Unidentified	28.9 ± 18.2

Values refer to mean abundances (\pm standard deviations) from surface and depth samples. Bold refers to taxonomic class; Bacillariophyceae is further divided into taxonomical order.

Some of these parameters, like oxygen, remained constant throughout the depth profile, whilst others were more variable. For instance, chl *a* increased gradually in concentration between the surface and 4.8 m before dropping sharply to half the concentration of the surface at 8.1 m. Temperature and salinity tended to increase with depth, although there were no readings for salinity at 4.8 and 8.1 m. The initial concentrations of the nutrients in these samples are shown in Table 2. The P concentration was relatively constant with depth, whilst N was more irregular with: the highest and the lowest concentrations of ammonium occurring between 8.1 and 4.8, respectively; and the highest concentration of nitrate at the surface declining rapidly until it was undetectable at 4.8 and 8.1 m. The Redfield ratio for N and P was consistently below 16.

Table 1 also shows that dinoflagellates were the most numerous group throughout the euphotic zone, except at 2.4 m depth, where diatoms were more abundant (Table 1). The nanoflagellates and ciliates had a more regular distribution throughout the water column than the other two groups. The taxa identified from the initial samples are shown in Table 3. In descending order of abundance, the most numerous taxa were *Gymnodinium*+*Gyrodinium* spp., *Nitzschia* spp. Cryptomonads, *Prorocentrum* spp., unidentified nanoflagellates and *Leptocylindrus* spp.

3.3. Treatment effects

Fig. 4 compares the changes in NCP after nutrient enrichment. The median coefficient of variation of the initial and incubated oxygen

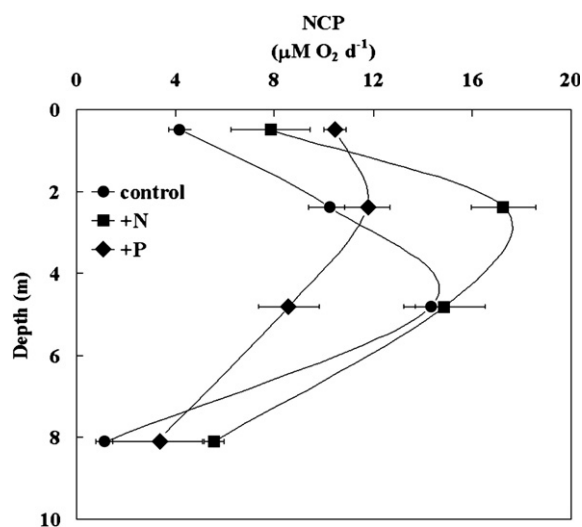


Fig. 4. Profiles of NCP (Net Community Production); bars represent standard errors.

samples was 0.22% ($n=20$) and 0.62% ($n=36$), respectively. The mean of the standard errors of NCP measurements was $0.94 \mu\text{M O}_2 \text{ d}^{-1}$. On the basis of the Mann–Whitney U test ($p<0.05$), N enrichment significantly increased production rates at every depth, except at 4.8 m, whereas P enrichment only significantly stimulated surface samples.

Fig. 5 shows the changes in nutrient concentrations between the initial and the final sample. Ammonium was consistently lower in the final samples, except at 4.8 m in the control and the N assay, and at 8.1 m in the P assay. Nitrate was generally higher in the final samples of the N assay and also in the control down to 4.8 m; it was undetectable in the control at 8.1 m. In the case of the P assay, the final nitrate values were very low or undetectable. The variation in phosphate concentration was minimal throughout the experiment. The Redfield ratio showed consistently lower values ($\text{N:P}<16$) for the

control and the P assay. However, with nitrate enrichments, there was a positive increase in the Redfield ratio ($\text{N:P}>16$) between the initial and the final samples, due to the accumulation of N relative to a constant value for P.

Fig. 6 shows the relative changes in the abundance of the main groups of microplankton throughout the experiments. No significant differences were observed between the initial and the control samples. However, diatoms showed a significant positive response ($p<0.05$, Kruskal–Wallis followed by post-hoc Mann–Whitney U test) to N enrichment, particularly, in the taxa *Nitzschia* spp., *Leptocylindrus* spp. and *Pseudo-Nitzschia* spp. (data not shown). These increases were associated with a decrease in the contribution of dinoflagellates to the total microplanktonic composition. The relative distribution of the microplankton groups was similar between the controls and P-assay.

