Universidade do Algarve
Faculdade de Ciências e Tecnologia

Primary Production in systems subject to Natural and Anthropogenic Eutrophication

(Tese para obtenção do grau de doutor
no ramo de Química,
especialidade de Química do Ambiente)

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IN SYSTEMS SUBJECT TO

NATURAL AND ANTHROPOGENIC EUTROPHICATION

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FARO
2005

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DEDICATION

To my Mother,
and all we've shared,
and all we'll share,
Here, and Everywhere!

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Este trabalho foi possível graças à valiosa colaboração de um grande número de pessoas. A todos elas desejo expressar os meus sinceros agradecimentos!

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Sofia Vitória Penela Sotto-Mayor Loureiro

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ABSTRACT

The input of nutrients to the aquatic system can have both natural (e.g. upwelling events) and human (e.g. sewage discharges, run-off of inorganic fertilizers from agricultural land) origins. The level of anthropogenic (human) eutrophication has grown in the past century. Primary production rates have increased significantly as a response, which can lead to harmful effects when the balance between the production and decomposition of organic matter is affected. These include the depletion of oxygen as excessive organic matter decomposes and the migration or death of surrounding life forms. Other consequences of nutrient enrichment may include changes in the natural autotrophic community structure by altered nutrient ratios, reduced biological diversity, increase in harmful algal blooms, poisoning of organisms, concluding in deterioration of the ecological and chemical status of waters. A decrease in fisheries, aquaculture and recreational resources may follow. To control and reverse eutrophication it is important to understand the dynamics between nutrients and the algal assemblage, identifying which nutrient is primarily limiting algae biomass and production. Within the context of the European Union (EU) several policies aim to reduce eutrophication and ensure the protection and improvement of the European aquatic environment. These include the Urban Waste Water Treatment (UWWT) Directive (91/271/EEC), the Nitrate Directive (91/676/EEC) and the recent Water Framework Directive (WFD) (2000/60/EC).

The general objective of this thesis was to investigate the relation between nutrients (specifically nitrogen and phosphorus) and the activity and structure of the pelagic assemblage of microalgae in the two selected study areas, recently included as part of the intercalibration sites for the Common Implementation Strategy of the WFD. The underlying hypothesis is that the microphytoplankton assemblage is affected by nutrient concentrations and ratios. This was tested both by field and experimental work. In management terms, this study evaluates whether the microphytoplankton assemblage (and its selected supporting elements) are good tools for the identification and management of anthropogenic eutrophication within the context of the WFD. Are these elements sufficient to distinguish between Natural and Anthropogenic Eutrophication? Can we use these tools for management?

The Sagres station (SW. Portugal), adjacent to the upwelling centre of Cabo S. Vicente and close to an oyster-culture was selected for the study of natural eutrophication, whereas

the Ria Formosa coastal lagoon (S. Portugal) was representative of a system subject to both natural and anthropogenic nutrient enrichment. The sampling in Sagres was performed during the upwelling season, from May to September (2001). The Ria Formosa was sampled according to the frequency recommended by the WFD (every 3 months) during representative conditions: close to the summer and winter solstice, and spring and autumn equinox, coinciding with both high (HW) and low water (LW), between June 2001 and July 2002. The water was collected from three contrasting stations on the western lagoon. Nitrogen and phosphorus enrichment experiments were conducted additionally over short-time scales on small volumes of water collected during a period of relaxation of upwelling conditions in Sagres (September 2002), and during the growing season (summer solstice of 2001 and 2002), as well as the autumn equinox (September 2001), in the Ria Formosa.

The results indicated that during the upwelling season the cold nutrient-rich waters of Sagres were dominated by the presence of diatoms. Flagellate forms developed by the end of the season when stratification occured, which was probably associated with the intrusion of the warm coastal countercurrent from the Gulf of Cádiz. *Chaetoceros* spp., *Thalassiosira* spp., *Lauderia* spp., *Detonula* spp., and *Pseudo-nitzschia* spp. were identified as a summer upwelling proxy. Production rates (seasonal average gross production: 25.4±19.8 μM O_2 d^{-1}) were comparable to other productive systems. Respiration, on the contrary, was low during the survey. Nitrogen seemed to be the major nutrient regulating the microalgae activity, both during the upwelling season in the form of "new" nitrogen (essentially nitrate), and during relaxation stages in regenerated forms (essentially ammonium). Diatoms were the most sensitive group to nitrogen enrichment, including *Pseudo-nitzschia* spp. that may include toxic species. Altogether, the main factors influencing the development and structure of the algal community in Sagres were of physical nature, which in turn determined the chemical supply of nutrients to the photic zone.

The microplankton community peaked in the summer solstice samplings (June 2001 and July 2002) in the Ria Formosa lagoon, with diatoms as the greatest contributors to the total numbers, whereas flagellates generally dominated the carbon biomass. The statistical analysis clustered the microplankton samples into their seasonal sampling groups. Physical factors such as temperature, salinity and solar radiation, together with the availability of nitrogen (especially in reduced forms) appear to be important parameters contributing for the microplanktonic evolution. The oceanic assemblage differed from the internal lagoon community on certain occasions. Events such as coastal upwelling in the adjacent waters and land run-off episodes within the Ria can contribute to a greater differentiation between the

Ria-ocean systems, and thereby to the selection of distinct biological forms. The Ria had an important role as an exporter of both algal biomass and nutrients to adjacent waters during the sampling campaigns. The importance of coastal upwelling events as a contributor for the trophic level of the lagoon was also highlighted. Further work may clarify the relative degree of natural versus anthropogenic processes for the eutrophication of the lagoon. High concentrations of nutrients and algal biomass can accumulate in regions prone to restricted water renewal such as upstream channels. High numbers of microalgae can also occur adjacent to UWWT plants. Transport mechanisms ultimately regulate the final location of the accumulated material. Nitrogen is suggested to be the potential limiting nutrient of the pelagic microalgae community of the lagoon during the growing season according to enrichment experiments, with diatoms as the most sensitive group. In temperate ecosystems, a switch in limiting factors is expected due to the seasonal change of biological and environmental variables.

The systems appear to differ significantly during the growing season, both in biological and physico-chemical parameters, but these differences seem to be nevertheless not sufficient to distinguish between Natural and Anthropogenic Eutrophication. Similar responses to enrichment bioassays were also observed in both systems. Harmful Algal Blooms (HAB) taxa seem to develop mainly due to natural events (upwelling), suggesting that this parameter should be used with caution as an indicator of anthropogenic eutrophication in Portuguese coastal waters. Altogether, although the fact that the microphytoplankton assemblage responded to changing trophic conditions supports the need to incorporate these data in monitoring programmes, these tools seem not to be sufficient for the management of anthropogenic eutrophication in the selected areas.

The two scenarios were in the ranges of mesotrophic conditions during the samplings, which is likely a consequence of the mechanisms of mixing and transport present in both areas. The high potential of dilution from the Atlantic Portuguese coastal waters contributes to its low degree of eutrophication problems. This works contributes to the on-going process of implementation of the WFD, and to the database of knowledge of the productive systems of Sagres and Ria Formosa.

RESUMO

A introdução de nutrientes no sistema aquático pode ter origem natural (exº eventos de afloramento) e humana (exº descargas de esgotos, escorrimento de fertilizantes inorgânicos de terrenos agrícolas). O nível de eutrofização antropogênica (humana) cresceu no século passado. Como resposta verificou-se um aumento significativo da taxa de produção primária, o qual pode induzir efeitos nocivos sempre que o equilíbrio entre a produção e a decomposição da matéria orgânica for afectado. Estes efeitos incluem o esgotamento de oxigénio devido à decomposição da matéria orgânica excessiva, e a migração ou morte das formas de vida circundantes. Outras consequências do enriquecimento de nutrientes podem incluir mudanças na estrutura natural da comunidade autotrófica devido à alteração da proporção de nutrientes, redução da diversidade biológica, aumento do crescimento de algas nocivas, envenenamento de organismos, concluindo na deterioração do valor ecológico e químico da água. Pode suceder-se uma diminuição nos recursos de pescas, de aquacultura e recreativos. Para controlar e reverter a eutrofização torna-se importante compreender a dinâmica entre os nutrientes e a comunidade de algas, identificando qual o nutriente que limita primariamente a sua produção e biomassa. No contexto da União Europeia vários planos de acção visam reduzir a eutrofização e garantir a protecção e melhoria do ambiente aquático Europeu. Estas medidas incluem a Directiva 91/271/CEE, relativa ao Tratamento de Águas Residuais Urbanas, a Directiva Nitratos (91/676/EEC) e a Directiva-quadro da Água (DQA) (2000/60/EC).

O objectivo geral desta tese foi o de investigar a relação entre nutrientes (especificamente azoto e fósforo) e a actividade e estrutura da comunidade pelágica de microalgas nas áreas de estudo seleccionadas, recentemente incluídas na lista de áreas de intercalibração para a estratégia de implementação comum da DQA. A hipótese subjacente é que a comunidade de microfitoplâncton é afectada pelas concentrações e proporções de nutrientes, o que foi testado por trabalho de campo e experimental. Em termos de gestão, este estudo avalia se a comunidade microfitoplanctónica (e os parâmteros de suporte seleccionados) são bons instrumentos para a identificação e administração da eutrofização antropogénica no contexto da DQA. Serão estes elementos suficientes para distinguir entre Eutrofização Natural e Antropogénica? Podemos usar estes elementos para a gestão da eutrofização?

A estação de Sagres (SO. Portugal), junto ao centro de afloramento do Cabo. S. Vicente e perto de uma cultura de ostras, foi seleccionada para o estudo da eutrofização natural, enquanto que a lagoa costeira da Ria Formosa (S. Portugal) foi representativa de um sistema

sujeito ao enriquecimento natural e antropogênico de nutrientes. A amostragem em Sagres foi efectuada durante a época de afloramento, de Maio a Setembro (2001). A Ria Formosa foi amostrada de acordo com a frequência recomendada pela DQA (3 em 3 meses): perto do solstício de verão e de inverno, e o equinócio de primavera e outono, coincidindo com maré alta e baixa, entre Junho 2001 e Julho 2002. A água foi recolhida de três estações na parte oeste da lagoa. Adicionalmente foram feitas experiências de enriquecimento de azoto e fósforo de pequena-escala e curta-duração, em água recolhida num período de relaxamento de condições de afloramento em Sagres (Setembro 2002), e durante a época de crescimento (solstício de verão 2001 e 2002), assim como no equinócio de outono (Setembro 2001), na Ria Formosa.

Os resultados indicaram que durante a época de afloramento as águas de Sagres, frias e ricas em nutrientes, foram dominadas pela presença de diatomáceas. O desenvolvimento de formas flageladas aquando da ocorrência de estratificação esteve provavelmente associada à intrusão da corrente contra-costeira quente vinda do Golfo de Cádiz. A análise estatística de comunidades identificou uma comunidade indicadora do afloramento de verão constituida por *Chaetoceros* spp., *Thalassiosira* spp., *Lauderia* spp., *Detonula* spp., e *Pseudo-nitzschia* spp.. As taxas de produção (produção primária bruta média: 25.4±19.8 $\mu M\ O_2\ d^{-1}$) foram comparáveis com a de outros sistemas produtivos. Pelo contrário, a respiração foi baixa durante o período de estudo. Como esperado para ambientes marinhos, o azoto parece ser o nutriente regulador da actividade microplanctónica, quer durante a época de afloramento sob a forma de azoto "novo" (essencialmente nitrato), quer durante estágios de relaxamento sob formas regeneradas (essencialmente amónia). As diatomáceas foram o grupo mais sensível ao enriquecimento por azoto, incluindo a *Pseudo-nitzschia* spp. que pode abranger espécies tóxicas. Ao todo, os principais factores que influenciaram o desenvolvimento e estrutura da comunidade pelágica de microalgas em Sagres foram de natureza física, que por sua vez determinaram o fornecimento químico de nutrientes à zona eufótica.

Na Ria Formosa, a comunidade microplanctónica atingiu o seu máximo nas amostragens dos solstícios de verão (Junho 2001 e Julho 2002) sendo as diatomáceas dominantes em termos de números totais, enquanto que os flagelados dominaram duma maneira geral a biomassa carbónica. A análise estatística de comunidades agrupou as amostras sazonalmente. Factores físicos como a temperatura, a salinidade e a radiação solar, juntamente com a disponibilidade de azoto (especialmente em formas reduzidas) parecem ser importantes parâmetros para a evolução do microplâncton. A comunidade oceânica diferiu da da lagoa em certas ocasiões. Eventos como o afloramento costeiro nas águas adjacentes e episódios de

escorrimento das terras na Ria, podem contribuir para uma maior diferenciação entre o sistema Ria-oceano, e consequentemente para a selecção de distintas formas biológicas. Durante as campanhas de amostragem foi evidenciado o papel da Ria como exportador de algas e nutrientes para as águas adjacentes. A importância dos eventos de afloramento costeiro para a contribuição do nível trófico da lagoa foi também realçada. O grau relativo dos processos naturais versus antropogénicos que contribuem para a eutrofização da lagoa pode ser clarificado por trabalhos futuros. Em regiões com tendência para uma renovação restrita de água podem acumular-se altos níveis de nutrientes e de algas. Elevados números de microalgas podem também ocorrer em áreas perto de ETARs (Estações de Tratamento de Águas Residuais). Os mecanismos de transporte podem em última instância regular a localização final do material acumulado. Os resultados das experiências de enriquecimento sugeriram o azoto como nutriente potencialmente limitante da comunidade pelágica de microalgas da lagoa durante a estação de crescimento, sendo as diatomáceas o grupo mais sensível a este enriquecimento. Em ecossistemas temperados espera-se uma mudança temporal de factores limitantes devido à variação sazonal dos parâmetros biológicos e ambientais.

Os sistemas estudados parecem diferir significativamente durante a estação de crescimento em parâmetros biológicos e físico-químicos mas, estes parecem no entanto insuficientes para distinguir entre Eutrofização Natural e Antropogénica. Resultados similares foram também observados aquando das experiências de enriquecimento. Os “blooms” tóxicos de microalgas parecem desenvolver-se principalmente devido a causas naturais (afloramento), sugerindo que este parâmetro deveria ser usado com cautela como indicador de eutrofização antropogênica. No todo, se bem que o facto da comunidade de microalgas responder às mudanças de condições tróficas apoia a necessidade de incluir este dado em programas de monitorização, esta parece não ser suficiente para a administração de eutrofização antropogénica nas áreas seleccionadas.

Durante as amostragens os dois cenários enquadraram-se na escala de condições mesotróficas, provavelmente em consequência dos mecanismos de mistura e transporte presentes em ambas áreas. O alto potencial de diluição das águas Atlânticas costeiras Portuguesas contribui para o seu baixo grau de problemas de eutrofização. Este trabalho contribui para o processo de implementação da DQA e, para a base de dados dos produtivos sistemas de Sagres e da Ria Formosa.

LIST OF ACRONYMS AND ABBREVIATIONS

ANOSIM = Analysis of Similarities

ANOVA = Analysis of Variance

ASP =Amnesic Shellfish Poisoning

ATP = Adenosine Triphosphate

AVHRR = Advanced Very High Resolution Radiometer

CCC = Coastal Countercurrent

Chl *a* = Chloropyhll *a*

CUI = Coastal Upwelling Index

DCR = Dark Community Respiration

DIN = Dissolved Inorganic Nitrogen

DSP = Diarrhetic Shellfish Poisoning

DPSIR = Driving Force - Pressure - State - Impact - Response

EEA = European Environmental Agency

EC = European Commission

EU = European Union

GP = Gross Production

HAB = Harmful Algal Bloom

HELCOM = Helsinki Commission Convention, Convention on the Protection of the Marine Environment of the Baltic Sea Area

HW = High Water

IM = Instituto de Meteorologia

IPIMAR = Instituto de Investigação das Pescas e do Mar

LW = Low Water

MDS = Multi-Dimensional Scaling

NADP = Nicotinamide Adenine Di-nucleotide Phosphate

NCP = Net Community Production

NEEA = U.S. National Estuarine Eutrophication Assessment

NOAA = U.S. National Oceanic and Atmospheric Administration

OSPAR = Oslo and Paris Convention, The Convention for the Protection of the Marine Environment of the North-East Atlantic

PAR = Photosynthetically Available Radiation

PLN = Proximate Limiting Nutrient

PSP = Paralytic Shellfish Poisoning

SIMPER = Similarity Percentages

SST = Sea Surface Temperature

U.S. = United States of America

UWWT = Urban Waste Water Treatment

WFD = Water Framework Directive

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1. GENERAL INTRODUCTION

All flesh is grass.

Prophet Isaiah

1.1. PRIMARY PRODUCTION

The autotrophs (Gr. *autos*: self; *trophe*: nutrition/food) are organisms capable of synthesizing high-energy organic compounds from low-energy inorganic precursors. They compose the first level of the food chains and are, as such, also called primary producers. The first trophic level includes photo-autotrophs (Gr. *photos*: light), which use light as energy source, and chemo-autotrophs, which use the chemical energy derived from the oxidation of inorganic compounds (such as ammonium, methane, and sulphur) as the energy source. This chemosynthesis made by bacteria corresponds, in general terms, to a minor fraction of the whole primary production. Primary producers will refer to photoautotrophs in this work, unless specified otherwise. The heterotrophs (Gr. *heteros*: different) are organisms that use organic compounds for their nutrition. Additionally, there are mixotrophs, which are autotrophic life forms capable of taking organic substances for supplementary nutrition (Raymont, 1980; Falkowski & Raven, 1997).

Photosynthesis is the process by which the photo-autotrophs use radiant energy to produce organic matter. This synthesis involves the reduction of carbon dioxide (Raymont, 1980). It takes place in small organelles called chloroplasts, that contain pigments capable of absorbing light in the visible band (400 to 700 nm range). This photosynthetic band, or photosynthetically available radiation (PAR), corresponds roughly to 40-45% of the total solar radiation at sea level (Kirk, 1994). Photosynthetic pigments includes chlorophylls, carotenoids and biliproteins. All plants contain chlorophyll *a* (chl *a*), which is the dominant pigment present in photosynthetic plants, and carotenoids. Red algae, blue-green algae (cyanobacteria) and cryptophytes also contain biliproteins (Kirk, 1994). The production of new plant material (from photosynthesis) over time is termed primary production.

The photosynthetic process is composed by light and dark reactions. The light reactions can be summarised by the equation:

$$2\,H_2O + 2\,NADP^+ \xrightarrow[3\,ADP + 3\,Pi \rightarrow 3\,ATP]{light} O_2 + 2\,H^+ + 2\,NADPH \qquad (1)$$

that describes the electron transport from water to NADP (Nicotinamide Adenine Di-nucleotide Phosphate), associated to ATP (Adenosine Triphosphate) synthesis and oxygen liberation, by means of light energy.

Regarding the dark reactions, the carbon dioxide is fixed by means of a cycle of reactions named the Calvin (or Calvin-Benson) cycle. The energy of ATP and the reducing power of NADPH, which were produced in the light reactions, promote this reduction of CO_2 to the level of carbohydrates:

$$CO_2 + 2\,NADPH + 2\,H^+ \xrightarrow[3\,ATP \rightarrow 3\,ADP + 3\,Pi]{} (CH_2O) + H_2O + 2\,NADP^+ \qquad (2)$$

Thus, the overall photosynthetic process could be summarized by:

$$CO_2 + 2\,H_2O \xrightarrow{light} (CH_2O) + H_2O + O_2 \qquad (3)$$

(Parsons *et al.*, 1984; Gregory, 1989; Kirk, 1994).

Primary production can be distinguished into gross and net production. The gross primary production is the total amount of organic material produced by photosynthesis. The net primary production deducts the costs of respiration from the total photosynthetic output. It therefore represents the gain in plant biomass that is available for consumption by other trophic levels. Conversely, the energy required for the metabolic processes of all organisms is provided by the respiration of organic matter, the reverse reaction to photosynthesis. Respiration involves the breakdown of complex molecules (such as carbohydrate) associated with the consumption of oxygen to yield energy, carbon dioxide and water (Williams *et al.*, 2002).

Primary Production in marine ecosystems

The photo-autotrophs within the marine environment, include mainly algae and photosynthetic bacteria (Parsons *et al*., 1984). However, the principal primary producers in the water column are unicellular algae collectively called phytoplankton (Gr. *phytos*: plant; *plankton*: drifting). Some of these may form colonies. There are approximately 28 000 species of phytoplankton, characterized by size, shape and pigmentation (Kennish, 2001). The main taxonomic groups include diatoms (class Bacillariophyceae), dinoflagellates (class Dinophyceae), coccolihophores (class Prymnesiophyceae) and silicoflagellates (class Dictyochophyceae). Euglenoids (class Euglenophyceae), cryptomonads (class Cryptophyceae), and green algae (class Chlorophyceae) may also be important in coastal areas. The response of each algae species to the surrounding environmental conditions determines the patterns of planktonic succession. Algal diversity regulates the structure of marine systems and photosynthetic rates, since the algae are at the base of the food chain (Margalef, 1978; Smayda, 1980; Reynolds, 1989).

Table 1 describes the main size classes of phytoplankton.

Size Category	Size Range
Macroplankton	2 - 20 cm
Mesoplankton	0.2 - 20 mm
Microplankton	20 - 200 µm
Nanoplankton	2 - 20 µm
Picoplankton	0.2 - 2 µm

Table 1. Classification of phytoplankton according to size. (from Kennish, 2001)

The phytoplankton contributes to 90% of the total photosynthesis in the marine environment whereas benthic (referring to the organisms that live on the seafloor) macro- and micro-algae and vascular plants (salt marsh plants, mangroves, and seagrasses), present in shallow systems, are the main responsible for the remaining share (Kennish, 2001).

Limiting factors of primary production

The light availability influences the amount of phytoplankton production. Significant photosynthesis occurs essentially in the euphotic (Gr. *eu*: good, well; *photos*: light) zone, considered to be the layer within which PAR falls to 1% of the surface value. The benthic algae community is, as such, geographically restricted to shallow coastal areas where the light penetration allows their development (Nybakken, 1997).

The variation of the photosynthetic rate with light is represented in Fig. 1 by the photosynthesis-irradiance (P-E) curve. There is no photosynthesis in the dark, and only respiration occurs. The amount of respiration at low light intensities overcomes photosynthesis until the compensation point (when respiration balances photosynthesis)

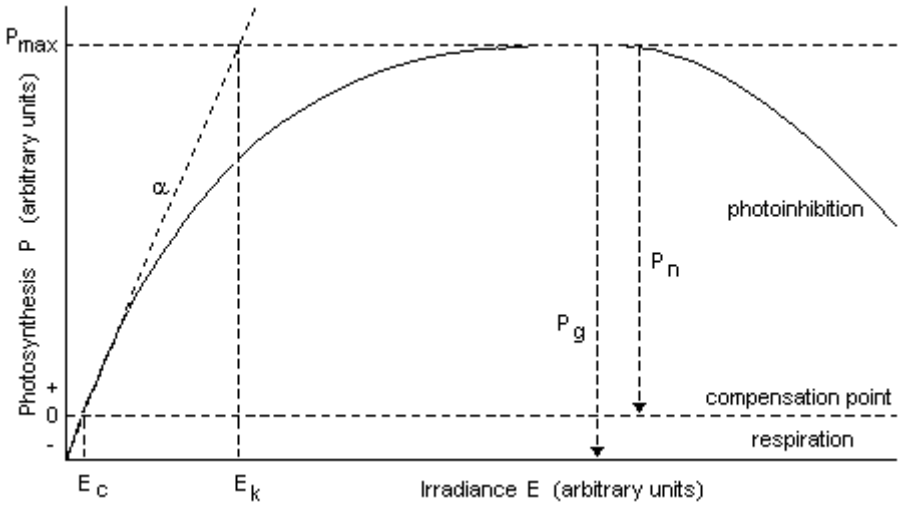

Fig. 1. Photosynthesis as a function of irradiance (E). P_{max} represents the maximal photosynthesis, E_k the saturation onset parameter, E_c the light compensation point, P_g the gross photosynthesis, P_n the net photosynthesis. (from Parsons *et al.*, 1984)

is reached, which occurs at the light compensation point (E_c). Photosynthesis increases with irradiance until a maximum rate is reached (P_{max}, the assimilation number related to the dark reactions and its capacity to fix carbon). E_k characterizes the onset of saturation. It is determined by the intersection of P_{max} with the extension of the initial slope of the P-E curve. The initial slope, generally parameterized as α, is defined as the quantum yield and it is related to the light reactions. These parameters are species-specific. Light becomes inhibitory at high intensities, provoking a decline in P as E increases (Parsons *et al.*, 1984; Lalli & Parsons, 1997).

The vertical mixing of the water column determines the phytoplankton exposure to light, thus its photosynthetic rate. The photosynthesis of a cell equals its respiration (Pc = Rc) at the compensation depth (Fig. 2); above this depth there is a net photosynthetic gain, and below it there is a net loss. The cells will be exposed to an average light intensity in the water column during mixing. The critical depth (D_{cr}) is the depth at which the average light intensity equals the compensation light (I_c); it corresponds to the depth where photosynthesis throughout the water column (P_w) equals phytoplankton respiration throught the water column (R_w). At the

D_{cr}, the area contained in the points 1, 2, 3, 4 (Fig. 2) (corresponding to the phytoplankton respiration) will equal the area contained in the points 1, 2 and 5 (corresponding to the photosynhtesis). If the depth of water mixing (D_m) is greater than the D_{cr}, then $P_w < R_w$ and no net production takes place. If the $D_{cr} > D_m$, the average light intensity in the mixed layer

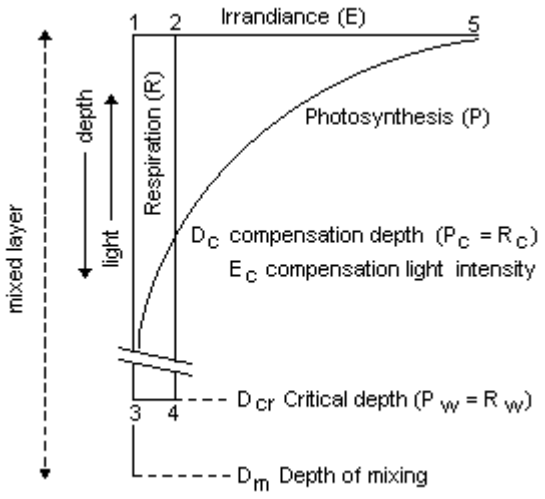

Fig. 2. Relationship between the compensation and critical depth, and the depth of mixing (see text). (modified from Lalli & Parsons, 1997)

(through which the phytoplankton is circulating) will be enough so that a positive net production in the water column ($P_w > R_w$) will be achieved (Parsons *et al*., 1984; Lalli & Parsons, 1997).

The amount of solar radiation that reaches the sea surface varies with latitude, decreasing from the equators towards the poles. This factor contibutes to the seasonal differences of primary production between the polar, temperate and tropical regions. However, the light is not the sole factor regulating plant growth. Both light and nutrients must be available for the phytoplankton production to take place. All primary producers require inorganic elements for their nutrition (nutrients). Some generalizations can be made regarding the latitute variation of primary production, which is related to light and nutrient availability, together with seawater mixing conditions. For instance, in temperate regions two peaks of production are commonly observed: one in spring, when light increases and one in autumn, after the breakdown of the thermal stratification developed during summer. The summer surface stratification is broken by the increasing wind-driven mixing, which induces a resuspension of the nutrients (Lalli & Parsons, 1997; Nybakken, 1997).

Essential elements like nitrogen, phosphorus, silicon, iron and manganese can limit primary production in seawater when present in lower concentrations than the required by phytoplankton. Other nutrients (carbon, calcium, magnesium, oxygen, potassium, sodium,

copper, molybdenum, etc), including organic ones (biotin, thiamine, etc), are also required by autotrophs, but are usually available in excess and therefore are generally not limiting (Hecky & Kilham, 1988). Nutrient inputs to the aquatic environment includes land drainage, sewage discharges and river outflow (Boesch & Brinsfield, 2000). The elements that become incorporated in the organisms are recycled after their death by decomposers (bacteria), and returned to the environment in inorganic forms that can be used by primary producers (Nixon, 1981). The concentrations of nutrients present in the euphotic zone depends greatly on the degree of mixing of the water column.

The uptake of carbon, nitrogen and phosphorus by phytoplankton generally follows the ratio 106:16:1 (the Redfield ratio by atoms; Redfield *et al*., 1963). Nutrient-sufficient phytoplankton produces biomass with a mean C:N:P Redfield ratio. Variations in the stoichiometry of dissolved inorganic nutrients may predict which nutrient will potentially limit biomass production. The Liebig's Law of the Minimum (Liebig, 1840), originally applied to plant or crop growth, stresses the importance of limiting factors in ecology. This law states that growth is not controlled by the total of resources available, but by the scarcest (limiting) one in relation to the need. Only by increasing the amount of the limiting nutrient would the growth of a plant be improved.

According to their content of mineral nutrients the aquatic systems can be classified as:

- oligotrophic (Gr. *oligo*: few), referring to regions with low concentration of nutrients and correspondingly low biological activity.
- mesotrophic (Gr. *meso*: middle), regions with intermediate concentrations and productivity.
- eutrophic (Gr. *eu*: good), regions with high nutrient concentrations supporting a high biological activity.
- hypertrophic (Gr. *hyper*: excessive, highest), regions with excessive concentration of nutrients (Kennish, 2001).

Grazing (the consumption of plants by herbivores) can also control biomass. The link between phytoplankton and zooplankton (the most numerous herbivores of the aquatic system) varies with latitude. Grazing generally lags behind phytoplankton peaks in middle latitudes. The phytoplankton population decreases as the grazing pressure increases, leading to an eventual decline on the zooplankton population (Sverdrup *et al*., 2003). Some mechanisms in the photosynthetic process, such as enzyme activity and electron transport, are influenced by temperature. However, the response of photosynthesis to temperature is

ultimately dependent upon the light availability (Davison, 1991). The dominant factors regulating primary production are as a conclusion: light and nutrient availability (Kirk, 1994).

Measuring Primary Production

Primary production is of major importance regarding carbon and energy budgets of ecosystems. The estimation of primary production rates in the sea, and the proper methods to be used, is a fundamental issue in oceanographic studies. However, no currently used method gives an exact measure of photosynthesis. Ambiguity includes factors as the distinction between gross and net photosynthesis, impact of metabolic processes not directly related to photosynthesis and artifacts associated with incubation (Platt & Sathyendranath, 1993).

To measure primary production one can measure the rate of increase in plant biomass or the rate of photosynthesis. To estimate the plant material in a sample of seawater one may count the cells, or extract the chlorophyll. The evolution of these parameters over time under a fixed area gives the temporal change of biomass estimating the net production (Ryhter, 1956; Sverdrup *et al*., 2003).

The two main approaches to estimate primary production follow the uptake of carbon dioxide or the generation of oxygen (see equation 3). The changes in dissolved oxygen over time are generally measured by the light-dark bottle incubation method (Strickland & Parsons, 1972). Initial seawater samples are titrated to determine the oxygen concentration at the beginning of the sampling. The remaining samples are incubated during a suitable period in clear ("light") and opaque ("dark") bottles. The rates are then estimated by the following equations:

$$\text{Net Community Production} = [O_2]_{light} - [O_2]_{initial} \quad (4)$$

$$\text{Respiration} = [O_2]_{initial} - [O_2]_{dark} \quad (5)$$

$$\text{Gross Production} = [O_2]_{light} - [O_2]_{dark} \quad (6)$$

This method allows the discrimination between net and gross production, and the quantification of respiration. Primary production may be expressed in volumetric units or per unit area. Per unit area primary production can be estimated by taking samples from different depths, which are then depth-integrated to obtain the water column production

expressed in m^2 of sea surface. The more sensitive radiocarbon technique is generally preferred when primary production is low.

Carbon uptake and fixation is commonly monitored by adding the radioactive isotope ^{14}C to light and dark bottles that are then incubated (Steeman Nielsen, 1952). The sample is filtered after incubation and the ^{14}C incorporated in the phytoplankton is measured in a scintillation counter. Carbon uptake is calculated from the amount of radioactivity and the original concentration of unlabelled inorganic carbon in the water. The high sensitivity of this method allows short incubation times, therefore reducing the potential bottle artifacts associated to *in vitro* techniques. However, it cannot determine unequivocally which term is actually measured, generally expressing a rate between net or gross production.

Modelling can be used as a tool to simulate and predict primary production, although it is not a measurement in *sensu stricto*. Most predictive models relate the rate of production to light intensity (P-E curves), in which the initial slope, α, and the light-saturated rate of photosynthesis (P_{max}) are the fundamental parameters. Rates are usually determined by laboratory incubations over a light gradient. Curve-fitting is thereafter used to model the light dependence of photosynthesis. The vertical gradient of photosynthesis may be predicted by transferring this data to the underwater light profile. Per unit area production is then estimated by vertical integration (Parsons *et al*., 1984; Tilzer *et al*., 1993).

Bio-optical models attempt to predict phytoplankton photosynthesis from light and biomass data. Satellite-borne radiometers can measure the back-irradiance from the upper optical depth of the sea in selected wavelengths. Empirical algorithms are established between surface chl *a* and water-leaving irradiances, allowing remote sensing estimates of chlorophyll on global scales and further calculation of primary production (Smith & Baker, 1978; Balch *et al*., 1989).

There are several other estimation methods, none of which widely used. This includes the use of stable oxygen-18 that allows discrimination of photosynthesis and respiration, double-flash fluorometry that uses the natural chlorophyll fluorescence to make instantaneous photosynthesis estimates avoiding the need for containment and incubation, $^{15}NO_3$ assimilation *in vitro* allowing the distinction between "new" versus "regenerated" production (Platt & Sathyendranath, 1993), and the carbon-13 technique that has the advantage of using the stable isotope ^{13}C (Hama *et al*. 1983) rather than the radioactive isotope ^{14}C. Combination of techniques such as flow cytometry with molecular biology (like anti-body molecular probes) allow the estimation of cell-specific photosynthetic rates (Maestrini *et al*., 1993).

1.2. EUTROPHICATION

Eutrophication can be simply defined as the process of increasing the nutrient concentrations (particularly nitrogen and phosphorus) of a water body through natural or artificial means (Clark *et al*., 1997). Other definitions are introduced below in the "Anthropogenic Eutrophication" section. Cultural or anthropogenic (Gr. *anthropo*: man; La. *genic*: origin) eutrophication refers to eutrophication attributed to human actions.

Despite the fact that eutrophication can be a natural occurring phenomenon, the general usage is now related to the excessive anthropogenic eutrophication that is affecting several ecosystems throughout the world, including lakes, rivers, estuaries and coastal areas (Schindler, 1974; Diaz & Rosenberg, 1995; Andrén, 1999; Billen *et al*., 1999; Cloern, 2001). More than half of the world population lives in the coastal region inducing an increase in the levels of disposal of waste products from agriculture, domestic and industrial activities to coastal waters (Paerl, 1999). The evolution of the concept of eutrophication parallels as such the intensified pressure of cultural eutrophication. However, within the context of this work, eutrophication will be referring to both natural and human (anthropogenic) sources. This work will try to characterize these different types of eutrophication.

An Example of Natural Eutrophication: The Upwelling Event

An offshore water mass transport is produced when the wind blows nearly parallel to the coastline. This Ekman transport (Ekman, 1905) is a result of the Coriolis effect (Coriolis, 1835) in which the surface water is deflected ≈ 45º to the right of the wind's direction in the Northern hemisphere and to the left in the Southern hemisphere. The Coriolis effect can be summarized as the action of the centrifugal forces (set up by the Earth's rotation) in deflecting the bodies in motion to the right of their original direction in the Northern hemisphere, and conversely in the South. The deeper water of lower temperature and greater density replaces the surface water moving away from the coastline, creating a coastal upwelling current (Fig. 3). Winds can also drive the water toward the coast or make it converge with another water mass. These waters pile up and move downward to create a downwelling current (Barber & Smith, 1981; Sverdrup *et al*., 2003).

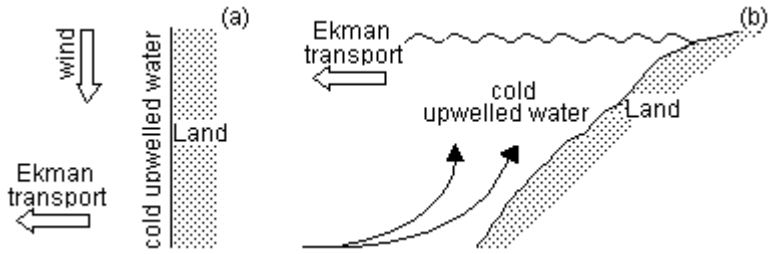

Fig. 3. Cross section: North winds along a west coast on the northern hemisphere causes an offshore Ekman transport of water (a). Plan view: The displaced water is replaced by cold nutrient-rich upwelled water (b). (from Stewart, 2004)

The deeper cold waters have high concentrations of nutrients essential to plant growth. Therefore, although upwelling regions make up only about 0.1% of the surface area of the ocean, they support high levels of primary production on a per unit area basis (Table 2).

Table 2. Global primary production in the marine environment.

Regions	PP * g C m^{-2} yr^{-1}	Ocean Area km^2	Area %	Total PP *
Upwellings	640	0.4 $x10^6$	0.1	0.2 $x10^9$
Coasts	160	54 $x10^6$	15	8.6 $x10^9$
Open oceans	130	307 $x10^6$	85	39.9 $x10^9$

*PP stands for Primay Production (from Smith & Hollibaugh, 1993).

This enhanced biological productivity by natural eutrophication processes can support a large biomass of commercially valuable fish. Upwelling also exerts a considerable influence on the local weather of the adjacent coastal regions. The cooler waters coming to the surface causes both surface water and land temperatures to drop significantly in periods of active coastal upwelling. Weather onshore of regions of upwelling tends to have fog, low stratus clouds, a stable stratified atmosphere, little convection, and little rain (Stewart, 2004).

Upwelling can be found along the west coasts of continents, along the equator, beneath cyclonic (counter clockwise) winds and adjacent to Langmuir-type cells (linear convergences in the open ocean). The major coastal upwelling regions are found along the eastern ocean boundaries, and the Somali and Arabian coasts (Fig. 4). The ocean currents that drive the main coastal upwelling include: the California current system off western North America, the Peru-Humboldt current system off western South America, the Canary and Iberian current system off northwestern Africa and Iberian Peninsula, the Benguela current system off southwestern Africa and the Somali current in the Indian ocean (Barber & Smith, 1981; Kudela *et al*., 2005).

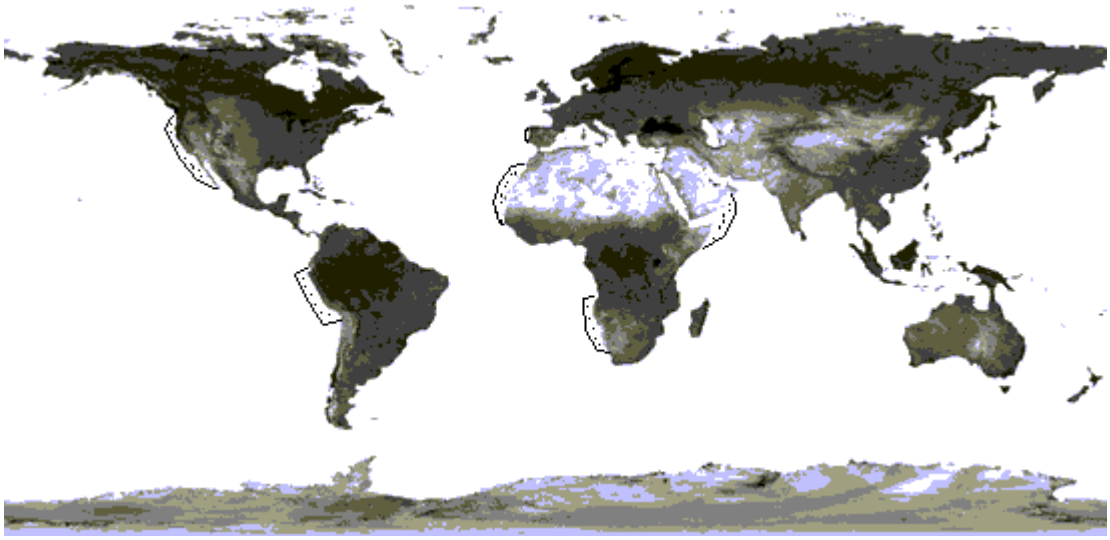

Fig. 4. Major upwelling regions of the world (shown by stippled areas). (modified from Barber & Smith, 1981).

