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Effects of nutrient enrichments on primary production in the Ria Formosa coastal lagoon (Southern Portugal)

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Abstract

Small-scale, short-term enrichment experiments were conducted in the Ria Formosa coastal lagoon (southern Portugal), to assess the effects of nutrient availability on primary productivity, biomass (as chlorophyll *a*), and algal composition. Samples were collected from natural communities at three different sites in the western lagoon: Barra, oceanic inlet; Ramalhete, adjacent to a urban waste water treatment plant; and Ponte, an upstream channel used for recreation and bivalve farming. These samples were enriched separately with nitrogen and phosphorous during the extreme neap tides of the summer solstice at both high (HW) and low water (LW). The experiment was repeated during the autumnal equinox to test for seasonality, and during the following summer solstice to test for replication. The addition of nitrogen consistently stimulated the productivity and biomass during summer experiments at the two sites within the lagoon, identifying N as the most likely primary "potentially limiting nutrient" in the western part of the lagoon for this period. No stimulation of biomass and productivity occurred in September at the same two sites indicating the importance of other factors such as light, sedimentation or grazing pressure, as controlling the pelagic community. However, these outcomes were reversed at the oceanic inlet (Barra-HW) where there was no stimulation by nitrogen during the summer months, but there was in September, suggesting that there is a different nutritional requirement for the coastal community in comparison with the lagoon community. In samples where productivity was stimulated, diatoms were the group most sensitive to enrichment.

Introduction

The Water Framework Directive (WFD; Directive 2000/60/EC) and a programme for Integrated Coastal Zone Management (ICZM) have been developed by the European Commission (EC) to reduce the deterioration of water quality in coastal zones and their adjacent watersheds. In particular, areas of restricted water exchange (Tett et al., 2003), such as coastal lagoons, are vulnerable to human (anthropogenic) pressures produced by urbanization, industry, agriculture, fish and shellfish culture, dredging, sewage discharges, and recreational activities (Vallejo, 1982).

One of the potential consequences of these pressures is eutrophication, which is defined in the Directives (91/271/EEC) of the European Union (EU) as an enrichment of waters by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae that may induce an undesirable disturbance to the balance of the living organisms and water quality. Degradation of water quality will depend upon the assimilative capacity of a system (Bricker et al.,

1999) that is modulated by the relative changes in algal production, consumption and decomposition (Malone et al., 1996), as well as by the degree of advective transport in open systems. The consequences of eutrophication can only be minimised by identifying the specific nutrient that is limiting to algal growth and primary productivity. In the case of freshwater systems, it is phosphorus (Schindler, 1974), whist in marine systems it is generally nitrogen (e.g., Tyrrell & Law, 1997). However, a seasonal switch from phosphorus (P) to nitrogen (N) limitation is observed in coastal transition areas, such as estuaries (Malone et al., 1996) and lagoons (Fong et al., 1993), where there is a salinity gradient (Caraco et al., 1987; Ault et al., 2000), and a seasonal release of P from sediments (Conley, 2000). Additionally, as a result of their intricate topography, coastal lagoons can have more complex response to enrichment than other coastal regions (Taylor et al., 1999).

The Ria Formosa is a mesotidal lagoon which extends 55 km along the southern coast of Portugal (Fig. 1). It is of sufficient ecological importance that it has been recognized by as a National Park, as well as a Ramsar and Natura 2000 site. Anthropogenic nutrients are supplied to the lagoon mainly from treated and untreated domestic effluents, and non-point source agricultural run-off (Ferreira et al., 2003; Newton et al., 2003). However, the coastal waters (Falcão & Vale, 2003) and the lagoon sediments (Falcão & Vale, 1998; Asmus et al., 2000) are also important nutrient sources. Previous observations of dissolved inorganic N to P ratios suggest that P is the limiting nutrient (Falcão & Vale, 1990). This lagoon is subject to remarkable human pressures, particularly in summer due to the increase in tourism and recreational activities. Nonetheless, it is a productive system supporting substantial aquaculture and fishing activity, as well as providing an important habitat and nursery for commercially important fish and shellfish species (Gamito, 1997a). In recent years, there have been reports of some environmental problems including: fish mortalities occurring in enclosed areas and aquaculture ponds, related apparently to algal blooms and consequent oxygen depletion (Gamito, 1997a); intoxication of humans by bivalve biotoxins from clams and oysters in the eastern lagoon (Vale & Sampayo, 1999); and the development during the winter months of extensive algal mats that smother the clam beds (Reis & Sprung, 1995).

Sprung (1994) suggests that primary production within the Ria Formosa is dominated by macrophytes and macroalgae, whilst planktonic production is mainly imported into the lagoon by tidal exchange. However, tidal flushing is not uniform throughout the lagoon and in the upstream locations it may not be well mixed, thereby increasing the residence time of the water (Newton & Mudge, 2003; Tett et al., 2003). At these upstream locations, the contribution of resident

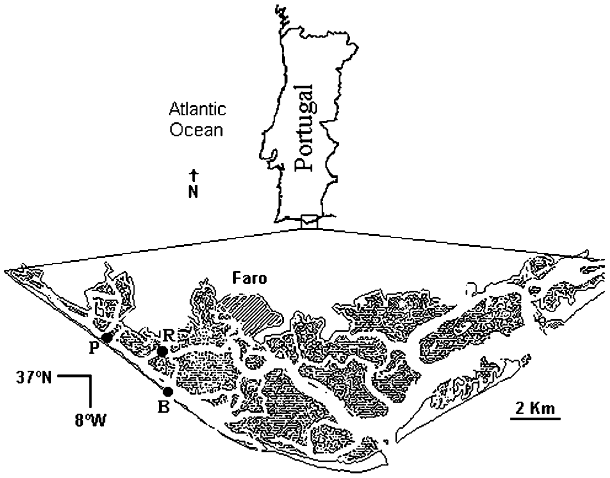

Figure 1. Ria Formosa lagoon and sampling stations: P = Ponte, upstream channel; R = Ramalhete, adjacent to a domestic sewage outlet: B = Barra, oceanic inlet.

plankton to primary production and nutrient competition is likely to be more important than in those regions of the lagoon with high daily rates of exchange. Several studies of the benthic community in the Ria Formosa have been reported (e.g., Austen et al., 1989; Sprung, 1994; Gamito, 1997b), whereas information on the phytoplankton population and processes is still scarce (Marques et al., 1996; Falcão & Vale, 2003). Integrated management is only possible with a complete understanding of all the influences on this ecosystem (Reis & Sprung, 1995).