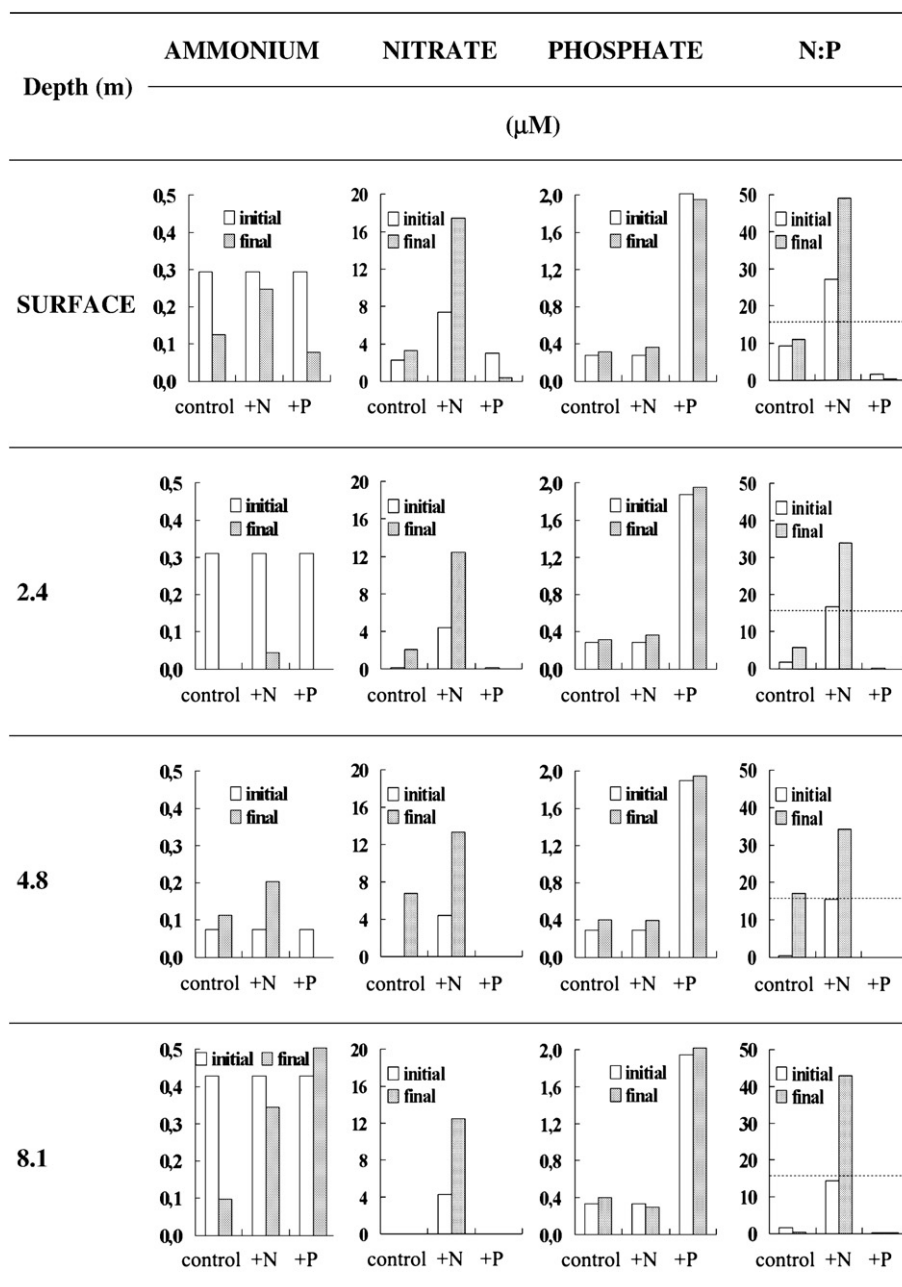


Fig. 5. Nutrient evolution during the enrichment experiments at different depths; where bars are not visible, nutrient concentration was below detection limit; dashed line on N:P figure represents the Redfield ratio ($\text{N:P}=16$).

4. Discussion

4.1. Initial conditions

The physical data taken during September as well as the satellite images confirm the influence of the cold upwelled waters on the sampling site at Sagres, originating from the circulation patterns along both the west and south coast (e.g. Fiúza, 1984; Loureiro et al., 2005a) as well as the influence of the warmer waters from the CCC (Relvas and Barton, 2002).