Upwelling tends to be seasonal in temperate latitudes, where it peaks in the spring-summer, but tends to be year-round in the more tropical zones. Seasonal or longer variations in wind patterns (such as El Niño) lead to changes in upwelling that can impact fisheries. Also, the spatial variability of this process, as affected by coastline geometry and bottom topography, produces highly heterogeneous environments types. A coastal upwelling index (CUI) based on an estimate of the alongshore wind stress (the driving force for upwelling) has been used to indicate the variations of intensity (Bakun, 1973).

The primary production based on newly available N (nitrogen), i.e., N that originates from below the euphotic zone, is defined as "new" production. The dominant source for new production is nitrate. In upwelling areas the rate of new production is high. The recycled N within the euphotic zone (primarily in the form of ammonium and urea) is the basis for "regenerated" production (Dugdale & Goering, 1967).

Anthropogenic Eutrophication

Anthropogenic eutrophication has become one of the most serious threats to aquatic ecosystems around the world since the last half of the 20th century (Nixon, 1995; Andrén, 1999; Cloern, 2001; Jonge *et al*., 2002). Lakes and rivers are vulnerable areas but estuarine, coastal and marine ecosystems may also be affected. Problematic regions in Europe include the Baltic Sea (Cederwall & Elmgren, 1990; Bonsdorff *et al*., 1997; Kononen, 2001), the Greater North Sea (estuaries and fjords, the Wadden Sea, German Bight, Kattegat and eastern Skagerrak; EEA, 2001), the Irish sea (Allen *et al*., 1998) and estuaries and coastal lagoons of

the Iberian coast and the Mediterranean sea (Arhonditsis *et al*., 2000; Moncheva *et al*., 2001; Newton *et al*., 2003; Nuccio *et al*., 2003). The European Commission (EC) have issued several directives as a response, including the Urban Waste Water Treatment Directive (UWWT; Directive 91/271/EEC; EC, 1991a), the Nitrates Directive concerning the protection of waters against pollution caused by nitrates from agricultural sources (Directive 91/676/EEC; EC, 1991b), and the recent Water Framework Directive (WFD; Directive 2000/60/EC; EC, 2000), in order to prevent further deterioration and protect and enhance the environmental status of European aquatic systems.

Several definitions regarding anthropogenic eutrophication can be found. The Helsinki Commission (HELCOM, 2000) states eutrophication as:

> "*a condition in an aquatic ecosystem where high nutrient concentrations stimulate the excessive growth of algae, which leads to an imbalanced function of the ecosystem*".

The European Environmental Agency (EEA) defines eutrophication (EEA, 2001) following Nixon's criteria (Nixon, 1995):

> "*increase in the rate of supply of organic matter to an ecosystem, which most commonly is related to nutrient enrichment enhancing the primary production in the system*".

The EU definition of eutrophication is incorporated in the key Directive on UWWT (EC, 1991a):

> "*enrichment of water by nutrients, especially compounds of N and/or P, causing an accelerated growth of algae and higher forms of plant life, to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned*."

The original definition of eutrophication from the U.S. National Estuarine Eutrophication Assessment (NEEA) study (Bricker *et al*. 1999), developed by the National Oceanic and Atmospheric Administration (NOAA), was recently updated (Bricker *et al*. 2003b):

> *"a natural process by which productivity of a water body, as measured by organic matter, increases as a result of increasing nutrient inputs. These inputs are a result of natural processes but in recent decades they have been greatly supplemented by various human related activities. Cultural eutrophication, or nutrient overenrichment, is the enhanced accumulation of organic matter, particularly algae, that is caused by human related increases in the amount and composition of nutrients being discharged to the water body. A variety of impacts may result,*

including nuisance and toxic algal blooms, depleted dissolved oxygen, and loss of submerged aquatic vegetation and benthic fauna. These impacts are interrelated and usually viewed as having a negative effect on water quality, ecosystem health, and human uses. Management concerns should address the human, or cultural, portion of nutrient additions insofar as the additions are detrimental to the environment."

The degree of water quality will depend upon the system's assimilative capacity, namely, the coupling between algae production and its consumption and decomposition, and the degree of advective transport, in the case of open systems.

The main causes of anthropogenic eutrophication include (Paerl, 1997; Boesch & Brinsfield, 2000; Cloern, 2001):

- run-off of inorganic fertilizers from agricultural land (containing nitrates and phosphates).
- run-off of manure from farms (containing nitrates, phosphates and ammonia).
- run-off from erosion (following mining, construction work or poor land use).
- discharge of partially treated or untreated sewage (containing nitrates and phosphates).
- atmospheric deposition of N (originating from ammonia from animal husbandry and from combustion of fossil fuels in traffic, industry and households),

all of which result in deviation from the Redfield ratio (Redfield *et al*., 1963).

A cascade of possible consequences may follow, like (Paerl, 1997, Vollenweider, 1998; Danilov & Ekelund, 1999; Gacia *et al*., 1999; Moncheva *et al*., 2001) (Fig. 5):

- excessive and frequent phytoplankton blooms (enhanced primary production).
- increase in turbidity (cloudiness) of water.
- death of underwater rooted plants (seagrass).
- increase in the rate of sedimentation, as the uneaten organic material decays.
- excessive consumption of oxygen for decomposition processes by bacteria.
- development of hypoxic (Gr. *hypo*-: deficient) (Fig. 6) or anoxic (Gr. *an*-: absence of) conditions (referring to dissolved oxygen concentrations: anoxia < 0 mg l^{-1} O_2; hypoxia $> 0, \leq 2$ mg l^{-1} O_2; biologically stressful $> 2, \leq 5$ mg l^{-1} O_2) (Diaz & Rosenberg, 1995).
- death of fish and other organisms.
- decrease in species diversity.
- change in dominant biota (e.g. from diatoms to flagellates because of the decrease of silicate in Redfield proportions, essential to the development of diatoms; Conley *et al*., 1993).

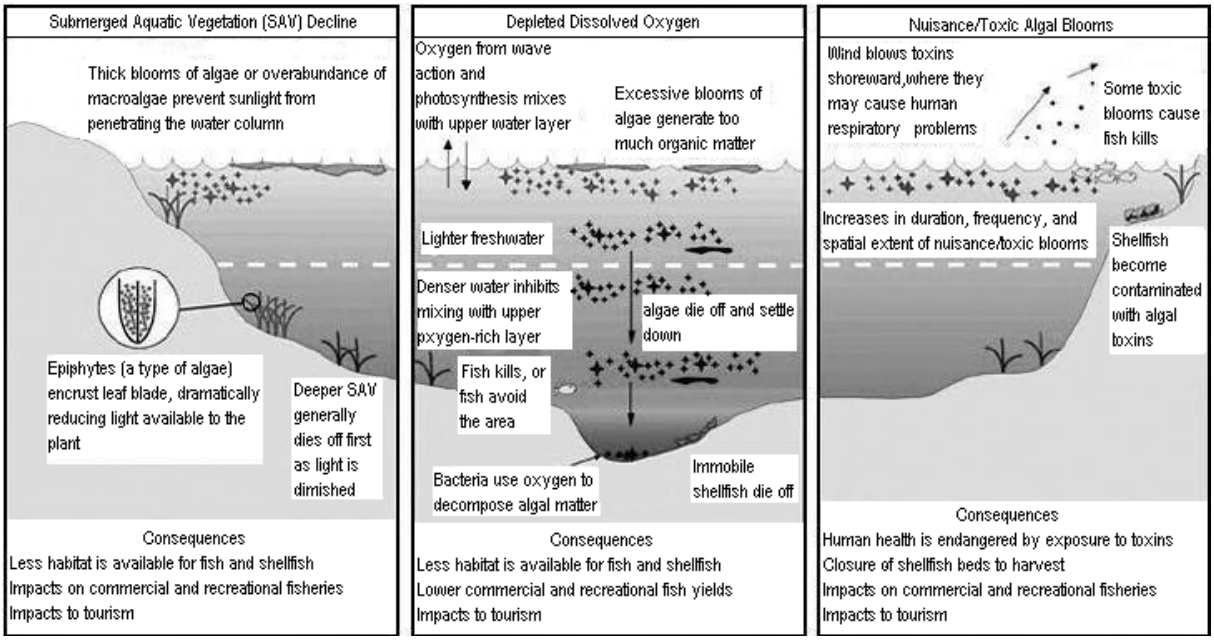

Fig. 5. Effects of eutrophication (Bricker *et al*., 1999).

- occurrence of toxic algal blooms.
- poisoning of fish, shellfish, birds and mammals (including humans).

Fig. 6. Global distribution of known oxygen depletion zones related to cultural eutrophication (modified from Diaz & Rosenberg, 1995).

- decrease in the amenity value of the water (e.g. it may become unsuitable for drinking or recreational activities).

Some phytoplankton blooms can also cause huge ugly foams on beaches. These nuisance algal blooms (e.g. *Phaeocystis* spp.) although not toxic, may temporarily ruin the beach reducing its recreational value and tourism incomes (Fogg & Thake, 1987).

Several models, indicators and indices have been proposed and used to assess the degree of eutrophication, e.g. the United States National Estuarine Eutrophication Assessment (NEEA; Bricker *et al.,* 1999), the Assessment of Estuarine Trophic Status (ASSETS; Bricker *et al*., 2003a), the European Environmental Agency (EEA) criteria (EEA, 1999a), the new trophic index (TRIX; Vollenweider *et al*., 1998), the estuarine quality index EQUATION (Ferreira, 2000) and the Oslo and Paris Conventions (OSPAR) Common Procedure (OSPAR, 1997) (see also chapter 3.1, pag. 111). The measurement of variables such as nutrients (inorganic+organic forms), chlorophyll *a*, dissolved oxygen in bottom water, water clarity (Secchi depth), levels of primary production, nuisance and toxic algal blooms, macroalgae and epiphytes, total organic carbon in sediments, are considered as related water quality parameters and its different combinations are part of the models mentioned above. The measured data can be displayed on maps, according to categories defined by pre-established criteria. These criteria are under evaluation by the environmental managers and policy makers of the EU member states. The development of relevant key indicators generally follows the DPSIR (Driving Force - Pressure - State - Impact - Response) framework for environmental reporting adopted by the EEA (EEA, 1999b), which makes a linkage between the resources, their pressures, states and responses, to detailing the management systems and policies that combine with these (Fig. 7) (Turner *et al*., 1998). Driving force indicators are related to the socio-economic factors leading to the pressures on the environment. State indicators highlight

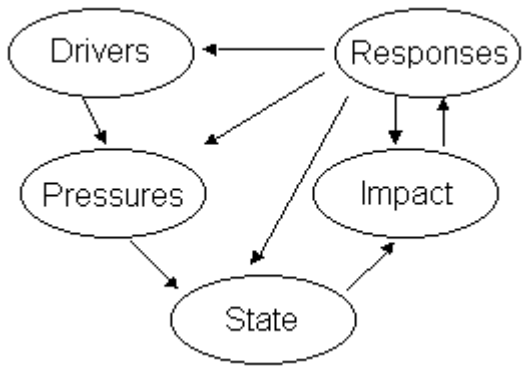

Fig. 7. Schematic model of the DPSIR framework for environmental reporting (from EEA, 1999b).

the environmental issues of concern. Impact indicators concern secondary effects caused by the environmental issues. Response indicators signify societal responses to the environmental problem.

Measures to reduce nutrient pollution of water bodies already attained some degrees of success in the EU, namely:

- reduction of N discharges from agriculture, due to the transposition of the Nitrates Directive to the national laws.
- reduction of N oxide emissions, by the use of catalytic converters on new cars**.**
- reduction of ammonia emissions, due to better management of animal wastes.
- reductions of P discharges, due to the transposition of the UWWT Directive to the national laws and the use of phosphate free detergents.

Some European countries already reduced in 50% their phosphorus discharges (OSPAR, 2003). However, it appears that phosphorus stored in sediments from earlier inputs is now being slowly released back into the water in some areas (e.g. Baltic waters) during hypoxic conditions (HELCOM, 2001). Nitrogen inputs to rivers from agricultural and sewage sources are still high (OSPAR, 2003). Although there was a decline of 25% in nitrate inputs in Europe's rivers from 1999 to 2001, 15% of the EEA selected monitoring stations showed an increasing trend in nitrate concentrations over the same (EEA, 2004). Pollutants can travel a long way from the place where they are emitted to the place where they are deposited, which can be in a different country. Such is the case of atmospheric deposition of nitrogen, which contribute 20-50% for the overall nitrogen loads to coastal waters (Rosenberg *et al*., 1990; Paerl *et al*. 1993). The nitrogen deposition to the Baltic Sea has a pronounced south – north gradient, owing to transport from the areas with high emission density in the northern part of the European continent (Hertel *et al*., 2003). Nitrogen oxide emmissions in the Baltic Sea area decreased 2% to 7.3% in six countries from the year 2001 to 2002, whereas no specific trend was found for ammonia emmisions (HELCOM, 2004). Transboundary environmental issues can only be solved by the development of European global actions in order to reduce pollution in coastal waters at an international level (Editorial, 2003).

1.3. OBJECTIVES AND THESIS STRUCTURE

Taking into consideration that one of the primary symptoms of eutrophication is the increase in algal biomass and primary production, the general objective of this work was to evaluate the dynamics between nutrient concentrations and ratios (specifically of nitrogen and phosphorus), and the activity and structure of the microphytoplankton assemblage. This was tested in the filed and by experimental work. The assemblage composition and the

relationship with other environmental factors were also assessed. In management terms, this work assesses the usage of the microphytoplankton assemblage and the selected supporting parameters as tools for the management of anthropogenic eutrophication in the studied systems. Are these elements sufficient to distinguish between Natural and Anthropogenic Eutrophication? Can these tools be used for management? Two scenarios, recently integrated in the intercalibration sites for the Common Implementation Strategy of the WFD, were selected. The Sagres area (SW Portugal) belongs to the mesotidal moderately exposed Atlantic coast type (Bettencourt *et al.*, 2004) surrounded by waters with high-dilution degree. The region is subject to minimal anthropogenic eutrophication impacts due to the low resident population (7000 inhabitants in an area of 179.76 km^2, 107.80 km^2 of which belongs to the National Park of the "Sudoeste Alentejano e Costa Vicentina"), which implies minimum levels of sewage and agricultural inputs to the seawater. There are also no permanent rivers in this area but only torrential streams (Peliz & Fiúza, 1999). Natural eutrophication processes, represented by the seasonal upwelling of cold nutrient-rich waters from May to September, are as such the main seawater fertilisation event in this location. The sampling station (chapter 2.1, Fig. 1, pag. 33) was in between the upwelling centre off Cabo S. Vicente and an offshore installation for bivalve aquaculture. The Ria Formosa (S. Portugal) is representative of a mesotidal shallow lagoon type (Bettencourt *et al.*, 2004) subject to both natural and anthropogenic influences. These include coastal adjacent upwelling episodes, agriculture and farming practice in the surrounding area, bivalve culture, urban discharges, recreational activities, dredging, etc. Three stations were selected to reflect contrasting conditions (chapter 3.1, Fig 1, pag 92): the Barra station was an oceanic inlet used as a control; the Ramalhete station was representative of a location subject to sewage discharges but with minimal land run-off influences; finally, the Ponte station was located in an upstream channel subject to land run-off from intense agriculture and chicken farming as well as golf resorts, bivalve aquaculture and recreational activities.

This dissertation is divided into five chapters. In this first chapter, background information on the general topics regarding primary production and eutrophication is provided. Chapter 2 is related to the study of natural eutrophication in Sagres, and it includes two sub-divisions. Chapter 2.1 introduces the published manuscript "Microplankton composition, production and upwelling dynamics in Sagres (SW Portugal) during the summer of 2001", Scientia Marina 2005, 69 (3): 323-341, presenting the results of the survey performed during the upwelling season, from May to September (2001), in the Sagres sampling station. The dynamics between the activity and structure of the microplanktonic assemblage with local

environmental factors, including upwelling conditions and consequent nutrient concentrations were evaluated. The relation between algal community and nutrients was further assessed in Chapter 2.2 (Enrichment Experiments and Primary Production at Sagres (SW. Portugal)), which describes the effects of nutrient additions over short-time small-volume scale experiments, performed in the Sagres station during an upwelling relaxation period (September 2002). Chapter 3 deals with the study set in the Ria Formosa coastal lagoon, and it includes two sub-divisions. Chapter 3.1 presents the published manuscript: "Boundary conditions for the European Water Framework Directive in the Ria Formosa lagoon, Portugal (physico-chemical and phytoplankton quality elements)", Estuarine Coastal and Shelf Science 2006, 67 (3) 382-398. The influence of nutrients and other environmental variables on the microplanktonic assemblage of the three selected stations was evaluated. The sampling was performed every 3 months following the requirements of the WFD, close to the summer and winter solstice, and the spring and autumn equinox, associated to large and small tidal exchanges, between June 2001 and July 2002. Sampling coincided with high and low water conditions. Chapter 3.2 corresponds to the published manuscript "Effects of nutrient enrichments on primary production in the Ria Formosa coastal lagoon (Southern Portugal)", Hydrobiologia 2005, 550: 29-45, in which the effect of nutrients on the algal production, biomass (as chl *a*) and composition is investigated, by conducting enrichment experiments over short-time small-volume scale, close to the summer solstices (June 2001 and July 2002) and autumn equinox (September 2001). A comparison between the two systems under study is presented in Chapter 4. The thesis finishes with Chapter 5 where the main conclusions of the investigation are outlined.

Comparative studies between places with different degrees of disturbance improve the understanding of the fundamental processes governing the ecosystem response to eutrophication mechanisms, thereby leading to the development of appropriate management strategies to conserve and avoid their over-exploitation and destruction, and also to the stimulation of new research and exploration of approaches. This investigation included several relevant quality elements required by the European WFD (EC, 2000) to assess the ecological status of coastal waters, including biological quality parameters (composition, abundance and biomass of the microphytoplankton community) and supporting physico-chemical parameters (nutrient concentrations, temperature, salinity, oxygen and water transparency). European countries are currently developing ecological quality ratios (EQR) in order to classify the status of waters into high, good, moderate, poor or bad classes. This study contributes to the on-going process for the implementation of the WFD along with

information to optimise monitoring plans for the WFD. It also contributes to the database of knowledge of the productive systems under study: Sagres and the Ria Formosa.

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2. NATURAL EUTROPHICATION: SAGRES

Ó mar salgado, quanto do teu sal
São lagrimas de Portugal!
Por te cruzarmos, quantas mães choraram,
Quantos filhos em vão resaram!
Quantas noivas ficaram por casar
Para que fosses nosso, ó mar!
Valeu a pena? Tudo vale a pena
Se a alma não é pequena.
Quem quere passar além do Bojador
Tem que passar além da dor.
Deus ao mar o perigo e o abysmo deu,
Mas nelle é que espelhou o céu.
Fernando Pessoa, Mar português, Mensagem, 1934

This chapter describes the studies performed in Sagres. Chapter 2.1 corresponds to the published manuscript: Loureiro, S., A. Newton and J.D. Icely. 2005. Microplankton composition, production and upwelling dynamics in Sagres (SW Portugal) during the summer of 2001, Scientia Marina, 69 (3): 323-341. Chapter 2.2 refers to the short-term small-scale enrichment experiments performed during a relaxation stage (September 2002) by the end of the upwelling season.

2.1. MICROPLANKTON COMPOSITION, PRODUCTION AND UPWELLING DYNAMICS IN SAGRES (SW PORTUGAL) DURING THE SUMMER OF 2001

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SUMMARY: Microplankton community, production, and respiration were studied alongside physical and chemical conditions at Sagres (SW Portugal) during the upwelling season, from May to September 2001. The sampling station was 5 km east of the upwelling centre off Cabo S. Vicente, and 2 km west of an offshore installation for bivalve aquaculture. Three major periods were distinguished according to sea surface temperature (SST): period 1 (P1; May and June), characterised by high temperature values (17.0±1.8 ºC); period 2 (P2; July), characterised by lower temperatures (14.6±0.3 ºC), identified as an upwelling-blooming stage; and period 3 (P3; August), characterised by a high temperature pattern (16.25±1.14 ºC). *Chaetoceros* spp., *Thalassiosira* spp., *Lauderia* spp., *Detonula* spp., and *Pseudo-nitzschia* spp. were the major taxa contributing to the dissimilarities between P2 (July) and the other periods. In July (P2), the average gross production (GP; 52.5±12.3 µM O_2 d^{-1}) and net community production (NCP; 46.9±15.3 µM O_2 d^{-1}) peaked with the maximal concentrations of diatom-chl *a*. Dark community respiration (DCR) remained low and more constant throughout (4.6±3.6 µM O_2 d^{-1}). Diatoms throughout the survey dominated the plankton assemblage. Physical events were the primary factors determining the microplankton structure and distribution at this location.

Keywords: Production, respiration, microplankton community, Iberian Peninsula, Cabo S. Vicente

RESUMEN. Composición del microplancton, producción y dinámica del aflojamiento en Sagres (Suroeste de Portugal) durante el verano de 2001. La comunidad microplanctónica, la

producción y la respiración, fueron estudiadas en Sagres (SE Portugal) durante la época de afloramiento, de Mayo a Septiembre 2001, junto con parámetros físicos y químicos. La estación de muestreo está a 5 km Este del centro de afloramiento del Cabo S. Vicente, y a 2 km Oeste de una instalación para el cultivo de bivalvos. Según los patrones de la temperatura del agua de superficie (SST) se diferenciaron tres periodos: periodo 1 (P1; Mayo y Junio), caracterizado por temperaturas altas (17.0±1.8 ºC); periodo 2 (P2; Julio), caracterizado por temperaturas más bajas (14.6±0.3 ºC), identificado como un estado de -afloramiento; periodo 3 (P3; Agosto), caracterizado por un patrón de temperaturas altas (16.25±1.14 ºC). *Chaetoceros* spp., *Thalassiosira* spp., *Lauderia* spp., *Detonula* spp., y *Pseudo-nitzschia* spp., fueron los principales grupos que contribuyeron a la diferenciación entre P2 (Julio) y el resto de periodos. Durante Julio (P2) la media de producción primaria bruta (GP; 52.5±12.3 μM O_2 d^{-1}) y de producción primaria neta (NCP; 46.9±15.3 μM O_2 d^{-1}) alcanzaron sus valores máximos, simultáneamente con el pico de diatomeas-chl *a*. La respiración de la comunidad en la oscuridad (DCR) permaneció baja y constante durante el muestreo (4.6±3.6 μM O_2 d^{-1}). La comunidad estaba dominada por diatomeas durante todo el muestreo. Los eventos físicos fueron el factor principal en la determinación de la estructura de la comunidad microplanctónica en esta localidad.

INTRODUCTION

Coastal fertilisation by cold nutrient-rich upwelled waters stimulates productivity and phytoplankton blooms (Barber and Smith, 1981). These blooms are dominated initially by non-motile diatoms (Officer and Ryther, 1980) that are preferentially selected under the turbulent conditions produced by strong winds, which are responsible for the upwelling. As the turbulence is reduced, optimal conditions develop for the more motile dinoflagellates, establishing the plankton succession pattern (Margalef, 1978; Smayda, 2000). The ocean biota is sustained by the balance between the autotrophic (i.e. production) and heterotrophic (i.e. respiration) processes (e.g., Williams, 1984, 1998). In coastal systems where inputs from terrestrial sources are limited, such as the studied location, phytoplankton primary production represents the main source of organic matter. Size fractionation studies (Williams, 1981) have associated the dominant respiratory activity in coastal waters with small non-photosynthetic organisms, such as heterotrophic bacteria and microflagellates.

Northerly winds along the west coast of the Iberian Peninsula produce conditions for seasonal upwelling from early spring to late summer (e.g. Wooster *et al*., 1976; Fiúza *et al*.,

1982), whilst occasional upwelling occurs along the southern coast of Portugal (Algarve) with favourable westerly winds. After a prolonged period of northerly winds, fertile water can circulate around the Cabo S. Vincente, the southwestern tip of the peninsula, and flow eastwards along the southern coastal shelf (Fiúza, 1983; Sousa and Bricaud, 1992; Relvas and Barton, 2002). In contrast, a warm counter current, originating in the Gulf of Cadiz (Fig. 1) flows westwards to the Algarve coast and, during periods of prolonged southeasterly winds, can circulate around Cabo S. Vincente and flow northwards (Relvas and Barton, 2002). In relation to the overall patterns of ocean circulation in the eastern Atlantic, the northern part of the west coast of the Iberian Peninsula is influenced by the subpolar branch of the Eastern North Atlantic Central Water (ENACWsp), whereas the southern upwelled waters have characteristics of the ENACW subtropical branch (Fiuza, 1984; Ríos *et al*., 1992).

The variations in phytoplankton abundance and composition between the northern and southern part of the west coast are primarily a consequence of the distinct topography of the continental shelves and river runoff (Peliz and Fiúza, 1999). In winter, the freshwater runoff induces salinity stratification on the wider and shallower shelf of the northwest coast, favouring the development of phytoplankton blooms. The peak for seasonal phytoplankton abundance occurs in spring and summer. The summer upwelling community is composed of chain-forming diatoms such as *Pseudo-nitzschia* spp. and *Chaetoceros* spp. (Moita, 2001).

These upwelling systems have supported an important fishery resource for the west coast of the Iberian peninsular. In the case of the Algarve, 12.1% of the total licensed fleet is located at Sagres (Martins and Carneiro, 1997; Pita *et al*., 2002). Furthermore, in recent years, a significant contribution to the local economy has come from the production of 300 tons of oysters at Sagres (Cachola, 1995; European Commission, 1999; pers. comm. Tessier). This aquaculture is dependent on the enrichment of the coastal waters by upwelling as there are no permanent rivers or streams in the area and the anthropogenic contribution is minimal because of the low resident population and limited agriculture.

Despite the importance of the Sagres region for Portuguese fisheries and bivalve culture, studies of production and associated phytoplankton community are scarce. Villa *et al*. (1997) reported a peak in May and September for phytoplankton based on estimates of chlorophyll *a* (chl *a*), and maxima for zooplankton between July and September based on plankton tows. Moita *et al*., (1998) have observed episodic blooms of toxic dinoflagellates east of Cabo S. Vicente along the Algarve coast. Sampayo *et al*. (1997) have detected biotoxins, leading to the temporary closure of oyster sales from Sagres.

This study was undertaken during the upwelling season, from May to September 2001, at Sagres, in order to understand the influence of the circulation and upwelling events on the local microplanktonic population and primary production. The monitoring includes several of the elements required by the European Water Framework Directive (WFD, 2000) to assess the ecological status of coastal waters including physico-chemical parameters (temperature, salinity, oxygen and transparency data) and biological parameters (composition, abundance and biomass) of the phytoplanktonic community.

MATERIAL AND METHODS

Study area

The Algarve coast along southern Portugal extends between 7º20'W and 9ºW, along 37ºN indented by two major canyons: S. Vicente and Portimão. The west coast off Algarve has an even narrow shelf, about 10 km wide. The sampling station (Fig. 1) was 5 km east of the upwelling centre off Cabo S. Vicente, at the entrance to the Porto Baleeiera at Sagres ($37^{o}00'63$" N and $8^{o}55'62$"W), and 3 km west of an offshore "long-line" system for oyster culture ($37^{o}00'40$"N and $8^{o}53'75$"W). Following the requirements of the Water Framework Directive (WFD, 2000), this area is classified as a mesotidal, moderately exposed, coastal water of the Atlantic type (Bettencourt *et al.*, 2004). The location was recently selected as an intercalibration site for the Common Implementation Strategy of the WFD.

Sampling

The Sagres station was sampled weekly, between the end of May and the beginning of September, with an interruption of 19 days in June. Surface water was collected early in the morning, independently of the tidal phase, and filtered through a 200µm mesh size net, to select for the microplankton community and remove the larger grazing organisms and particles. Aliquots for nutrients determination were frozen at -20 ºC for later analysis of ammonium, nitrite, nitrate, phosphate, and silicate, according to the methods described in

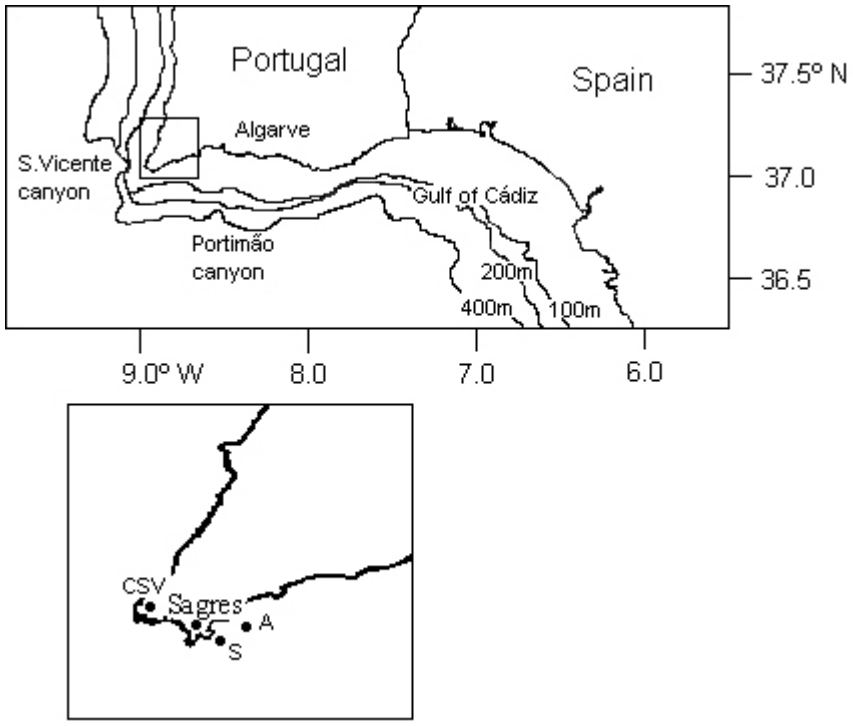

FIG. 1. Location of the sampling station (S). Cabo S. Vicente (CSV), oyster aquaculture (A).

Grasshoff *et al*. (1983). Chl *a* concentration was determined by further filtering 1 l of water sample, through a Whatman GF/F filter, for measurement with a Jasco FP-777 based on the fluorometric methods described by JGOFS (1994).

Water transparency was determined by Secchi-disc depth and used for the estimation of the percentage irradiation depth profile. In general, the euphotic zone (defined as the depth at which the light intensity is 1% of the intensity of the surface) was greater than the overall depth of the sample site, which averaged 20 ± 3m depending on tidal fluctuations. Water for the determination of the dissolved oxygen concentration was collected with a Niskin bottle from depths at which the light intensity was 100, 50, 25 and 10% of that at the surface. Oxygen concentrations were determined with triplicates of each sample by the Winkler method (Strickland and Parsons, 1972; Bryan *et al.*, 1976) using a Brand microburette for the titrations and expressing the final concentrations as µM O_2 (± SE).

Sea surface temperature (SST) was recorded with a Tinytalk PT 100 logger attached to a "long-line" for oyster culture. Total daily solar irradiance (KJ m^{-2}) was recorded by the Portuguese Instituto de Meteorologia (IM) at the Sagres station (8°57'W, 37°00'N, 25 m). Irradiance was converted to photosynthetically available radiation (PAR) using the criteria that PAR roughly represents 45% of total solar radiation (Kirk, 1994). PAR values for the surface layer were estimated based on the equation:

$I_z = I_0 e^{-k z}$ (1)

where I_0 is the incident radiation, I_z the radiation at z depth, and k the Secchi extinction coefficient (Kirk, 1994).

Apart from the 24 July, temperature and salinity profiles were recorded with a Seacat SBE 19 CTD between July and the end of the survey in September. The density (σ_t) was calculated from temperature and salinity data according to the algorithms of Fofonoff and Millard (1983).

Upwelling index

The Ekman transport of surface water was estimated according to Bakun's (1973) method, and used as a coastal upwelling index:

$$q_{x,\,y} = \frac{\tau_{x,\,y}}{f\rho_w} = \frac{\rho_a C_D |V| V_{x,\,y}}{f\rho_w} \qquad (2)$$

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 (8.78×10^{-5} s^{-1} for Sagres), and ρ_w is the density of seawater (~1025 Kg m^{-3}).

Wind direction and magnitude were obtained from the IM station at Sagres. The wind stress vector was divided into its two components (τ_x the eastward component, and τ_y the northward component), giving an estimation of q_x and q_y (m^3 s^{-1} km^{-1}) for Ekman transport. Positive values for q_x indicate upwelling-favourable offshore Ekman transport along the south coast, whereas negative values of q_x represent inshore Ekman transport on the south coast. Conversely, positive values of q_y indicate downwelling on the west coast, whilst negative values of q_y indicate upwelling-favourable offshore Ekman transport along the west coast.

Production and respiration rates

Production and respiration rates were estimated by the oxygenlight-dark bottle technique (Strickland and Parsons, 1972). The filtered samples were siphoned carefully into 300 ml Winkler bottles with silicon tubing to reduce turbulence. Triplicates were fixed immediately for measurement of initial dissolved oxygen concentrations. Triplicates of light and dark bottles were suspended along a 'long-line' and incubated for 24 h, after which they were fixed.

Gross production (GP), net community production (NCP) and dark community respiration (DCR) were determined from the difference between the means of the light, dark, and initial time replicates; rates are expressed as $\mu M\ O_2\ d^{-1}$ (±SE). Rates were converted to carbon units using 1.4 as the photosynthetic quotient (Laws, 1991).

Microplankton identification and carbon content

Microplankton samples were preserved with acidified Lugol's iodine solution. Each sample was placed in a 100 ml sedimentation chamber and settled for observation with a Zeiss Axiovert 25 inverted microscope. Qualitative and quantitative analyses of the samples were based on the methods of Utermöhl (1958). Smaller cells were identified (Tomas, 1997) and counted at 400x magnification up to a total of 100 optical fields, whereas the less abundant and larger organisms were observed over the entire chamber at 100x magnification. Organisms were generally identified down to genus and whenever possible to species level; whenever this classification was not possible cells were included in wider groups (see Table 3). Cell volumes were determined by approximation to the nearest geometric shape (Hillebrand *et al.*, 1999), and converted to biomass carbon units on the basis of formulae devised by Verity and Langdon (1984) and Verity et al. (1992).

Analysis of microplankton assemblage

A statistical study of the microplankton community was completed with PRIMER© software (Plymouth Routines In Multivariate Ecological Research) for a multivariate analysis of the microplankton community. An assessment of natural groupings within the community was completed by multi-dimensional scaling (MDS) ordination using the Bray-Curtis similarity matrices of square-root abundance and biomass data. Significance tests for differences between the *a priori* established groups were carried out using one-way analysis of similarities (ANOSIM in Clarke and Warwick, 2001). The contribution of taxa to dissimilarities between the different periods (see results for period's definition) were evaluated using the routine for similarity percentages (SIMPER in Clarke, 1993). The non-parametric statistical tests were done with the STATISTICA© 6 program.

RESULTS

Stages of the upwelling season

Three periods were distinguished on the basis of the changes in SST during the survey (Table 1): period 1 (P1), from 24 May to 10 July, corresponded to a high temperature stage prior to a persistent upwelling event; period 2 (P2), from 11 July to 31 July, was marked by lower temperatures corresponding to a major upwelling event; finally, period 3 (P3), from 1 August to 3 September, corresponded to a further stage of higher temperatures.

Wind and hydrographic conditions

Figure 2 summarises both the speed distribution and the direction of the wind, and Fig 3b

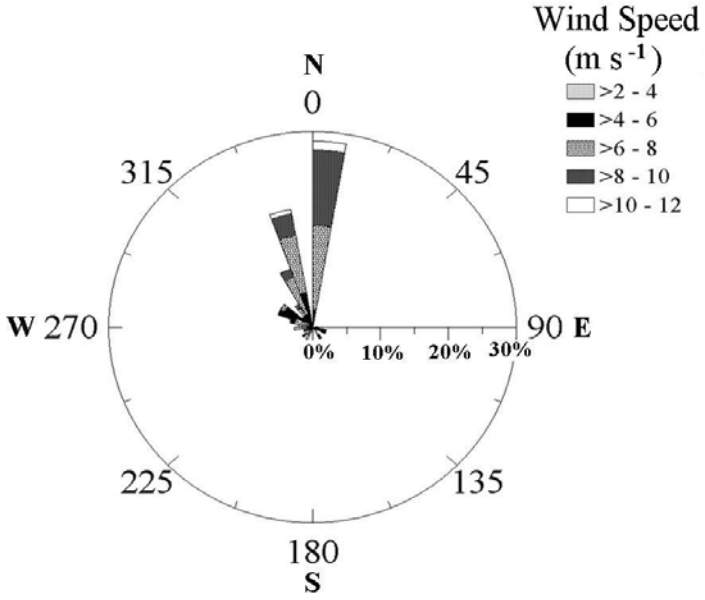

FIG. 2. Chart of wind direction and speed distribution (%), from May to September 2001, at Sagres.

is a stick vector diagram of the time-series for coastal wind speeds. Both figures show the prevailing northerly wind regime, from May to September, with average velocities of 6-10 m s^{-1}. At the beginning of May (P1), favourable conditions for upwelling on the south coast (inferred by q_x >0, Fig. 3a) induced a period of low SST (14 ºC), followed by conditions favourable for upwelling on the west coast (inferred by q_y < 0). The increasing SST by the end of May was related to a brief reversal in wind direction, leading to the replacement of cold water by warmer waters from the intrusion of the counter current from Cadiz towards the study site at Sagres. In June (P1), the generally high wind velocities and the persistent upwelling on the west coast (q_y decreased to -700 $m^3 s^{-1} km^{-1}$) were linked to a decline in local

TABLE 1. Surface values of physical, biological, and chemical parameters (± SD); *n* = number of observations; min - minimum value; max - maximum value; PAR - photosynthetically available radiation; Sal. – salinity; σ_t – density; photic = euphotic layer; T- temperature; NCP- net community production; GP- gross production; DCR - dark community respiration; chl *a* - chloroyphyll *a* concentration; $N\text{-}NH_4^+$ - ammonium concentration; $N\text{-}NO_2$ - nitrite concentration; $N\text{-}NO_3^-$ - nitrate concentration; $Si\text{-}SiO_4^{2-}$ - silicate concentration; O_2 - oxygen concentration.