The use of nutrient ratios as a tool for predicting the limitation to algal production by nutrients (Smith, 1984) is impaired both by difficulties in measuring accurately nutrients and their availability to phytoplankton (Robertson, 1999), and by the affect on the N to P ratio of fluctuations of nutrient loadings in the water column (Fong et al., 1993). The experimental approach measures the response of phytoplankton to nutrient additions, to show directly how nutrient limitation may affect algal growth rate, net primary production and net biomass accumulation, or net ecosystem production (Smith, 1984; Granéli et al., 1990).

The present study adopts Tyrrell's definition (Tyrrell, 1999) of "proximate limiting nutrient" (PLN), which represents the local limiting nutrient according to the Liebig's law (Liebig, 1840). To achieve this goal, short-term and small-scale experiments were performed to assess the effects of N and P enrichments on water collected from three different stations in the western Ria Formosa lagoon. This kind of experiment lies between level I and II of the hierarchical test systems (Hecky & Kilham, 1988), and allows us to infer the PLN.

Materials and methods

Study site

The Ria Formosa is a shallow lagoon with a mean depth of about 3.5 m (Fig. 1). Tidal range is 2.8 m at spring tide and approximately 1.3 m at neap tide. It is permanently connected to the ocean by six inlets, with no significant input of fresh water (Falcão & Vale, 1990). Salinity ranges from 13 to 36.5 all year round, with lower values after winter rainfall, whilst the water temperature varies from 12 to 27 °C (Newton & Icely, 2002; Newton & Mudge, 2003).

Three representative sampling stations were selected from the western lagoon: Barra, an oceanic inlet; Ramalhete, an inner channel close to a urban waste water treatment (UWWT) plant; and Ponte, an upstream channel used for bivalve culture and subjected to tourism pressure.

Sampling and analysis

Sampling were performed during the extreme neap tide of the summer solstice (June 2001), at both high (HW) and low water (LW). The sampling was repeated at the extreme spring tide of the autumnal equinox (September 2001) to test for seasonal variability, and at the following summer solstice (July 2002) to test for replication. All water samples were collected at 0.5 m depth and filtered through a 200 μm mesh to conserve the microplankton and exclude larger particles and zooplankton. Water samples were frozen at −20 °C for subsequent determinations. Nutrients were analysed according to Grasshoff et al. (1983). Samples for chlorophyll *a* (chl *a*) were filtered through Whatman GF/F filters and frozen. Chl *a* was analysed with a fluorimetric technique (JGOFS, 1994). Dissolved oxygen concentration was estimated with the iodometric method using a microtitration technique (Strickland & Parsons, 1972; Bryan et al., 1976). Temperature and salinity data were recorded with a WTM-LF197-S conductivity meter. Total daily solar irradiance (KJ m^{-2}) was supplied by the Portuguese Instituto de Meteorologia (IM) at Faro meteorological station (07° 58′ W, 37° 01′ N, 8 m).

Microscopic identification

Microplankton samples were preserved with Lugol's solution and subsequently identified (Tomas, 1997) and counted (Utermöhl, 1958), using an inverted Zeiss Axiovert 25 microscope. Identification was generally carried out to the genus level. When this was not possible, organisms were classified into wider taxonomic groups. Four groups were distinguished: diatoms, dinoflagellates, ciliates and nanoflagellates. Wherever possible the small nanoflagellates (0–20 μm) were separated into three classes: Cryptophyceae, Dictyochophyceae and Euglenophyceae (Tomas, 1997).

Net production

Natural water samples for production experiments were filtered and siphoned into ~300 cm^3 Winkler bottles within 15 min after collection. Initial concentrations of dissolved oxygen were determined in triplicate, whilst a further five bottles were incubated for 24 h on a disc suspended 0.5 m below the surface from a flotation system anchored in the lagoon. Three of the bottles were fixed for the determination of oxygen, and the contents of the other two were treated for the subsequent analysis of chl *a* and nutrients. Net Community Production (NCP), expressed as mg $O_2\ l^{-1}\ d^{-1}$ (±SE), was estimated by the difference between the means of the incubated and the initial samples. The mean of the NCP standard error was: 0.04 mg $O_2\ l^{-1}\ d^{-1}$ (number of samples = 53).

Enrichment experiment

Nitrogen (as NH_4NO_3) was added as a single pulse to a set of 5 Winkler bottles filled with the sampled water, whilst phosphorus (as KH_2PO_4) was added to a different set of five samples (Table 1). The N to P ratios for these experiments are reported on Table 2. Incubation procedures were similar to those described for the production estimates. N and P additions were based on average concentrations observed during the previous 2 years, for the same locations and seasons. Nutrient additions were maintained at sufficiently small concentrations so as not to modify the assimilation mechanisms or to induce toxic effects (Dufour & Berland, 1999). Identification of microplankton to evaluate community changes, was only carried out in September 2001 and July 2002, when production was stimulated by nitrogen. The potential PLN was identified by the effect of nutrient additions on net primary production and biomass.

Statistical analysis

For statistical purpose, each sample was assumed to be representative of its sampling site and time (Clarke & Warwick, 2001). Statistical analysis for the microplankton data was done with PRIMER© software. Bray–Curtis similarity matrices of square root transformed data were used to produce multi-dimensional scaling (MDS) ordination for abundance. SIMPER, a similarity percentage routine (Clarke and Warwick, 2001), was used to evaluate the contribution of taxa between distinct groupings. STATISTICA© 6 was used for the statistical analysis of the rest of the experimental data, including a non-parametric Mann–Whitney *U*-test that evaluate the effects of experimental stimulation with nutrients. These effects were tested on the combined results of nitrogen, or phosphorus, stimulated samples for each season (Granéli, 1987). On the basis of the results obtained for these experiments, samples were assumed to be stimulated when either production or biomass was at least 1.5 times greater than the controls.