The low values of nitrate suggest that there has been active uptake of this nutrient by the algal community, likely associated with a bloom event previous to the sampling campaign. Well-developed blooms often reduce nitrate to undetectable levels in shallow upwelling regions (Horner et al., 1997). The chl *a* and NCP maxima at 4.8 m is associated with minimal ammonium concentrations, which probably reflects a primary production based on regenerated forms of N (Dugdale and Goering, 1967) at this depth. Biological subsurface maxima are generally linked to nutrient gradients established in stratified water conditions (Cullen, 1982). The maintenance of these maxima depends upon factors such as enhanced growth rates and buoyancy regulation of the algal assemblage (Innes and Walker, 1991). The moderate values attained for NCP (Moncoiffé et al., 2000), despite the low values of nutrients, suggests that transient pools of nutrients, usually formed during nutrient-rich periods, could have partially

supported this additional production. The formation of such pools is expected to be maximal in upwelling regions where nutrients are delivered in pulses (Dortch et al., 1984). Values of chl *a* and NCP from this study are similar to those observed previously at Sagres during periods of upwelling relaxation (Loureiro et al., 2005a).

The succession from diatoms to dinoflagellates is typically observed during relaxation periods when turbulence decreases (Margalef, 1978). The extension of this succession will depend upon the duration of the quiescent stage (Smayda, 1980). The presence in this study of a mixed microplanktonic population have been previously observed during weak upwelling/transition conditions at the south west of Portugal (Moita, 2001). These assemblages are dominated by small diatoms, which are generally associated with active upwelling conditions, and small flagellates, which are indicators of coastal stratification. This overlap of communities occurs due to the gradients of vertical mixing and nutrient availability (Reynolds, 1996), especially visible in upwelling regions (Estrada and Blasco, 1985).

There are a number of taxa observed in this study with the potential to produce HABs, with some of the most numerous including the dinoflagellates *Gymnodinium* spp. and *Prorocentrum* spp. that have been included in both habitat-types I and II (Smayda, 2000), normally associated with nutrient-enriched, nearshore waters. The acclimation abilities of these taxa allow them to be retained in the surface water during periods of relaxation/downwelling. For example, *Gymnodinium* spp. can form long chains, that increase their swimming