Period	PAR	Sal.	σ_τ	Secchi	Photic	T	NCP	GP	DCR	Chl *a*	$N\text{-}NH_4^+$	$N\text{-}NO_2^-$	$N\text{-}NO_3^-$	$P\text{-}PO_4^{3-}$	$Si\text{-}SiO_4^{2-}$	O_2
	$10^3 KJm^{-2}d^{-1}$		Kg m^{-3}	m	m	° C		$\mu MO_2\ d^{-1}$		mg m^{-3}			μM			mg l^{-1}
P1 (*n*=5)	12.6±4.2	35.9±0.0 (*n*=2)	26.1±0.2 (*n*=2)	10±3	26±9	17.0±1.8	12.5±7.5	16.2±8.7	3.7±2.0	1.8±0.5	0.6±0.6	0.2±0.1	8.4±3.1	0.3±0.1	1.1±0.7	7.6±0.3
min	11.9	35.9	26.0	7	19	14.7	5.1	8.3	2.2	1.2	u.d.l.*	0.1	4.3	0.2	0.4	7.3
max	12.9	35.9	26.3	14	40	19.2	20.9	28.1	7.2	2.4	1.5	0.3	11.6	0.4	1.9	7.9
P2 (*n*=4)	11.1±1.5	35.8±0.1 (*n*=3)	26.6±0.2 (*n*=3)	8±1	21±3	14.6±0.3	47.5±14.5	53.0±9.9	5.5±5.2	5.6±0.6	0.3±0.2	0.2±0.1	12.7±5.9	0.3±0.1	0.6±0.4	8.2±0.5
min	8.9	35.7	26.4	7	19	14.3	26.3	39.6	1.8	4.8	0.1	0.1	6.2	0.2	0.1	7.9
max	12.2	35.8	26.7	9	24	14.9	57.1	60.6	13.3	6.2	0.5	0.2	19.3	0.4	1.0	8.9
P3 (*n*=5)	10.1±1.3	35.8±0.1 (*n*=5)	26.2±0.3 (*n*=5)	8±2	22±5	16.2±1.1	8.5±8.0	12.6±7.7	4.1±2.0	3.1±0.9	0.3±0.1	0.3±0.1	10.2±3.8	0.3±0.1	1.2±0.9	7.7±0.3
min	8.0	35.7	25.8	7	19	14.8	-3.9	2.4	1.0	1.7	0.2	0.1	5.9	0.2	0.3	7.2
max	11.1	35.8	26.5	11	30	17.5	16.4	19.9	6.3	4.0	0.4	0.4	16.0	0.4	2.4	7.9
Total (*n*=14)	11.3±15.3	35.8±0.1 (*n*=10)	26.6±0.4 (*n*=10)	9±2	23±6	16.0±1.6	21.1±19.7	25.4±19.8	4.3±3.1	3.3±1.7	0.4±0.4	0.2±0.1	10.3±4.3	0.3±0.1	1.0±1.7	7.8±0.4
min	8.0	35.7	25.8	7	19	14.8	-3.9	2.4	1.0	1.2	u.d.l.*	0.1	4.3	0.2	0.1	7.2
max	12.9	35.9	26.6	14	40	19.2	57.1	60.6	13.2	6.2	1.5	0.4	19.3	0.4	2.4	

u.d.l.* denotes under detection limits (NH_4^+<0.02 μM)

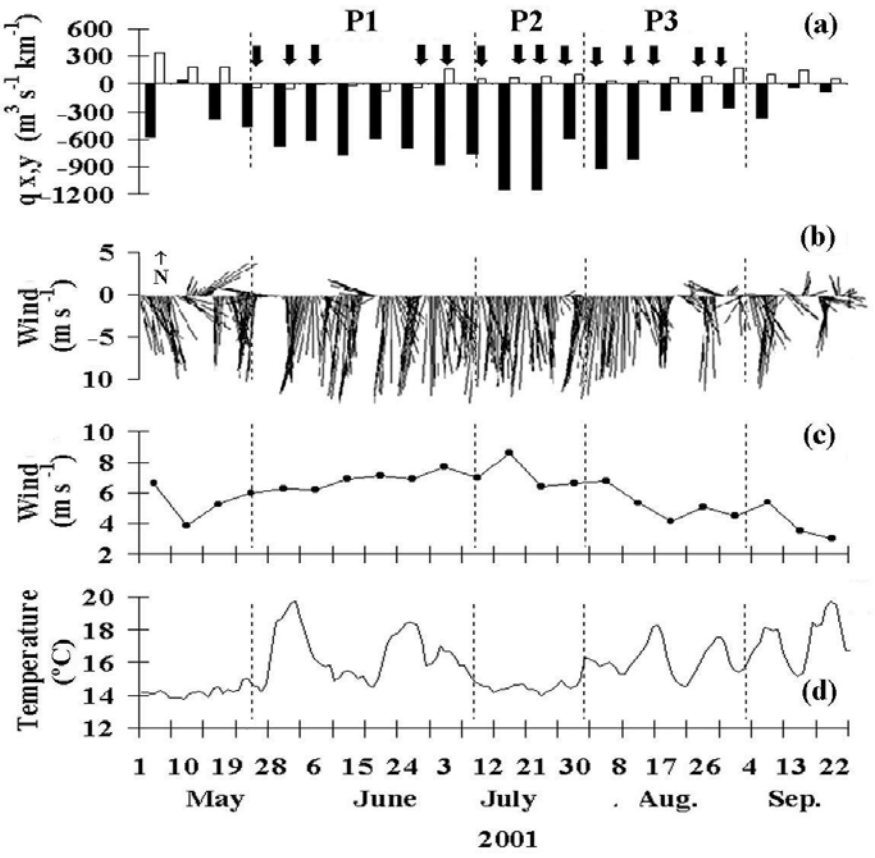

FIG. 3. Temporal evolution, of (a) mean weekly eastward (q_x , white bar) and northward (q_y, black bar) Ekman transport ($m^3\ s^{-1}\ km^{-1}$); positive q_x values indicates upwelling favourable conditions for the south coast, whereas negative q_y represents upwelling favourable conditions for the west coast; (b) wind vectors time series between May and September 2001; (c) mean weekly wind speed, and (d) sea surface temperature (SST; ºC) at the sampling location. Arrows indicate sampling dates. Period 1, 2 and 3 (P1, P2 and P3, respectively) mark stages of the upwelling season, defined according to the temperature ranges (see results).

SST (min. 15 ºC), suggesting the influence of western, upwelled cold waters on the Sagres site. An increase in SST was recorded in the last week of June (max. 18 ºC), probably reflecting the relaxation of the upwelling conditions on the south coast ($q_x < 0$) followed by the intrusion of the warmer coastal counterflow (07 and 25 June in Fig. 4). During the first few days of July (P2) an upwelling plume extended eastward from Cabo S. Vicente (03 June in Fig. 4) but had still not arrived at Sagres sampling station. In July (P2), SST reached the minimal values, associated with q_x and q_y favourable to offshore transport. On 11 July cold waters were mainly located south of the Cabo S. Vicente region, with a slight eastward advection extending up to Sagres (Fig. 4). During the rest of the month cold waters extended along the shelf off the Algarve. In August (P3), the SST at Sagres (Fig. 3d) reflected the variability of the wind regime with a cycle of upwelling / relaxation events with a duration of

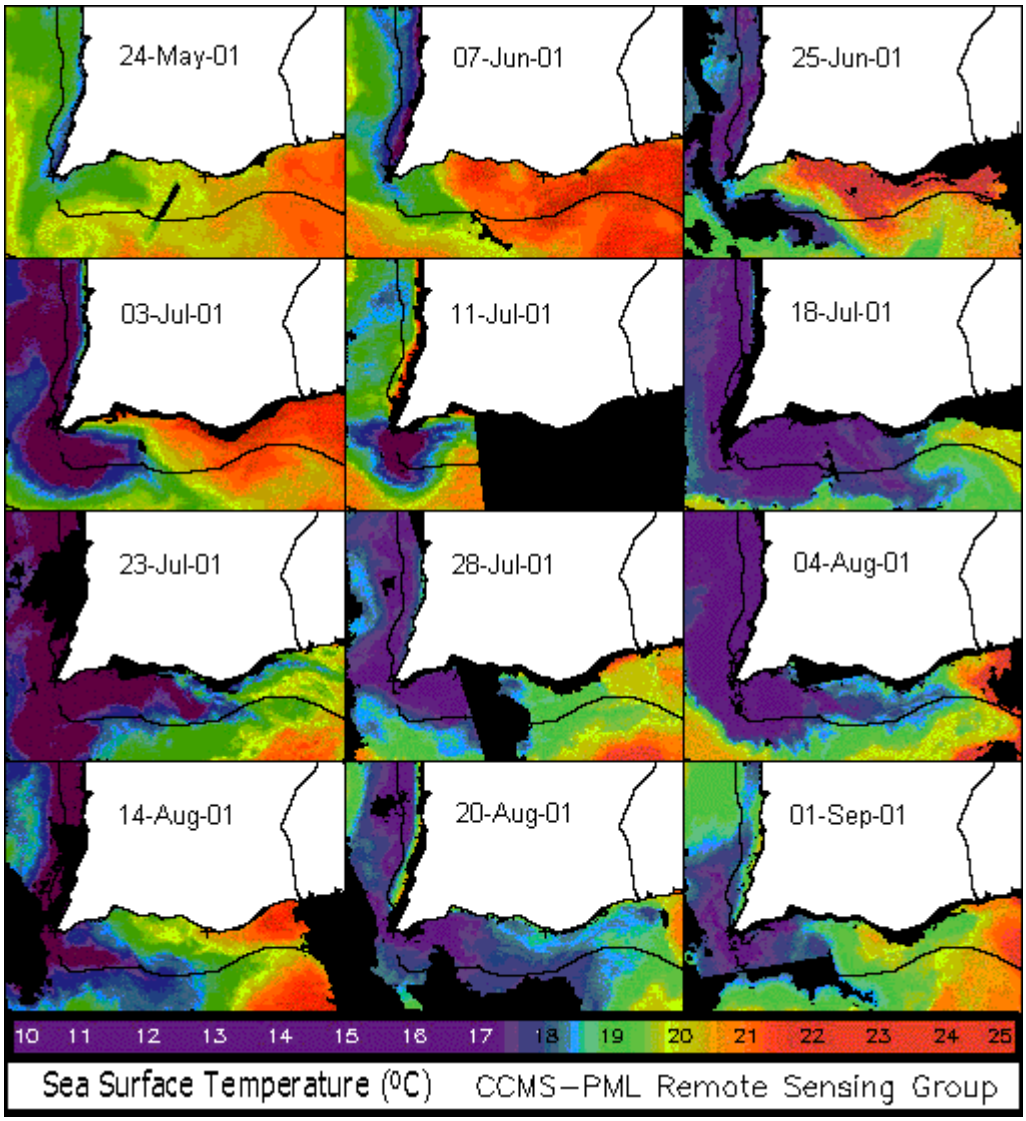

FIG. 4. Sea Surface Temperature (SST) satellite images (NOAA/AVHRR) from the South of Portugal (Algarve), processed at the Plymouth Marine Lab, UK. Dates are indicated in the images.

14 days. Along the south continental shelf, episodes of relaxation were associated with the influence of the warm counter current. The selected SST satellite images (Fig. 4) are representative of P1 (24 May – 3 July), P2 (11 July – 28 August) and P3 (4 August – 1 September).

Local SST was negatively correlated (Spearman; $p<0.05$) with average weekly values for q_x taken from the previous 7 days, and positively correlated with average weekly values for q_y (Table 2) over the period of the survey. Overall, the upwelling events adjacent to Sagres seemed to be influenced by the interplay between water circulation driven by the winds along the west and south coasts.

TABLE 2. Spearman rank-order correlation between biological, chemical and physical parameters determined during the sampling season: net community production (NCP), gross production (GP), dark community respiration (DCR), chlorophyll *a* concentration (Chl *a*), temperature (T), nitrate (NO_3^-), diatom ([diat]), dinoflagellate ([dino]), nanoflagellate ([nano]), ciliate ([cilia]) and total microplankton abundance ([total]) and biomass (BMdiat, BMdino, BMnano, BMcilia, Bmtotal), oxygen concentration (O_2), eastward (q_x) and northward (q_y) Ekman transport component. Bold underlined figures are significant at $p < 0.05$; *n* represents the number of samples.

n = 14	NCP	GP	DCR	Chl *a*	T	NO_3^-	[diat]	[dino]	[nano]	[cilia]	[Total]	BM diat	BM dino	BM nano	BM cilia	BM total	O_2	q_x	q_y
NCP	1																		
GP	**0.99**	1																	
DCR	0.03	0.08	1																
Chl *a*	**0.78**	**0.77**	0.15	1															
T	**-0.74**	**-0.70**	0.45	**-0.70**	1														
NO_3^-	0.32	0.25	**-0.56**	0.30	**-0.63**	1													
[diat]	**0.78**	**0.82**	0.33	**0.87**	**-0.60**	0.18	1												
[dino]	-0.44	-0.48	0.50	-0.20	**0.65**	-0.36	-0.30	1											
[nano]	-0.52	**-0.54**	-0.06	-0.41	0.31	0.11	-0.32	0.35	1										
[cilia]	-0.31	-0.24	**0.62**	-0.31	**0.64**	**-0.71**	-0.17	0.49	-0.20	1									
[Total]	**0.76**	**0.78**	0.45	**0.89**	-0.47	0.01	**0.96**	-0.05	-0.30	0.04	1								
BMdiat	0.44	0.44	0.20	**0.79**	**-0.55**	0.32	**0.75**	-0.21	-0.16	-0.27	**0.67**	1							
BMdino	-0.19	-0.25	0.27	-0.18	0.42	-0.38	-0.37	**0.65**	-0.14	0.38	-0.20	-0.24	1						
BMnano	-0.33	-0.38	0.03	-0.24	0.30	0.11	-0.24	0.49	**0.66**	-0.27	-0.17	-0.13	0.24	1					
BMcilia	-0.26	-0.23	0.25	-0.37	0.38	-0.47	-0.27	0.30	-0.02	**0.72**	-0.24	-0.15	0.43	-0.13	1				
BMtotal	0,43	0.41	0.21	**0.78**	-0.45	0.11	**0.63**	-0.12	-0.34	-0.19	0.59	**0.91**	0.06	-0.18	-0.08	1			
O_2	**0.66**	**0.65**	0.22	**0.78**	-0.51	-0.13	**0.66**	-0.13	-0.50	-0.06	**0.71**	**0.54**	0.08	-0.17	-0.09	**0.66**	1		
q_x	0,47	0.42	-0.22	0.53	**-0.67**	0.19	0.31	-0.22	-0.40	-0.30	0.30	0.31	0.07	-0.36	-0.12	0.40	0.43	1	
q_y	-0,48	-0.45	-0.00	-0.52	**0.56**	-0.42	**-0.57**	0.45	0.10	0.38	-0.41	**-0.75**	0.28	0.01	0.16	0.16	-0.45	0.01	1

Time-series of depth profiles

Figure 5 shows a series of depth profiles for temperature and O_2 measured during the survey.

24 May – 10 July (P1). At the beginning of the study the water column was homogeneously oxygenated (248±0.3 µM O_2, n = 12). On 31 May, the SST maximum was complemented by a minimal oxygen concentration at the surface (233±0.2 µM O_2). In June, the two available oxygen profiles presented similar distribution patterns, and by 3 July a subsurface (9.5 m) minimal oxygen value (222±0.2 µM O_2) indicated the possibility of intrusion at Sagres of oxygen-deficient, upwelled waters.

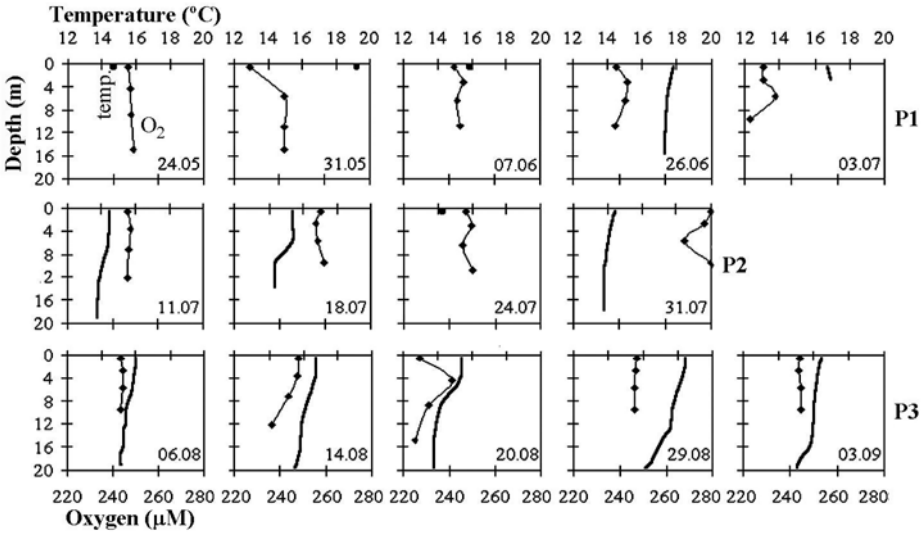

FIG. 5. Vertical profiles of oxygen concentrations (µM O_2), and temperature (ºC) taken from available CTD casts; when CTD casts were not available, temperature data are from a logger (see Material & Methods). Sampling date is shown on each plot. P1, P2 and P3 denote the three distinct upwelling periods.

11 July – 31 July (P2). This period of mature-upwelling was characterised by colder temperatures at all depths. On 11 July, the thermocline (0.2 ºC m^{-1}) was at a depth of 11 m. By 18 July, the surface water was warmer, and a steeper (0.4 ºC m^{-1}), shallower (6 m) thermocline had developed: salinity and density (σ_t) profiles (Fig. 6) demonstrated a stratification on this date. However, oxygen profiles were generally homogeneous. On 31 July, high pelagic oxygen concentrations (277±2 µM O_2, *n* = 12) probably reflected a recent active blooming phase. The pycnocline was associated with a less saline surface layer.

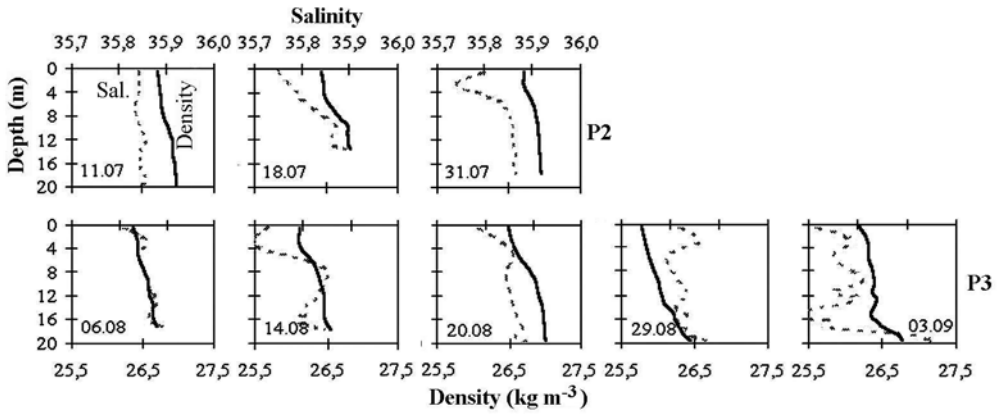

FIG. 6. Vertical profiles of salinity (dashed line), and density (dark line) taken from available CTD casts. Sampling date is shown on each plot. P2 and P3 denote the distinct upwelling periods.

1 August – 3 September (P3). The oxygen decreased, as is typical of post-blooming periods. On 14 August there was an increase in surface stratification (pycnocline of 5-10 m) associated with a warmer, less saline layer. This probably reflects the intrusion of the warm coastal countercurrent coming from the Gulf of Cádiz. On 20 August there was a steep shallow thermocline (4 m), below which was a layer of both low oxygen (227±1 μM O_2, n = 6) and low temperature (13.8 ºC). The subsurface oxygen maximum (241±0.2 μM O_2) was at 3 m adjacent to the thermic surface layer. The water column temperature rose towards the end of August (max. 18 ºC), but declined rapidly in September (min. 16 ºC), reflecting the relaxation / upwelling cycles referred to previously.

Physical, biological and chemical parameters

Table 1 summarises the ranges of surface physical, chemical and biological parameters. PAR was high throughout the survey, with a maxima during P1 (Fig. 7b). The density attained a maximum (26.7 kg m^{-3}) in July (P2), confirming the upwelling of denser water masses. The highest transparency values for the water column (Fig. 7a) were recorded at the beginning of the study and on 20 August (11 m). The depth of the euphotic layer, calculated from Secchi disk data, was 19-40 m in May-June (P1). This was reduced to 19-24 m during the July (P2) upwelling/blooming event and then increased in August (P3) to 19-30 m.

In May-June (P1), chl*a* surface values averaged 1.8±0.5 μg l^{-1}, followed by a significant increase during the July (P2; ANOVA $p < 0.0001$, *post hoc* LSD Fisher test) upwelling episode (Fig. 7a), with the maximum of 6.2 μg l^{-1}. The August (P3) decline (min. 1.7 μg l^{-1})

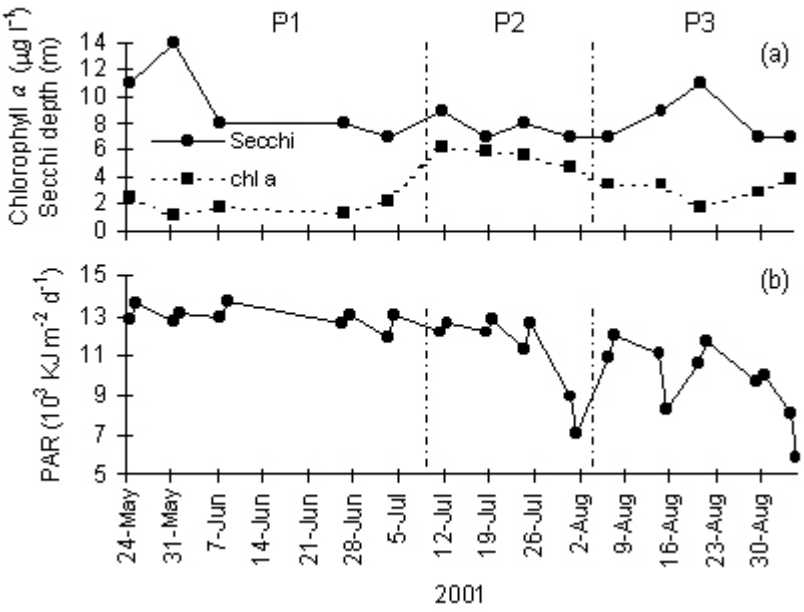

FIG. 7. Temporal distribution of surface (a) chlorophyll *a* concentration (µg l^{-1}), Secchi depth (m), and (b) photosynthetically available radiation (PAR; 10^3 KJ $m^{-2}d\text{-}^{1}$) from 24 May to 3 September 2001 at the Sagres station.

was followed by a steady rise until the end of the survey, implying the development of a new bloom.

The mean coefficient of variation (CV) for initial, "light", and "dark" oxygen bottles was 0.56% (*n* = 42), 0.49% (*n*=42), and 0.72% (*n* = 42) respectively. The mean of the standard errors for primary production and respiration rates were: 0.84 µM O_2 d^{-1} (*n*=14) for NCP, 1.02 µM O_2 d^{-1} (*n*=14) for GP, and 1.07 µM O_2 d^{-1} (*n*=14) for DCR. The distribution of production rates (Fig. 8a) exhibited a similar pattern to chl *a* (max. GP, 61±1.4 µM O_2 d^{-1}; NCP, 57±1.5 µM O_2 d^{-1}), although minimal values were observed in August (P3) instead of May-June (P1), both for NCP (-4±0.7 µM O_2 d^{-1}) and GP (2±0.8 µM O_2 d^{-1}). The NCP minimum corresponded to a period of net heterotrophy (negative NCP) on 14 August. A high significant correlation (Spearman, $p< 0.05$) between production and chl *a* (Table 2) was observed. DCR remained low throughout the survey (Fig. 8b), reaching its peak (13±1 µMO_2 d^{-1}) on 31 July. This date marked the end of a major bloom and was concurrent with a decrease in chl *a* and a minimal value for PAR. Rates in carbon units averaged 180± 169 mg C m^{-3} d^{-1} for NCP, 218± 170 mg C m^{-3} d^{-1} for GP, and 37± 26 mg C m^{-3} d^{-1} for DCR (*n*=14).

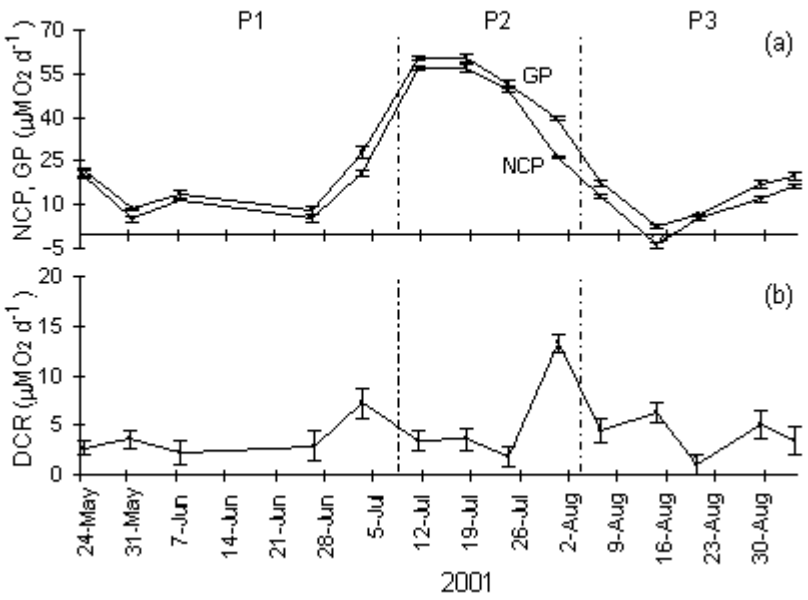

FIG. 8. Temporal distribution of surface (a) net community production (NCP), gross oxygen production (GP) and (b) dark community respiration (DCR) rates (μM O_2 d^{-1}), based on the light-dark bottle method, 24 h incubation, from 24 May to 3 September at the Sagres station. Bars correspond to standard errors; where the bars are not visible the data hid the small errors.

Pulses of nitrate-rich waters (> 12 μM) fertilised the surface from July to August (P2 and P3; Fig. 9a). Each pulse was followed by a decrease in concentration, suggesting an autotrophic consumption. There was a significant negative correlation (Spearman, $p < 0.05$) between nitrate and SST (Table 2). Ammonium, silicate, and phosphate (Fig. 9 a, b) remained low throughout the study; silicate reached a minimal value (0.1 μM) during the diatom-bloom in July (P2), and ammonium peaked twice in May-June (P1). Surface oxygen concentrations were significantly higher (ANOVA, $p = 0.041$, *post hoc* LSD Fisher test) in July (P2; max. 281±0.2 μM O_2).

Microplankton abundance, biomass and composition

A total of 58 microplankton taxa were identified (Table 3) during the survey. From the analysis of microplankton composition, four groups were distinguished: diatoms, dinoflagellates, ciliates, and nanoflagellates; the latter included Crytophyceae, Dictyochophyceae, and nanoflagellates. Bacillariophyceae were the best represented (32), followed by Dinophyceae (17), Ciliatae (5), Dictyochophyceae (2), Cryptophyceae (1) and

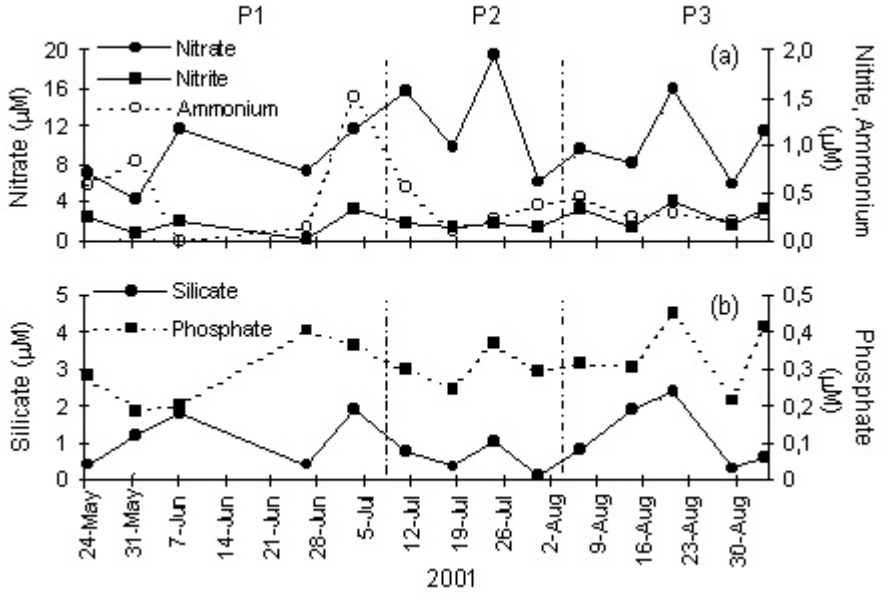

FIG. 9. Temporal surface distribution of (a) nitrate,ammonium, nitrite, (b) phosphate and silicate concentration (µM) from 24 May to 3 September at the Sagres station.

nanoflagellates (1). The highest occurrences (> 50%) were recorded for five diatoms *(Lauderia* spp., *Leptocylindrus* spp., *Rhizosolenia* spp., *Nitzschia* spp. and *Pseudo-nitzschia* spp.), two dinoflagellates *(Gymnodinium+Gyrodinium* spp., and *Protoperidinium* spp.), one ciliate (Oligotrichida) and the nanoflagellate group.

The temporal evolution of diatom abundance (Fig. 10a) showed a similar trend to those reported for chl *a* and production rates, reflecting the high significant ($p < 0.05$) correlation

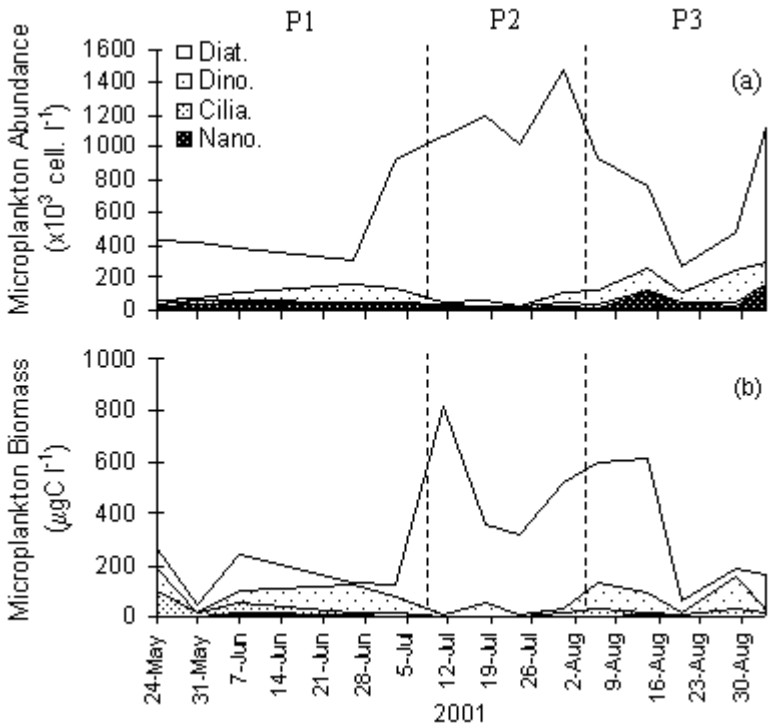

FIG. 10. Temporal distribution of (a) abundance (10^3 cell. l^{-1}) and (b) biomass (µg C l^{-1}), from the identified functional groups (diat = diatoms, dino = dinoflagellates, nano = nanoflagellates, cilia = ciliates) from 24 May to 3 September 2001 at the Sagres station.

TABLE 3. List of identified microplankton taxa, its codes, and frequency of occurrence during the survey. Most frequent taxa (≥50%) in **bold** underlined type.

Code	Taxa	Frequency (%)	Code	Taxa	Frequency (%)
	Bacillariophyceae (Diatoms)			**Dinophyceae (Dinoflagellates)**	
	Centrales		Ale	*Alexandrium* spp.	14
Ast	*Asteromphalus* spp.	14	Amp	*Amphidinium* spp.	**79**
Bac	*Bacteriastrum* spp.	21	Cer	*Ceratium* spp.	**79**
Cha	*Chaetoceros* spp.	**86**	Dic	*Dicroerisma psilonereiella*	14
Cos	*Coscinodiscus* spp.	36	Din	*Dinophysis* spp.	**57**
Dac	*Dactyliosolen* spp.	**71**	Gon	*Gonyaulax* spp.	7
Det	*Detonula* spp.	36	Gym	*Gymnodinium* spp.	**86**
Euc	*Eucampia* spp.	**57**	GmGr	*Gymnodinium+Gyrodinium* spp.	**100**
Gui	*Guinardia* spp.	**64**	Gyr	*Gyrodinium* spp.	**86**
GuiF	*Guinardia flaccida*	**57**	Kat	*Katodinium* spp.	36
GuiS	*Guinardia striata*	21	Oxy	*Oxytoxum* spp.	7
Hem	*Hemiaulus* spp.	**50**	ProC	*Prorocentrum* spp.	**57**
Lau	*Lauderia* spp.	**93**	ProP	*Protoperidinium* spp.	**93**
Lep	*Leptocylindrus* spp.	**100**	Scr	*Scrippsiella* spp.	**57**
Lic	*Licmophora* spp.	43	Tor	*Torodinium* spp.	36
Mel	*Melosira* spp.	29	DNs	Small < 20µm Unidentified	**79**
Odo	*Odontella* spp.	**57**	DNb	Big > 20µm Unidentified	36
Rhi	*Rhizosolenia* spp.	**100**		**Ciliatae**	
Ske	*Skeletonema* spp.	**57**	Hap	Haptorida	**50**
ThaS	*Thalassiosira* spp.	**64**	Mes	Mesodiniidae	**57**
DCs	Small <20 µm Unidentified	**79**	Oli	Oligotrichida	**100**
DCb	Big >20 µm Unidentified	**79**	Tin	Tintinnina	36
	Pennales		Cil	Unidentified	43
AstP	*Asterionellopsis* spp.	21		**Cryptophyceae**	
Dip	*Diploneis bombus*	29	Cry	Cryptomonadales	**86**
Fra	*Fragilariopsis* spp.	29		**Dictyochophyceae**	
Man	*Manguinea* spp.	29	Dic	Dictyochaceae(Sillicoflagelates)	29
Meu	*Meuniera membranacea*	29	Ped	Pedinellaceae	36
Nav	*Navicula* spp.	**71**		**Nanoflagellates**	
Nit	*Nitzschia* spp.	**100**	Nan	Unidentified	**100**
Ple	*Pleurosigma* spp.	21			
PSN	*Pseudo-nitzschia* spp.	**93**			
ThaN	*Thalassionema* spp.	21			
DPb	Big (>20 µm) Unidentified	**71**			

between these variables (Table 2). Maximum diatom abundance was recorded in July (P2; range: 936-1366 x10^3 cell. l^{-1}), and at the end of August (P3). Highest abundances for dinoflagellates were observed on 26 June (110 x10^3 cell. l^{-1}) and at the end of the sampling season (> 130 x10^3 cell. l^{-1}). Diatom abundance was significantly higher in July (P2; ANOVA $p = 0.002$, *post hoc* LSD Fisher), whereas dinoflagellate abundance was significantly higher in August (P3; ANOVA $p = 0.02$, *post hoc* LSD Fisher). Ciliate abundances remained low (<

30 x10^3 cell. l^{-1}) throughout the study, whereas nanoflagellates peaked (> 100 x10^3 cell. l^{-1}) during P3, on 14 August and 3 September.

Temporal variations in biomass are shown in Fig. 10b: diatom biomass reached mean values of 62±53 (n = 5), 479±238 (n =4) and 240±236 (n = 5) μgC l^{-1} for May-June (P1), July (P2) and August (P3) respectively. Dinoflagellate biomass reached higher values in May-June (P1) and August (P3). The contribution of nanoflagellates to the biomass was low throughout the study (maximum < 15 μgC l^{-1}), whilst the biomass of the oligotrichida and tintinnina ciliates was dominant in May-June (P1) and August (P3).

The changes in relative composition of the systematic groups in each period are evaluated in Fig. 11 a, b. There was a clear dominance of diatom abundance throughout the survey,

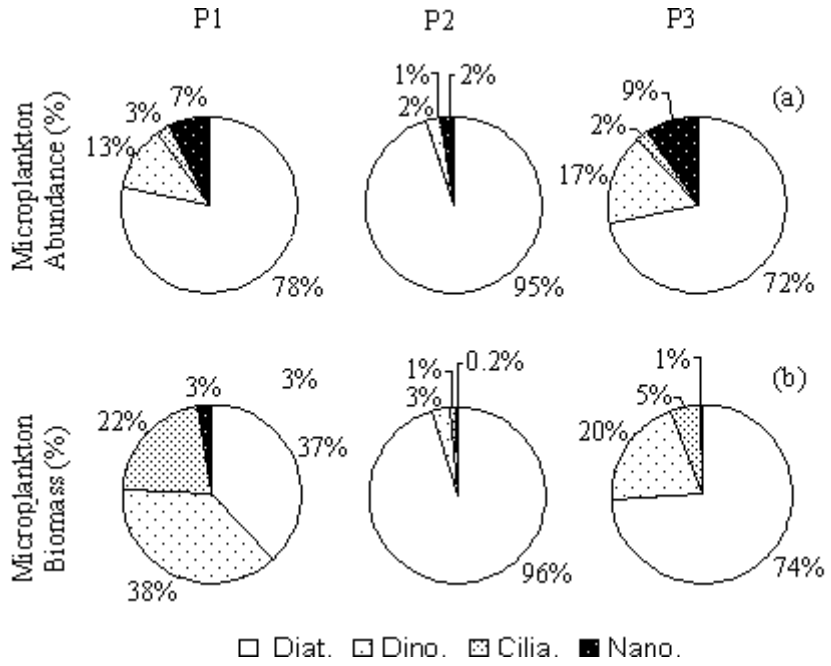

FIG. 11. Relative (a) abundance and (b) biomass of the microplankton groups in each period (P1, P2 and P3). Each segment represents the total percentage of the respective group (diat = diatoms, dino = dinoflagellates, nano = nanoflagellates, cilia = ciliates).

reaching a peak (95%) in July (P2). May-June (P1) showed a biomass with a balanced composition of diatoms (37%), dinoflagellates (38%) and ciliates (22%). The biomass contribution of dinoflagellates in May-June (P1) was mainly due to *Protoperidinium* spp. and

Ceratium spp. Tintinnina and oligotrichida were confirmed as the principle contributors to the biomass of ciliates .

Statistical assemblage analysis

The MDS plots evidenced three distinct groupings corresponding to the May-June (P1), July (P2), and August (P3) stages, both for abundance (Fig. 12 a, b) and for biomass (Fig. 12 c, d). The global R (a statistical measure of the degree of separation of groups) resulting from

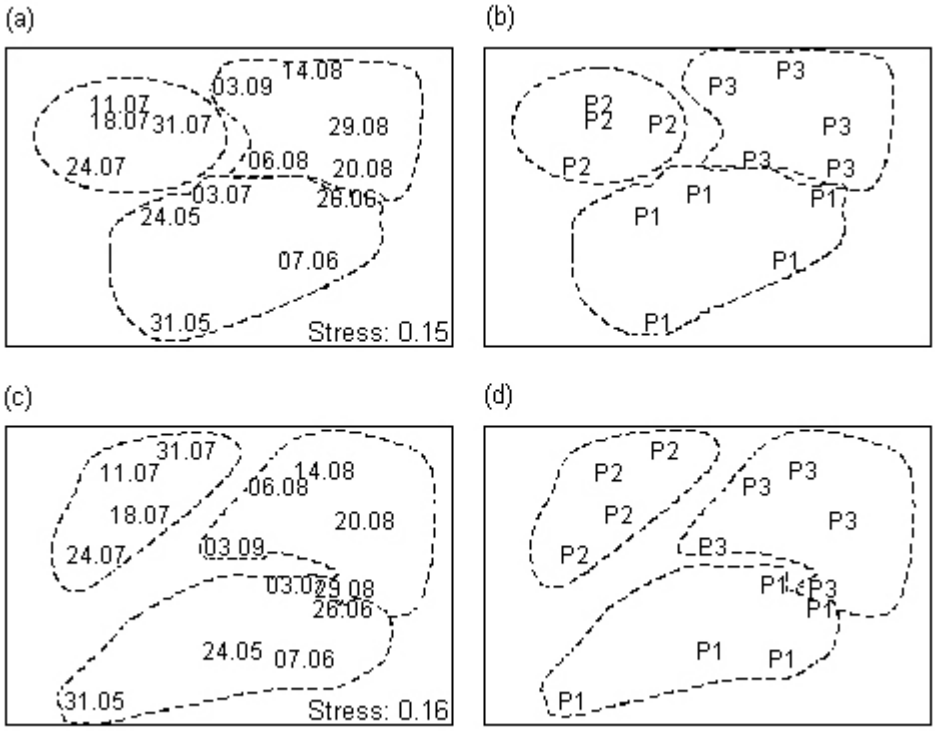

FIG. 12. Two dimension MDS ordination of Bray-Curtis similarities, from square root transformed abundance (a), (b) and biomass (c), (d). Numbers correspond to sampling dates (day.month). P1, P2, and P3 (the defined sampling periods) groupings are delimited on each plot.

the one-way ANOSIM tests for abundance data (Table 4) implied the rejection of the null hypothesis (no assemblage differences between P1, P2 and P3) at the 0.002 significance level. However, the pairwise R values (resulting from the comparison of the specific pairs of groups) showed a weak separation (R = 0.37) between the community structures in May-June (P1) and August (P3). The May-June (P1) and July (P2) groups were significantly different (R > 0.5); finally, July (P2) and August (P3) showed a well-separated community composition

TABLE 4. One-way ANOSIM test for microplankton assemblage differences (in square root transformed abundance and biomass data) between the three *a priori* groups (P1, P2 and P3).