Results

Biotic and abiotic factors

Initial parameters of the natural seawater used in the experiment are shown in Table 3. Lagoonal water was warmer than the oceanic water (Barra HW station), with a maximum temperature (25 °C) attained at the upstream station (Ponte LW). Salinity differences were more pronounced during the summer, with higher salinity values

Table 1. Nutrient concentrations added to natural seawater for the enrichment experiments

Date/Station	N (μM)			P (μM)		
	Barra	Ramalhete	Ponte	Barra	Ramalhete	Ponte
June (2001)	2.1	4.4	6.3	0.5	0.3	0.2
September (2001)	1.6	1.8	10.5	0.3	6.6	0.2
July (2002)	10.9	13.7	8.6	7.1	7.4	7.5

Table 2. Initial N to P ratios in control and enriched samples

Date	Station/Tide	Initial N:P		
		Control	+N	+P
June 01	B/LW	11	23	6
	B/HW	28	41	23
	R/LW	20	26	14
	R/HW	15	24	11
	P/LW	6	11	4
	P/HW	22	39	18
Sep 01	B/LW	25	31	12
	B/HW	9	12	7
	R/LW	7	11	0.6
	R/HW	10	17	0.4
	P/LW	28	50	20
	P/HW	27	77	14
July 02	B/LW	7	34	0.3
	B/HW	3	33	0.1
	R/LW	6	21	0.5
	R/HW	10	29	0.7
	P/LW	7	13	0.7
	P/HW	4	9	0.5

N= nitrate + nitrite + ammonia.

(max: 37.0) inside the lagoon due to evaporation. Chl *a* was generally higher at LW (max: 5.1 μg l^{-1} at Ponte LW during September), indicating that the lagoon could export phytoplankton to the adjacent coastal waters (Falcão & Vale, 2003). Summer chl *a* concentrations were higher in 2001 compared to 2002, with a similar trend observed for ammonium concentrations. The Spearman correlation showed a significant positive relationship between chl *a* and ammonium (0.65, $p < 0.05$), and chl *a* and salinity (0.91, $p < 0.05$). Ammonium concentrations were generally higher at LW during June 2001 (max: 4.9 μM at Ramalhete LW) compared to HW. However, during July 2002 there was a similar concentration over the entire tidal regime at all stations. In common with ammonium, nitrate was generally higher during the summer of 2001. At Barra HW (oceanic inlet) nitrate values (9.7 μM) were higher than in lagoonal waters during June 2001, which contrasted with July 2002 where the value (0.7 μM) was lower than those observed within the lagoon: these contrasting values may be related to the upwelling dynamics of adjacent coastal water. Phosphate showed only minor changes during the survey (0.5 ± 0.2 μM). In the control conditions, although the N to P ratio was occasionally higher than the Redfield ratio (Table 2), N:P < 16 was the most frequent condition, which suggests N as the limiting nutrient.

Microplankton abundance (Fig. 2) correlated with chl *a* values (Spearman correlation between chl *a* and total microplankton numbers: 0.50, $p < 0.05$) with the greatest numbers (1244×10^3 cells l^{-1}) occurring during June 2001. Ramalhete HW (sewage outlet) was the station with the greatest concentration ($808 \pm 395 \times 10^3$ cells l^{-1}), whereas Barra HW (oceanic inlet) was the station with lowest concentration ($257 \pm 50 \times 10^3$ cells l^{-1}). Diatoms dominated the summer community, whereas September 2001 had a mixed population. Different seasonal assemblages were confirmed by MDS ordination (Fig. 3). However, the Barra HW showed a distinct summer assemblage from the other sites characterized by small (<20 μm) unidentified dinoflagellates, *Gyrodinium* spp. and *Pseudo-nitzschia* spp. (SIMPER analysis).

NCP (Fig. 4) had a similar seasonal evolution as biomass, with higher rates in 2001 (range: 0.8–1.6 mgO$_2$ l^{-1} d^{-1}), compared with summer 2002 (range: 0.2–0.9 mgO$_2$ l^{-1} d^{-1}). No significant difference was observed in tidal regime or spatial variation.

Response to enrichment: production and Chl a

During the summer experiments, primary production was stimulated by N enrichment at most stations (Fig. 4a and c), except for Barra HW. The greatest response was up to eight times the control and occurred in both summer samples at Ponte LW, the upstream lagoon channel. In September, production was only stimulated by N enrichment at Barra HW (Fig. 4b). No data was available for control and N addition of Ponte HW for this period.

Biomass, measured as chl *a*, followed trends similar to those observed for production rates (Fig. 4c, d and e). A significant correlation between chl *a* and production was found (0.78, $p < 0.05$). In September, after 24 h incubation, a biomass increase was detected in the control, but differences between controls and the nutrient enriched cultures were not significant, except for Barra HW (Fig. 4b). The Mann–Whitney *U*-test

Table 3. Initial seawater conditions

Date	Station/Tide	Temperature (°C)	Salinity	Chl *a* (μg l^{-1})	NH_4^+ (μM)	NO_3^- (μM)	PO_4^{3-} (μM)
June 01	B/LW	23	36.5	2.1	4.6	0.4	0.5
	B/HW	20	36.0	1.9	1.1	9.7	0.4
	R/LW	23	36.7	3.0	4.9	9.2	0.7
	R/HW	22	37.0	3.1	2.1	9.3	0.8
	P/LW	25	36.7	3.5	3.7	3.8	1.3
	P/HW	22	36.5	1.7	3.2	7.2	0.5
Mean ± SD	June	22 ± 2	36.6 ± 0.3	2.5 ± 0.7	3.3 ± 1.5	6.6 ± 3.7	0.7 ± 0.3
Mean ± SD	LW	23 ± 1	36.6 ± 0.1	2.8 ± 0.7	4.4 ± 0.6	4.5 ± 4.4	0.8 ± 0.4
Mean ± SD	HW	21 ± 1	36.5 ± 0.5	2.2 ± 0.8	2.1 ± 1.1	8.7 ± 1.4	0.6 ± 0.2
Sep 01	B/LW	21	36.6	2.4	1.1	5.3	0.3
	B/HW	20	36.6	3.0	0.6	6.3	0.7
	R/LW	22	36.9	3.0	2.3	1.5	0.6
	R/HW	22	36.8	4.2	1.8	0.8	0.3
	P/LW	22	37.0	5.1	3.0	9.0	0.4
	P/HW	21	36.6	3.1	0.5	5.2	0.2
Mean ± SD	Sep	21 ± 1	36.8 ± 0.2	3.5 ± 1.0	1.6 ± 1.0	4.7 ± 3.1	0.4 ± 0.2
Mean ± SD	LW	22 ± 0.2	36.8 ± 0.2	3.5 ± 1.4	2.2 ± 1.0	5.3 ± 3.8	0.4 ± 0.1
Mean ± SD	HW	21 ± 1	36.7 ± 0.1	3.4 ± 0.7	1.0 ± 0.8	4.1 ± 2.9	0.4 ± 0.3
July 02	B/LW	23	36.0	1.4	0.3	2.1	0.3
	B/HW	18	35.5	0.1	0.3	0.7	0.3
	R/LW	22	36.0	0.7	0.2	3.6	0.7
	R/HW	23	36.2	0.8	0.3	4.3	0.5
	P/LW	24	36.4	1.3	0.3	5.0	0.7
	P/HW	20	35.8	0.4	0.3	2.7	0.8
Mean ± SD	July	22 ± 2	36.0 ± 0.3	0.8 ± 0.5	0.3 ± 0.0	3.1 ± 1.6	0.6 ± 0.2
Mean ± SD	LW	23 ± 1	36.1 ± 0.2	1.1 ± 0.4	0.3 ± 0.0	3.6 ± 1.5	0.6 ± 0.2
Mean ± SD	HW	20 ± 2	35.8 ± 0.4	0.4 ± 0.3	0.3 ± 0.0	2.6 ± 1.8	0.5 ± 0.2