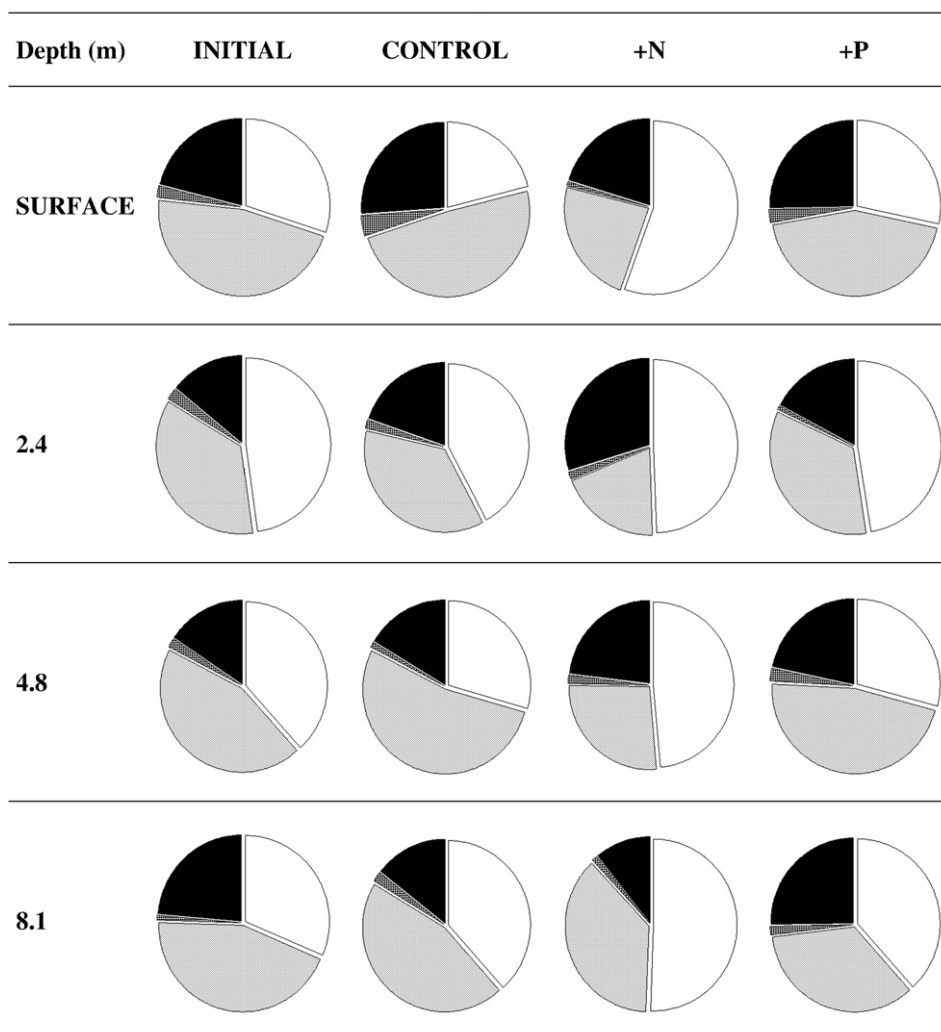


Fig. 6. Microplankton distribution at different depths and treatments; white represents diatoms, grey represents dinoflagellates, grey with squares represents ciliates, and black represents nanoflagellates.

ability, allowing them to migrate to nutrient-rich layers (Fraga et al., 1989). On the other hand, *Prorocentrum* spp. can increase both the size and number of photosynthetic units (Harding, 1988), increasing their ability to survive at lower irradiances, and thereby, at greater depths.

4.2. Methodological constraints

The constraints associated with this type of experimental work are discussed in detail in Loureiro et al. (2005b). Results from enrichment experiments indicated which nutrient had the potential to limit the growth of phytoplankton assemblages *in situ*, in the absence of other limiting factors (Elser and Kimmel, 1986; Ault et al., 2000). The outcomes of this study will be discussed on this basis.

4.3. Enrichment response

The stimulating effect of N on the oxygen photosynthetic rates in this study, suggests N as the most likely nutrient limiting algal production at Sagres (Elser and Kimmel, 1986). Similar conclusions have been made by Edwards et al. (2005) from a continuous culture microcosm experiment with samples taken from the same location a week before the current experiment and, also, from an earlier temporal study made by Loureiro et al. (2005a).

Other upwelling systems are also suggested to be primarily regulated by N availability (Kokkinakis and Wheller, 1987; Kudela and Dugdale, 2000). The accumulation of nitrate has been previously observed in N-enriched samples from other upwelling regions (MacIsaac et al., 1985). Nitrate uptake can decrease when ammonium exceeds a threshold of approximately 0.5 μM (Eppley et al., 1969). There is a preference for the uptake of ammonium as N can only be assimilated when nitrate has been reduced to nitrite and ammonium; this reduction would consequently imply a high energetic cost with consequences for the algal photosynthetic performance (Smith et al., 1992). The exudation of newly synthesized organic N-rich compounds by phytoplankton is sometimes observed a few hours after nitrogen addition (Dortch and Postel, 1989). Amongst other mechanisms, dissolved organic nitrogen (DON) release may also result from apoptosis (autolysis) (Agusti et al., 1998) and viral lysis (Weinbauer, 2004 and references therein). Accumulation of nitrate could then be explained by phenomena such as mineralisation of DON by bacteria (Søndergaard et al., 2004), together with preferential uptake of ammonium by the microalgae community. However, the mechanism for the complex interactions between ammonium and nitrate uptake in multi-specific assemblages of algae has yet to be studied fully (Dortch et al., 1991).

The stimulation of diatoms by N addition concurs with observations from other microcosm experiments at Sagres (Edwards et al., 2005), as well as in the Ria Formosa Lagoon, further to the east of the Algarve coast (Edwards et al., 2005; Loureiro et al., 2005b), and other coastal areas (Schülter, 1998). Diatoms are known to have high growth and uptake rates, which are important mechanisms to attain a higher biomass than other algae (Kudela and Dugdale, 2000). Some algal species are better uptake specialists, whereas others are better storage specialists. Nutrient additions in this study are likely to favour species adapted to the uptake of nutrient pulses (Granéli et al., 1999).

5. Conclusions

The fertilisation of surface waters in Sagres is mainly regulated by natural eutrophication events (upwelling). The results suggest that nitrogen is the most likely nutrient regulating microalgal growth at the time of the experiment, in the absence of other limiting factors. The mechanisms of nutrient dynamics are nevertheless still to be fully understood. Reduced forms of nitrogen, namely ammonium, seem to play an important role in the maintenance of the observed production rates, probably because of the elevated energetic cost associated with

nitrate assimilation. A significant change in the relative composition of the microplankton assemblage is induced by nitrogen addition, which stimulates the growth of diatoms.

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