Periods	R pairwise test	Possible Permutations	Significance level
(a) Abundance			
Global R	0.574	999	0.002
P1-P2	0.563	126	0.024
P1-P3	0.368	126	0.008
P2-P3	0.825	126	0.008
(b) Biomass			
Global R	0.589	999	0.001
P1-P2	0.625	126	0.016
P1-P3	0.452	126	0.016
P2-P3	0.756	126	0.008

for abundance ($R > 0.75$). Biomass followed a similar statistical pattern to the community composition.

The result of SIMPER analysis is represented on Table 5. The highest average dissimilarities were found between July (P2) and August (P3) for abundance ($\delta = 54.45$), and between May and June (P1) and July (P2) for biomass ($\delta = 66.01$). May-June (P1) and August (P3) were the most similar periods for both abundance and biomass data, confirming the values obtained in the ANOSIM test and the MDS ordination. *Chaetoceros* spp., *Thalassiosira* spp., *Lauderia* spp., *Detonula* spp., and *Pseudo-nitzschia* spp. were the main taxa contributing to the dissimilarities between July (P2) and the other periods. Figure 13 shows the temporal distribution of the main taxa contributing to the abundance and biomass dissimilarities between the different periods.

Potentially HAB organisms

Identification was mainly done down to genus level, so differentiation of harmful species within each taxa was not detected. Nevertheless, Table 6 presents the temporal distribution of algal taxa associated with harmful algal bloom (HAB) events identified during the survey at Sagres. *Pseudo-nitzschia* spp., a taxon that includes toxic species associated with amnesic shellfish poisoning (ASP; Bates *et al.*, 1998), had the highest values for abundance in July (P2; $178 \pm 58 \times 10^3$ cell. l^{-1}). Water discolorations, commonly called red tides, are produced by *Ceratium* spp., *Gonyaulax* spp., and *Scrippsiella* spp., amongst other organisms (Pitcher and Calder, 2000; Smayda, 2000). These blooms, although non-toxic, are undesirable because they may cause fish and invertebrate killings due to oxygen depletion, following the decay of

TABLE 5. Taxa contribution (%) to the average (a) abundance and (b) biomass Bray-Curtis dissimilarity (δ), between the three defined sampling periods (P1, P2 and P3). Data were square root transformed. Taxa were selected until ~50% of the cumulative dissimilarity was attained (for taxa codes see Table 3).

Taxa Code	P1 and P2 (%)	Taxa Code	P1 and P3 (%)	Taxa Code	P2 and P3
(a) Abundance	δ = 51.01		δ = 48.60		δ = 54.87
Cha	16.79	Lep	8.05	Cha	13.38
ThaS	7.34	Cha	6.00	ThaS	5.89
Ske	5.73	Rhi	5.85	Lep	5.84
PSN	5.03	Ske	5.45	Ske	5.62
Lau	3.62	PSN	5.03	PSN	4.70
Det	3.31	Cry	4.01	Rhi	4.52
DPb	3.15	DNs	3.37	Lau	3.77
Lep	3.08	GmGr	3.06	GmGr	3.19
GuiS	2.55	Gym	2.99	Cry	2.99
		ProC	2.98		
		DPb	2.94		
Cumulative δ %	50.61		49.73		49.92
(b) Biomass	δ = 66.01		δ = 58.98		δ = 62.60
Cha	9.27	Rhi	10.12	ThaS	7.73
ThaS	8.47	ProP	5.54	Rhi	7.60
Gui	5.35	Tin	4.42	Cha	7.50
Lau	5.35	DNb	3.78	Lau	5.73
Det	5.08	GuiF	3.72	Gui	5.44
ProP	4.28	Cha	3.54	Det	5.06
Cos	4.22	Cer	3.40	DNb	3.23
Tin	3.51	Oli	3.28	Cer	3.20
Cer	3.33	Cos	2.93	Cos	2.92
GuiF	2.59	DNs	2.89	ProP	2.82
		Lau	2.78		
		Gui	2.64		
		GmGr	2.60		
Cumulative δ %	51.44		51.64		51.23

the blooms. *Ceratium* spp. occurred at low values (< 4 x10^3 cell. l^{-1}) and was basically characteristic of May-June (P1) and August (P3). *Gonyaulax* spp. only occurred once in May-June (P1) and August (P3). *Gonyaulax* spp. only occurred once in May-June (P1), and *Scrippsiella* spp. was prominent in August (P3; 0.3 - 16.8 x10^3 cell. l^{-1}). Organisms with the potential to cause paralytic shellfish poisoning (PSP), such as *Alexandrium* spp. and *Gymnodinium* spp., were also recorded. *Alexandrium* spp. occurred only in low numbers in August, whilst *Gymnodinium* spp. occurred throughout the survey, with the greatest abundance in May-June (P1) and August (P3; 47 x 10^3 cell l^{-1}). *Dinophysis* spp. and *Prorocentrum* spp., related to diarrhetic shellfish poisoning (DSP), were absent in July (P2), but occurred in May-June (P1) and August (P3).

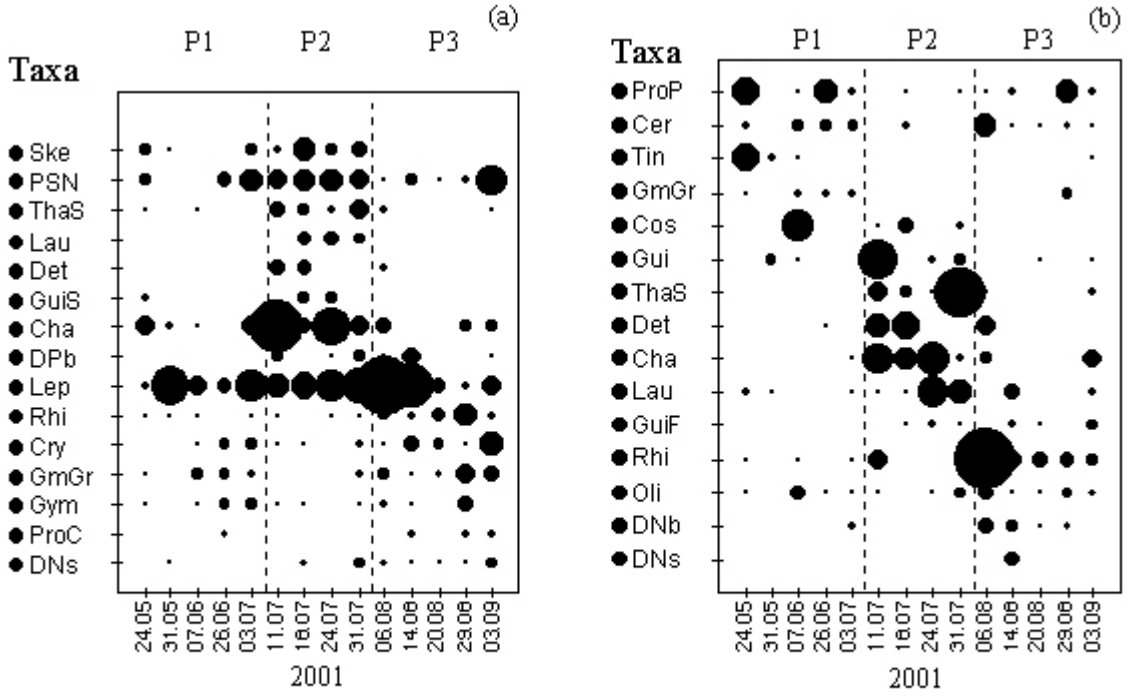

FIG. 13. Temporal distribution of (a) abundance and (b) biomass, of the main taxa contributing to Bray-Curtis dissimilarities between the defined sampling periods: P1, P2 and P3 (see Table 3 for taxa codes). Circles are proportional to abundance (max. 637 10^3 cell. l^{-1}) and biomass (max. 390 µgC l^{-1}) values; to avoid overlapping of circles, they represent 50% of their original size, as such, the absence of a bubble does not necessarily mean no occurrence, but that the relative abundance is low.

DISCUSSION

Physical events and microplankton assemblage

Although the lack of sufficient vertical data limits an understanding of the whole dynamics in three-dimensions of the study site, the results show that during the upwelling season the Sagres region is influenced by the wind-driven circulation along the south and west coast, which forces cold, upwelled water into the surface layer. Upwelled water masses have characteristics of the ENACW subtropical branch (temperature > 13 ºC, σ_t < 27.1 kg m^{-3}). These findings are consistent with the patterns already described for the Algarve coast (Fiúza, 1983, 1984; Sousa and Bricaud, 1992). Winds are mostly moderate (6-8 m s^{-1}), and relatively intense velocities (8-10 m s^{-1}) are only registered in July (P2), revealing a decrease in wind stress conditions in comparison with previous years (Relvas and Barton, 2002). The influence

TABLE 6. Abundance of potentially HAB organisms (Hallegraeff, 1995; Pitcher and Calder, 2000; Smayda, 2000) from May to September (2001) at Sagres station. See Table 3 for taxa codes.

Sampling Period	Sampling Date	Taxa Codes							
		PSN	Ale	Cer	Din	Gon	Gym	ProC	Scr
		10^3 cell. l^{-1}							
P1	24-May	32.9	-	0.7	0.7	-	6.7	-	-
	31-May	-	-	0.7	-	-	-	-	-
	07-Jun	2.2	-	3.3	0.7	1.9	7.4	1.3	1.3
	26-Jun	45.0	-	1.7	1.0	-	26.7	14.3	1.7
	03-Jul	105.1	-	2.6	2.6	-	39.7	1.3	-
P2	11-Jul	76.7	-	-	-	-	6.5	-	-
	18-Jul	104.1	-	0.7	-	-	3.9	-	-
	24-Jul	128.9	-	-	-	-	-	-	-
	31-Jul	71.8	-	-	-	-	3.3	-	3.8
P3	06-Aug	7.8	-	3.6	1.3	-	21.7	1.2	3.3
	14-Aug	29.6	11.2	2.4	-	-	4.9	19.8	3.6
	20-Aug	4.0	0.7	0.7	0.3	-	1.3	1.5	0.3
	29-Aug	22.3	-	2.3	2.3	-	47.2	19.3	16.8
	03-Sep	209.4	-	1.9	1.3	-	1.3	23.3	8.4

- defines no occurrence

of the warm counterflow on the south coast during episodes of relaxation (Relvas and Barton, 2002) has been noticed on several occasions.

Chl *a* peaks earlier (July) than has been reported (September) for the same area by Villa *et al*. (1997), probably owing to the interannual variability of physical factors (Peliz and Fiúza, 1999). The seasonal values for chl *a* and chemical parameters are in general agreement with the ranges described for the Cabo S. Vicente region (Moita, 2001). However, lower values of phosphate and silicate may imply the occurrence of a spring-bloom before the beginning of the survey. The maximal values for chl *a* (6.2 μg l^{-1}) attained in July are similar to those reported for the upwelling regions of NW Spain – La Coruña (6.7 μg l^{-1} Casas *et al*., 1999) and Chile (6.2 μg l^{-1}, Daneri *et al*., 2000), but lower than those of other upwelling systems such as Orgeon (1-57 μg l^{-1}, Dickson and Wheeler, 1995), Benguela, NW Africa, and off Peru (5-50 μg l^{-1}, Andrews and Hutchings, 1980; Estrada, 1974; Blasco, 1971 respectively). The lack of correlation between the Secchi-depth and chl *a* (Fig. 7), particularly during the bloom stage in July (P2) when Secchi values did not decrease as expected, may be due to several factors. The Secchi-disk depth is a measure of the concentration of light attenuating particles

in the water column, whether from phytoplankton or non-phytoplankton sources. Factors contributing to the variation in Secchi-depth include the sun angle, sea surface reflectance and tidal height (Edmonson, 1980; Preisendorfer, 1986, Borkman and Smayda, 1998). In the current study, observations have been made independently of tidal phase. It may also be associated with variability in changes in chl *a* content per cell, carbon:chl *a* ratio, or chl *a* to accessory pigment ratio (Falkwoski and LaRoche, 1991).

Several upwelling pulses were registered from late spring to late summer (Fig. 3). The first pulse in June (P1) fertilises the surface water with nutrients, but its evolution was not followed by this survey. A more persistent-active upwelling event develops in July (P2), fertilising the surface with concentrations of nitrate up to 19 μM. This value is higher than that reported for NW Spain (La Coruña, 9.8 μM, Casas *et al.*, 1999; Ria de Vigo, 12μM, Moncoiffé *et al.*, 2000).

Diatom biomass and density is dominant throughout the survey, and its temporal evolution is positively correlated (Spearman, $p<0.05$) with chl *a* and negatively correlated with SST, implying an association with cold waters supplied by upwelling. The maximal diatom abundance (1366 $x10^3$ cell. l^{-1}) is typical for other upwelling regions (10^6 cell. l^{-1}, refs. in Moita, 2001): NW Iberian-Galicia (Estrada, 1984), NW Africa (Blasco *et al*., 1980), Peru (Blasco, 1971) and Benguela (Giraudeau *et al*., 1993). The persistent diatom-chl *a* peak (≈ 21 days in July, P2) is related to prolonged conditions favourable to upwelling. This group is adapted to turbulent conditions (Margalef, 1978). The fact that ammonium peaks are not coincident with oxygen minima, together with the predominance of low ammonium levels (< 0.5 μM), may imply pelagic nutrient regeneration as a secondary process during the survey period. Positively or neutrally buoyant diatoms could also partially explain the persistent bloom (refs. in Tremblay *et al*., 2002).

The bloom collapse seems to be associated with a decrease in conditions favourable to upwelling, together with episodes of stratification in the water column, probably caused by the influence of the warm countercurrent. Nevertheless, the transition to a well-established stratified surface layer, which is a condition for the development of the classical diatom-dinoflagellate succession (Margalef, 1978), does not occur because of the fortnightly cycles of upwelling and relaxation, typical of temperate upwelling conditions (Walsh *et al.*, 1977).

Dinoflagellate abundance is positively correlated (Spearman, $p<0.05$) with temperature, suggesting an association with the warm waters of the countercurrent. *Lingulodinium polyedrum* has been described for this location by Amorim *et al.* (in press). Its absence from the samples in this study may be due to the sampling hour (early morning), when diel vertical

migration limits its presence in surface waters, or to the inclusion of this species in higher classification groups. This species seems to be associated with coastal retention conditions in the Sagres area that may develop at times of relaxation when the cold waters are replaced by the warm waters of the countercurrent. Water retention has been reported in several upwelling areas (Graham and Largier, 1997; Demarcq and Faure, 2000; Marín *et al.*, 2003). Coccolithophorids have been observed in the Cabo S. Vicente region (Abrantes and Moita, 1999; Cachão and Moita, 2000), but they have not been quantified because the calcareous plates may be damaged by preservation with acidic Lugol's solution.

Statistical analysis shows a distinct planktonic assemblage for the major upwelling-bloom stage (July, P2). *Chaetoceros* spp. (max. 567 $x10^3$ cell. l^{-1}), *Thalassiosira* spp. (max. 95 $x10^3$ cell. l^{-1}), *Pseudo-nitzschia* spp. (max. 129 $x10^3$ cell. l^{-1}), *Lauderia* spp. (max. 67 $x10^3$ cell. l^{-1}), and *Detonula* spp. (max. 53 $x10^3$ cell. l^{-1}) are the main taxa contributing to the dissimilarities between the July (P2) upwelling-blooming period and the other sampling periods (P1 and P3). This is in agreement with Moita (2001), who classifies these taxa as coastal upwelling indicators during spring and summer for the Portuguese coast.

Potentially HAB organisms

The *Pseudo-nitzschia* spp. reached high abundances (171 $x10^3$ cell. l^{-1}) during this study. Nevertheless, this taxon includes toxic and non-toxic organisms. In order to evaluate the potential harmful effects of this species, a joint study of occurrence of organisms and detection of total biotoxin and biotoxin per cell must be undertaken. In Portugal, IPIMAR is the National Reference Laboratory for biotoxins. Potentially harmful dinoflagellate taxa (*Alexandrium* spp., *Ceratium* spp., *Dinophysis* spp., *Gonyaulax* spp., *Gymnodinium* spp., *Prorocentrum* spp., and *Scripsiella* spp.) were also recorded. Since 1994, *Gymonidinium catenatum* blooms have been registered east of Cabo S. Vicente, and their presence seemed to be dependent on upwelling nutrient enrichment (Moita *et al.*, 1998). Regarding *Dinophysis* spp., concentrations of < 500 cell. l^{-1} were already reported as agents of human intoxication in Portugal, leading to the closure of bivalve harvest (Vale, 1999). During this survey, higher concentrations were attained (max. 2600 cell. l^{-1}). These values fall within ranges previously described for the Portuguese coast (Moita and Silva, 2000; Palma *et al.*, 1998).

In a region such as as Sagres where bivalve culture occurs, precautionary closure of the zone should be carried out for abundances of 200-1000 cell. l^{-1} for *Dinophysis* spp., *Gymnodinium catenatum,* and *Alexandrium minutum* and > 100 000 cell. $l^{-1}$for *Pseudo-*

nitzschia spp. (European Commission, 2002). The closure should be maintained until the respective biotoxin analysis is found to be negative.

Production and respiration rates

Production maxima are attained in July, concurrent with the diatom-chl *a* peak. The seasonal average of volumetric GP (25.4±19.8 μM O_2 d^{-1}) is higher than for the systems of Chile (11.5 μM O_2 d^{-1}; Daneri *et al*., 2000), Arabian Sea (6.8 μM O_2 d^{-1}; Robinson and Williams, 1999), NW Africa and Benguela (15.2 μM O_2 d^{-1}, 14.4 μM O_2 d^{-1} respectively, Robinson *et al*., 2002), but lower than for the Ría de Vigo – NW Spain area (37.3±30.7 μM O_2 d^{-1}, Moncoifée *et al*., 2000). DCR, on the other hand, is generally lower than reported for the above systems, representing only 17% of the GP, which reflects the predominance of the autotrophic component throughout the survey. The high significant correlations between total microplankton, chl *a*, diatoms, production and oxygen data (Table 2) also suggest a dominant and active community of diatom-producers.

Following the approach of Blight *et al.* (1995), GP was plotted against respiration to study the phasing of these parameters (Fig. 14). It is generally observed that the autotrophic peaks

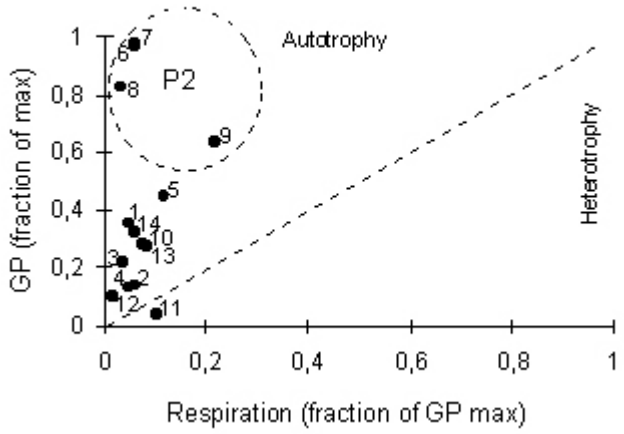

FIG. 14. Phase plot of gross production (GP) versus respiration. Numbers indicate the temporal sequence of sampling dates (1= 24 May; 2= 31 May; 3= 7 June; 4= 26 June; 5= 3 July; 6= 11 July; 7= 18 July; 8= 24 July; 9= 31 July; 10= 6 August; 11= 14 August; 12= 20 August; 13= 29 August; 14= 3 September). P2 indicates the diatom-blooming period.

are not coupled with heterotrophic maxima, denoting a temporal lag between the two processes. This feature has been reported for other coastal areas (e.g. Blight *et al.*, 1995; Robinson *et al.*, 1999) and is probably associated with natural physical loss mechanisms (dispersion, sedimentation) in upwelling areas. However, from date 4-5 (May-June, P1), and date 12-13 (August, P3), the increase in GP is related to an increase in respiration rates. Although the lack of bacterioplankton data limits the interpretation of these findings, high

temperatures were recorded on day 5 and 13, which usually favours picoheterotrophic activity (Wiebe *et al.*, 1993). Additionally, on both occasions there was a peak for ciliate abundance, the best biological predictor of DCR according to Spearman's correlation. A more efficient transfer from the auto- to the heterotrophic communities can be associated with a low molecular weight (LMW) pool of organic matter, originating from algal exudation, readily assimilated by heterotrophs (Blight *et al.*, 1995).

The autotrophic maximum (18 July) is coincident with the diatom bloom in July (P2). The heterotrophic maximum (31 July) is associated with a ciliate peak, together with a diatom maximum, a silicate minimum and a low PAR value, which suggests a co-limitation of light and nutrient on the diatom-photosynthetic rate (Kudela and Dugdale, 2000). The net heterotrophic period (NCP < 0) on 14 August occured during an episode in which nutrients were not limiting (nitrate: 8.5 μM; phosphate: 0.3 μM; silicate: 1.9 μM) but the value for PAR is low. This can be interpreted as a light limitation of the production rate (e.g. Ryther, 1956; Kirk, 1994). Cloud coverage can affect rates of production by a factor of up to 4.5 (Riegman and Colijn, 1991). Also, the decline in diatoms by this date is accompanied by an increase in the remaining functional groups (ciliate, dino- and nanoflagellate), contributing to a higher heterotrophic component. This transition period of the microplankton composition is probably associated with the intrusion of the warm coastal counterflow and the consequent stratification described above. As suggested for other systems (Moncoifée *et al.*, 2000; Robinson *et al.*, 2002), the observed heterotrophy could have been sustained by the accumulation of organic substrates from a recent bloom. The persistently high oxygen saturation (107%) measured at this time corroborates this hypothesis (Robinson *et al.*, 2002). A contribution to the dissolved organic matter pool from the excretion of hanging mussels has also been reported (Álvarez-Salgado *et al.*, 1996).

CONCLUSIONS

The Sagres area is subjected to the upwelling of cold waters in spring to late summer, originating in the wind-driven circulation patterns off the south and west coast. The temporal variation of these physical events regulates the influx of nutrients to the surface waters and subsequent microalgal growth, sustaining the phytoplankton biomass and production of the system. The long-lived diatom-chl *a* peak throughout July is probably associated with the persistence of the upwelling event. The collapse of this diatom bloom appears to be related to the decrease in upwelling conditions and the stratification of the water column, probably

induced by the intrusion of the warm inshore water mass. These features imply a physical control of the biological development. *Chaetoceros* spp., *Thalassiosira* spp., *Lauderia* spp., *Detonula* spp., and *Pseudo-nitzschia* spp. can be considered as an upwelling proxy for this site. The progression of an upwelling / relaxation cycle determined the attained succession stage, therefore regulating the composition of the microplankton assemblage and the subsequent nature of transfer to higher trophic levels, sediments and export. Low respiration rates (17% of GP) and uncoupling with production peaks appear to stem mainly from the interplay of the predominant autotrophic component and physical loss factors. Altogether, physical events seem to be the main factor influencing microplankton structure and production in this area.

More work needs to be done to understand the whole dynamic of this ecological productive system, including water-column studies of new production, bacterial rates, regeneration processes and grazing pressure. Also, the benthic and atmospheric domain awaits further study to improve the understanding of the ecosystem behaviour. Nevertheless, the present study brings a valuable insight into the productive waters of the Sagres area.

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2.2. Enrichment Experiments and Primary Production at Sagres (SW. Portugal)

Abstract

Water was collected from the Sagres station, next to the upwelling centre of Cabo S. Vicente, during a relaxation of upwelling inducing winds. Samples were enriched with nutrients in order to evaluate their influence in microalgae production, biomass (as chlorophyll *a*) and composition. Small-scale short-term bioassays involved *in vitro* additions of nitrogen and phosphorus, separately and simultaneously. Enrichments involving nitrogen (N and N+P treatments) led to a general enhancement of algal production and microplankton numbers, especially of diatoms. However, biomass was only stimulated in N+P treatment. These results suggest a nitrogen limitation of microalgal growth. No significant change in microplankton composition was observed. The stimulation of *Pseudo-nitzschia* spp. (a potentially Harmful Algal Bloom taxon) by nutrient enrichment could be of concern due to the presence of an oyster-farm in the studied area. These bivalves may accumulate domoic acid produced by certain species of *Pseudo-nitzschia*, which may in turn cause human intoxication upon ingestion.

Introduction

Coastal upwelling areas are amongst the most productive habitats of marine ecosystems (Smith & Hollibaugh, 1993). Production rates are primarily enhanced by nitrate supply (new production, *sensu* Dugdale & Goering, 1967) to the photic region during the active stages of upwelling (Barber & Smith, 1981). This supply decreases in times of relaxation of upwelling inducing winds in which the phytoplanktonic activities are mainly supported by regenerated forms of nutrients (e.g. ammonium and urea) (Codispoti, 1983; Bode *et al*., 1997). Phytoplankton must be able to adapt to environmental heterogeneities such as sharp spatial and temporal variability of physical and chemical factors (Hutchinson, 1961; Cloern & Dufford, 2005). Nitrogen compounds can be stored in intracellular pools during nitrogen-sufficient periods, when luxury uptake exceeds growth rates, allowing further development after external nutrient exhaustion (Dortch *et al*., 1984). It is generally found that nitrogen availability controls primary production and biomass accumulation (as chlorophyll *a*) in upwelling systems (Kudela & Dugdale, 2000).

Potentially HAB organisms are prone to develop during upwelling relaxations. The presence of potentially HAB dinoflagellates in the studied area was mainly associated with warm water and quiescent or weak upwelling periods (Amorim *et al*., in press), whereas potentially toxic diatoms (*Pseudo-nitzschia* spp.) were mainly associated with cold upwelled waters. The occurrence of red tides depends on the duration of the relaxation stage in relation to cellular growth rates (Smayda, 2000). Red tide dinoflagellates of upwelling systems generally present a preference for high nutrient conditions (Smayda, 1997; Smayda & Reynolds, 2001).

Short-term small-scale N and P enrichment essays were performed during a relaxation period in the Sagres station, in order to assess the influence of nutrients in algal production rates and biomass accumulation. The response of the organisms was also evaluated in terms of changes in composition.

Material & Methods

Sampling and Analysis

Water samples were collected at the Sagres station (see Chapter 2.1, pag. 33), in the mid morning of 29 September (2002), from surface and at depths representing 50%, 25% and 10% light-depth (Secchi-depths: 2.4 m, 4.8 m, and 8.1 m, respectively). Samples were filtered with a 200 μm mesh size net to select for the microplankton community and remove the larger grazing organisms and particles. The last euphotic depth (defined as the depth where the light intensity is 1% of the intensity of the surface) was not considered due to the shallowness (20 ± 3 m due to tidal fluctuations) of the sampling station. Integrated rates from surface to 10% light-depth are assumed to be representative of the euphotic column of the studied location. Sea surface temperature (SST; ºC) 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 (see chapter 2.1, pag. 34 for details). Samples for nutrient determination were frozen at - 20ºC. Ammonium, nitrite, nitrate, phosphate and silicate were determined by the methods described in Grasshoff *et al*. (1983) modified according to Newton (1995) (see annex A.2, pag. 178). Chlorophyll *a* (chl *a*) concentration was determined by filtering the seawater through a Whatman GF/F filter

and further freezing until fluorometrical analysis by acetone extraction, following the JGOFS's method (JGOFS, 1994). The fluorescence was determined with a Jasco FP-777 spectrofluorometer previoulsy calibrated (Strickland & Parsons, 1972). The seawater dissolved oxygen concentration was estimated by the Winkler method, following modifications by Strickland & Parsons (1972) (see annex A.1, pag. 175 for details). The samples were carefully siphoned into 300 cm^3 Winkler bottles with silicon tubing and overflowed with two to three volumes of sample. The temperature of the sample was determined by means of a quick response digital thermometer. The triplicates were then fixed with manganese chloride and alkaline iodide reagents and stored underwater until analysis. Further acidification produced iodine in stoichiometric proportion to the original oxygen concentration. The iodine was then starch-titrated with a standartised thiosulfate solution using a Brand microburette. Concentrations are expressed as μM O_2. Oxygen saturation (%) was calculated using the standard equations for the solubility of oxygen in seawater (Aminot & Chaussepied, 1983).

Microscopic identification

As described in chapter 2.1 (pag. 35), and annex A.3 (pag. 192).

Net Production

Water samples for primary production determination were filtered through a 200 μm mesh, and siphoned into ~300 cm^3 Winkler bottles with silicon tubing. Triplicates 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" during 24 h. Triplicates were then fixed and analysed for dissolved oxygen concentration as described above. Net community production (NCP) was calculated as the difference in oxygen concentration between the means of the incubated and the initial samples. The samples from the two remaining bottles were used for chl *a* and nutrient determination, and microscopic identification.

Enrichment Experiment

The oxygen bottles (~300 cm^3) were filled with pre-filtered water samples from the surface, 50%, 25% and 10% light-depth and were enriched with a single-pulse of nitrogen (as

$NaNO_3$) and/or phosphorus (as Na_2HPO_4). Added quantities of nutrients were based upon the mean values found at the sampling location during previous years, in order to obtain a N:P >16 for N additions (4 disks in total, one for each depth), < 16 for P additions (4 disks in total, one for each depth) and ≈16 for N+P addition (4 disks in total, one for each depth). Four more disks (one for each depth) were incubated without additions for experiment control. The disks were incubated *in situ* during 24 h. Production was estimated as described above.

Univariate Indices

Each sample was assumed to be representative of its sampling depth for statistical purposes (Clarke & Warwick, 2001). PRIMER© software (Plymouth Routines In Multivariate Ecological Research) was used to perform univariate analysis of the microplankton assemblage. Several univariate indices were calculated to evaluate community structural attributes, including: total number of taxa, total number of individuals ($x10^3$ cell l^{-1}), Margalef's richness index, Pielou's evenness and Shannon-Wiener index. Statistical differences of the ecological indices were assessed by means of one-way analysis of variance (ANOVA) test. The identification of cells down to genus level does not account for the intraspecific variability, which may lead to an underestimation of the univariate ecological indices. However, the relative comparison of these indices will still reflect the depth and treatment variations of the microplanktonic community structure (Nuccio *et al*., 2003).

Results

Wind and hydrographic conditions

Favourable wind conditions for upwelling on both coasts (q_x >0 and q_y <0) were recorded in the beginning of September (Fig. 1a). A decrease on the northerlies caused a favourable downwelling period for the west coast (q_y >0) in the middle of the month. Nevertheless, favourable upwelling conditions for the south coast, evidenced by positive eastward Ekman transport (q_x), associated with high wind-speed values, probably contributed to the low sea surface temperature (SST) recorded until 24 September (Fig. 1c). A steady rise in SST was observed after this date. A high temperature (18.8 ºC) was attained by the time of sampling, associated with relaxation conditions (min wind speed ≈ 2 m s^{-1} and q_x, q_y ≈ 0). SST satellite imagery (Fig. 2) for the two days previous to the sampling date shows 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, reaching the sampling station on 28 September.

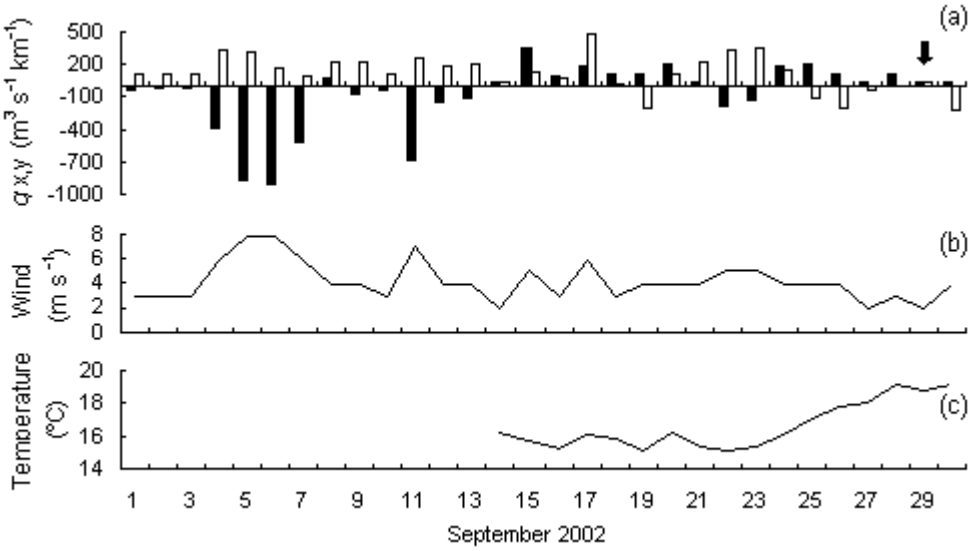

Fig. 1. Eastward (q_x , white bar) and northward (q_y, black bar) Ekman transport (a) during September 2002 at Sagres; positive q_x values indicates favourable upwelling conditions for the south coast, whereas negative q_y represents favourable upwelling conditions for the west; the black arrow indicates the sampling date. Temporal evolution of wind speed (b) and temperature (c).

No good quality satellite imagery was available for the 29 September. Because of technical problems, the available CTD cast only recorded data until 2 m depth (Fig. 3). However, it is

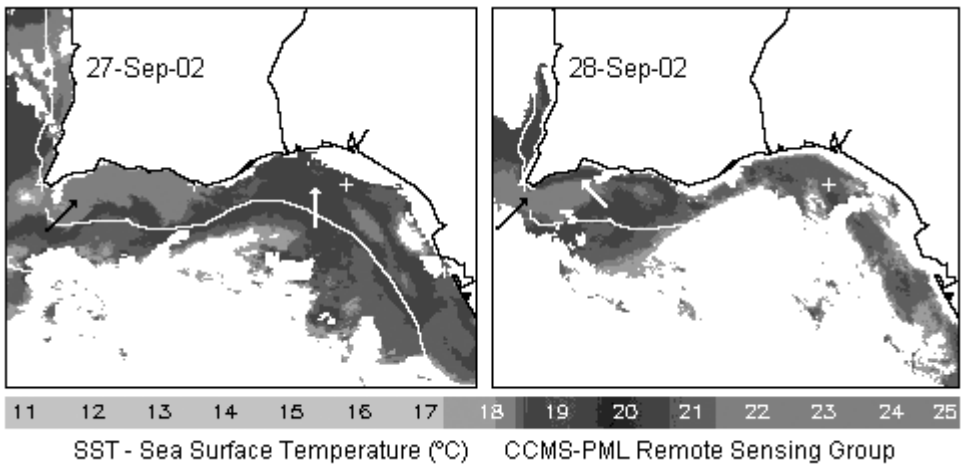

Fig. 2. Sea Surface Temperature (SST) satellite images (NOAA/AVHRR) processed at the Plymouth Marine Lab, UK. Dates are indicated in the images. Black arrows show colder waters, whereas white arrows indicate the influence of the warm water band coming from the Gulf of Cadiz to the south continental shelf of Portugal.

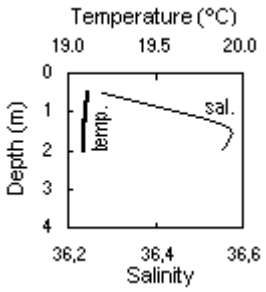

Fig. 3. Available CTD cast for temperature and salinity data, on the 29 September 2002 at Sagres station.

visible a less saline surface layer, already suggested to be associated with the warm CCC (see chapter 3.1).

Initial biological and chemical parameters

Chlorophyll *a* and net community production (NCP) vertical data followed a parallel evolution (Fig. 4), with maximal values at 4.8 m (2.9 µg l^{-1} and 14.3±0.6 µM O_2 d^{-1},

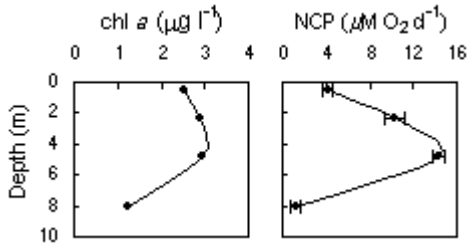

Fig. 4. Chl *a* and NCP vertical data at Sagres station on the 29 September 2002. Bars on NCP plot represent ±SE of NCP rate.

respectively) and minimal at 8.1 m (1.2 µg l^{-1} and 1.2±0.5 µM O_2 d^{-1}, respectively). Dissolved oxygen concentrations shown in Figure 5 evidence a general homogeneous vertical distribution (237±1 µM) with consistent super-saturation values. Figure 6 highlights the vertical variation of nutrients. Phosphate and nitrite were evenly depth-distributed (0.30±0.02 µM and

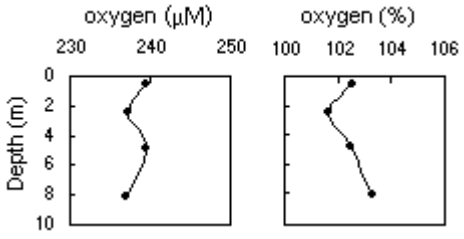

Fig. 5. Vertical data of dissolved oxygen concentration and dissolved oxygen saturation on 29 September 2002 at Sagres station.

0.07±0.02 µM, respectively). Ammonium had a subsurface minimum at 4.8 m (0.07 µM), whereas nitrate was only detected at surface (2.2 µM) being below limit detection (< 0.1 µM)

at the remaining studied depths. N:P ratio reveals a Redfield nitrogen limitation (N:P < 16) at every sampling depth.

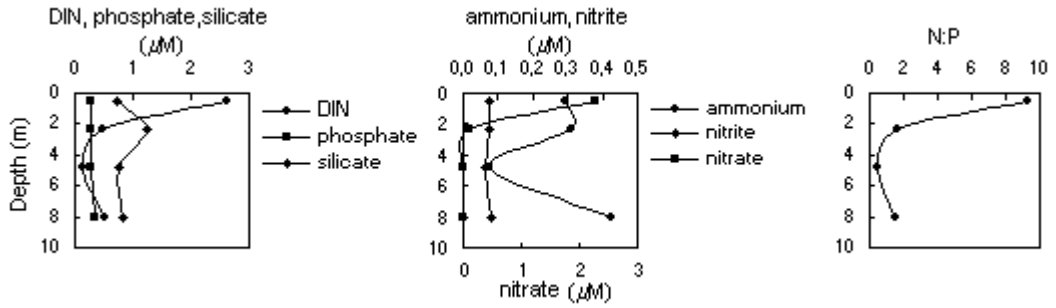

Fig. 6. Vertical nutrient profiles at Sagres station on the 29 September 2002. DIN = dissolved inorganic nitrogen.

Dinoflagellates were the dominant group (max: 216 x10^3 cell. l^{-1}, surface, Fig. 7), except at 2.4 m depth where diatoms were more abundant (203 x10^3 cell. l^{-1}). Nanoflagellates had a lower vertical variability (mean: 74±20 x10^3 cell. l^{-1}), followed by the ciliate group (mean: 7±3 x10^3 cell. l^{-1}). The most abundant taxa (>50 x10^3 cell. l^{-1}) were consistently

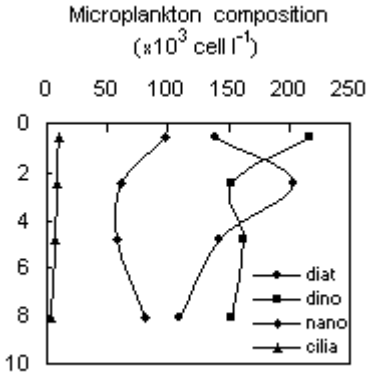

Fig. 7. Depth-distribution of microplankton abundance on 29 September 2002 at Sagres station (diat = diatoms, dino = dinoflagellates, nano = nanoflagellates, cilia = ciliates).

Gymnodinium+Gyrodinium spp. and *Nitzschia* spp.. Cryptomonads, unidentified nanoflagellates and *Leptocylindrus* spp. followed in numbers at surface and 2.4 m depth. The same pattern was found at 4.8 and 8.1 m, except that *Leptocylindrus* spp. was replaced by *Prorocentrum* spp.

Potentially HAB taxa from initial (time zero) samples are described in Table 1. *Pseudo-nitzschia* spp. were more abundant at surface (15 x10^3 cell. l^{-1}). *Ceratium* spp. and *Dinophysis* spp. were the least abundant potentially HAB dinoflagellates (max: 0.4 x10^3 cell. l^{-1} and 1.3 x10^3 cell. l^{-1}, respectively).