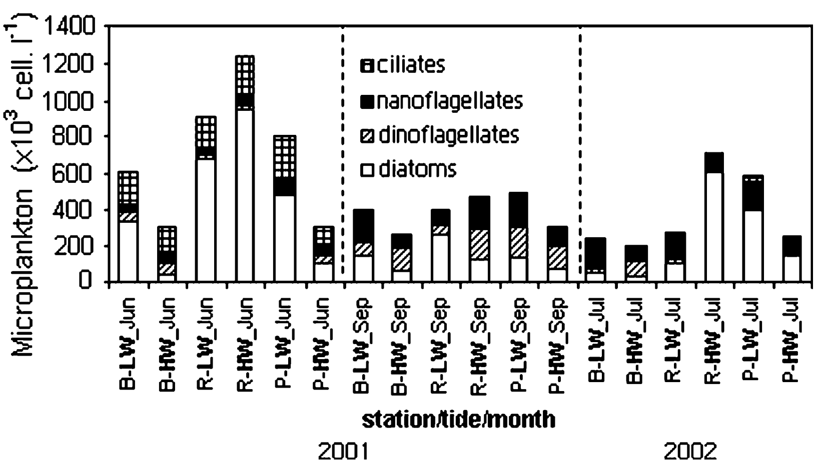

Figure 2. Microplankton abundance (×10^3 cells. l^{-1}) of natural seawater from the Ria Formosa stations: B = Barra; R = Ramalhete; P = Ponte; LW = Low Water (in bold); HW = High Water (in bold).

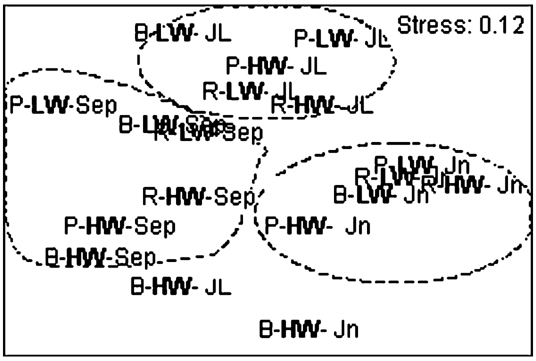

Figure 3. Multi-Dimensional Scaling of the microplankton analysis from the Bray–Curtis similarity matrix of the square root transformed abundance data: B = Barra; R = Ramalhete; P = Ponte; LW = Low water (in bold); HW = High water (in bold); Jn = June2001; Sep = September 2001; JL = July 2002.

confirmed the significant ($p < 0.05$) response of microplankton production (Fig. 5) and biomass (Fig. 6) to the N enrichment, during summer 2001 and 2002.

P additions had an effect on production only in June 2001 at Ramalhete HW and at Ponte HW (Fig. 4a). Overall, the Mann–Whitney *U*-test revealed that P stimulation was not significant in the other cases. P treatment produced a significant (Mann–Whitney *U*-test, $p < 0.05$) increase in algal biomass in June and September 2001 at high tide at all sampling stations (Fig. 4d).

Nutrient dynamics

In the incubated samples, although there was a general decrease of phosphate, P was never depleted and attained a minimum of 0.15 μM (Figs. 7, 8 and 9). During July 2002 there was an accumulation of phosphate in all N enriched samples after 24 h, suggesting a preferential uptake for nitrogen.

Nitrate was generally depleted in P treated samples. In June 2001, in some N enriched cultures (Ramalhete HW, Ponte LW, and Ponte HW), the nitrate decrease was slower and nitrate was not completely exhausted. Accordingly, ammonium concentrations declined with a similar trend, but it was only depleted in July 2002 at Ponte HW, after P enrichment. Unlike phosphate and nitrate, which accumulated in N treatments during July 2002, there was a depletion of ammonium during this period, probably associated with a preferential uptake for this nutrient owing to its low concentration in surface waters.

Microplankton composition changes

Microscopic identification of microplankton in N stimulated samples in September 2001 and July 2002 showed evidence of some common trends (Fig. 10a, b). Nitrogen enrichment promoted a general increase of centric diatoms (mainly *Thalassiosira* spp., *Chaetoceros* spp., *Skeletonema* spp. and *Leptocylindrus* spp.) and also pennate diatoms (mainly *Pseudo-Nitzschia* spp. and *Nitzschia* spp.). The highest increase of diatoms was observed during July 2002 at Ponte LW (Fig. 10a), where the final assemblage was dominated by *Thalassiosira* spp. and *Chaetoceros* spp. However, at Ponte HW there was no observable community change, with differences occurring between controls and treatments because of relative abundances and not species composition. Dinoflagellates were less abundant in controls compared to N treatments. Nanoflagellates followed a similar trend, except in September at Barra HW, where cryptomonads were responsible for an increase of the nanoflagellate group.

Discussion

Methodological constraints

Outcomes of small-scale enrichment experiments should be extrapolated with caution to natural ecosystems (Howarth, 1988). *In vitro* incubations have been criticized because of the potential "bottle effects". Artifacts can be introduced due to exclusion of natural factors such as sedimentation, advection, grazing pressure, isolation from nutrient sources (e.g., sediments and atmospheric deposition), and reduction of other biogeochemical processes like nitrogen fixation (e.g., Smith, 1984; Hecky & Kilham, 1988; Oviatt et al., 1995; Ault et al., 2000). This decoupling from controlling processes may not allow feedback mechanisms present in the natural assemblage to operate, which can influence N and P availability (Granéli et al., 1990; Malone et al., 1996).

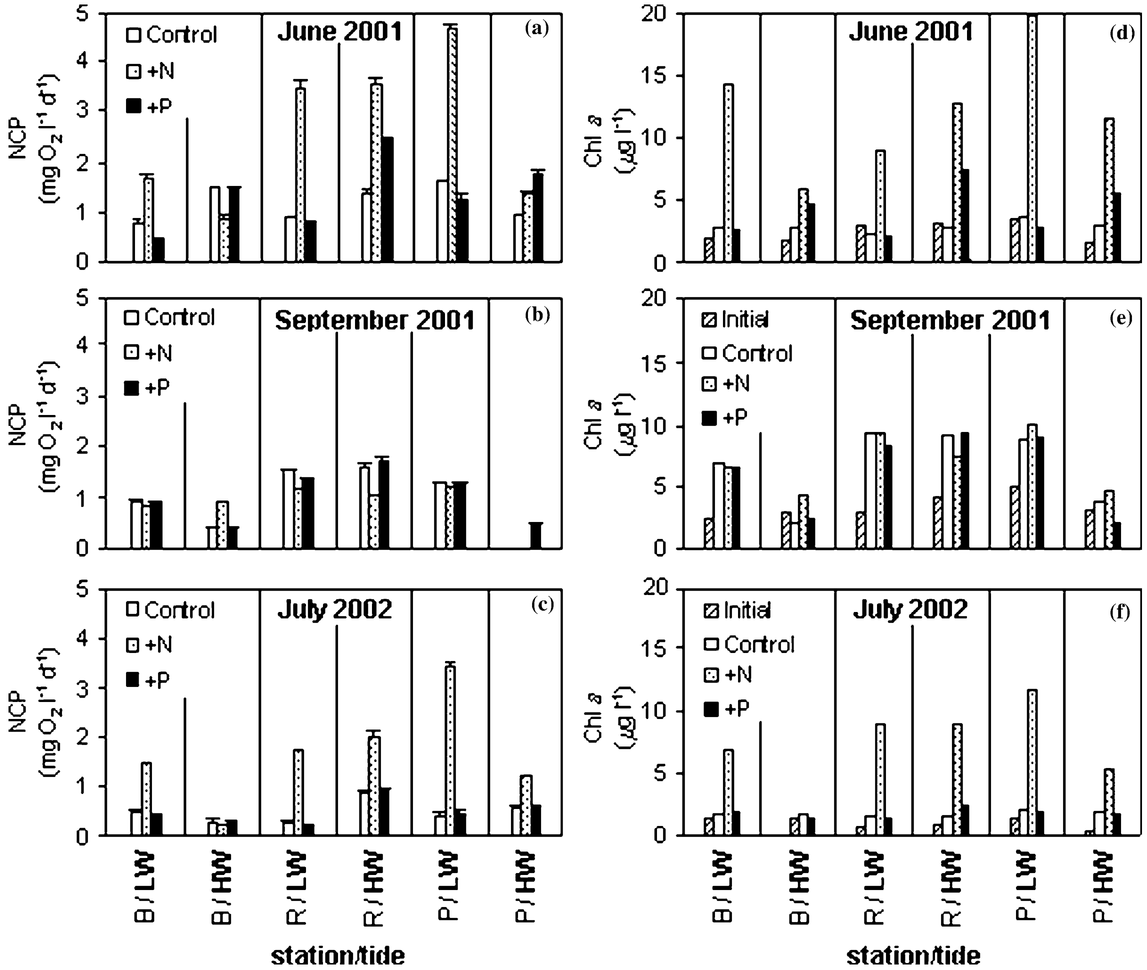

Figure 4. Response to enrichment of Net Community Production (mg O_2 l^{-1} d^{-1}) in (a) June 2001, (b) September 2001 and (c) July 2002 experiments; each column is the mean of three replicates; error bars are standard errors, where the bars are not visible it represents small errors hidden by the data columns. Response to enrichment of biomass, as chlorophyll *a* (μg l^{-1}), in (d) June 2001, (e) September 2001 and (f) July 2002. B = Barra; R = Ramalhete; P = Ponte (inner channel); LW = Low Water (in bold); HW = High Water (in bold).

However, despite these limitations, enrichment bioassay experiments still provide a valuable insight into nutrient dynamics. By using natural assemblages to investigate the likely effects on the original seawater community, these assays act as a bridge between controlled laboratory cultures and field experiments, where limited manipulation is possible due to the interference of environmental factors (Berdalet et al., 1996). Also, several results from enclosure experiments have been reported to accurately describe processes in natural assemblages (Pitcher et al., 1993; Maranon et al., 1995; Oviatt et al., 1995).

Methodological drawbacks from prolonged containment of small water volumes were minimized by performing short-term (one day) experiments. Additionally, *in situ* incubation provided the ambient light and temperature conditions of the system. Results of enrichment experiments indicated which nutrient had the potential to limit the growth of assemblages *in situ*, in the absence of other limiting factors (Elser & Kimmel, 1986; Ault et al., 2000). The outcomes of the Ria experiments will be discussed on this basis, in order to identify the PLN (Tyrrell, 1999). The estimation of algal production by the oxygen method must also take

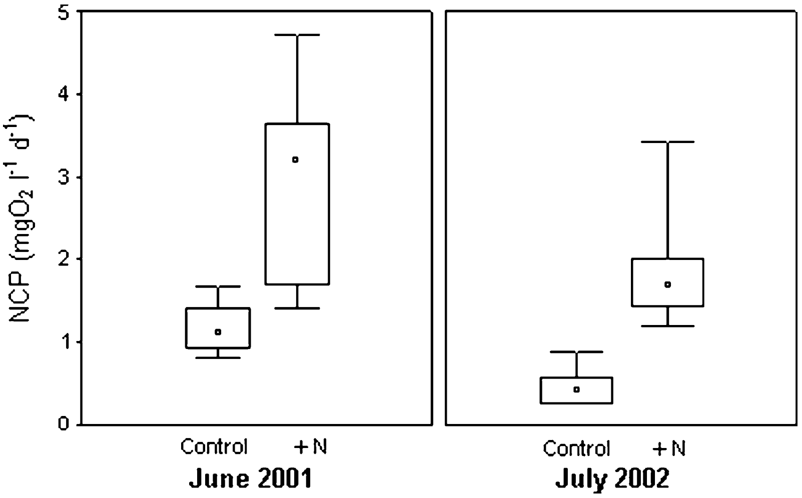

Figure 5. Box-plot distribution of the NCP (mg O_2 l^{-1} d^{-1}) from natural water stimulated with N during the summer.

into account that all components of the microplankton, including heterotrophic plankton, cyanobacteria and bacteria will contribute to respiration thereby affecting the final estimate for net production of the autotrophic component of the microplankton.