Table 1. Abundance of potentially HAB organisms. See chapter 2.1, Table 3 pag. 46 for taxa codes.

Depth (m)	Sample	Taxa Codes							
		PSN	Ale	Cer	Din	Gon	Gym	ProC	Scr
		10^3 cell. l^{-1}							
Surface	Initial	15.3	-	0.3	1.3	34.5	34.5	32.0	14.1
	Control	11.3	-	0.2	-	2.3	12.3	7.7	27.0
	+N	40.4	-	0.3	0.1	-	12.9	10.0	36.1
	+P	19.2	-	0.6	0.1	5.8	32.7	26.3	75.6
	N+P	83.5	-	0.8	0.1	2.6	33.4	21.8	113.0
2.4	Initial	8.7	-	0.3	0.1	1.7	1.7	7.5	8.3
	Control	27.0	-	0.3	0.7	68.1	41.7	5.1	10.9
	+N	97.9	-	0.2	-	0.9	11.9	3.7	64.0
	+P	93.9	-	0.3	0.1	18.0	24.0	7.3	131.2
	N+P	39.2	-	0.3	0.1	-	28.7	7.8	88.1
4.8	Initial	6.4	-	0.4	0.1	-	14.1	48.6	7.0
	Control	27.6	0.6	0.6	0.2	-	21.8	59.1	140.6
	+N	43.7	-	0.2	-	16.1	18.0	17.3	71.3
	+P	14.7	-	0.2	-	7.8	23.0	7.5	47.7
	N+P	31.1	-	0.7	0.1	71.0	24.0	13.3	126.9
8.1	Initial	9.3	-	0.4		0.1	15.3	30.3	7.3
	Control	16.4	-	0.5	0.1	4.0	22.6	21.3	87.8
	+N	39.4	-	0.4	0.1	18.9	27.7	37.7	35.5
	+P	12.6	-	0.1	0.1	3.0	3.3	16.6	18.3
	N+P	22.3	-	0.3	-	16.6	4.7	39.3	0.7

- defines no occurrence

Higher numbers were reached at the surface by *Scrippsiella* spp., *Gonyaulax* spp. and *Gymnodinium* spp. (14.1 x10^3 cell. l^{-1}, 34.5 x10^3 cell. l^{-1} and 34.5 x10^3 cell. l^{-1}, respectively). However, the maximum abundance was reached by *Prorocentrum* spp. (48.6 x10^3 cell. l^{-1}) at 4.8 m.

Treatment effects

NCP and chl *a* enrichment responses to the simulated upwelling pulse are reported as depth-integrated rates in Figure 8. The median coefficient of variation of the initial and light analyses was 0.22% (n = 16) and 0.63% (n = 48), respectively. The mean of the standard

errors of NCP measurements was 0.95 μM O_2 d^{-1} (n = 16). A modest positive stimulation of NCP was visible at N and N+P experiments.

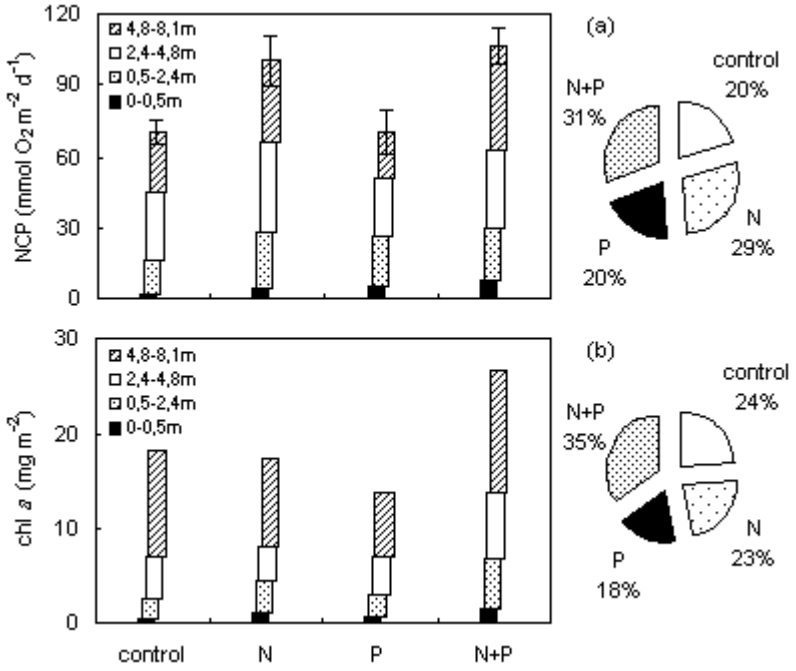

Fig. 8. NCP (a) and chl *a* (b) depth-integrated values, along with relative contributions. Bars represent error ranges on integrated NCP rates (calculated by integrating the respective standard errors from each depth). The segments within each circle correspond to the percentage shares of control and enrichment experiments.

Chl *a* concentrations were only stimulated with N+P addition. Ammonium was consistently lower in final samples (Fig. 9), whereas nitrite had a small change during the experiments. Nitrate accumulation together with phosphate minimal variation, contributed to a final N:P higher than the initial values. Final nitrate values were lower than initial ones only during P treatment.

Depth-integrated values of microplanktonic components (Fig. 10) revealed a major response from the diatoms group for N and N+P enrichments (mainly of *Nitzschia* spp., *Leptocylindrus* spp. and *Pseudo-Nitzschia* spp.). A minor stimulation was observed in dino- (mainly of *Scrippsiella* spp. and *Gymnodinium+Gyrodinium* spp.) and nanoflagellates (mainly of cryptomonads and unidentified nanoflagellates) numbers, also in N and N+P essays. Ciliates responded modestly at N addition. Representation of relative contributions (Fig. 11) emphasizes the higher diatoms stimulation at N and N+P enrichments.

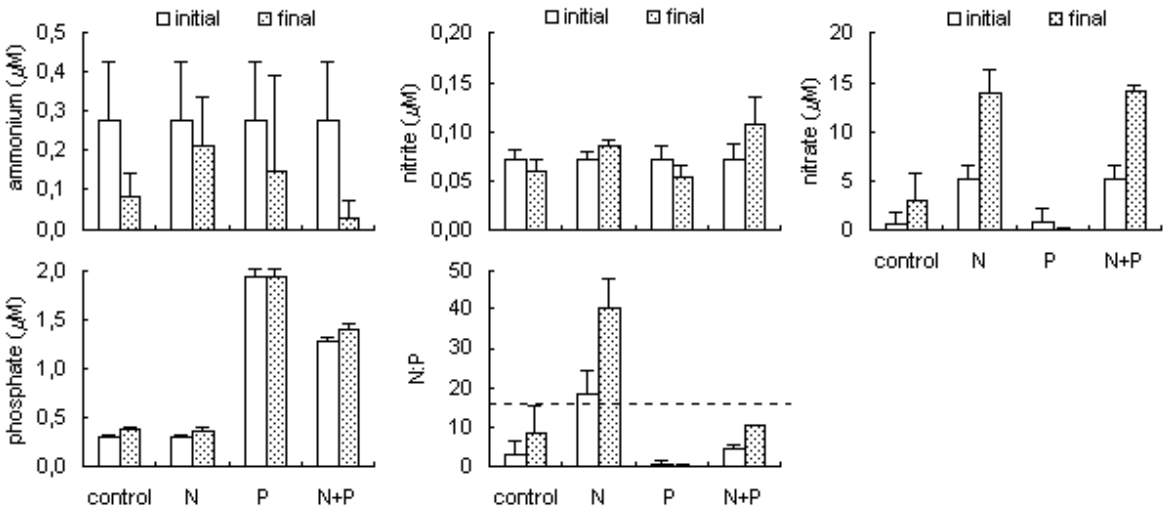

Fig. 9. Depth-average of initial (time zero) and final (24 H) nutrients concentration. Bars represent standard deviations. Broken line on N:P plot indicates N:P = 16 (Redfield ratio).

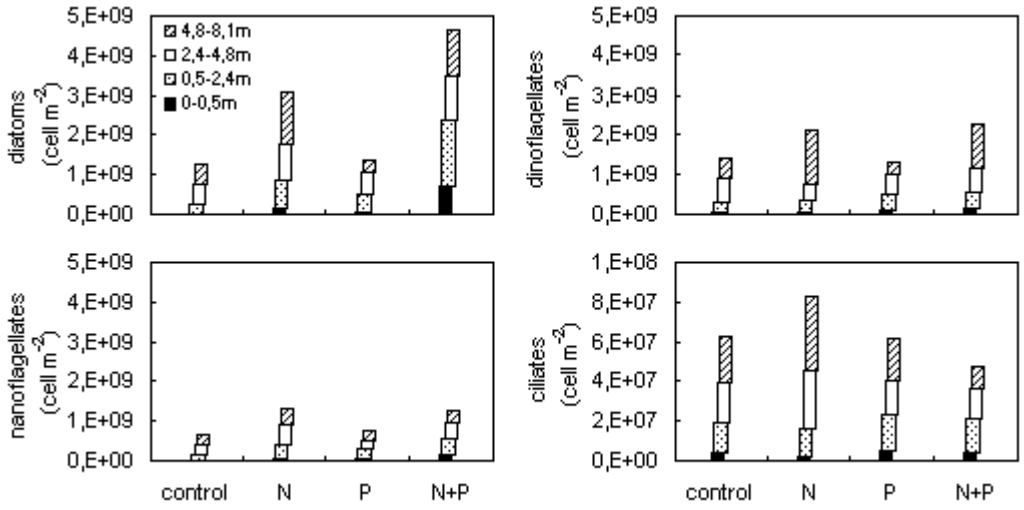

Fig. 10. Depth-integrated values of microplankton groups (diatoms, dinoflagellates, nanoflagellates and ciliates).

This increase was associated with a decrease of the dinoflagellates share. Despite the higher number of individuals on N and N+P enriched samples, one-way ANOVA tests performed on community univariate indices showed no significant differences between the initial, control and enriched sets (Fig. 12).

Potentially HAB organisms responded to nutrient additions in variable ways (Table 1). At the surface, *Pseudo-nitzschia* spp. were stimulated by nitrogen enrichments (both N and N+P treatments), whereas the potentially HAB dinoflagellates *Gymnodinium* spp., *Prorocentrum*

spp. and *Scripsiella* spp. were stimulated by the presence of P (both P and N+P treatments). *Pseudo-Nitzschia* spp. higher stimulation was generally associated with N addition in depth samples.

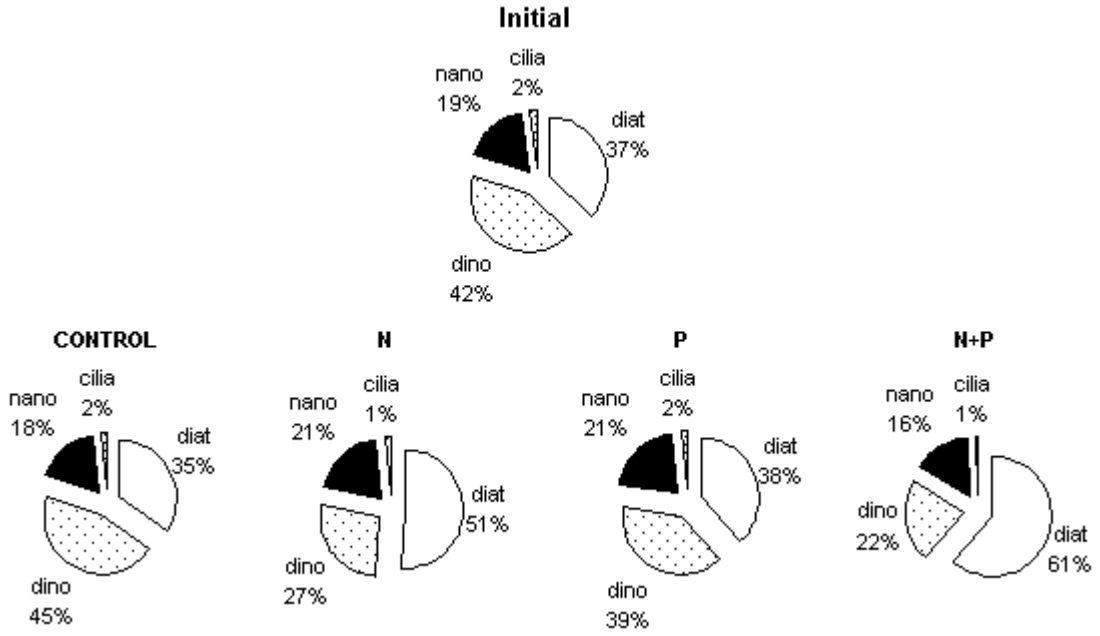

Fig. 11. Relative composition of microplankton assemblage at initial (time zero), control, and enriched samples. Segments in each circle represents percentage shares of diatoms (diat), dinoflagellates (dino), nanoflagellates (nano) and ciliates (cilia).

P addition also induced a high increase of this species at 2.4 m depth. *Alexandrium* spp. was mostly absent, whereas *Ceratium* spp. was evenly distributed at all samples (natural and enriched) in low concentrations (mean: 0.4±0.2 x10^3 cell. l^{-1}). *Dinophysis* spp. followed a similar distribution pattern (mean: 0.2±0.3 x10^3 cell. l^{-1}). *Gonyaulax* spp. had their higher response at 4.8 and 8.1 m in the presence of nitrogen. *Scripsiella* spp. had a high response in all additions at 2.4 m depth.

Discussion

Initial conditions

Chl *a* and NCP maxima at 4.8 m, associated with the ammonium minima, likely reflect a regenerated-based primary production (regenerated production, *sensu* Dugdale & Goering, 1967) at this depth. Biological subsurface maxima are generally linked to chemical (nutrients)

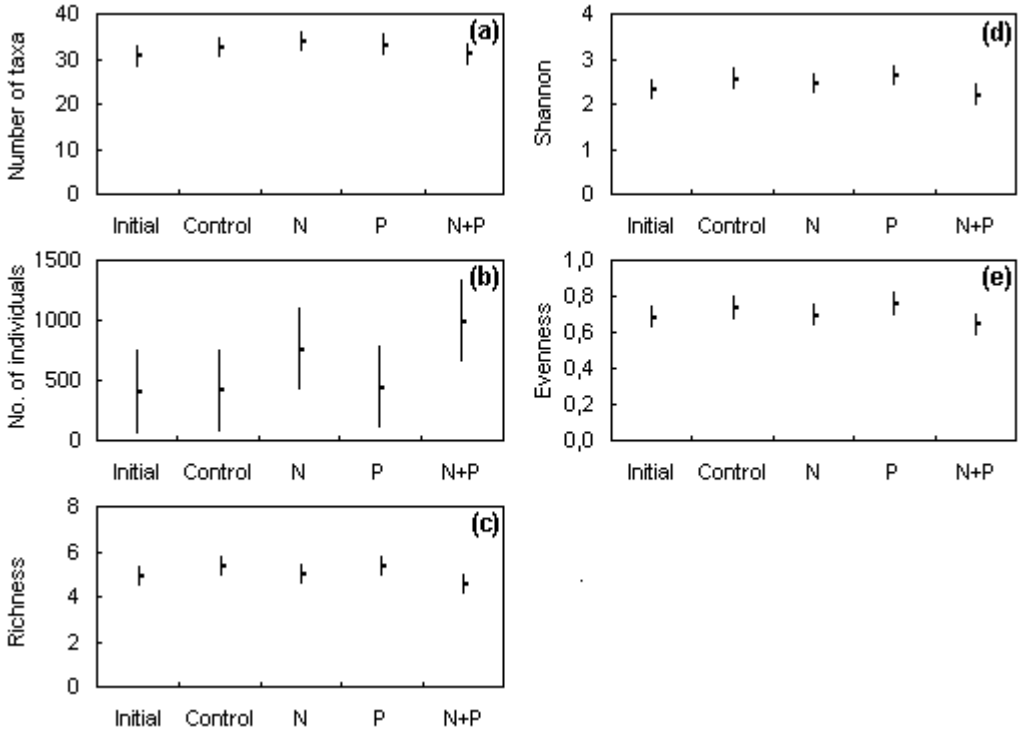

Fig. 12. Univariate indices of initial, control, and experiment additions (N, P, N+P). Bars are means and 95% confidence intervals (based on pooled standard deviations) for studied depths.

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 & Walker, 1991). Values of chl *a* and NCP are in agreement with those found for the studied area during weak-upwelling and relaxation periods (see chapter 3.1). Low values of nitrate suggest a previous active uptake of this nutrient by the algal community, probably 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 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 production. The formation of such pools is expected to be maximized in upwelling regions where nutrients are pulsed-delivered (Dortch *et al*., 1984).

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 dominated by small diatoms, generally associated with active upwelling conditions, and small flagellates, indicators of coastal stratification, have

been previously observed during weak upwelling/transition conditions (Moita, 2001). Such assemblage overlap occurs due to the gradients of vertical mixing and nutrient availability (Reynolds, 1996), especially visible in upwelling regions (Estrada & Blasco, 1985).

Prorocentrum spp *and Gymnodinium* spp. were the most abundant potentially HAB taxa (depth-mean: 29±17 $x10^3$ cell. l^{-1} and 16±13 $x10^3$ cell. l^{-1}, respectively). *Gymnodinium* spp. and *Prorocentrum* spp. are included in habitat-type I and II (Smayda, 2000) related to shallow, nutrient-enriched nearshore waters. *Gymnodinium* spp. reached its maxima at the surface layer, whereas *Prorocentrum* spp. were more abundant at 4.8 m. The flexible behaviour of some *Gymnodinium* spp. (namely, *Gymnodinium catenatum*, a toxic species) to form long chains, which increases its swimming ability, allows the species to be retained during relaxation/downwelling periods and to migrate to nutrient rich layers (Fraga *et al*., 1989). On the other hand, *Prorocentrum* spp. photoadaptive capacity of increasing both size and number of photosynthetic units (Harding, 1988) results in an enhancement of photosynthesis at lower irradiances.

Methodological constrains

See chapter 3.2 pag. 136, for a detailed discussion. The results of 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 & Kimmel, 1986; Ault *et al*., 2000). The outcomes of the experiments will be discussed on this basis.

Enrichment response

The observed stimulation of oxygen photosynthetic rates suggests nitrogen as the most likely nutrient limiting algal production, under the specific conditions of the experimental incubations (Elser & Kimmel, 1986), as already observed in other upwelling systems (Kokkinakis & Wheeler, 1987; Kudela & Dugdale, 2000). Typical responses of bottle assays include productivity stimulation by nitrogen alone, and a still greater stimulation when both N+P are added (Dufour & Berland, 1999), suggesting that enrichment by nitrogen (alone) can lead to a secondary P limitation. No such secondary limitation seemed to develop during this survey in which N and N+P treatment stimulated production in a similar magnitude, which is in accordance with mesocosmos observations for temperate coastal marine regions (Oviatt *et al*., 1995). The observed stimulation of chl *a* by simultaneous addition of nitrogen and

phosphorus, together with the non-stimulation by the separate addition of nutrients, suggests no limitation of algal biomass (as chl *a*). However, the enhancement of production rates by nitrogen addition in the absence of biomass increases happens when the growth rate of algae is nutrient-limited (Malone *et al*., 1996).

The accumulation of nitrate in nitrogen-rich incubated samples has already been observed in nutrient uptake experiments in upwelling regions (MacIsaac *et al*., 1985). Nitrate uptake can drop when ammonium present in the medium exceeds a certain threshold (e.g. 0.5 μM) (Eppley *et al*., 1969). Given the energetic cost of nitrate reduction (necessary for its assimilation) the onset of such metabolic pathway can affect the algal photosynthetic performance (Smith *et al*., 1992), leading thus to an ammonium preferential uptake. A decrease of nitrate concentration in this study (P treatment, Fig. 9) was in fact not associated with an increase in algal production. Nevertheless, it was nitrate (the added nitrogen source) that induced the mechanisms leading to the stimulation of NCP rates in N and N+P treatments. The supply of nutrients is necessary for the induction of uptake mechanisms and enzyme systems, according to Wilkerson & Dugdale (1987). The loss of newly synthesized N-rich compounds is observed a few hours after nitrate addition (Dortch & Postel, 1989). Some phytoplankters may use dissolved organic nitrogen without initial transport into the cell, by using cell surface enzymes to previously degrade these forms into ammonium (Palenik *et al*., 1992). It may hypothesized, given such data, that the observed N-enhanced NCP rates could have been partially sustained by the excretion of organic nitrogen compounds, stimulated by nitrate addition. The lack of detailed studies of the interaction between ammonium and nitrate uptake in different algal species makes it difficult to fully understand such complex mechanisms in multi-specific assemblages (Dortch *et al*., 1991).

The stimulation of diatoms by nitrogen addition is in accordance with results observed in microcosmos experiments for this location (Edwards *et al*., 2005), and other coastal areas (Schülter, 1998). Diatoms are known to attain high growth and uptake rates, which are important mechanisms to reach higher biomass than other algae (Kudela & Dugdale, 2000). Some algae species are better uptake specialists, whereas other are better storage specialists. Nutrients addition in this study likely favoured species adapted to the uptake of nutrient pulses (Granéli *et al*., 1999). Nevertheless, although there was an increase in microplankton numbers at N and N+P experiments, there was not a significant difference between control and treatment samples regarded assemblage composition, as evidenced by one-way ANOVA tests of ecological indices.

Pseudo-nitzschia spp., followed by *Scripsiella* spp. were the potentially HAB taxa most stimulated by nutrient enrichment. *Pseudo-nitzschia* spp. stimulation by nutrient inputs is in accordance with findings that include this taxon as part of the Portuguese coastal upwelling indicators (Moita, 2001) associated with enriched upwelled waters at the Sagres area. Harmful effects may arise from *Pseudo-nitzschia* spp. because of the current oyster-farm practice in this region. The oysters contaminated with domoic acid, which is produced by some *Pseudo-nitzschia* species, can cause human intoxication (ASP = amnesic shellfish poisoning) upon ingestion. High growth rates, from 1.25-2.1 day^{-1}, have been reported for the red tide *Scripsiella* spp. (Smayda, 1997). This species is an invasive competitor type reaching high abundances, whose blooms are suggested to be primarily associated with high-nutrient stratified conditions (Smayda, 2000). No reports of harmful blooms of *Scripsiella* spp. were nevertheless found for the studied region. This may reflect the influence of physical and biological loss factors present in the natural environment, that were not present in the experimental enclosures. Overall, factors triggering red tide blooms in upwelling systems are still not clear (Smayda, 2000).

Conclusions

The fertilisation of surface waters in Sagres is mainly regulated by natural eutrophication events (upwelling). The results suggest nitrogen as the most likely nutrient controlling microalgae growth at the time of the experiment, especially diatoms, 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 assemblage composition was not induced by nitrogen additions, although there was an increase in microplankton numbers. The unpredictability of red tides in upwelling areas reflects the great variability of physical and chemical factors within these systems. *Pseudo-nitzschia* spp. and *Scripsiella* spp. were the potentially HAB taxa more sensitive to nutrient enrichment in this study. *Pseudo-nitzschia* spp. is the taxon more prone to cause negative effects in this region because of the bivalve farming practice.

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3.1. BOUNDARY CONDITIONS FOR THE EUROPEAN WATER FRAMEWORK DIRECTIVE IN THE RIA FORMOSA LAGOON, PORTUGAL (PHYSICO- CHEMICAL AND PHYTOPLANKTON QUALITY ELEMENTS)

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Abstract

The dynamics between nutrients, the microplankton assemblage and physical factors were evaluated in the Ria Formosa (S. Portugal) coastal lagoon. Water samples were collected from Ramalhete and Ponte within the lagoon and compared with the conditions at Barra, an oceanic inlet. The two lagoon stations represent the boundary conditions of two different water bodies that have been registered as intercalibration sites for the European Water Framework Directive. Sampling coincided with high and low water conditions, at the summer and winter solstice, and at the spring and autumn equinox between June 2001 and July 2002. Chlorophyll a values, with a maximum of 5.1 $\mu g\ l^{-1}$ during growing season, were lower than those reported for similar systems. The maximal winter values of 5.99 μM for total inorganic nitrogen, 0.53 μM for phosphate, and 6.34 μM for silicate, were also lower than previously reported for this area. Microplankton peaked during the summer solstices of June 2001 and July 2002, with maximal abundances of 12×10^5 cells l^{-1} and 7×10^5 cells l^{-1} for total microplankton, respectively: these communities were dominated by diatoms. At the autumn and spring equinox (September 2001 and April 2002), the maximal abundances were 4.9×10^5 cells l^{-1} and 2.6×10^5 cells l^{-1} total microplankton, respectively: these communities were more evenly distributed between diatoms, dinoflagellates, nanoflagellates and ciliates. At the winter

solstice (December 2001), the microplankton were at their lowest with a maximal abundance of 1.0 x 10^5 cells l^{-1} : these communities were dominated by small organisms, particularly nanoflagellates. The oceanic microplankton community at the Barra inlet was generally less numerous and differed in composition from the lagoonal communities at Ramalhete and Ponte. Multivariate analysis clustered the microplankton assemblage according to season. Changes in the microplankton community were related mainly to variations in temperature, solar radiation and salinity, and to the availability of the reduced forms of nitrogen. The differences between the parameters observed at the entrance of the lagoon during the summer solstice of 2001 and 2002 may be due, respectively, to the colder upwelled water during 2001 and the much warmer water observed in 2002. Nutrient enrichment was possible both from coastal waters and from internal lagoonal processes. Consequent accumulation of biomass may occur in inner regions where water circulation is restricted, which may lead to episodes of water quality degradation. This study does not alter the boundary conditions for Ramalhete and Ponte registered at the European Commission, respectively, as 'good/moderate' and 'high/good'.

Keywords:
Water Framework Directive, microplankton assemblage, nutrients, coastal lagoon

1. Introduction

Coastal lagoons have high physico-chemical and biological diversity over both spatial and temporal scales. Strong salinity and temperature gradients, limited volumes, shallow waters, close coupling between benthic and pelagic domains, and restricted connections to the adjacent sea are important aspects of lagoons that contribute to variability (Nixon, 1982; Nuccio et al., 2003). Lagoon habitats are increasingly vulnerable to uncontrolled human activity (Vallejo, 1982), such as increasing nutrient loads from terrestrial watersheds (Bricker et al., 1999; Boesch and Brinsfield, 2000; Skei et al., 2000; Cloern, 2001) that can induce anthropogenic eutrophication. Indeed, one of the first examples of the detrimental effects of eutrophication in marine ecosystems was from the shallow coastal lagoon of Moriches Bay, on the south shore of Long Island, New York (Nixon, 1995).

Regulatory pressure is increasingly an option taken by many countries (NRC, 2000; EC, 2000) to redress the environmental degradation of water resources. In the European

Community (EC), the Water Framework Directive (WFD; EC, 2000) is the legal mechanism for maintaining and improving the ecological quality of fresh and coastal waters. This improvement of quality includes preventing, or limiting, anthropogenic eutrophication, that has been defined under the Urban Waste Water Directive (EC, 1991) as the "enrichment of water by nutrients especially compounds of nitrogen and phosphorus causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms and the quality of the water concerned". The WFD proscribes monitoring programmes to establish the typology and the quality of water resources. For surveillance monitoring, the Directive suggests an interval of three months for physico-chemical quality elements including salinity, temperature, oxygen and nutrients, but for biological quality elements the intervals are much greater, six months for phytoplankton and three years for macroalgae, invertebrates and fish. However, frequencies should be increased at sites where environmental objectives may not be achieved (operational monitoring). In cases where accidental pollution has occurred, or the reasons for poor ecological status are unknown, investigative monitoring should be instigated at the frequencies necessary to provide the solutions. Finally, the Directive states that monitoring must be carried out at all sites with protected status.

Borja (2005) has noted that relatively little has been published on the European WFD in relation to coastal waters. This study examines the implementation of the WFD for the Ria Formosa(Fig. 1), an important lagoon with protected status in southern Portugal. Under the Common Implementation Strategy (CIS) of the WFD (Vincent et al., 2002), the Ria typology corresponds to a mesotidal, sheltered Atlantic coast (Bettencourt et al., 2004). There is a body of historical data (see references in Falcã oand Vale, 2003; Newton and Mudge, 2005) that has been evaluated:

(1) to identify the susceptibility to eutrophication of the lagoon (Newton et al., 2003);

(2) to establish the boundaries for water bodies within the lagoon (Ferreira et al., 2005).

On the basis of this historical data, the sites of Ramalhete (No. 1261, Site Code C3979[1]) and Ponte (No. 1259, Site Code C3978[1]) in the Ria Formosa (Fig. 1) have been registered by the Commission of European Communities[1] as part of the European list of inter-calibration sites for the WFD, and have been classified with an ecological status of good/moderate and high/good, respectively. In this study, the boundary conditions for physico-chemical

[1] The Commission of the European Communities took the decision on the 17th August 2005 to establish a register of sites to form an intercalibration network in accordance with Directive 2000/60/EC of the European Parliaments and of the Council.

parameters have been compared at these two sites with the conditions at the oceanic inlet of Barra (Fig. 1) to assess whether the frequency of sampling proposed by the WFD is adequate to monitor changes in the Ria Formosa.

Phytoplankton is an important component of the biological quality elements considered by the WFD. In coastal waters, phytoplankton are generally efficient filters for nutrient inputs from terrestrial watersheds (Jickells, 1998) and will respond rapidly to biotic and abiotic changes (Harris, 1986; Niemkiewicz and Wrozloek, 1998). However, in the case of the Ria Formosa there is little published work on changes in the phytoplankton community (Moita and Vilarinho, 1999; Marques et al., 1996). Although the WFD recommends sampling phytoplankton at six monthly intervals, in this study the phytoplankton were sampled at the three monthly intervals from the same locations used for the physico-chemical elements, with the objective of providing an initial baseline for the community structure of the phytoplankton at Ramalhete, Ponte and Barra (Fig. 1); and also to evaluate the dynamics between the micro-pelagic community and nutrient concentrations.

1.1 Study area

Fig 1 shows the location of the Ria Formosa lagoon along the south coast of Portugal. Nationally it has been recognised as a Natural Park since 1987. Internationally, it forms part of the Natura 2000 European network for nature conservation, it is a Ramsar wetland and it is included in the Special Bird Protection Area (European Directive 79/409/EEC). It is 55 km long with an area reaching 160 km^2, of which one third is intertidal, with an average channel depth of 3.5 m (Falcão and Vale, 1990). The lagoon is bordered by a string of sand dunes and interacts with the surrounding oceanic waters via six inlets. At each tide there is a 50-75% exchange of water mass (Tett et al., 2003). Tidal range varies from 2.8 m to 1.3 m at spring and neap tides, respectively. There are no major fresh water discharges in the western lagoon, although episodic run-off occurs from rainfall during the winter season. Salinity ranges from 13 to 36.5, and temperature from 12 ºC to 27 ºC (Newton and Mudge, 2003).

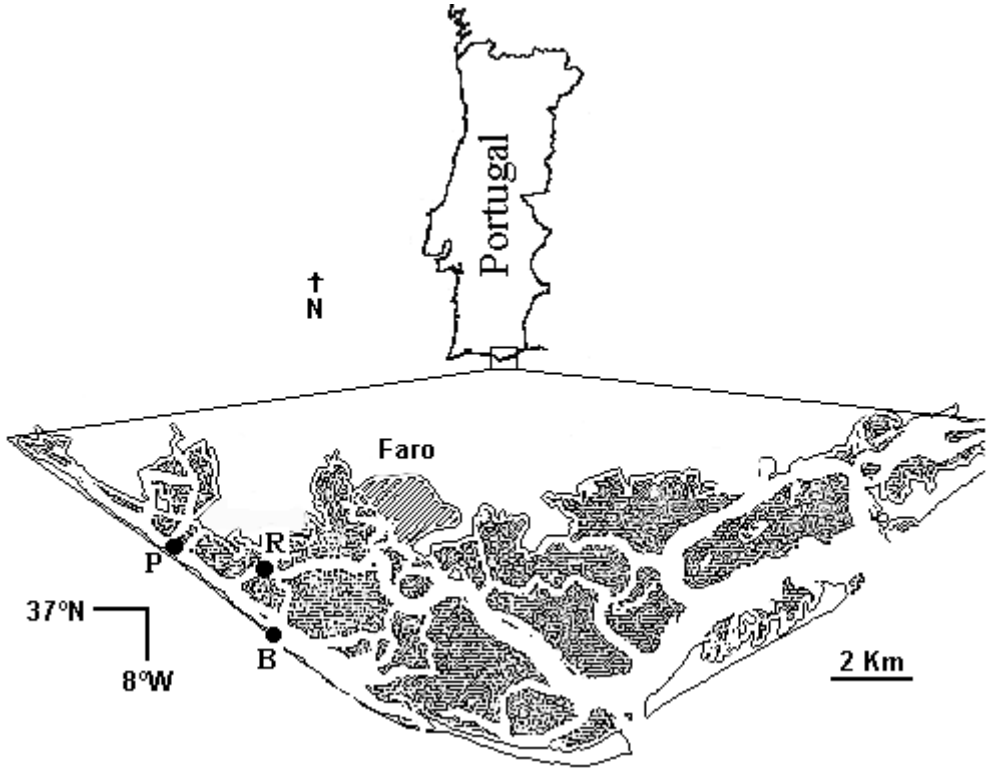

Fig. 1. Sampling stations in the Ria Formosa coastal lagoon (S. Portugal): B=Barra (oceanic inlet); R=Ramalhete (next to an urban waste water treatment plant), P=Ponte (upstream channel, bivalve culture, recreational activities).

The concentrations of nutrients in the lagoon and the coastal waters are probably influenced by fluctuations in currents along the southern coastal shelf of Portugal. An upwelling of nutrient-rich water is induced along this shelf by westerly winds. There is also seasonal upwelling, from May to September driven by northerly winds down the west coast. These nutrient-rich waters (e.g. Loureiro et al., 2005a) may flood the southern shelf after flowing counter-clockwise around Cabo SãoVicente, at the south-western tip of Europe (Fiúza, 1983; Sousa and Bricaud, 1992). The southern shelf is also influenced by the presence of a warm coastal countercurrent (CCC) that originates from the Gulf of Cádiz. The progress of the CCC along the shelf towards Cabo São Vincente depends on pressure gradients and wind forcing (Relvas and Barton, 2002). Apart from the exchange with adjacent coastal waters (Falcão and Vale, 2003; Newton and Mudge, 2005), nutrients are also imported from urban wastewater treatment plants as point sources, and from agricultural run-off as non-point sources (Ferreira et al., 2003; Newton et al., 2003). Nutrients are additionally supplied by

tidal pumping from sediments within the lagoon (Falcão and Vale, 1990).

The lagoon is an important economic resource for the Algarve region because of the intensive fishing, aquaculture, salt extraction and touristic activities (Newton and Mudge, 2003; Santos et al., 2004). Anthropogenic pressure increases during the summer touristic season. Aquaculture practice can be affected by episodes of anoxia (Gamito, 1997a) and by extensive mats of green algae formed during the winter (Reis and Sprung, 1995). Accumulation of biotoxins in clams and consequent human intoxication has been observed in the eastern lagoon (Vale and Sampayo, 1999). Macroalgae and macrophytes are reported as the major sources of organic carbon in the Ria (Sprung, 1994; Santos et al., 2004).

The sampling stations represent contrasting conditions:

(1) Ramalhete has a muddy substrate, with a complex circulation pattern where the water exchanges are slower than at the other two sites (Newton and Mudge, 2003). The Ramalhete channel receives the effluent from the Urban Waste Water Treatment (UWWT) for the urban conurbation of Faro, and is adjacent to a busy airport (R in Fig. 1). The site is affected by recreational activities between Faro City and the barrier islands of the lagoon; and by bivalve culture;

(2) Ponte has a sandy-muddy substrate. It is located on a channel that links the Barra oceanic inlet with the Ancão basin at the blind-end of the western lagoon (P in Fig. 1). The Ancãoo basin receives the effluent from the golf courses of the luxury tourist development at Quinta do Lago, and from intensive agriculture. This site is also affected generally by recreational and touristic activities, and by bivalve culture.

(3) Barra has a sandy substrate and is the site for the opening of an artificial inlet in 1997 on the west lagoon (B in Fig. 1) during the INDIA (Inlet Dynamics Initiative: Algarve) project (Williams et al., 2003).

2. Material and Methods

2.1 Sampling

The sampling interval was based on the three monthly period proposed in Annex V by the WFD for surveillance monitoring. Sampling coincided with high (HW) and low water (LW), close to the extreme “neap” tides of the summer (13.06.2001; 03 .07.2002) and winter (08.12.2001) solstice, and the extreme “spring” tides of the spring (17.04.2002) and autumn

equinox (18.09.2001). HW samples illustrated the influence of the adjacent coastal waters, whereas LW samples characterised residual lagoon water influenced by internal lagoon dynamics. These situations were chosen to represent seasonal and tidal "extremes", thus exploring the variability of conditions to optimize sampling effort in WFD monitoring plans.

Surface water was collected and filtered through a 200µm mesh to exclude large organisms and particles. Samples for chlorophyll *a* (chl *a*) were filtered using Whatman GF/F filters, and frozen until pigments were extracted by acetone for determination by fluorometrical analysis (JGOFS, 1994). Nutrient samples were frozen at –20ºC and subsequently analysed by standard colourmetric techniques (Grasshoff et al., 1983) for nitrate ($N\text{-}NO_3^-$), nitrite ($N\text{-}NO_2^-$), ammonium ($N\text{-}NH_4^+$), phosphate ($P\text{-}PO_4^{3-}$) and silicate ($Si\text{-}SiO_4^{2}$). Dissolved oxygen (D.O.) was estimated by a standard Winkler titration (Strickland and Parsons, 1972; Bryan et al., 1976) using a Brand microburette. Oxygen saturation (%) was derived from standard equations (Aminot and Chaussepied, 1983). Temperature and salinity data were recorded with a WTM-LF197-S profiline conductivity meter with a TetraCon 325 standard conductivity cell. Standard seawater (International Oceanographic Commission) was used for calibration at the beginning and end of each sampling period. Total daily solar irradiance (KJ m^{-2}) was recorded by the Portuguese Instituto de Meteorologia (IM) at the Faro Airport meteorological station (07° 58' W, 37° 01' N, 8m).

2.2 Microscopic identification

Samples for microscopy were fixed with Lugol's solution and subsequently settled in sediment chambers for identification of the microplanktonic assemblage (Tomas, 1997) and the determination of their abundance (Utermöhl, 1958) using a Zeiss Axiovert 25 inverted microscope. Organisms were generally classified to genus level with the assemblage divided into four major taxonomic components: diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae), ciliates (Ciliatae) and nanoflagellates. Wherever possible the nanoflagellates were separated into Cryptophyceae, Dictyochophyceae, and Euglenophyceae but, where this was not possible, they were enumerated as unidentified nanoflagellates.

2.3 Statistical treatment

PRIMER© software (Plymouth Routines In Multivariate Ecological Research) was used for statistical analysis of the microplanktonic assemblage (Clarke, 1993). Each sample was

assumed to be representative of its time of collection, station and tidal condition (Clarke and Warwick, 2001). Hierarchical agglomerative clustering (group-average linking) and multi-dimensional scaling (MDS) of the Bray-Curtis similarity matrix of square-root transformed abundances was used to assess natural groupings of samples and its relation to abiotic factors. The ANOSIM routine evaluated the statistical difference between the *a priori* sampling groups (June, September and December 2001; April and July 2002). The SIMPER routine tested for the contribution of taxa to the dissimilarities between these groupings. Several ecological indices were calculated to evaluate the characteristics of the community structure, including: total number of taxa, total number of individuals, Margalef's richness index, Pielou's evenness, and Shannon-Wiener index. The identification of cells down to genus level and wider groups did not account for the intraspecific variability of taxa, which could have led to an underestimation of diversity values. Nevertheless, relative comparison of such indices still reflected the seasonal diversity oscillation and consequent modification of the microplanktonic community structure (Nuccio et al., 2003). STATISTICA© software was used for parametric and non-parametric tests.