Microplankton productivity and biomass limitation

During the summer, in most cases, nitrogen stimulated significant increase in algal metabolism and biomass (as chl *a*). In P treatments, N was preferentially taken up by phytoplankton until it was depleted. These observations suggest nitrogen as the most likely primary PLN (Tyrrell, 1999) of the phytoplankton community in the western part of the lagoon. Denitrification processes in bottom sediments are usually associated with nitrogen shortage in coastal waters (Nixon, 1981; Granéli et al., 1990). Such processes have also been reported in the Ria Formosa lagoon (Falcão & Vale, 1990). Additionally, the new action program for the Faro region (Diário da República, 2001), based upon the transposition to the national legislation of the Nitrates Directive (ND, 91/676/EEC), is expected to have reduced the nitrogen

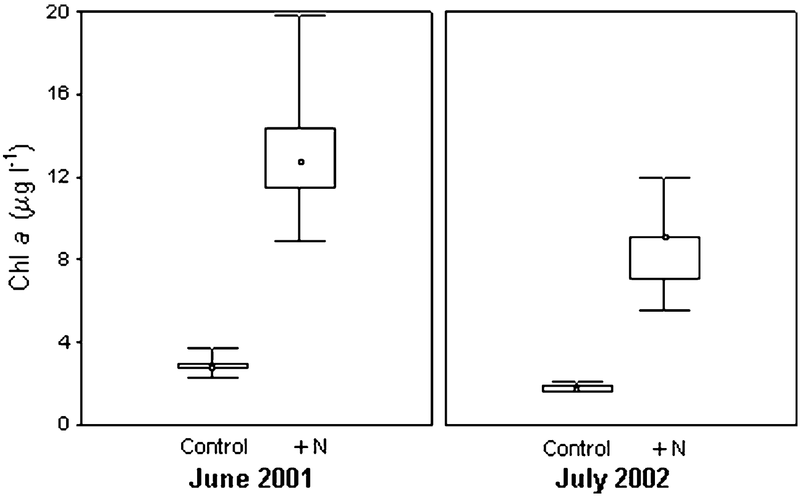

Figure 6. Box-plot distribution of the biomass, as chl *a* (µg l^{-1}), from natural water stimulated with N during the summer.

input in recent years. This may explain the previous expectation of phosphorus limitation of lagoonal waters all the year round (Falcão & Vale, 1990). However, N fixation by micro-organisms such as N fixing cyanobacteria can make up for *in situ* N deficits (Smith, 1984), although in systems with short water-residence time (as is the case with the Ria Formosa), N:P ratios are not likely to be affected by gaseous N fluxes (Granéli & Sundbäck, 1985). Nevertheless, in inner channels where water has a longer residence time (Tett et al., 2003) this process can be more relevant. The lagoon stations considered in this study have a higher response to enrichment during low water regimes, when the dilution effect of oceanic water is minimized. The upstream channel station (Ponte), is the most sensitive to summer nitrogen addition, supporting the observations that the upstream end of the Ria, where water renewal can be restricted (Newton & Mudge, 2003), may be more susceptible to water quality deterioration.

Nitrogen limitation is reported for several coastal areas (Bishop et al., 1984; Granéli et al., 1990; Oviatt et al., 1995), including estuaries (Pennock & Sharp, 1994; Ault et al., 2000), bays (Granéli, 1987; Kudela & Dugdale, 2000), and coastal lagoons (Nixon, 1982; Fong et al., 1993). N limitation is often evident during the summer growing season (Sanders et al., 1987; Fong et al., 1993; Conley, 2000). In the Ria Formosa, the increase in diatoms due to nitrogen stimulation is consistent with previous findings, which reported on significant increases in diatom growth rates after N additions (Table 4, Sanders et al., 1987; Schülter, 1998; Carlsson & Granéli,

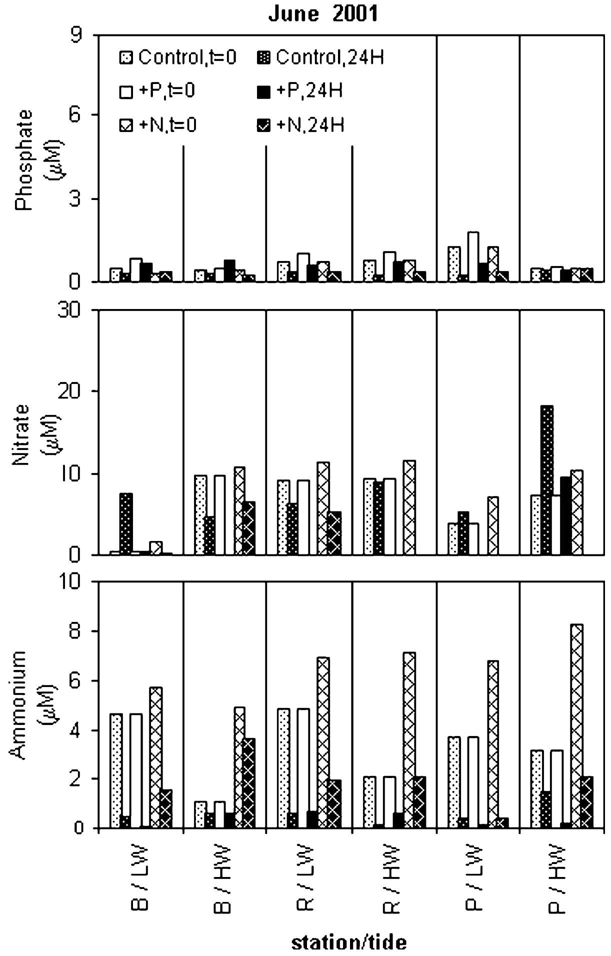

Figure 7. Evolution of inorganic nutrient concentrations (μM) during June 2001: B = Barra ; R = Ramalhete; P = Ponte; LW = Low water; HW = High water.