3. Results

3.1 Variations of measured parameters

3.1.1 Absolute variations

The range and mean values of physical, chemical and biological parameters measured, as well as chlorophyll a, measured during the survey are summarised in Table 1 for comparison with historical data. Variations between the overall mean values and those estimated for the individual stations demonstrated differences between the three sites. The highest mean values were observed: at the Ponte upstream station for temperature, chl *a*, $N\text{-}NO_3^-$, DIN (Dissolved Inorganic Nitrogen), $P\text{-}PO_4^{3-}$, $Si\text{-}SiO_4^{2-}$ and N:P; at the Ramalhete lagoon station for salinity, chl *a* , $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and N:Si; and at the Barra oceanic inlet for dissolved oxygen and oxygen saturation. The lowest mean values occurred at the Barra for most of the parameters, except for dissolved oxygen, oxygen saturation and DIN, which were lowest at Ramalhete.

3.1.2 Sampling variations

Table 1. Mean, minimum and maximum values of the parameters measured at the Ria Formosa lagoon during the whole studied period (June 2001 to July 2002), and at the sampling stations described in the text. Chl *a* = chlorophyll *a*; DIN = Dissolved Inorganic Nitrogen (NO_3^-+NO_2^-+NH_4^+) Temp. = temperature; D.O. = dissolved oxygen; O_2 sat. = oxygen saturation.

Parameters	Whole study period			Sampling stations								
				Barra			Ramalhete			Ponte		
	Mean	min	max	Mean	min	max	Mean	min	max	Mean	min	max
Temp. (ºC)	20.3	14.5	24.7	19.8	15.9	22.9	20.4	14.5	23.2	20.6	15.5	24.7
Salinity	36.4	35.5	37.0	36.3	35.5	36.9	36.5	35.6	37.0	36.4	35.7	37.0
Chl *a* ($\mu g\ l^{-1}$)	1.7	0.1	5.1	1.4	0.1	3.0	1.8	0.7	4.2	1.8	0.4	5.1
$N\text{-}NO_3^-$ (μM)	4.1	0.4	9.7	3.2	0.4	9.7	4.2	0.8	9.3	5.0	2.7	9.0
$N\text{-}NO_2^-$ (μM)	0.13	0.02	0.41	0.11	0.02	0.41	0.14	0.08	0.25	0.12	0.06	0.25
$N\text{-}NH_4^+$ (μM)	1.15	0.08	4.89	0.87	0.08	4.64	1.31	0.09	4.89	1.26	0.09	3.69
DIN (μM)	5.4	1.0	14.3	4.2	1.0	11.2	5.6	2.3	14.3	6.3	3.1	12.2
$P\text{-}PO_4^{3-}$ (μM)	0.49	0.14	1.25	0.35	0.14	0.75	0.52	0.27	0.80	0.60	0.21	1.25
$Si\text{-}SiO_4^{2-}$ (μM)	4.0	0.4	15.4	2.3	0.4	5.3	3.5	1.2	7.6	6.1	0.5	15.4
N:P	12	3	28	12	3	28	10	5	20	14	4	28
N:Si	2.3	0.5	9.7	2	0.7	4.3	2.6	0.5	9.7	2.2	0.5	6
D.O. ($mg\ l^{-1}$)	7.1	5.4	8.3	7.3	6.3	8.2	6.9	5.7	8.2	7.0	5.4	8.3
O_2 sat. (%)	97	76	111	99	89	109	95	81	111	96	76	108

Figures 2, 3 and 4 illustrate spatial and tidal values measured for the physical, chemical and biological parameters. In general the seasonal difference between most of the parameters was statistically significant on the basis of the Kruskal-Wallis test including: temperature and salinity ($p < 0.001$), chlorophyll *a* ($p = 0.0002$), reduced forms of nitrogen ($p < 0.03$), and oxygen ($p < 0.05$). There were no seasonally significant differences between the values for phosphate and silicate.

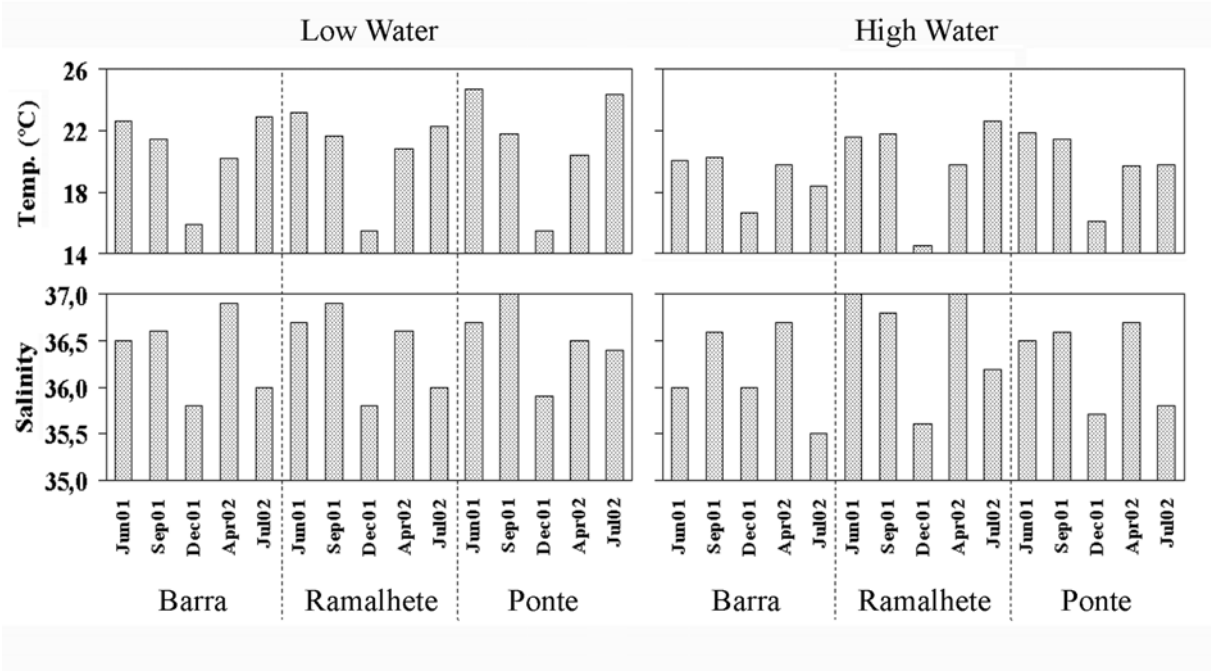

Fig. 2. Temperature and salinity values registered during sampling at the Barra, Ramalhete and Ponte stations of the Ria Formosa lagoon, during high and low water.

Spatial variation has been demonstrated in Section 3.1.1 for the absolute mean values of the data set, but there are also patterns in the tidal variation within the periodic data from the sampling campaigns. In the case of temperature and salinity (Fig. 2), the greatest variation between LW and HW occurred during the summer solstice in 2001 and 2002, with the warmest water observed at LW at all the sites, apart from Ramalhete in July, where the values were similar between LWand HW. The coldest waters were observed at LW for Barra and Ponte, and HW for Ramalhete during the winter solstice. In general, salinity was less variable than temperature but the pattern for LW and HW for the three sites was relatively similar between the two parameters. When there were differences, they tended to occur at Ramalhete where, for example, at LW in June 2001 the temperature was higher and the salinity was lower than at HW.

In the case of chl *a* (Fig. 3), the maximal values and the greatest differences at different tidal conditions occurred at the autumn equinox in 2001 (September). The inorganic nitrogenous compounds varied between LW and HW depending on the compound and the time of the year. Thus, ammonium was generally higher at LW at all the three sites throughout the year. Nitrite was higher at LW throughout the year at the Ponte, but only in December 2001, April 2002 and July 2002 at the Barra, and only in September 2001 at Ramalhete. Nitrate was higher at LW in December 2001, April 2002 and July 2002 at the

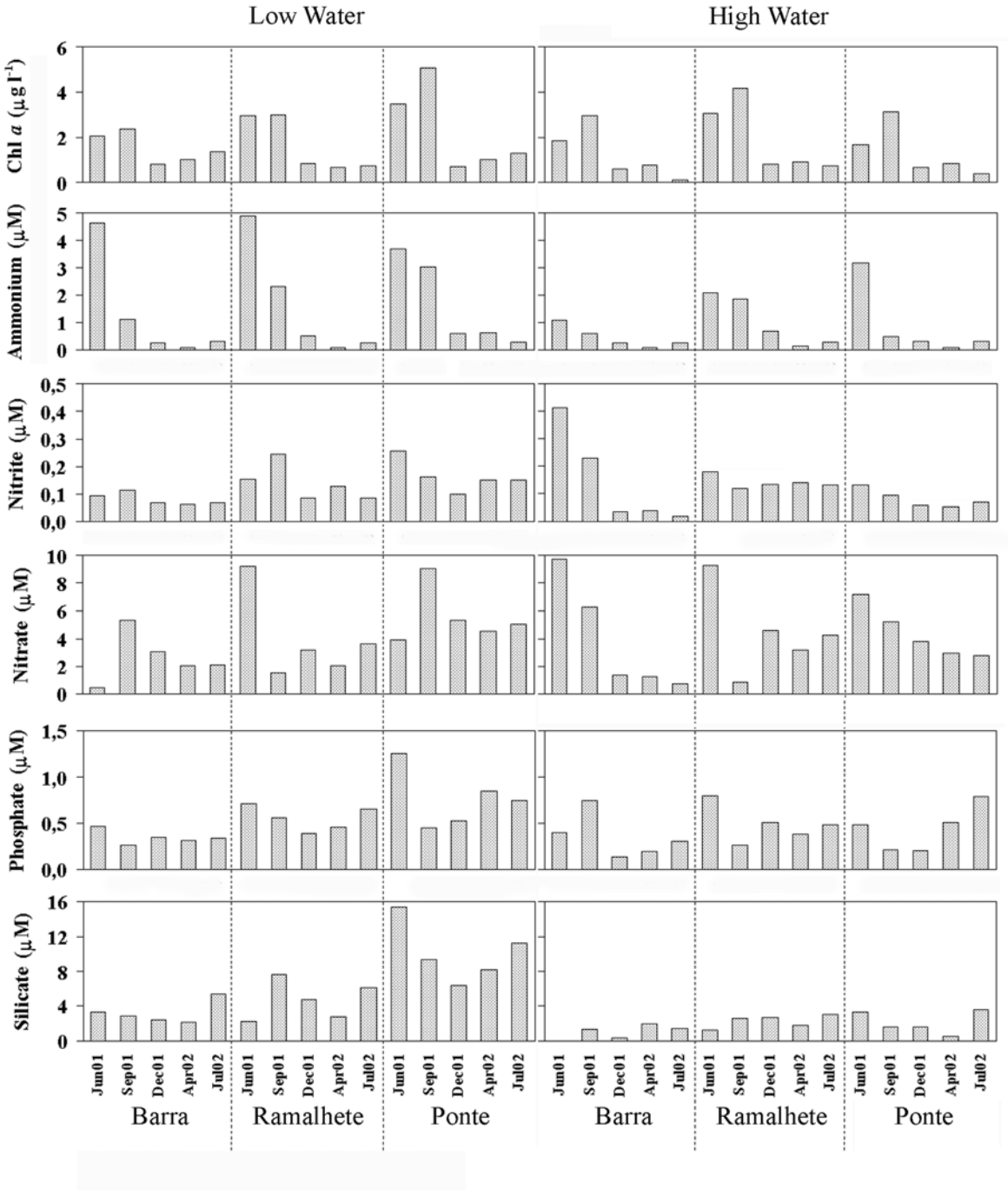

Fig. 3. Chl *a*, ammonium, nitrate, nitrite, phosphate and silicate concentrations at the Barra, Ramalhete and Ponte of the Ria Formosa lagoon, at high (HW) and low water (LW).

three sites, and additionally in September for Ponte and Ramalhete. It was striking that the lowest and highest values for nitrate throughout survey occurred at the oceanic inlet (Barra) at

LW and HW in June 2001, respectively. Phosphate values were generally higher at LW, whilst silicate values were consistently higher at LW throughout the survey.

Both oxygen concentrations and saturation (Fig. 4) also showed a consistent pattern between the three sites with higher values at LW for the three solstice sampling campaigns, and at HW for the two equinox samplings. These patterns were related to the time of sampling with the higher values observed during the afternoon and the lower values during the morning.

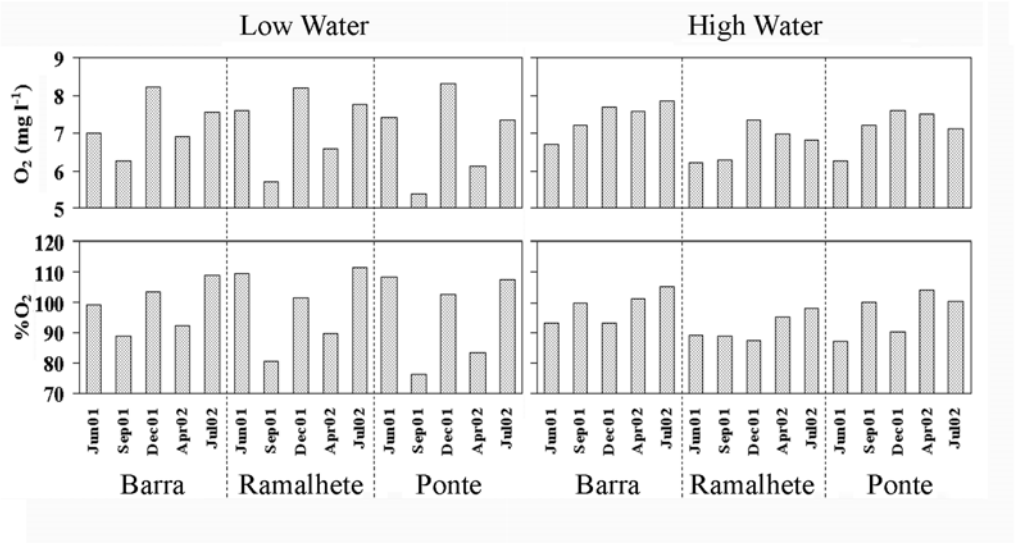

Fig. 4. Oxygen concentration and saturation measured during sampling at the Ria Formosa lagoon (stations: B = Barra, R = Ramalhete, P = Ponte; tide: LW = low water; HW = high water). Bars on oxygen concentration columns correspond to standard errors; where the bars are not visible the data columns hid the small errors.

3.2. Microplankton assemblage

3.2.1. Microplankton abundance changes

Table 2 shows the taxa identified during the survey of the Ria Formosa, together with their codes and their frequency of occurrence. Greater than, or equal to, 60 % of the total taxa were represented by 12 diatoms, 10 dinoflagellates, 3 ciliates and 3 nanoflagellates. Fig. 5 shows that the greatest abundance of microrganisms occurred generally during LW at boththe Barra nd Ponte, and at HW at Ramalhete. Peaks in abundance were observed at the summer solstices of June 2001 (12 $x10^5$ cell l^{-1}, Ramalhete-HW) and July 2002 (7.1 $x10^5$ cell l^{-1}, Ramalhete-HW). During June (2001) and July (2002), the samples were mainly diatom dominated (max: 9.5 $x10^5$ cell l^{-1}, June 2001; Fig. 5), although ciliates were at their most numerous during June 2001 (range: 98-221 x 10^3 cell l^{-1}). In September 2001 and April 2002,

Table 2. List of microplankton taxa found in Ria Formosa, its codes, and frequency of occurrence during the survey. Most frequent taxa (≥ 60%) in **bold** underlined type.

Code	Taxa	Frequency (%)	Code	Taxa	Frequency (%)
	Bacillariophyceae (Diatoms)			**Dinophyceae (Dinoflagellates)**	
	Centrales		Ale	*Alexandrium* spp.	50
Ast	*Asteromphalus* spp.	3	Amp	*Amphidinium* spp.	37
Bac	*Bacteriastrum* spp.	17	Cer	*Ceratium* spp.	57
Cha	*Chaetoceros* spp.	**93**	Din	*Dinophysis* spp.	40
Cos	*Coscinodiscus* spp.	53	Gon	*Gonyaulax* spp.	47
Dac	*Dactyliosolen* spp	30	Gym	*Gymnodinium* spp.	**83**
Euc	*Eucampia* spp.	3	Gm+Gr	*Gymnodinium+Gyrodinium* spp.	**97**
Gui	*Guinardia* spp.	43	Gyr	*Gyrodinium* spp.	**73**
GuiF	*Guinardia flaccida*	17	Kat	*Katodinium* spp.	23
GuiS	*Guinardia striata*	30	Oxy	*Oxytoxum* spp.	3
Hem	*Hemiaulus* spp.	10	ProC	*Prorocentrum* spp.	**77**
Lau	*Lauderia* spp	10	ProT	*Protoperidinium* spp.	**90**
Lep	*Leptocylindrus* spp.	**70**	Scr	*Scrippsiella* spp.	**80**
Lic	*Licmophora* spp.	50	Tor	*Torodinium* spp.	27
Mel	*Melosira* spp.	10	DNs	Small (< 20μm) Unidentified	**97**
Odo	*Odontella* spp.	10	DNb	Big (> 20μm) Unidentified	**67**
Rhi	*Rhizosolenia* spp.	**77**		**Ciliatae**	
Ske	*Skeletonema* spp.	20	Hap	Haptorida	13
Ste	*Stephanopyxis spp.*	3	Mes	Mesodiniidae	**63**
ThaS	*Thalassiosira* spp.	**60**	Oli	Oligotrichida	**100**
DCs	Small <20 μm Unidentified	**63**	Tin	Tintinnina	**77**
DCb	Big >20 μm Unidentified	**63**	Cil	Unidentified	**90**
	Pennales			**Cryptophyceae**	
Ast	*Asterionellopsis* spp.	7	Cry	Cryptomonadales	**97**
Dip	*Diploneis bombus*	27		**Dictyochophyceae**	
Fra	*Fragilariopsis* spp.	7	Dic	Dictyochaceae (Silicoflagellates)	53
Man	*Manguinea* spp.	33	Ped	Pedinellaceae	13
Meu	*Meuniera membranacea*	3		**Nanoflagellates**	
Nav	*Navicula* spp.	**80**	Nan	Unidentified	**93**
Nit	*Nitzschia* spp.	**97**		**Euglenophyceae**	
Ple	*Pleurosigma* spp.	**73**	Eug	Euglenaceae	27
Psn	*Pseudo-nitzschia* spp.	**77**	Eut	Eutreptiaceae	**77**
ThaN	*Thalassionema* spp.	17			
Str	*Striatella* spp.	20			
DPs	Small (<20μm)Unidentified	23			
DPb	Big (>20 μm) Unidentified	47			

the assemblage was evenly distributed between diatoms (mean: 135 x10^3 cell l^{-1}, 64 x10^3 cell l^{-1}, respectively), dinoflagellates (mean: 121 x10^3 cell l^{-1}, 47 x10^3 cell l^{-1}, respectively) and nanoflagellates (mean: 122 x10^3 cell l^{-1}, 36 x10^3 cell l^{-1}, respectively). Nanoflagellates generally dominated the plankton in December 2001 (max: 62 x10^3 cell l^{-1}) when the microplankton abundance at all three sites was at ita lowest (min: 34 x10^3 cell l^{-1}).

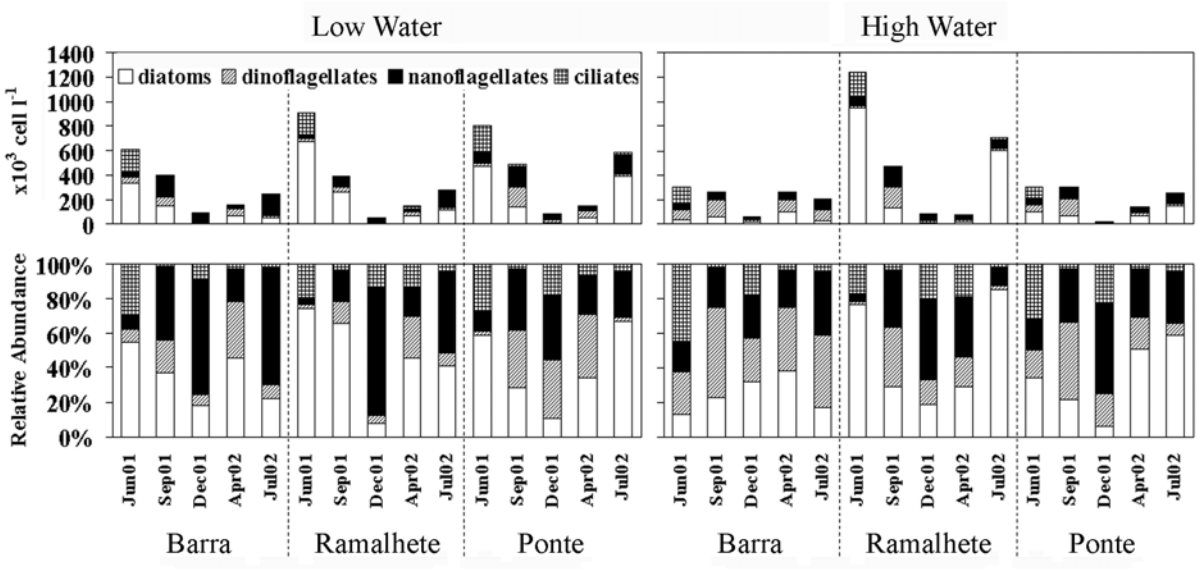

Fig. 5. Abundance and relative numbers of the defined microplanktonic groups identified during the studied period (June 2001 to July 2002), at the three stations (B = Barra, R = Ramalhete, P = Ponte) of the Ria Formosa lagoon, at both high (HW) and low water (LW) conditions.

3.2.2. Statistical community analysis

The dendrogram obtained by cluster analysis (Fig. 6) associated the samples on the basis of seasonal differences, with the assemblage for the winter solstice (December 2001) showing a strong separation from the other groups. At 50% of the Bray-Curtis similarity index three clusters were evident: the summer solstice 2001 (June), the winter solstice, and a third cluster which linked samples from the autumn equinox (September 2001), the spring equinox (April 2002) and the summer solstice 2002 (July). When the similarity level was increased to 55%, the assemblage divided further into a total of six groups. The Barra-HW assemblage separated from its seasonal cluster during the solstice campaigns (June, December, July).

One-way ANOSIM tests (Table 3) on the similarity matrices of the square root transformed abundances of the distinct *a priori* group of samples (June 2001, September 2001, December 2001, April 2002 and July 2002), demonstrated that the differences in the community structure were statistically significant (global R = 0.82). Specific R values for each pairwise comparison showed that the largest community separation was between summer solstice 2001 and spring equinox 2002 samples (R = 0.99), whereas the weakest community separation was between the autumn equinox 2001 and the summer solstice 2002 (R = 0.53).

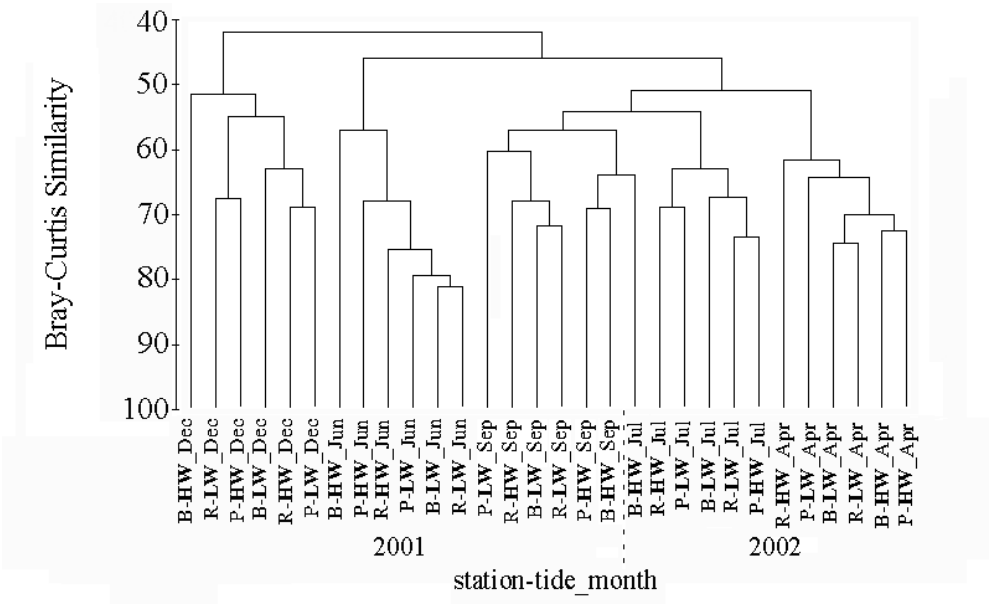

Fig. 6. Cluster dendrogram of the Bray-Curtis similarity matrix of the square root transformed abundances. B = Barra station, the oceanic inlet; R = Ramalhete station, next to an urban waste water treatment plant; P = Ponte station, upstream channel used for touristic activities. LW = low water, HW = high water.

The similarity percentage analysis (SIMPER) on the abundances (transformed to square root) revealed the most discriminating taxa between the different microplanktonic groups, as well as the indicator taxa for each of the groups. The spatial and seasonal distribution of the principle taxa are represented in Fig. 7. Thus, the characteristic groups for: the summer

Table 3. Results from the one-way ANOSIM test of microplankton assemblage differences (Bray-Curtis similarity matrices of square-root transformed abundances) between the *a priori* seasonal groups (2001: June, September and December; 2002: April and July).

Periods	R pairwise test	Number of Permutations	Significance level
Jun - Sep	0.88	462	0.002
Jun - Dec	0.95	462	0.002
Jun - Apr	0.99	462	0.002
Jun - Jul	0.68	462	0.002
Sep - Dec	0.96	462	0.002
Sep - Apr	0.84	462	0.002
Sep - Jul	0.53	462	0.006
Dec - Apr	0.85	462	0.002
Dec - Jul	0.89	462	0.002
Apr - Jul	0.84	462	0.002
Global R	0.82	999	0.001

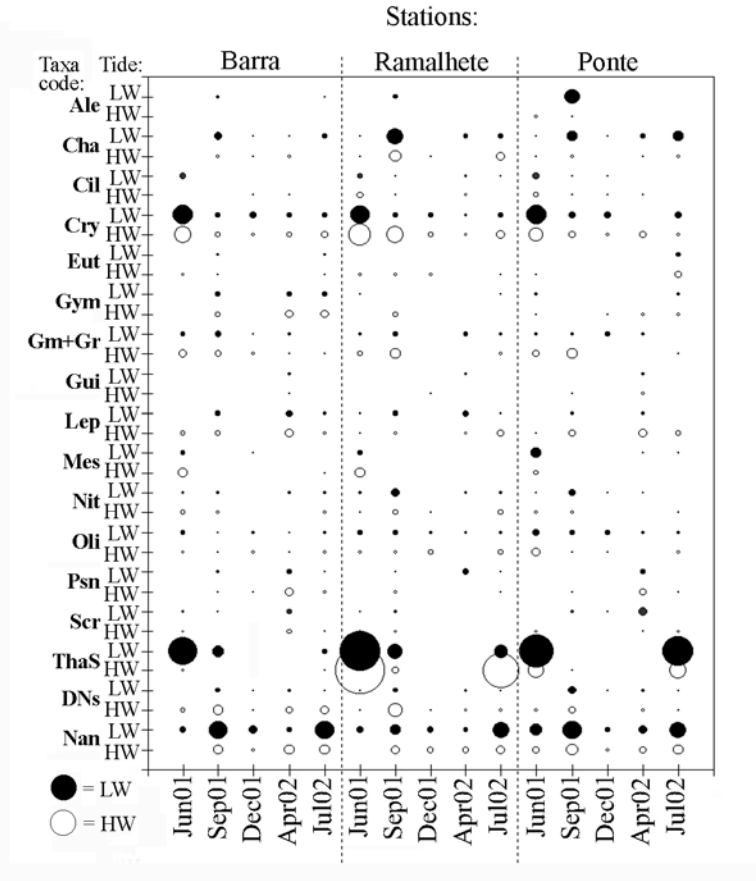

Fig. 7. Evolution of the main taxa contributing to the Bray-Curtis dissimilarities between the seasonal groups (2001: June, September and December; 2002: April and July). See Table 2 for taxa codes. Circles are proportional to abundance values. LW = low water; HW = high water.

solstice in 2001 were *Thalassiosira* spp., cryptomonads, and ciliates; the autumn equinox in 2001 were unidentified nanoflagellates, *Chaetoceros* spp., small (< 20 μm) unidentified dinoflagellates, *Gymnodinium+Gyrodinium* spp., *Nitzschia* spp. and *Alexandrium* spp.; the winter solstice in 2001 were nanoflagellates, cryptomonads, Oligotrichida and *Gymnodinium+Gyrodinium* spp.; the spring equinox in 2002 were *Leptocylindrus* spp., *Pseudo-nitzschia* spp., *Guinardia* spp. and *Scrippsiella* spp.; and finally, the summer solstice in 2002 were Eutreptiaceae taxa, *Thalassiosira* spp., (in common with the previous summer solstice), and unidentified nanoflagellates (in common with the previous autumn equinox).

3.3. Biotic and abiotic relations

In Fig. 8, environmental variables (as bubble plots) are superimposed on the MDS ordination, in order to relate the abiotic variables to the microplankton community. These plots confirmed the results of the cluster analysis (Fig.6) where the samples were grouped according to the sampling season. Temperature, salinity, solar radiation and ammonium parameters showed a similar distribution to total microplankton, dividing the biotic clusters. However, the remaining variables did not appear to relate closely with the overall biotic

structure. As a result of the patchy distribution of these residual variables, there was not a dominant gradient in these ordinations.

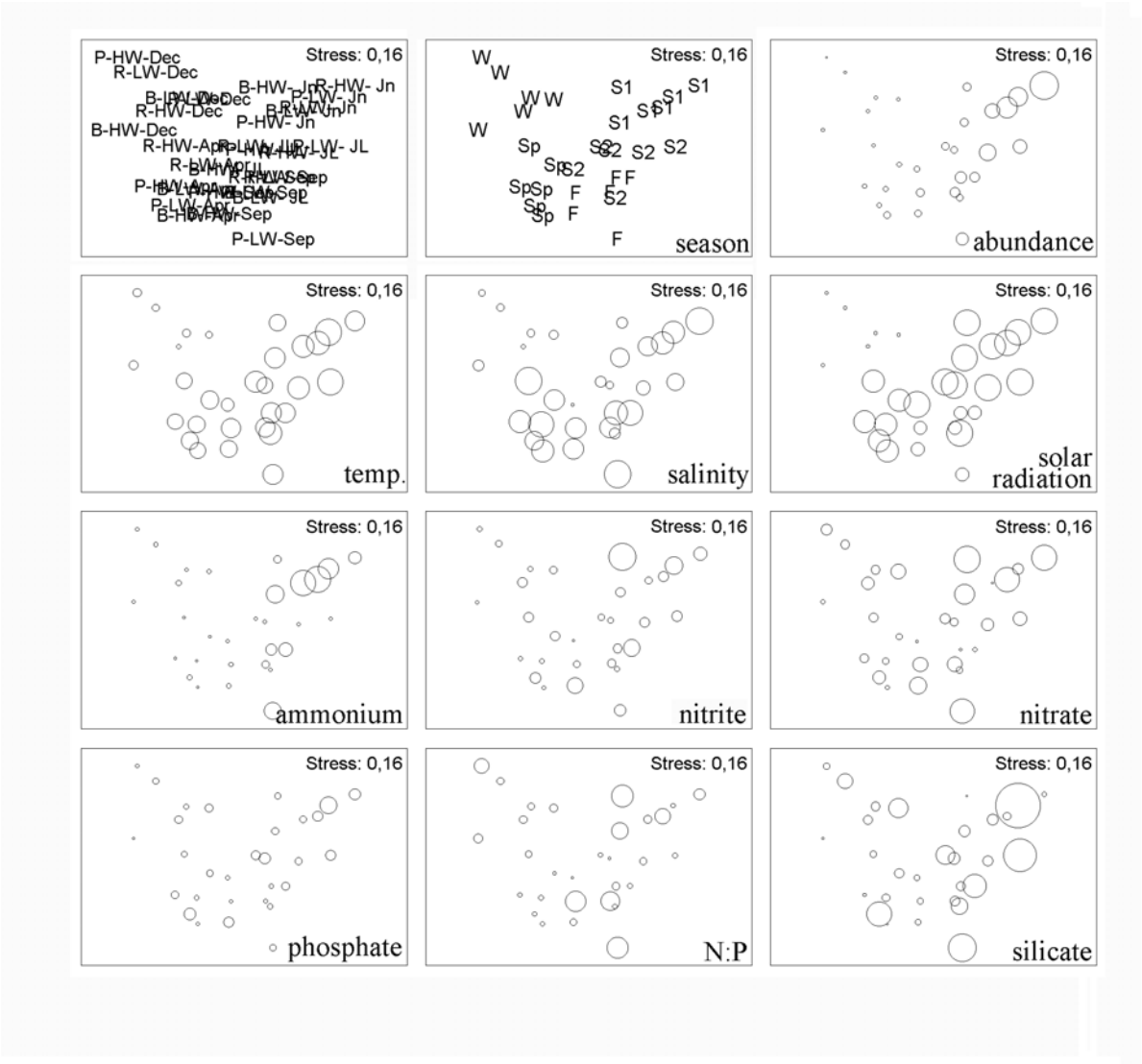

Fig. 8. Two dimension MDS (multi-dimensional scaling) ordination of Bray-Curtis similarities of square-root transformed abundance data; bubbles represent the super-imposition of biotic and abiotic factors. B = Barra station, the oceanic inlet; R = Ramalhete station, next to an urban waste water treatment plant; P = Ponte station, upstream channel used for touristic activities. LW = low water, HW = high water. S1=summer 2001 ; F=fall; W=winter; Sp=spring; S2=summer 2002.

The results of the Spearman rank-order correlations between the microplankton assemblage and physical, chemical and biological parameters are presented in Table 4. The total microplankton abundance was significantly ($p < 0.05$) correlated to temperature, solar radiation, and ammonium. A major autotrophic component would be expected by the strong correspondence between chl *a* and the total microplankton ($r_s = 0.72$, $P < 0.05$). Comparisons with chemical parameters suggested that reduced forms of nitrogen had a high correlation

Table 4. Pairwise Spearman correlations between biological, chemical and physical parameters determined during the survey: temperature (T), salinity (S), solar radiation (light); nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), silicate (SiO_4^{2-}), dissolved oxygen (D.O.) and chlorophyll *a* (Chl *a*) concentrations; diatom ([diat]), dinoflagellate ([dino]), nanoflagellate ([nano]), ciliate ([cilia]) and total microplankton ([total]) abundances. Bold underlined figures are significant at P<0.05; *n* represents the number of samples.

n = 30	T	S	Light	NO_3^-	NO_2^-	NH_4^+	PO_4^{3-}	SiO_4^{2-}	D.O.	Chl *a*	[diat]	[dino]	[nano]	[cilia]	[Total]
T	1														
S	**0.45**	1													
Light	**0.63**	0.04	1												
NO_3^-	0.17	0.10	-0.01	1											
NO_2^-	**0.45**	**0.42**	0.10	**0.64**	1										
NH_4^+	**0.40**	0.17	-0.02	**0.44**	**0.62**	1									
PO_4^{3-}	0.34	0.11	**0.37**	**0.39**	**0.62**	**0.37**	1								
SiO_4^{2-}	**0.44**	-0.04	0.16	0.07	0.32	0.34	**0.48**	1							
D.O.	**-0.39**	**-0.59**	-0.12	-0.23	**-0.57**	**-0.37**	-0.19	-0.15	1						
Chl *a*	**0.57**	**0.61**	-0.02	**0.36**	**0.64**	**0.68**	0.17	0.22	-0.56	1					
[diat]	**0.81**	**0.55**	**0.58**	0.13	**0.41**	**0.39**	**0.44**	0.27	**-0.46**	**0.43**	1				
[dino]	0.22	**0.42**	0.06	0.02	0.15	0.20	-0.14	-0.10	**-0.45**	**0.46**	0.22	1			
[nano]	**0.54**	0.11	**0.37**	0.12	0.19	0.26	0.10	**0.37**	-0.21	**0.44**	**0.50**	0.33	1		
[cilia]	**0.43**	0.14	0.32	0.27	**0.59**	**0.52**	**0.44**	0.25	-0.24	0.23	**0.44**	-0.10	-0.02	1	
[Total]	**0.84**	0.48	**0.52**	0.31	**0.54**	**0.58**	**0.37**	0.23	**-0.45**	**0.72**	**0.91**	**0.37**	**0.62**	**0.51**	1

with the microplankton assemblage (r_s = 0.54 and r_s = 0.58, for nitrite and ammonium, respectively), whereas phosphate had a weaker relation (r_s = 0.37, $p < 0.05$). Dissolved oxygen was inversely correlated with total microplankton abundance, diatoms and dinoflagellates, as well as with reduced forms of nitrogen.

3.4. Univariate indices

During the survey the total number of taxa were not significantly different (one-way ANOVA, p = 0.21; Fig. 9a). However, the variation of the total number of individuals (Fig. 9b) produced significant differences in the other ecological indices. The LSD Fisher test showed which of these groups differed statistically (Fig. 9). The high richness index in December (Fig 9c) was mainly caused by the low densities observed during these period. The dominance of *Thalassiosira* spp., cryptomonads and unidentified nanoflagellates was associated with the poorer evenness (Fig. 9d) and diversity (Fig. 9e) indices of the summer months (June 2001 and July 2002).

4. Discussion

4.1. Abiotic and biotic variability

Financial costs of monitoring programmes are high and should be therefore optimized. The current sampling programme for the Ria Formosa lagoon, based on the three monthly intervals proposed by the WFD for surveillance monitoring, complements the observations of the physical and chemical parameters made in previous studies (see references below). The measurements for temperature and salinity confirm the observations of Newton and Mudge (2003) where the trend is for warmer, hypersaline conditions in the summer due to high insolation and rapid evaporation and for cooler, less saline conditions in the winter produced by low insolation and freshwater runoff from the land. These conditions explain the higher temperatures and salinity at LW compared to HW, where LW reflect better the conditions within the inner lagoon, whilst HW reflects the intrusion of oceanic conditions from the adjacent coastal water. At Ramalhet,e the opposite trend occurs periodically, with the higher measurements observed at HW. This observation probably reflects the complex tidal mixing and circulation pattern described for this region (Newton and Mudge, 2003). The higher salinity values measured in June 2001 in comparison with July 2002 are probably due to the

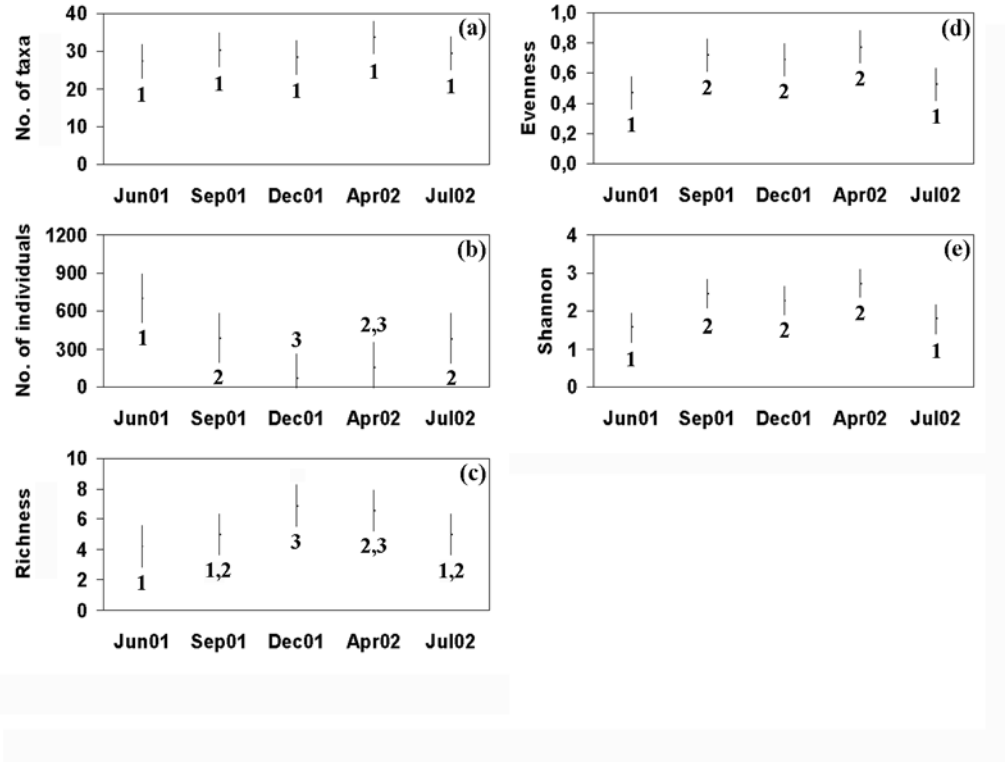

Fig. 9. Mean and 95% confidence intervals based on pooled standard deviations of univariate indices of the microplankton community. Numbers on bars represents the results of the *post hoc* test (LSD Fisher test): bars labeled with different numbers have significant different means; bars with the same numbers do not have significant, but different means.

the influence of different coastal water masses over these periods (Fig 10).

The ranges of chl *a* are in general agreement with previous findings for this region (Falcão and Vale, 1995; Asmus et al., 2000; Newton et al., 2003). The chl *a* pattern is mainly related to the availability of reduced nitrogen sources, temperature and salinity levels. Lower values of chl at LW indicate an export to coastal waters (Falcão and Vale, 2003), with concentrations during the growing season that are generally lower compared to other similar systems (Tett et al., 2003). Low values of summer phytoplankton biomass have been associated with the presence of benthic suspension-feeding organisms in estuarine ecosystems (Alpine and Cloern, 1992). In the Ria Formosa, a significant depletion of algal concentration in the water column at low current velocities has been related to bivalve suspension feeding (Sobral and Widdows, 2000).

The nutrient values observed in this study are within the range reported for various lagoons (Nixon, 1982), and are generally comparable with previous studies on the Ria Formosa (e.g. Newton et al., 2003, and references therein). However, DIN in this study is substantially

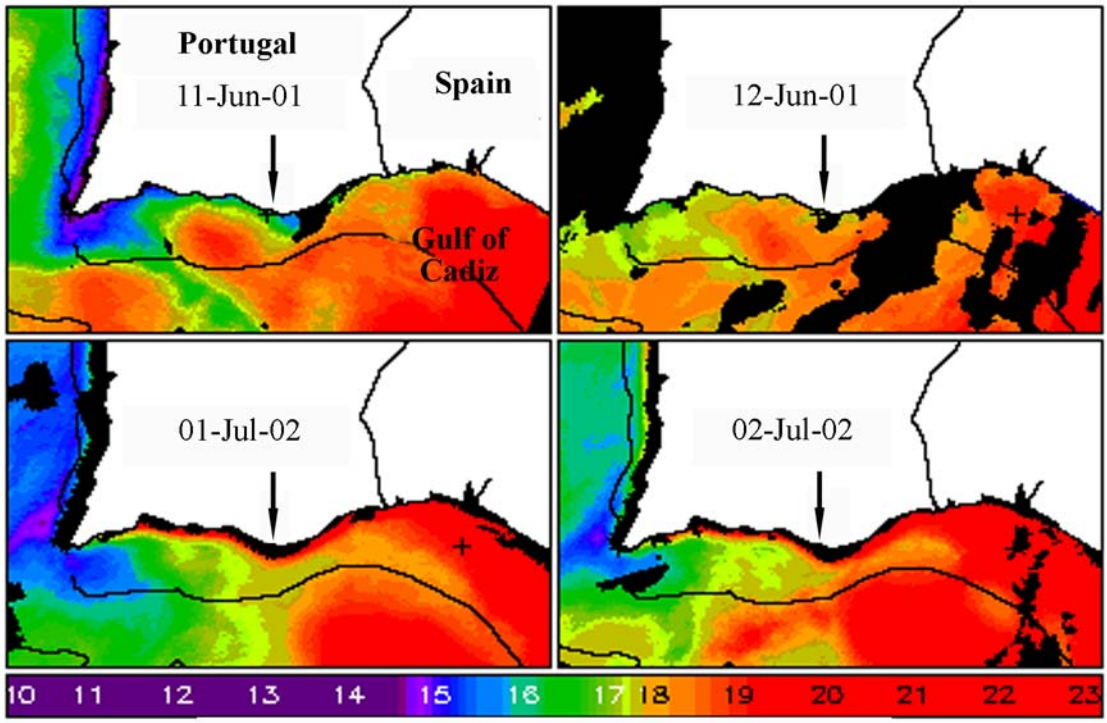

Fig. 10. Sea Surface Temperature (SST) satellite images (NOAA/AVHRR) from the South of Portugal (Algarve), processed at the Plymouth Marine Lab, UK. The dates are indicated in the images. The black arrow points to the sampling location.

lower than the values obtained by Newton and Mudge (2005) for the western lagoon, although in comparison with the historical data (Table 5) the values were high at Ramalhete and relatively similar at Ponte. A decrease of nitrogen inputa int the Ria Formosa waters, since the observations of Newton and Mudge (2005), could be attributed to the improvements in water circulation (Newton and Icely, 2002) after the opening of the artificial inlet at the Barra site in 1997 (Williams et al., 2003), and to the improvements in sewage treatment after the installation of an UWWT near Ramalhete. Also, the transcription to national legislation of the Nitrate's Directive (ND, 91/676/EEC) stimulated a new action program for the Faro region (Diário da República, 2001), with the objective to reduce water pollution by nitrates of agriculture origin.

In general, the higher concentrations of nutrients at LW support the previous observations that the lagoon exports nutrients to the coastal waters (Falcao and Vale 2003; Newton and Mudge, 2005). However, Newton and Mudge (2005) describe periods, particularly in spring and autumn, when DIN is imported into the lagoon. In this current study, DIN is imported into the lagoon during the summer solstice of 2001, but it is exported to the coastal water

Table 5. Boundary conditions for the registered WFD intercalibration sites of Ramalhete and Ponte*. Parameters are from the historical dataset for the Ria Formosa lagoon (available at http://www.barcaweb.com/). Figures in parentheses and italics are from means from Table I of the current study. PCE: physico-chemical elements; PQE: phytoplankton quality element expressed as chlorophyll *a* (chl *a*) concentrations; -, no available data

Boundary Condition		Ramalhete	Ponte
Boundary *		Good/Moderate	High/Good
Geographic location		37º0'22''N 7º58'11''W	37º0'34''N 7º59'38''W
PCE Historical records	Temperature (ºC)	21.5±2.4 *(20.4)*	18.2±3.6 *(20.6)*
	Salinity	36.3±0.6 *(36.5)*	36.2±2.6 *(36.4)*
	Nitrate (µM)	1.3±1.8 *(4.2)*	5.1±13.4 *(5.0)*
	Ammonium (µM)	1.4±1.1 *(1.3)*	2.03±2.40 *(1.26)*
	Nitrite(µM)	0.16±0.18 *(0.14)*	0.30±0.31 *(0.12)*
	DIN	2.8±2.4 *(5.6)*	7.3±17.4 *(6.3)*
	Phosphate (µM)	0.72±0.36 *(0.52)*	0.73±0.48 *(0.6)*
	Silicate (µM)	5.4±2.2 *(3.5)*	13.5±12.6 *(6.1)*
	D.O. (mg l^{-1})	7.3±0.8 *(6.9)*	7.7±1.9 *(7.0)*
	O_2 sat. (%)	-	94±11 *(96)*
PQE Historical records	chl *a* (µg l^{-1})	1.1±0.8 *(1.8)*	1.2±0.9 *(1.8)*

* See earlier footnote about European intercalibration sites for WFD.

during the summer solstice of 2002. A comparison of satellite images just prior to the summer samplings (Fig. 10) show that there is a colder water mass adjacent to the coast in 2001. Physical, chemical and biological patterns of coastal transitional areas can be influenced by the dynamics of coastal upwelling events (Duxbury, 1979; Taylor, 1992; Tilstone et al., 2000, Álvarez-Borrego, 2004), especially in the dry season when freshwater supply from the land is reduced.

Falcão and Vale (1990) have suggested that phosphorus might be the limiting nutrient in the Ria Formosa on the basis of fluxes from the sediments. However, the Redifield ratio (N:P) in this study and that of Newton and Mudge (2005) support evidence from estuaries (Pennock and Sharp, 1994; Ault et al., 2000) and other lagoons (Nixon, 1982; Fong et al, 1993) that nitrogen is the limiting nutrient, at least for the western Ria Formosa. Observations from microcosm experiments described later in Section 4 also support this conclusion (Edwards et al., 2005; Loureiro et al., 2005b).

The results for oxygen concentrations essentially reflect the time of day that the samples are collected. However, at LW in September at both Ponte and Ramalhete there is undersaturation of oxygen, which would be biologically critical if it persisted. Newton and Mudge (2005) have reported persistent undersaturation of oxygen at a station near to Faro town and the Ramalhete channel. Low oxygen saturation may be responsible for some of the symptoms of water degradation that have been reported from some of the restricted regions of the lagoon where there have been marked decreases in benthic organisms (Gamito, 1997b) and substantial mortality of macroalgae (Asmus, at al., 2000). The mortality of macroalgae and the consequent decomposition of the biomass may contribute to low oxygen.

4.2. Microplankton dynamics

The enumeration of the microplankton community does not account for the picoplanktonic assemblage. Pico-phytoplankton includes N_2 fixing cyanobacteria organisms, which can influence the N:P ratios (Smith, 1984). Nevertheless, in systems with short water residence time, like the Ria Formosa, this influence is likely to be low (Granéli and Sundbäck, 1985).

The maximum microplankton abundance is consistently found at Ramalhete during summer sampling (June 2001 and July 2002), although this location does not have the highest nutrient concentrations. However, recent findings have shown that some algae can use dissolved organic matter as a nutrient source (Graneli et al., 1999). Ramalhete is adjacent to a UWWT plant that discharges organic matter (Mudge and Bebbiano, 1997), which increases during the summer months with the influx of tourists to the area. In addition, the residence time of water at this site of the inner lagoon is much greater than at the sites on the outer lagoon (Newton and Mudge 2003; pers observ.). Other studies have shown that transport mechanisms can concentrate algal biomass at a single location from algal assemlages located at other sites in an estuary or lagoon (Lucas et al., 1999; Monsen et al., 2002).

Clustering analysis shows that there is an oceanic community at the Barra that is distinct from the lagoon communities at the Ponte and Ramalhete. Benthic studies of the Ria also point to a distinct lagoonal assemblage in relation to the adjacent Atlantic Ocean (Lock and Mees, 1999). Lagoonal waters frequently have a distinct phytoplanktonic assemblage, compared to the adjacent coastal waters (Sarno et al., 1993). During winter the Ria is exposed to run-off episodes induced by rainfall (Bröckel, 1989), whereas coastal waters are influenced by upwelling events (Fiúza, 1983) and the warmer currents from Cádiz (Relvas and Barton, 2002). Essentially, the Ria-ocean assemblages are exposed to different physical, trophic and

biological conditions that select for distinct biotic communities (Margalef, 1978; Dronkers & Zimmerman, 1982; Reynolds, 2001).

Community analysis groups the microplankton assemblage according to seasonal factors suggesting that the microplanbkton structure in the lagoon is mainly influenced by variability in seasonal parameters, as opposed to tidal and spatial effects. These are mainly: the physical parameters of temperature, solar radiation, and to a lesser extent salinity; and the chemical paramteres, in the form of reduced nitrogen compunds. The more extreme summer conditions support a lower diversity assemblage, probably because of the higher levels of stress selecting for the fittest life-forms (Hutchinson, 1961; Margalef, 1978). Under the milder environmental conditions of the autumn and spring, the mixed populations exhibit a more diverse community (Connell, 1978). The high diversity and equitability indices continue into winter, due to the decrease of individuals associated with the maintanence of taxa number.

4.3 Nutrient enrichment experiments

Over the same period as this current study, there have been short term experiments with nutrient enrichment of nitrogen and phosphorus to estimate how the pelagic microplankton community described in this current study might respond to eutrophic conditions during HW and LW around the summer solstice in 2001 and 2002 and around the autumn equinox of 2001 (Loureiro et.al., 2005). At the sites within the lagoon, the production and biomass is consistently stimulated by nitrogen during the summer but not during the autumn. In contrast, water entering the oceanic inlet at HW is only stimulated during the autumn.

A microcosm experiment using continuous culture techniques has also been carried out over 7 days during April 2002 using lagoon water collected during LW at the Ponte site (Edwards et al., 2005). After enrichment with nutrients, whereby nitrogen would become the limiting nutrient over time, both production and biomass of the microplankton attained a maximum within four days. In both sets of experiments and, in common with other observations in the literature (Loureiro et al., 2005b, references therein), diatoms are the main community that responds to nutrient enrichment.

4.4 Modelling and the application of monitoring indices for eutrophication

Nobre et al. (2005) have used data for the lagoon from a relational data base (Newton et al., 2003) to calibrate and validate EcoWin (Ferreira, 1995), a complex ecological model that simulates physical and biogeochemical state variables for multi-year runs. The water fluxes over the modelling domain have been calculated from the MOHID hydrodynamic model

(Silva et al., 2002). The output from the complex research model (EcoWin) has been used to drive ASSETS (Assessment of Estuarine Trophic Status; Bricker et al, 2003) a simple screening model for eutrophication that has been refined from the NEEA approach (United States National Estuarine Eutrophication Assessement; Bricker et al., 1999) that evaluates symptoms of eutrophication such as chlorophyll *a*, macroalgae, dissolved oxygen, loss of submerged aquatic vegetation, and development of nuisance or toxic blooms. The data set for the Ria Formosa, including data from this study, achieves a score of "good" from a eutrophication status of five classes ranging from "high" to "bad". This hybrid approach to eutrophication assessment has been used to predict the response of the lagoon to a range of nutrient loads, as well as investigate scenarios were there is currently no data. An example of this is night time anoxia in tide pools. Newton and Icely (in press) have also examined what the CSST screening model for eutrophication (Comprehensive Studies Task Team; Tett et al, 2003) would predict for the Ria Formosa. Results suggest that the effects would only be limited in the outer lagoon, but there are possibilities for problems in the inner lagoon, where water exchange is more restricted. An important aspect of this model is the development of biological parameters to assess the "balance of organisms" from the microplanktonic assemblage. These parameters include Eta (η; Tett and Wilson, 2000), which estimates the heterotrophic contribution to the total microplankton community, and Psi (ψ; Tett et al., 2003), which estimates the ratio of non-Si-requiring phytoplankters (flagellates, dinoflagellates and cyanobacteria). Eta is associated with oxygen related processes and psi to the presence of "undesirable" algae, and as such can assess symptoms of eutrophication. Eta and psi vary considerably for the Ria Formosa lagoon (Newton and Icely, 2005) particularly at Barra and Ponte where the residence time of the water masses is generally less than a day.

ASSETS and CSST are part of a suite of models that have been developed to evaluate trophic conditions in marine areas. Other examples include the new trophic index (TRIX) proposed by Vollenweider et al. (1998), which is based on chl *a*, oxygen saturation, mineral N and total P. The eutrophication risk index (EUTRISK) and the Physically Sensitive Area index (PSA) are indices for eutrophication sensitive areas in European coastal waters (Druon et al., 2002). EUTRISK is based on biomass flux estimated by remote sensing chl *a* maps, and PSA combines the physical factors influencing the primary production on the surface with the oxygen availability in the bottom layer. In 1997, OSPAR (Oslo and Paris Conventions) adopted a Common Procedure for the Identification of the Eutrophication Status of the Maritime Area (OSPAR, 1997) by ascribing to each area, one of three categories: problem, potential problem and non-problem.

The important point for this current study is that if these models and indices are to be effective, they require data, preferably, a time series of five years or more (EEA, 2001). Also, they must be compared critically, as Newton et al. (2003) have shown that different screening models can produce very different assessments for eutrophication from the data set.

5. Conclusions

Most of the data obtained for the physical-chemicals conditions (Table 1) are comparable to the historical data for the Ria Formosa (Table 5). There is also a striking difference in DIN concentrations between the 2001 and 2002 summer solstice that has been attributed to the inlfuence of different coastal water masses. There is an interaction between warmer water supplied by coastal currents from Cadiz, with cooler, nutrient-rich water supplied to the Algarve coast by upwelling events.

This study has provided a baseline of data for phytoplankton in the Ria Formosa. Statistical treatment of the data with PRIMER© software has identified microplanktonic assemblages in waters with oceanic origins that are distinct from those in waters with lagoonal origins. In addition, the dominant factor separating the various groups after cluster analysis is the sampling season. Temperature, solar radiation, salinity and reduced forms of nitrogen are the main parameters contributing to these clusters. Although sampling interval of three months is not ideal to establish an annual cycle for phytoplankton in the lagoon, it has been possible to add value to this baseline data through microcosm experiments, which have shown that nitrogen is the principle limiting nutrient in the water column of the lagoon, and that diatoms are the principle microorganisms that will respond to elevated concentrations of nutrients (Edwards et al., 2005; Loureiro et al., 2005b).

In relation to “surveillance” monitoring for the WFD (Water Framework Directive), the sampling interval of three months used in this study has provided adequate data on the physico-chemical quality elements needed for comparison with the historical data. The most developed of the screening models for euthrophication (e.g. CSTT, ASSETS) have used the data on the Ria Formosa to classify the chemical and ecological status of the lagoon as good. However, more detailed sampling, or investigative monitoring in terms of the WFD, is necessary to study those sections of the inner lagoon where residence time is much higher (2-3 days Newton and Mudge, 2003; Mudge pers. obser.) and, also, to establish in more detail the interactions between the lagoon and the adjacent coastal water masses. Investigative

monitoring is also necessary to establish the interactions between the atmospheric, pelagic, benthic and ground water domains in the lagoon.

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3.2. EFFECTS OF NUTRIENT ENRICHMENT ON PRIMARY PRODUCTION IN THE RIA FORMOSA COASTAL LAGOON (SOUTHERN PORTUGAL)

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Key words: nutrient limitation, primary production, bioassay, diatom stimulation, coastal lagoon

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 an 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; Directive2000/60/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

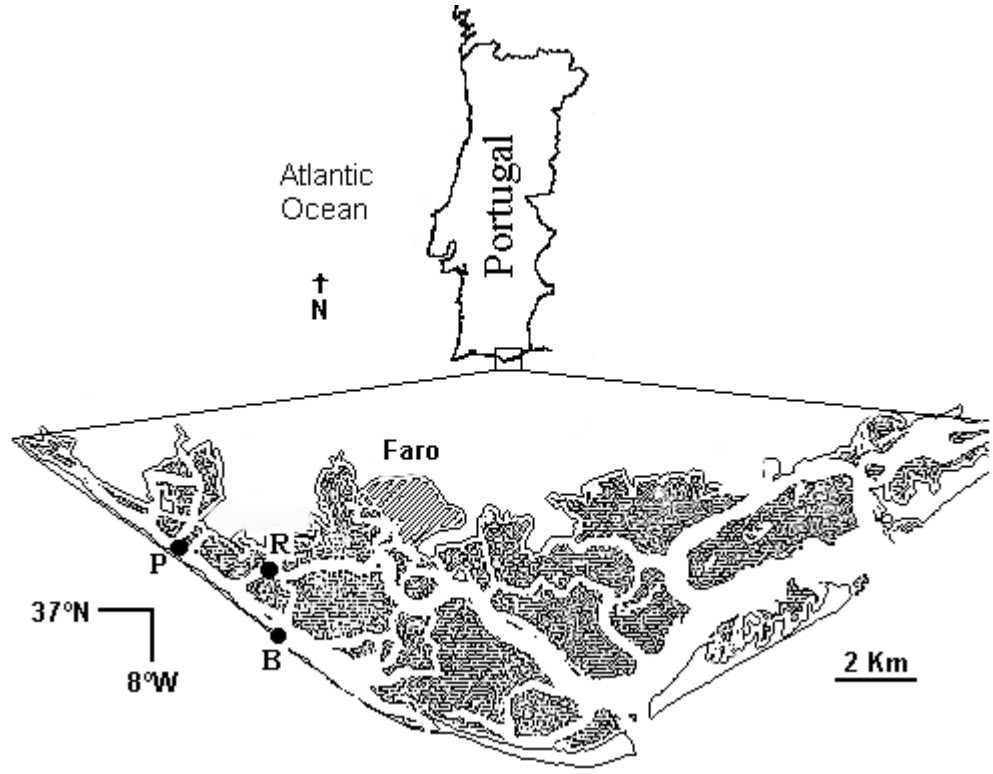

Figure 1. Ria Formosa lagoon and sampling stations: P = Ponte, upstream channel; R = Ramalhete, adjacent to a domestic sewageoutlet: B = Barra, oceanic inlet.

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 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 ºC (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 ºC 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 (07º 58' W, 37º 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

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

	N (μM)			P(μM)		
Date/Station	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

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

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

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 ob tained 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.

Table 3. Initial seawater conditions

Date	Station/Tide	Temp. ºC	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

Lagoonal water was warmer than the oceanic water (Barra HW station), with a maximum temperature (25 ºC) attained at the upstream station (Ponte LW). Salinity differences were more pronounced during the summer, with higher salinity values (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

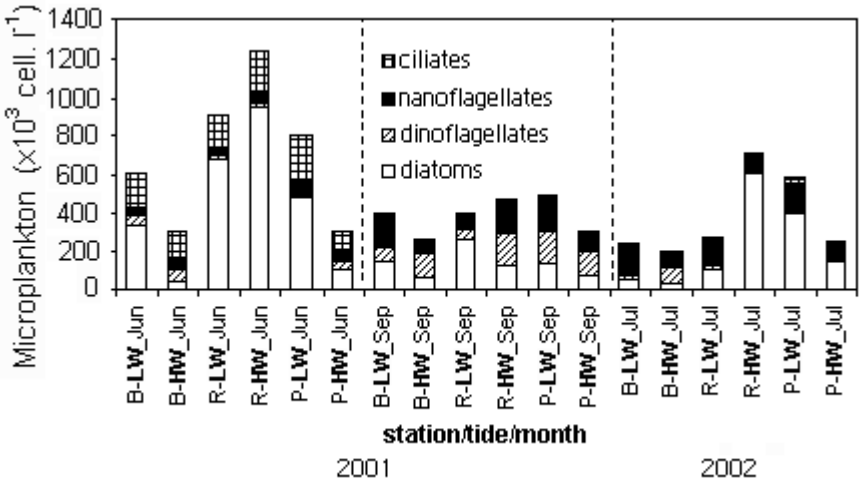

Figure 2. Microplankton abundance (x 10^3 cells l^{-1}) of natural seawater from the Ria Formosa stations: B = Barra; R = Ramalhete; P = Ponte; LW = Low Water (in bold); HW = High Water (in bold).

(1244 x 10^3 cells l^{-1}) occurring during June 2001. Ramalhete HW (sewage outlet) was the station with the greatest concentration (808 ± 395 x 10^3 cells l^{-1}), whereas Barra HW (oceanic inlet) was the station with lowest concentration (257 ± 50 x 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).

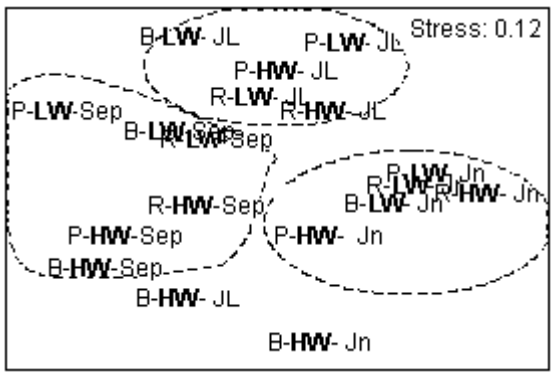

Figure 3. Multi-Dimensional Scaling of the microplankton analysis from the Bray–Curtis similarity matrix of the square root transformed abundance data: B = Barra; R = Ramalhete; P = Ponte; LW = Low water (in bold); HW = Highwater (in bold); Jn = June2001; Sep = September 2001;JL = July 2002.

NCP (Fig. 4) had a similar seasonal evolution as biomass, with higher rates in 2001 (range: 0.8– 1.6 mgO2 l^{-1} d^{-1}), compared with summer 2002 (range: 0.2–0.9 mgO2 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 Naddition of Ponte HWfor 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 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, $p <$

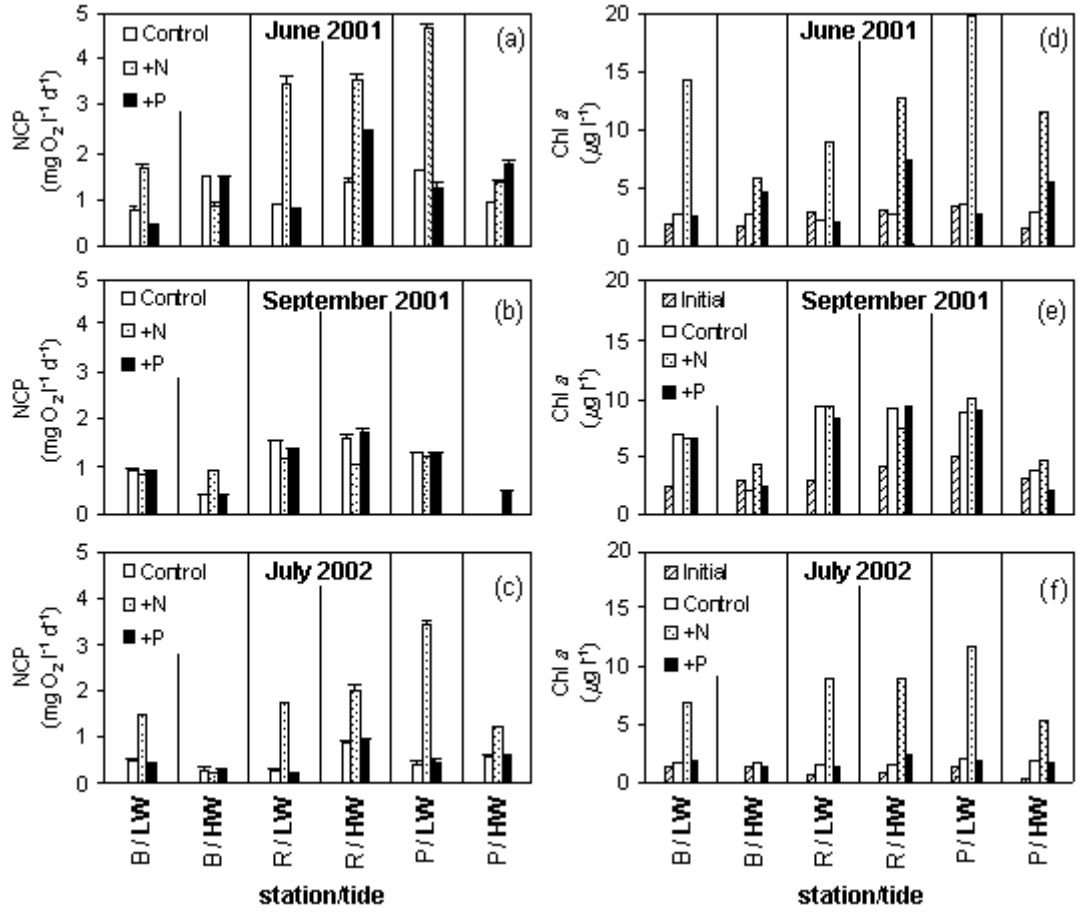

Figure 4. Response to enrichment of Net Community Production (mgO2 l^{-1} d^{-1}) in (a) June 2001, (b) September 2001 and(c) July 2002 experiments; each column is the mean of three replicates; error bars are standard errors, where the bars are not visible it represents small errors hidden by the data 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).

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

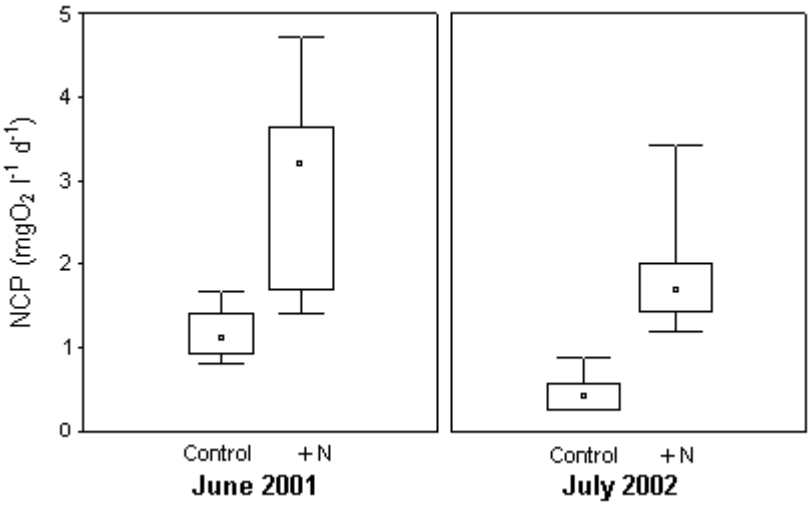

Figure 5. Box-plot distribution of the NCP (mgO2 l^{-1} d^{-1}) from natural water stimulated with N during the summer.

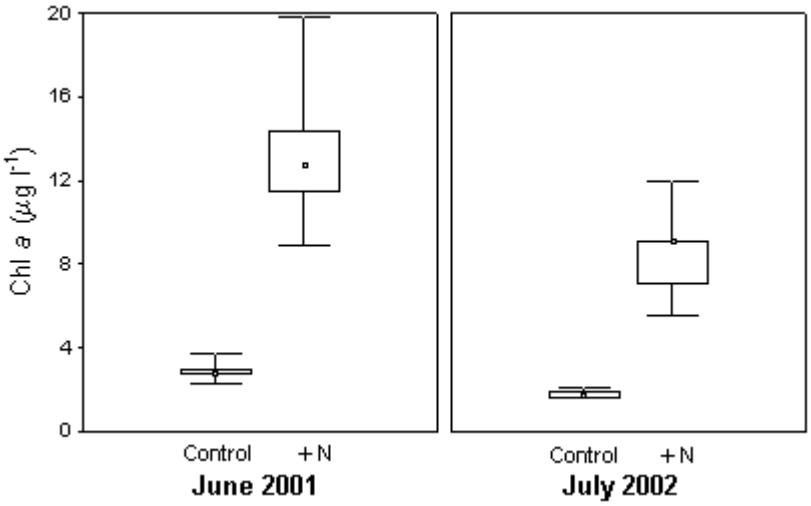

Figure 6. Box-plot distribution of the biomass, as chl a (µg l^{-1}), from natural water stimulated with N during the summer.

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 concen tration in surface waters.

Microplankton composition changes

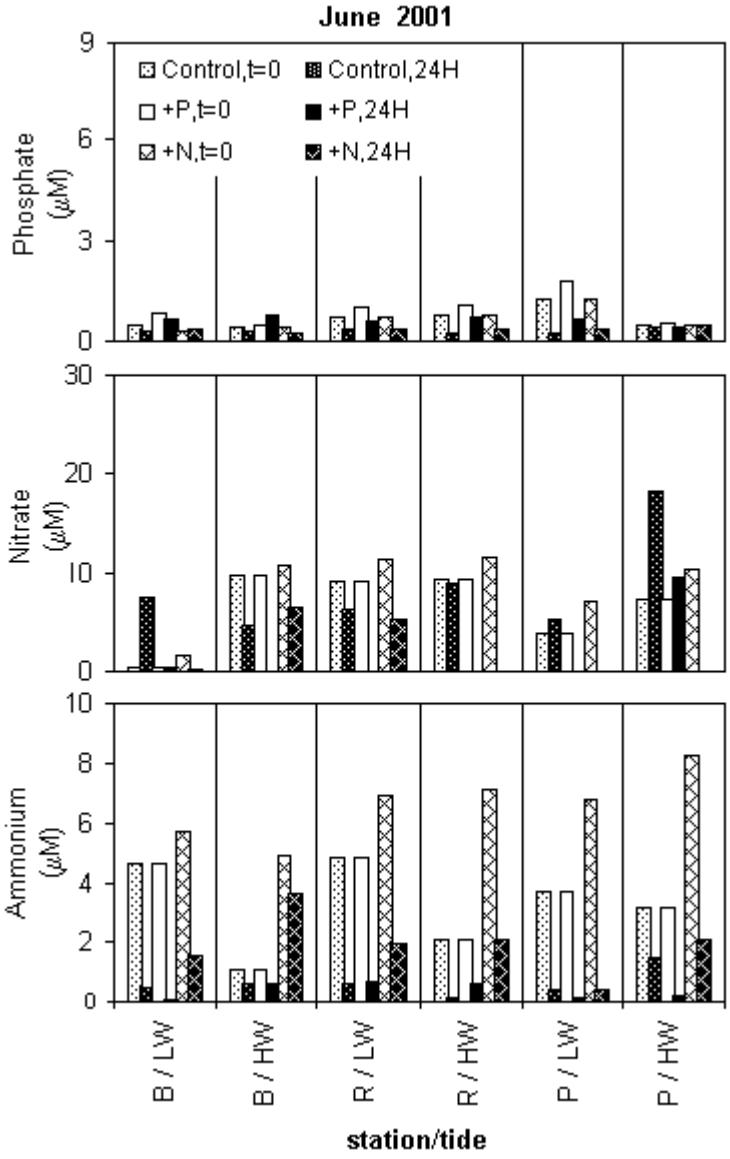

Figure 7. Evolution of inorganic nutrient concentrations (µM) during June 2001: B = Barra ; R = Ramalhete; P = Ponte;LW = Low water; HW = High water.

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

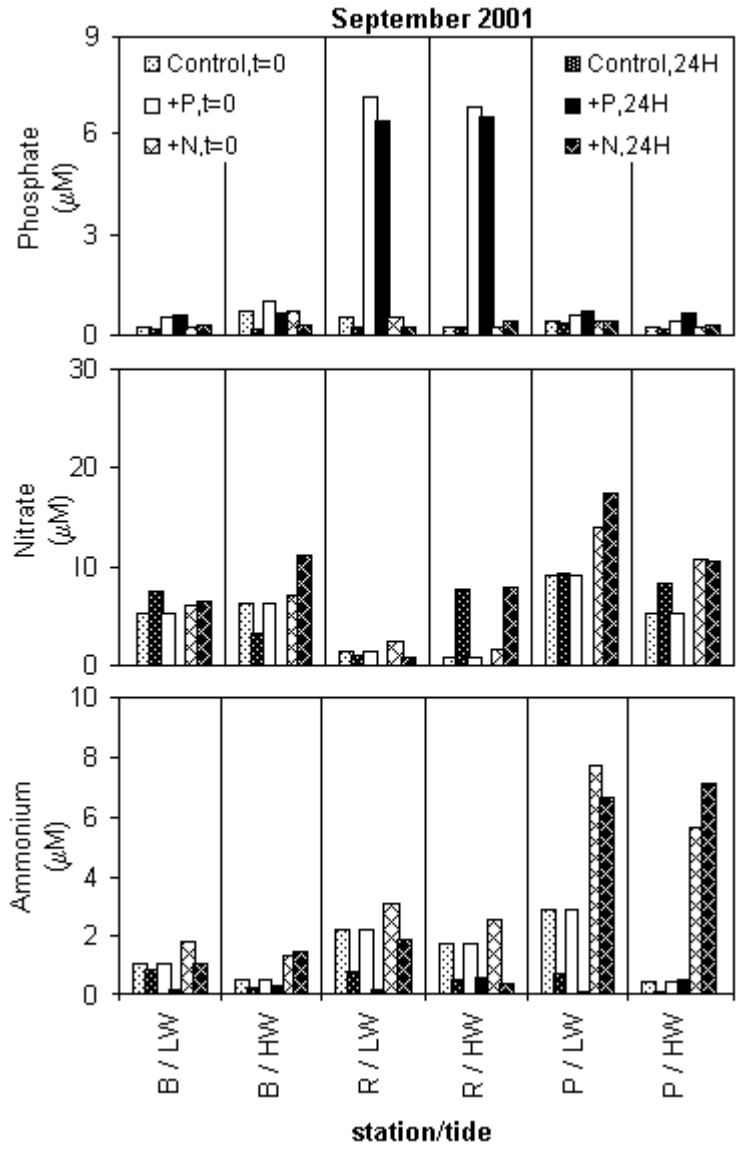

Figure 8. Evolution of inorganic nutrient concentrations (µM) during September 2001: B = Barra ; R = Ramalhete; P = Ponte;LW = Low water; HW = High water.

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

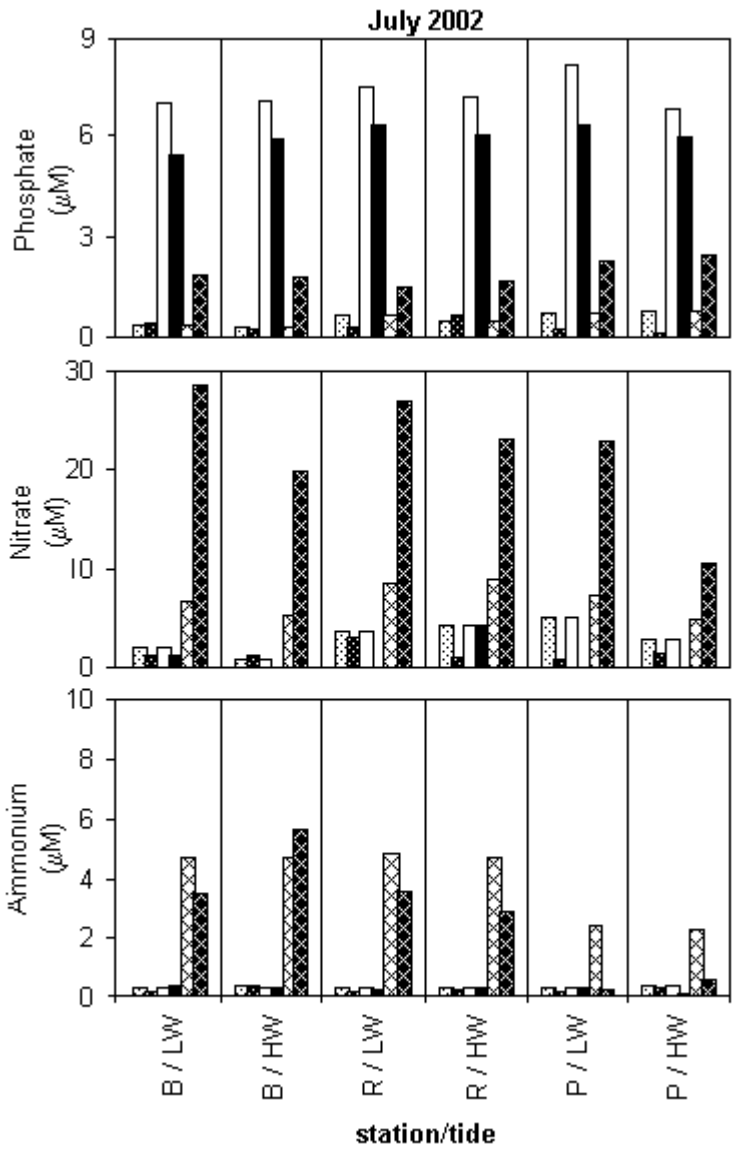

Figure 9. Evolution of inorganic nutrient concentrations (µM) during July 2002: B = Barra ; R = Ramalhete; P = Ponte;LW = Low water; HW = High water.

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).