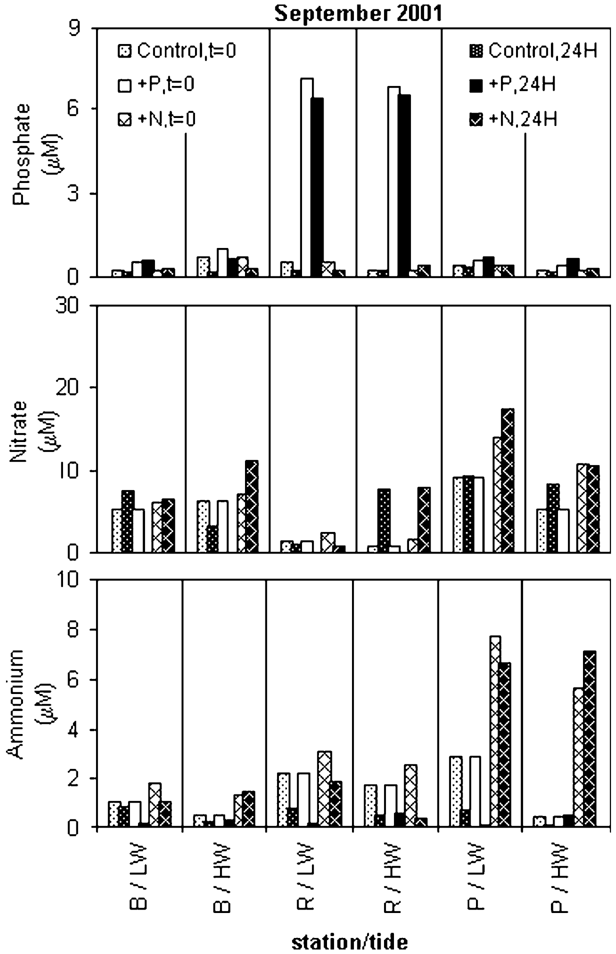

Figure 8. Evolution of inorganic nutrient concentrations (μM) during September 2001: B = Barra ; R = Ramalhete; P = Ponte; LW = Low water; HW = High water.

1999; Edwards et al., in press). Diatoms are generally regarded as beneficial for the aquatic ecosystem, because of their role in marine food chains and the fact that most of the species do not form toxic blooms with the exception of some *Pseudo-nitzschia* species (Officer & Ryther, 1980).

Cell counts did not include picophytoplankton, which may contribute in a significant way to the total autotrophic biomass and total rates of primary production in coastal areas (Affronti & Marshall, 1993; Marshall, 1995). Nevertheless, this smaller size class of the plankton usually dominates under nutrient-depleted conditions in enriched coastal systems (Agawin et al., 2000). The results of a mesocosm enrichment experiment in a coastal embayment suggest that the addition of N and P can favour large phytoplankton organisms (Jacquet et al., 2002).

Oceanic water, here represented by Barra HW, respond differently to enrichment compared to the other water types, emphasising the distinct chemical and biological features of coastal waters. During summer, the absence of nutrient stimulation is probably due to algal communities with different nutritional status and/or subjected to a lag phase (Healey, 1979), as well as it could depend on fertilization from upwelling conditions (Fiúza et al., 1982). In September 2001, consistent production and biomass stimulation by nitrogen in Barra HW suggests a limiting situation in coastal waters, which can correspond to bloom conditions after upwelling. By contrast,

Table 4. Results of bioassays with natural samples of seawater from a range of coastal areas

Location	Potential nutrient limitation	Chl *a* stimulation	Taxonomic stimulation	Method production estimation	Primary production stimulation	Potential limitation season	Reference
Georgia Coast, USA	Nitrogen	Yes	–	–	–	–	Bishop et al. (1984)
Laholm Bay, Sweden	Nitrogen	Yes	–	–	–	–	Granéli et al. (1986)
Patuxent River estuary, USA	Nitrogen	–	Small centric diatoms	–	–	Summer fall	Sanders et al. (1987)
Stege Bay, Denmark	Nitrogen	–	–	^{14}C	Yes	Summer	Lyngby & Mortensen (1995)
Narragansett Bay, USA	Nitrogen	Yes	–	Diel changes in oxygen	Yes	–	Oviatt et al. (1995)
Ria Formosa, Lagoon, Portugal	Nitrogen	Yes	Diatoms	Oxygen light-dark bottle	Yes	Summer	This study

– not analysed.

the lagoon waters did not respond to nutrient enrichment. Under these circumstances, one can assume non-limiting conditions, which probably resulted from strong rainfalls following a particularly dry summer (IM, http://www.meteo.pt/InformacaoClimatica/Index1.html). The consequent land run-off was expected to deliver a great nutrient amount to the lagoon waters. However, nutrient concentrations in the lagoon were lower than those detected in June 2001. This nutrient depletion can be associated to a fast consumption by primary producers and/or an export to coastal waters. Other factors affecting algal growth rates, such as light (31×10^3 KJ m^{-2} d^{-1} in June 2001 and July 2002; 19×10^3 KJ m^{-2} d^{-1} in September 2001), trace elements, grazing pressure, sedimentation and biomass washout, may be structuring the community dynamics (Hecky & Kilham, 1988; De Baar, 1994). The observed effect of incubation on algal biomass (chl *a*) in control cultures may reflect the importance of active *in situ* loss mechanisms that were not present in the experimental enclosures.

In June 2001, biomass stimulation by P at HW may be linked to P retention by sediments (Howarth, 1988; Falcão & Vale, 1998; Asmus et al., 2000). Combined N and P limitation can happen in a multi-species assemblage with different nutritional demands and substrate affinity (Hecky & Kilham, 1988; Dufour & Berland, 1999). The fact that nitrogen was depleted under N enrichments and that production was not significantly stimulated, still suggests N as the primary limiting nutrient.

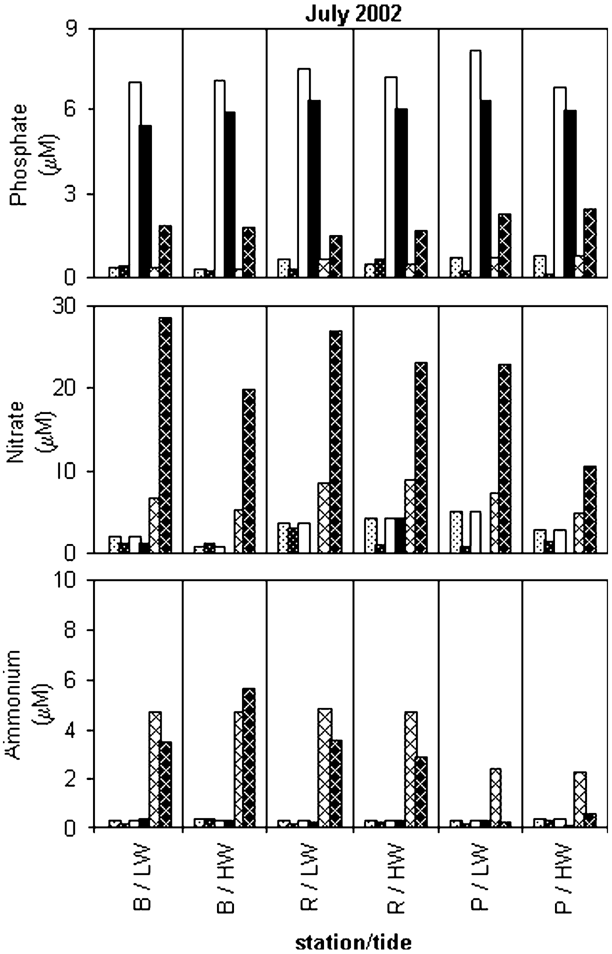

Figure 9. Evolution of inorganic nutrient concentrations (μM) during July 2001: B = Barra ; R = Ramalhete; P = Ponte; LW = Low water; HW = High water.

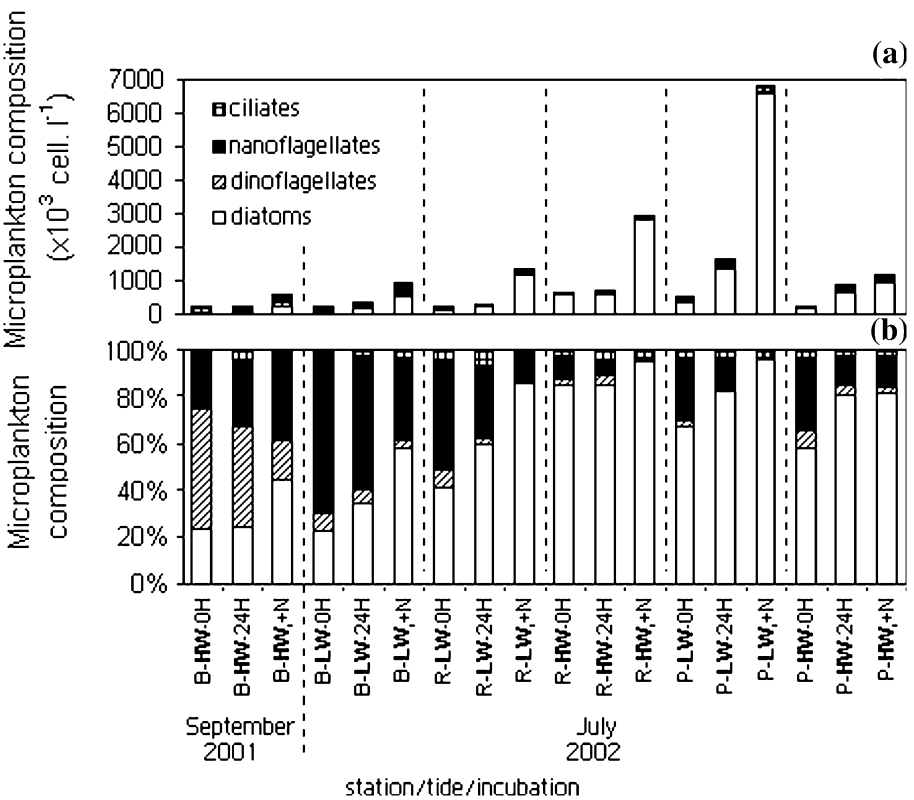

Figure 10. Microplankton composition from September 2001 and July 2002 experiments, expressed as abundance (×10^3 cells l^{-1}; (a)) and percentage (b). B = Barra; R = Ramalhete; P = Ponte; LW = Low water (in bold); HW = High water (in bold); 0H = initial composition of control samples; 24H = final composition of control samples; +N = final composition of N enriched samples.

Comparison with other studies

In April 2002 a microcosm study was carried out with natural water samples from the Ponte site of the Ria Formosa at low water, to estimate the yield of chlorophyll from nitrogen (Edwards et al., in press). This microcosm experiment was carried out under laboratory conditions with a duration of 7 days. A continuous culture technique was used in order to reduce some of the methodological constraints mentioned above. The objective of this microcosm study in the Ria Formosa was to test whether this approach was appropriate for ecological diagnosis and prediction of eutrophication (e.g., Tett et al., 2003). Similar studies have been carried in Scottish waters to develop a parameter that could be useful for predicting the dynamic relations between nitrogen supply and increased phytoplankton biomass (Gowen et al., 1992; Edwards et al. 2003).

Within 24 h of setting up the microcosm in the laboratory, nitrate was added to increase the ambient concentration by approximately 12 μM, together with other nutrients, vitamins etc., so that only the nitrogen would be limiting during the experiment duration. Samples were collected daily from the microcosm to analyse for a range of different parameters including chlorophyll, nutrients, and the microplankton community. The initial concentration of N in the lagoon water was 7.2 μM similar to the 8.8, 12.5 and 6.0 μM estimated for Ponte LW in the current study in June 2001, September 2001 and July 2002, respectively. The chl *a* increase after 24 h stimulation with N were relatively similar between the two studies, with values of 26.5 μg l^{-1} in April 2002 (Edwards et al., in press) and up to 20 μg l^{-1} in June 2001 (present study). A synthesis of studies on this subject is also given in Table 4. Edwards et al. (in press) observed a marked increase in the diatom population after N stimulation with an initial community dominated by centric diatoms (e.g., *Thalassiosira* sp.), but with an increase of pennate diatoms (*Pseudo-nitzschia* sp.) toward the end of the experiment. A substantial number of autotrophic flagellates, dinoflagellates and cyanobacteria were also observed as well as bacteria and protozoan grazers, but as might be expected the groups did not increase with N stimulation.

Conclusions

This study indicates that in summer, nitrogen can control phytoplankton production rates and algal biomass and, as such, it is the most likely "proximate limiting nutrient". Although present management strategies point to a positive future outlook regarding eutrophication of this coastal lagoon (Ferreira et al., 2003), inner areas where tidal mixing is limited (Newton & Mudge, 2003) can be more sensitive to over-enrichment, which can cause water quality deterioration in this part of the lagoon. This could affect the salt extraction, tourism, fisheries or aquaculture activities, which are the basis of the economy for this region. Although the stimulated algal community was mainly composed of diatoms, generally regarded as a "beneficial" group, uncoupling between production and decomposition can still lead to anoxia episodes. Further higher level (Hecky & Kilham, 1988) experimental studies, involving atmospheric, pelagic and benthic domain interactions, important for the control of nutrient availability in shallow ecosystems, should be carried out to confirm the findings. Nevertheless, this first assessment contributes to a better understanding of the dynamics between nutrients and pelagic algal processes and composition in the Ria Formosa lagoon.

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