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

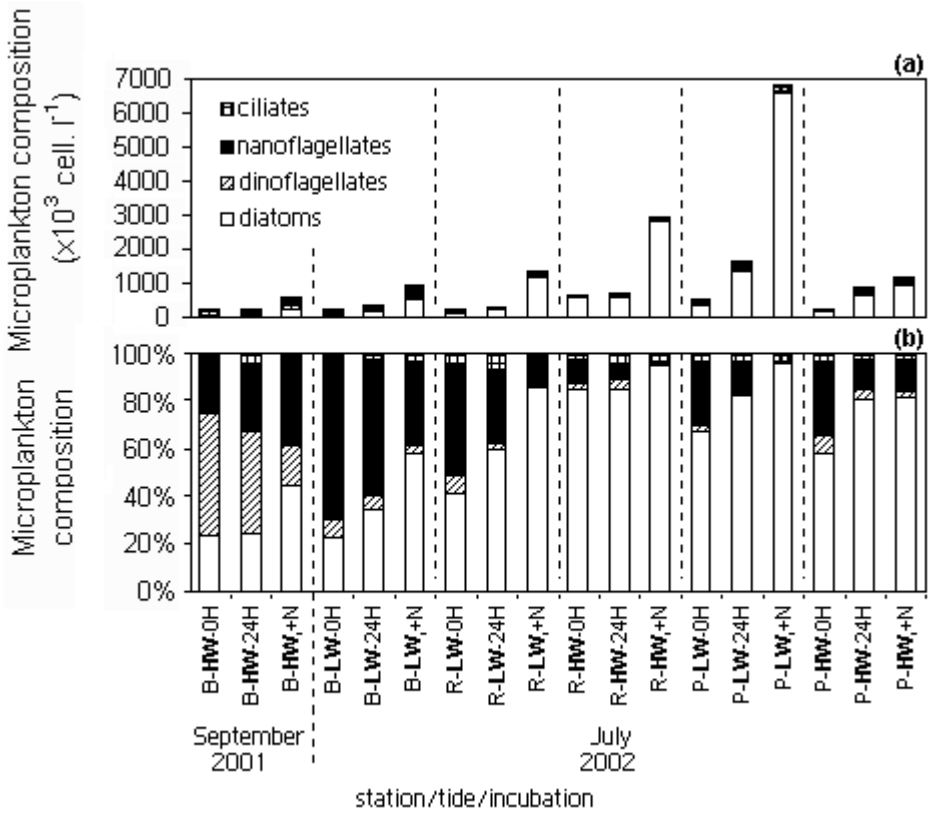

Figure 10. Microplankton composition from September 2001 and July 2002 experiments, expressed as abundance (x10^3 cells. l^{-1}; a) and percentage (b). B=Barra; R=Ramalhete; P=Ponte; LW=Low Water (in bold); HW=High Water (in bold); 0H=initial composition of control samples; 24H=final composition of control samples; +N=final composition of N enriched samples.

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 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 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 (Bishopet 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, 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).

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	-	-	Summerfall	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

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, 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 with a fast consumption by primary producers and/or an export to coastal waters. Other factors affecting algal growth rates, such as light (31 x 10^3 KJ m^{-2} d^{-1} in June 2001 and July 2002; 19 x 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.

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 *a*, 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 lg 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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4. COMPARISON BETWEEN THE TWO SYSTEMS: SAGRES & RIA FORMOSA

La mer joint les régions qu'elle sépare.
Alexander Pope (1688-1744)

The southern coast of Portugal (Algarve) is composed by contrasting systems including an estuarine area (River Guadiana), a coastal lagoon (Ria Formosa) and an upwelling centre (Cabo S. Vicente). Sagres (next to Cabo S. Vicente) and the Ria Formosa were the subject of this study. Sagres is part of the mesotidal moderately exposed Atlantic coast type, whereas the Ria Formosa is classified as a mesotidal shallow lagoon type (Bettencourt *et al*., 2004). Both locations (only 100 km apart) are North East Atlantic intercalibration sites for the Common Implementation Strategy (CIS) of the Water Framework Directive (WFD). In both areas, aquaculture and tourism acitivities are present, which could be negatively affected by eutrophication (e.g. HABs outbreaks and hypoxia events).

Anthropogenic nutrient inputs in Sagres are low due to the scant population (7000 inhabitants in a 179.76 km^2 area), reducing to a minimum sewage discharges and run-off from agriculture land. Fluvial inputs are also minimal because there are no permanent rivers on the area, only small torrential streams. The seasonal upwelling is therefore the main source of nutrients to the waters at this site. Fisheries and oyster-culture practice are both dependent upon upwelling enrichment.

The Ria Formosa is under both anthropogenic and natural influence. Treated and untreated domestic effluents and non-point source agricultural run-off are the main source of anthropogenic nutrient loadings. Benthic fluxes and tidal pumping from sediments are also important sources of nutrients (Falcão & Vale, 1990; Newton *et al*., 2003), together with the transport of rich-nutrient waters into the lagoon, enriched by coastal upwelling events.

The two systems were compared during the growing season, therefore excluding the Ria Formosa winter sampling (December 2001). The data discussed herein are presented on chapters 2 and 3, and annex B.

Natural samples

Despite the proximity of the two locations, the temperature at the Sagres station is significantly lower (Mann-Whitney U test, $p<0.0001$) than that of the lagoon (Table 1), due to the different typology and direct exposure to frequent upwelling events. Salinity is distinctly higher in the Ria Formosa, compared to Sagres (Mann-Whitney U test, $p<0.0001$), as a consequence of the negligible freshwater inputs, shallowness and strong insolation with consequent high evaporation rate (Newton & Mudge, 2003).

The water fertilisation by upwelling episodes in Sagres, induces higher concentration of chl *a* (Mann-Whitney U test, $p=0.017$) than the ones found in the Ria (Table 1), that acts as a chl *a* exporter to adjacent oceanic waters by tidal transport. Values of ammonium are generally higher in the lagoon, reflecting the mechanisms of regeneration therein. They are nevertheless not significantly different from the ones found in Sagres. Nitrate and nitrite concentrations are higher (Mann-Whitney U test, $p<0.03$) in Sagres. Coastal upwelling regions are among the greatest contributers of "new" nitrogen (essentially in the form of nitrate) into the euphotic zone (Dugdale *et al*., 1990). The total inorganic nitrogen (DIN = nitrate+nitrite+ammonium) is greater in Sagres (U test, $p=0.0007$). Phosphate and silicate reach higher concentrations (Mann-Whitney U test, $p<0.003$) in the Ria Formosa. Main sources of phosphate in the inner lagoon include sewage discharges and remineralization processes, whereas silicate seems to be associated with freshwater inputs (mainly induced by rainfall) and flux from sediments during summer (Falcão & Vale, 1990; Newton *et al*., 2003). The N:P ratio is higher in Sagres (Mann-Whitney U test, $p<0.0001$), whereas in the Ria the values are generally <16 indicating a Redfield nitrogen limitation. N:Si ratio is higher in Sagres (Mann-Whitney U test, $p<0.0001$) probably as a reflex of silicate uptake by diatoms and nitrate's upwelling inputs.

Lagoon oxygen concentrations and percent saturations are lower in comparison with Sagres (Mann-Whitney U test, $p<0.04$). The undersaturation of oxygen in the Ria waters is likely a consequence of the degradation of organic loadings from sewage discharges (Newton & Mudge, 2005). Gross oxygen production is higher at the Ria (see Ria Formosa production data in Annex B.1, pag. 195) than in Sagres (U test, $p=0.027$). However, the increased rate of heterotrophic activity in the former (U test, $p=0.0018$) gives rise to similar net community production in the two sites. The similar levels of net production at both locations, in conjunction with the increased chl *a* in Sagres, results therefore in a significantly higher chl *a*-specific rate of net production (P^{chl}=P/chl) in the Ria (U test, $p=0.0004$). This is probably a consequence of the different temperature patterns, as well as the relative higher proportion of nanoflagellates in the Ria Formosa (Fogg & Thake, 1987).

Table 1. Mean, minimum and maximum values of the measured parameters, during growing season. Temp. = temperature; Chl *a* = chlorophyll *a*; DIN = dissolved inorganic nitrogen (NH_4^++NO_2^-+NO_3^-); GP = gross production; DCR = dark community respiration; NCP = net community production; [diat] = diatom, [dino] = dinoflagellate, [nano] = nanoflagellate, [cilia] = ciliate, and [Total] = total microplankton abundance; BM = biomass; U-test = results of Mann-Whitney U test to evaluate the statistical differences of variables between the two groups.

Variables	Sagres			Ria			U-test
	Mean	min	max	Mean	min	max	
Temp. (ºC)	16.0	14.3	19.2	21.4	18.4	24.7	distinct
Salinity	35.8	35.7	36.3	36.5	35.5	37.0	distinct
Chl *a* (mg m^{-3})	3.3	1.2	6.2	2.0	0.1	5.1	distinct
N-NH_4^+ (µM)	0.4	u.d.l.	1.5	1.3	0.1	4.9	similar
N-NO_2^- (µM)	0.2	0.02	0.4	0.1	0.02	0.4	distinct
N-NO_3^- (µM)	9.7	2.2	19.3	4.3	0.4	9.7	distinct
DIN (µM)	10.3	2.2	21.2	5.7	0.5	15.0	distinct
P-PO_4^{3-} (µM)	0.3	0.2	0.5	0.5	0.2	1.3	distinct
Si-SiO_4^{2-} (µM)	1.0	0.1	2.4	4.0	0.5	15.4	distinct
N:P	33.8	9.3	58.7	11.9	3.2	28.4	distinct
N:Si	17.4	4.3	59.6	2.2	0.5	9.7	distinct
O_2 (mg l^{-1})	7.8	7.2	8.9	6.9	5.4	7.9	distinct
O_2 (%)	104	95	117	97	76	111	distinct
GP (µM O_2 d^{-1})	25.4	2.4	60.6	38.0	17.1	65.1	distinct
DCR (µM O_2 d^{-1})	4.3	1.0	13.3	10.6	-3.7	40.6	distinct
NCP (µM O_2 d^{-1})	21.1	-3.9	57.1	27.4	-1.9	50.9	similar
[diat] (x10^3cell l^{-1})	641	146	1366	214	24	953	distinct
[dino] (x10^3cell l^{-1})	71	5	190	59	13	165	similar
[nano] (x10^3cell l^{-1})	43	10	147	82	25	172	distinct
[cilia] (x10^3cell l^{-1})	12	1	30	50	4	222	similar
[Total] (x10^3cell l^{-1})	768	265	1472	405	82	1244	distinct
BMdiat (mg C m^{-3})	245	10	812	19	5	151	distinct
BMdino (mg C m^{-3})	50	1	119	22	1	83	similar
BMnano (mg C m^{-3})	2	1	14	20	1	96	distinct
BMcilia (mg C m^{-3})	20	1	99	14	1	108	similar
BMtot (mg C m^{-3})	317	47	816	76	23	226	distinct
Nº Taxa	33	19	39	30	21	38	similar
Richness	4.94	2.98	6.08	5.16	2.95	7.01	similar
Evenness	0.63	0.42	0.78	0.62	0.29	0.81	similar
Shannon	2.19	1.28	2.73	2.12	0.91	2.89	similar

u.d.l.* denotes under detection limits (NH_4^+<0.02 µM)

Diatoms dominate the microplankton abundance and carbon biomass in Sagres (U test, $p<0.0002$), whilst nannoflagellates are more prominent in the Ria (see Ria Formosa carbon biomass data in Annex B.2, pag. 196) (U test, $p<0.05$), as expected by the higher tolerance to

turbulence (present in Sagres waters) from diatoms (Margalef, 1978). Although dinoflagellates and ciliates data are similar, the total microplankton assemblage is significantly distinct in numbers (U test, $p<0.003$) with higher values registered in Sagres. Table 2 shows the mean abundance and carbon biomass of the most frequent (>50%) taxa. *Pseudo-Nitzschia* spp., *Ceratium* spp. and *Dinophysis* spp., which are potentially harmful microalgae are significantly higher in Sagres (Mann-Whitney U test, $p<0.0005$), whereas the opposite trend is found for *Gonyaulax* spp.

The separation of Sagres and Ria Formosa natural samples is evidenced by the cluster analysis of the Bray-Curtis similarity matrix of the square root transformed abundances (Fig. 1) and biomass (Fig. 2). The main taxa contributing for the intra-group similarities and inter-

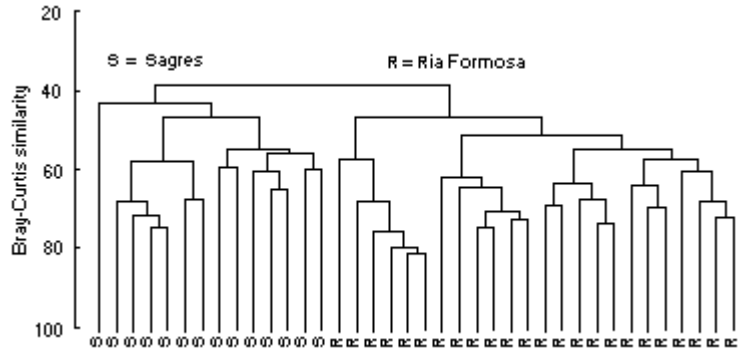

Fig. 1. Dendrogram showing the results of the cluster analysis of the Bray-Curtis similarity matrix of the square root transformed abundances.

group dissimilarities of the assemblages are shown in Table 3. It is found by SIMPER analysis that Sagres is mainly characterized by the presence of *Leptocylindrus* spp.,

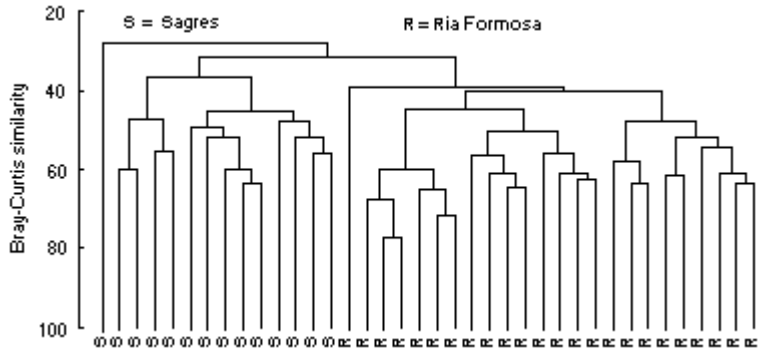

Fig. 2. Dendrogram showing the results of the cluster analysis of the Bray-Curtis similarity matrix of the square root transformed biomass.

Chaetoceros spp. and *Pseudo-Nitzschia* spp. taxa, whereas the Ria most typical components include unidentified nanoflagellates, cryptomonads and *Thalassiosira* spp.. These are also the primary taxa accounting for the dissimilarities between the two locations. *Rhizosolenia* spp., *Chaetoceros* spp., *Thalassiosira* spp., *Lauderia* spp., *Protoperidinium* spp. and *Leptocylindrus* spp. are the most important contribuitors for the observed assemblage

Table 2. Mean abundance (x10^3 cell. l^{-1}) and biomass (mg C m^{-3}) of the most frequent taxa (> 50%) in Sagres and Ria Formosa. For taxa codes see Chapter 3.1, pag. 101.

Taxa Code	Abundance		Biomass		Taxa Code	Abundance		Biomass	
	Sagres	Ria	Sagres	Ria		Sagres	Ria	Sagres	Ria
Diatoms					Amp	1.5	0.9	0.7	0.1
Centrales					Cer	1.4	0.2	9.7	0.8
Cha	187.4	14.6	25.8	0.4	Din	0.7	0.1	1.4	0.2
Cos	1.4	0.1	9.6	1.3	Gym	12.1	5.8	1.5	0.4
Dac	6.9	0.4	1.0	0.1	Gm+Gr	20.7	10.7	3.7	0.5
Euc	3.6	-	3.0	-	Gyr	4.6	2.3	2.3	0.5
Gui	4.9	1.1	14.5	0.5	ProC	6.5	2.7	1.4	0.4
GuiF	0.5	0.2	3.9	0.4	ProP	3.4	3.7	16.9	8.0
Hem	3.1	0.1	1.0	0.1	Scr	3.4	4.0	1.9	1.5
Lau	14.4	-	16.9	-	DNs	11.9	11.0	3.3	2.2
Lep	191	9.0	10.0	0.6	DNb	2.8	0.7	4.2	1.1
Lic	0.6	0.6	0.3	0.4	**Ciliatae**				
Odo	1.2	0.1	1.3	0.1	Hap	0.5	0.1	1.3	0.1
Rhi	26.1	1.9	40.3	1.0	Mes	0.8	5.9	0.7	6.5
Ske	43.5	2.6	0.9	0.1	Oli	9.1	8.3	9.6	12.8
ThaS	28.7	124.5	23.5	9.0	Tin	0.6	0.5	7.5	2.5
DCs	4.5	1.5	0.5	0.1	Cil	1.3	4.4	0.7	6.7
DCb	2.0	0.9	1.0	0.6	**Cryptophyceae**				
Pennales					Cry	22.8	43.5	0.6	0.3
Nav	1.1	1.2	0.4	0.1	**Dictyochophyceae**				
Nit	3.1	6.6	0.4	0.1	Dic	0.6	0.6	0.6	0.4
Ple	14.6	0.1	0.1	0.3	**Euglenophyceae**				
Psn	60.1	5.8	1.7	0.1	Eut	-	3.4	-	0.4
DPb	12.5	0.5	0.1	0.5	**Nanoflagellates**				
Dinoflagellates					Nan	18.6	46.4	0.8	1.7
Ale	0.8	4.5	0.2	2.1					

Table 3. Results of SIMPER analysis giving the contribution (%) from each taxa to the average similarity within each group, and the average dissimilarity between groups (Sagres and Ria Formosa). Av.Abund. = average abundance; Av.Sim. = average similarity of each taxa; Contrib.% = percent contribution of each taxa; Cum% = percent cumulative contribution; Av.Diss. = average dissimilarity of each taxa. Data were square root transformed. Taxa were selected until ~50% of the cumulative contribution was attained. For taxa codes see Chapter 3.1, Table 2 (pag. 101).

	SAGRES			
	Average similarity within the group: 51.44			
Taxa	Av.Abund.	Av.Sim.	Contrib.%	Cum.%
Lep	191.00	9.57	18.59	18.59
Cha	187.39	4.21	8.18	26.77
Psn	60.00	3.71	7.20	33.97
Nan	18.63	3.15	6.13	40.10
GmGr	20.74	2.48	4.81	44.92
Rhi	26.11	2.41	4.68	49.59
Oli	9.10	2.29	4.44	54.04
	RIA FORMOSA			
	Average similarity within the group: 52.86			
Taxa	Av.Abund.	Av.Sim.	Contrib.%	Cum.%
Nan	53.48	6.80	12.86	12.86
Cry	50.93	5.52	10.44	23.30
ThaS	155.67	4.19	7.93	31.23
Oli	8.29	3.07	5.81	37.04
Cha	17.82	2.92	5.52	42.56
DNs	13.39	2.91	5.51	48.07
Lep	11.30	2.85	5.39	53.46
	SAGRES & RIA FORMOSA			
	Average similarity within the group: 61.61			
Taxa	Av.Abund. Ria	Av.Abund. Sagres	Av.Diss.	Contrib.%	Cum.%
Lep	11.30	191.00	5.86	9.51	9.51
ThaS	155.67	28.67	4.67	7.59	17.10
Cha	17.82	187.39	4.66	7.56	24.66
Psn	1.29	60.00	3.29	5.34	30.00
Cry	50.93	22.80	2.32	3.76	33.76
Ske	3.30	43.52	2.27	3.69	37.45
Nan	53.48	18.63	2.06	3.34	40.79
ProP	0.07	14.55	1.83	2.97	43.76
Rhi	2.33	26.11	1.76	2.85	46.61
Lau	0.03	14.35	1.63	2.65	49.26
GmGr	12.46	20.74	1.51	2.46	51.72

differences (data not shown) regarding carbon biomass. However, despite the distinct number of individuals, the differences in the ecological indices between Sagres (see annex B.3, pag. 200) and the Ria are negligible implying a homogeneity in the structural attributes of the microplanktonic community in both locations.

The relation of the microplanktonic assemblage distribution with the studied environmental variables can be visualized by the superimposition of these variables on the MDS ordination, as bubble plots (Fig. 3). Temperature and salinity appear to consistently

divide the two biotic clusters. DIN and nitrate also displays a similar pattern as the total microplankton, denoting a relation between these variables and the biotic ordination.

Enriched samples

The key question of marine ecology in the last decades is what "limits" the development of phytoplankton. This question is of great practical interest regarding fish production and water quality (Granéli *et al*., 1986). The idea that the limiting nutrient is that which predicts the algal increase is important for the control of eutrophication.

The results of small-scale short-term enrichment experiments performed in Sagres (September 2002) and in the Ria Formosa (June 2001, September 2001 and July 2002) lead to similar conclusions: in these coastal systems there is a trend for potential limitation of net production and/or algal biomass (as chl *a*) by nitrogen. Sagres is potentially limited during relaxation of upwelling conditions, whereas the summer season is the sensitive period in the Ria. In both cases the nitrogen-enriched samples demonstrated a general inhibition of nitrate uptake associated with a preferential uptake of ammonium. Preference experiments with isotopes are needed to test which nitrogen source is the primary potential limiting nutrient.

Higher levels of response are attained in the Ria, which can be a consequence of the distinct environmental conditions in which the experiments were performed. Higher irradiance and temperature values are known to be more favourable to photosynthesis (Kirk, 1994). The predominance of fast growing diatoms in the Ria natural samples is probably reflected in the increased production rates, in comparison with the predominance of flagellates during the Sagres relaxation period. The nutritional status of the cells determined by the external and the internal (cell quota) amount of nutrients is also important in the dynamics of such bioassays.

There is a general stimulation of diatoms by nitrogen enrichment, which is in agreement with microcosmos experiments performed in these areas (Edwards *et al*., 2005), as well as with several other enrichments bioassays done in coastal regions (see Chapter 3.2, Table 4, pag. 140). The diversity (see annex B.3, pag. 200) of the resulting assemblage does not significantly differ from that of controls, although the number of individuals increases with enrichment. The nitrogen addition causes an increase in both centric (Sagres: *Leptocylindrus* spp.; Ria Formosa: *Thalassiosira* spp., *Chaetoceros* spp., *Skeletonema* spp. and *Leptocylindrus* spp.) and pennate (Sagres and Ria: *Pseudo-Nitzschia* spp. and *Nitzschia* spp.)

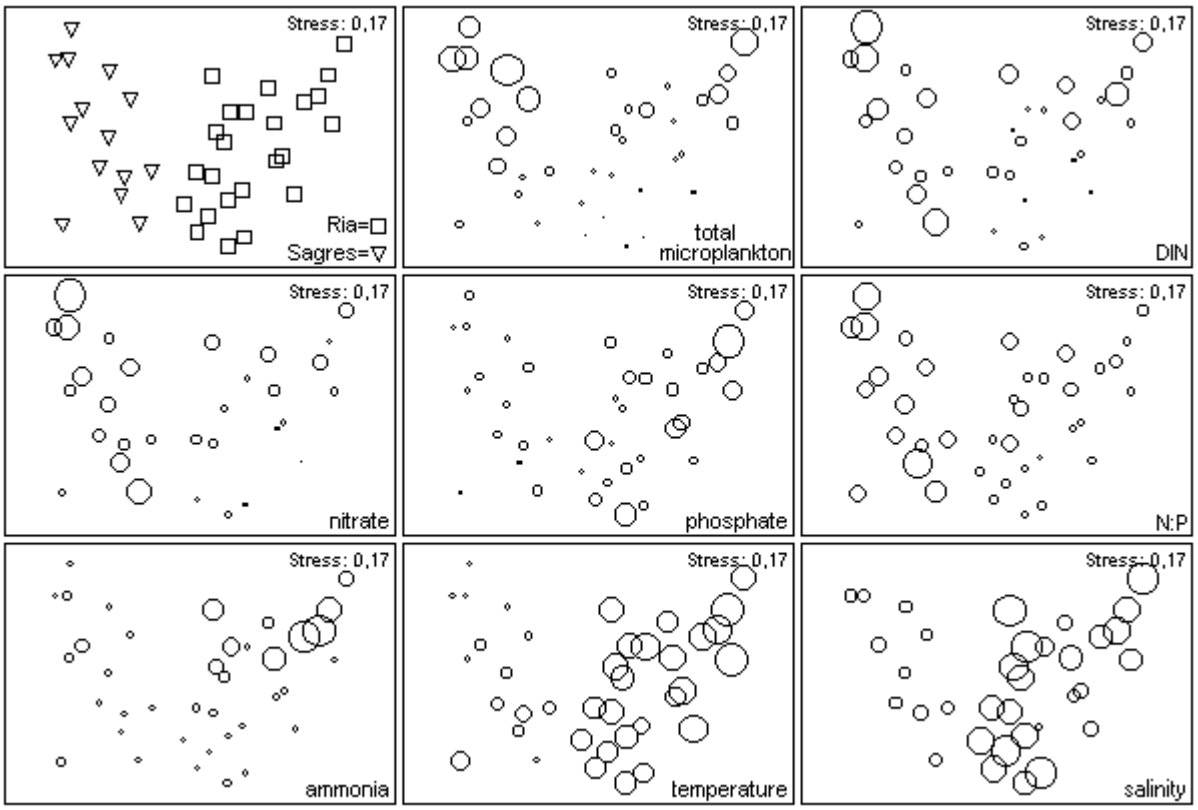

Fig. 3. MDS ordination of Bray-Curtis similarities from square root transformed abundances. Bubble plots represent the super-imposition of the total microplankton abundance and environmental variables (specified on each plot) to the biotic ordination. DIN = dissolved inorganic nitrogen (ammonium+nitrite+nitrate).

diatoms. *Pseudo-Nitzschia* spp. may include toxic species that can cause food poisoning after ingestion of contaminated shellfish. Nitrogen stimulation of potentially harmful dinoflagellates also includes *Gymnodinium* spp. and *Scripsiella* spp. in the two studied areas (see annex B.4, pag. 201). There are no reports of nuisance problems caused by *Scripsiella* spp. in the south of Portugal. Blooms of *Gymnodinium catenatum*, a paralytic shellfish-poisoning (PSP) producer, have been detected in this area since 1994, provoking the temporary closure of shellfish harvesting on several occasions. It seems that the intensity and frequency of these blooms have decreased since 1996 (Vale, 1999).

Conclusions

The comparison herein, based on statistical analysis of the studied variables, and regarding the active biological period (i.e., excluding winter sampling), reveals some differences between the two systems under study (Sagres & Ria Formosa) on the biological and physico-chemical level. It seems that the gradients of temperature and salinity, together with nutrient

concentrations (especially nitrogen forms), are important ecological factors contributing for the observed biotic dissimilarities. The differences in taxonomic dominance from diatoms in Sagres, to flagellates in the Ria have been commonly associated with growing anthropogenic eutrophication (Niemkiewicz & Wrzolek, 1998). The nature of nutrient inputs often implies a change in nutrient ratios that can induce a rearrangement in the biological composition of the systems (Moncheva *et al*., 2001). However, phytoplankton dynamics is a complex process frequently determined by the interaction of biological, chemical and physical processes (e.g. grazing, cell lysis, cell quota, nutrient availability, advection, turbulence, sinking, light regime), being difficult to relate the community structure to a single parameter at a time (Margalef, 1978; Cloern, 1991, 1996; Smayda, 1997; Lucas *et al*., 1999).

Similar results were observed regarding the number of taxa involved, diversity and evenness indices. Despite the different concentration of nutrients, and microplankton abundance and carbon biomass, the values of net production (the part that is available for transfer to higher trophic levels) are equivalent. It should be noted that due to the differences in water depth, a detailed regional comparison should include values of production per unit area and benthic production. Nevertheless, data on volumetric production of surface waters give a preliminary idea about the ranges of these rates.

Nitrogen seems to be the primary potential nutrient limiting phytoplankton production and/or biomass in both regions, during certain times of the year. Other factors such as light regime, temperature, grazing, turbulence and mixing, or top-down pressure from benthic feeders are expected to control algal rates in a seasonal basis in temperate regions (Margalef, 1978; Kirk, 1994; Pennock & Sharp, 1994; Carlsson & Granéli, 1999). Diatoms are found to be the most sensitive group to nitrogen enrichment in both systems.

During the studies herein, both sites generally fell within the ranges of mesotrophic conditions, although a higher sampling frequency is needed for a clear trophic classification (Wasmund *et al*., 2001). It seems that the frequent water exchange between the Ria and the oceanic waters keeps the lagoon in a low trophic status.

Overall, although statistical analysis suggest some differences in the biological and physico-chemical characteristics of the two studied systems, these seem to be not sufficient to distinguish between Natural and Anthropogenic eutrophication. E.g., nitrate inputs are associated to natural upwelling events and not just to human activities; HABs, which are beginning to be used as anthropogenic eutrophication indicators in some countries, seem to have mainly an oceanic origin on these areas; despite some cells are more numerous in one system then in other, most of the microplankton assemblage components are common to both

areas. As such, although the algal assemblage responded to changing trophic conditions, supporting the need to incorporate these data in monitoring programmes, this seem not to be sufficient for the management of anthropogenic eutrophication in the systems under study.

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5. GENERAL CONCLUSIONS

Cuando estuve por primera vez frente al océano
quede sobrecogido.
Allí entre dos grandes cerros (el Huilque y el Maule)
se desarrollaba la furia del mar.
No era sólo las inmensas olas nevadas
que se levantaban a muchos metros de altura sobre nuestras cabezas,
sino un estruendo de corazón colosal,
la palpitación del universo.
Pablo Neruda, Confieso que he vivido, 1974

The EU (European Union) states eutrophication as (EC, 1991):

> "*enrichment of water by nutrients, especially compounds of N and/or P, causing an accelerated growth of algae and higher forms of plant life, to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned*."

NOAA (U.S. National Oceanic and Atmospheric Administration) recently updated their definition (Bricker *et al*. 2003):

> *"a natural process by which productivity of a water body, as measured by organic matter, increases as a result of increasing nutrient inputs. These inputs are a result of natural processes but in recent decades they have been greatly supplemented by various human related activities. Cultural eutrophication, or nutrient overenrichment, is the enhanced accumulation of organic matter, particularly algae, that is caused by human related increases in the amount and composition of nutrients being discharged to the water body. A variety of impacts may result, including nuisance and toxic algal blooms, depleted dissolved oxygen, and loss of submerged aquatic vegetation and benthic fauna. These impacts are interrelated and usually viewed as having a negative effect on water quality, ecosystem health, and human uses. Management concerns should address the human, or cultural,*

portion of nutrient additions insofar as the additions are detrimental to the environment."

(for more definitions see chapter 1, pag. 11)

Eutrophication in coastal waters originates from both natural (coastal upwelling) and anthropogenic sources (e.g. discharges from waste water treatment plants, run-off of inorganic fertilizers from agricultural land, deposition of atmospheric nitrogen from automobile and power plant emissions) (Barber & Smith, 1981; Cloern, 2001). The final nutrient stoichiometry in the marine environment frequently differs according to the nature of the supply. The alteration of nutrient ratios may induce the selection of particular biological forms thereby affecting the structure of ecosystems (Moncheva *et al*., 2001; Nuccio *et al*., 2003). One of the primary symptoms of eutrophication is the increase in the rates of primary production (Oviatt *et al*., 1986). The impact of eutrophication will depend upon the attained levels of primary production and its degree of coupling with decomposition and transport processes (Bricker *et al*., 1999). Hence, the study of the dynamics between algal assemblages and environmental variables (including nutrient availability) is important to comprehend the behaviour of coastal ecosystems in relation to eutrophication. New scientific knowledge will support the application of future management actions in order to assure the preservation and effective use of the multiple coastal resources.

Through this dissertation several biological quality elements and their supportive physico-chemical elements refered in the European Water Framework Directive (WFD), have been assessed in two representative locations in the south coast of Portugal (Algarve). The fertilisation of Sagres waters (adjacent to an upwelling centre) is based upon natural eutrophication processes, whereas the Ria Formosa (a mesotidal shallow coastal lagoon) is subject to both natural and anthropogenic nutrient enrichment. Additional data from nutrient enrichment bioassays contributed to further elucidate the influence of nutrient concentration and ratios, on the microphytoplankton community in the systems under study.

The Sagres area is influenced by the wind-driven circulation patterns off the south and west coasts. Main physical features include a seasonal upwelling from May to September and the intrusion during relaxation stages of the warm coastal counter-current originating in the Gulf of Cádiz. The results herein (chapter 2.1) show that the cold upwelled waters are associated with a diatom-dominated assemblage, whereas flagellate forms predominate during relaxation stages when the water column becomes stratified. This stratification seems to be induced by the intrusion of the warm coastal countercurrent. Nevertheless, the classical

pattern of succession - diatoms-flagellates - (Margalef, 1978; Smayda, 1980) does not fully unfold until the end of the upwelling season when stratification of the water column occurs (Reynolds, 1989). Before the development of such conditions a progression of a fortnightly cycle of upwelling-relaxation is observed, typical of temperate coastal upwelling areas.

Statistical assemblage analysis identified *Chaetoceros* spp., *Thalassiosira* spp., *Lauderia* spp., *Detonula* spp., and *Pseudo-nitzschia* spp. as a summer upwelling proxy for this site. The occurrence of potentially harmful microalgae in the phytoplankton samples allowed to infer some general trends about its distribution during this investigation. The presence of potentially toxic and nuisance taxa includes *Pseudo-nitzschia* spp., *Alexandrium* spp., *Ceratium* spp., *Dinophysis* spp., *Gonyaulax* spp., *Gymnodinium* spp., *Prorocentrum* spp., and *Scripsiella* spp. Of these, *Pseudo-nitzschia* spp., *Gymnodinium* spp., *Prorocentrum* spp., and *Scripsiella* spp. are the most abundant. However, *Dinophysis* spp. can cause diarrhetic shellfish poisoning (DSP) even at very low concentrations (< 500 cell. l^{-1}); during this survey *Dinophysis* spp. reached 2600 cell. l^{-1}. *Pseudo-nitzschia* spp. may include toxic species associated with amnesic shellfish poisoning (ASP) which may have a negative impact in the oyster-culture practised in this area. Since 1994 blooms of *Gymnodinium catenatum* (paralytic shellfish-poisoning -PSP- producer) caused the temporary closure of shellfish harvesting on several occasions, but the intensity and frequency of these blooms have decreased since 1996. Intense blooms of *Lingulodinium* spp., a potentially harmful taxon, have nevertheless been detected during September 2004 along the Portuguese South shelf (Newton, pers. comm.). No harmful effects regarding *Prorocentrum* spp., and *Scripsiella* spp. have been reported. In conclusion, the presence of nuisance or toxic microalgae in this area is mainly the consequence of natural phenomena associated with upwelling dynamics and not with anthropogenic eutrophication. This has implications for the implementation of the WFD, which is essentially concerned with the deterioration of water quality from human activities.

The values of new nitrogen, chlorophyll *a* (chl *a*) and primary production are comparable to other productive upwelling areas (see chapter 2.1 for comparison with similar systems) and are associated with the persistence of favourable upwelling conditions. The low respiration rates together with the decoupling between autotrophic and heterotrophic processes seem to be an expression of the predominant autotrophic component and mechanisms of physical losses. New nitrogen decreases during quiescent periods (chapter 2.2), when the system is mainly sustained by a regenerated-based primary production. The formation of transient pools of nutrients during algal luxury uptake probably also contributes for the levels of rates observed but they were not evaluated on this study. Nitrogen seems to be the potential

nutrient limiting algal growth during relaxation stages. Diatoms are the most sensitive group to nitrogen addition in enrichment experiments. The nitrogen stimulation of *Pseudo-nitzschia* spp. can be of concern due to the presence of an oyster-culture in the area.

Overall, the results suggest that the structure and activity of the microplankton community in Sagres is mainly a reflexion of the dynamics of the physical events occurring in this area, which in turn regulates the supply of nutrients to the pelagic domain.

The Ria Formosa coastal lagoon is subject to a frequent and intense tidal exchange with the adjacent Atlantic waters. The shallow lagoonal waters are generally saltier and warmer than the deeper oceanic ones due to high insolation and evaporation processes during summer. This pattern is reversed during winter due to the freshwater inflow, caused by episodes of rainfall, and the cooling of the shallow waters (Newton & Mudge, 2003).

The microplankton assemblage peaked during the summer solstices samplings (June 2001 and July 2002) (chapter 3.1), numerically dominated by diatoms. Flagellates usually dominated the carbon biomass in both occasions. Picophytoplankton is generally abundant in similar systems but they were not quantified in this work. The hierarchical agglomerative clustering technique grouped the microplankton assemblage into the seasonal sampling groups. The seasonality of temperature, solar radiation and salinity, together with the variation of nitrogen (essentially in reduced forms) seem to be the main parameters influencing the microplanktonic evolution. The more extreme environmental conditions can select for a less diverse community during the summer season.

The low concentration obtained for chl *a* during the growing season may be a consequence of bivalve suspension-feeding and/or export to oceanic waters. The lagoon also acts as a nutrient exporter, which contributes to maintain a low trophic level in the Ria. Sources of nutrients to lagoonal waters include sewage discharges, agricultural run-off, sediment fluxes and tidal pumping (Falcão & Vale, 1990; Newton *et al*., 2003). Nutrients, especially nitrogen, can also be imported into the lagoon by the transport of coastal upwelled waters. The interactions between the Ria and the adjacent coastal events should be the focus of further studies involving broader scales of investigation, so that a better understanding of the relative effects of natural versus anthropogenic processes contributing to the eutrophication of this system is attained.

The oceanic assemblage differs from that of the lagoon in certain occasions, which is likely due to the influence of external upwelling events during the summer and internal winter run-off episodes, that contribute to a greater contrast between the two systems and thereby for the selection of distinct life-forms. Results suggest that during the survey, potentially harmful

taxa were mainly imported into the Ria from the adjacent Atlantic waters. Tidal flushing will likely minimize the presence of such organisms in the lagoon, unless they are driven into areas of reduced water renewal where they might accumulate. *Alexandrium* spp., *Pseudo-nitzschia* spp., *Scrippsiella* spp. and *Gymnodinium* spp. were the most abundant potentially harmful microalgae observed during the survey. This also leave important consequences to water quality management. Even if nutrient inputs into the lagoon are managed, HABs (Harmful Algal Blooms) may still be imported from natural offshore events.

The monitoring of HABs and phycotoxins are used as an additional indicator in the context of anthropogenic eutrophication in relevant areas (e.g. shellfish production areas) of European countries (e.g. OSPAR, 1997; EEA, 2004). Nevertheless, the dynamics between physical, chemical and biological processes triggering the development of harmful algal events is still poorly understood and are the focus of several international programmes such as ECOHAB (Ecology and Oceanography of Harmful Algal Blooms), EUROHAB (European Initiative on Harmful Algal Blooms), and GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms). Although HABs development may be linked to changes in nutrient inputs to coastal ecosystems as a consequence of human activities (Anderson et al., 2002; Vila & Masó, 2005), many blooms also occur in areas where input of nutrients have origin in natural events such as in coastal upwelling areas (GEOHAB, 2003). HABs develop mainly as a consequence of natural phenomena (upwelling) in the Portuguese Atlantic coast (e.g. Moita *et al*, 1998; Moita, 2001), and should be used with caution as an anthropogenic eutrophication indicator in thia area. Natural events cannot be prevented or reduced by feedback societal management response as in the usual context of the DPSIR (Driving Force - Pressure - State - Impact - Response) reporting environmental framework (EEA, 1999). Activities such as aquaculture, a growing economical sector due to the overexploitation of natural fish stocks (EEA, 2003), and tourism-related businesses can be affected by the development of HABs because of contamination of cultured species and public avoidance of tourist affected areas. Effective surveillance methods that ensure the closure of contaminated-cultured organisms harvesting in due time are needed. Appropriate assessement should include the prediction of these events by models based on the area history, metereological data, and physical factors, and its early detection by remote sensing and molecular probes. Regional algorithms for remotely sensed chlorophyll *a* should be developed. An effective reporting system, so that information is quickly transmited has also to be ensured (EU-US HAB, 2003; GEOHAB, 2003) in order to mitigate the consequences of this problematic phenomenon.

Within the lagoon, areas prone to restricted water exchange due to circulation patterns tend to accumulate dissolved nutrients and algal biomass, which may cause episodes of hypoxia and consequent water degradation decreasing the amenity value of the water. This could affect the fishing, aquaculture, and recreational activities that rely on good water quality. Increased algal numbers may also be found next to regions of urban waste water treatment (UWWT) discharges. However, transport mechanisms including tidal currents and wind hydrodynamic forcing can induce migration processes thereby influencing the final location of the accumulated material.

Results regarding N:P ratios and enrichment experiments (chapter 3.2) suggest nitrogen as the potential nutrient limiting the algal biomass and activity during the summer growing season. This contradicts the previous expectation of phosphorus limitation in the Ria Formosa. Factors such as the opening of UWWT plants, dredging, opening of new inlets, reduction of nitrogen from agriculture fertilisers by implementation of the Nitrates Directive along with denitrification in bottom sediments may explain the decrease of nitrogen in the lagoon. Experimental nitrogen addition stimulated the growth of diatoms, including the *Pseudo-nitzschia* spp., which may include ASP producers. Enrichment experiments suggest that the development of phytoplankton is associated with the seasonal switch of limiting factors, which is traditionally observed in temperate regions due to the seasonal variability of environmental parameters.

The comparison between Sagres and the Ria Formosa during the growing season (chapter 4) show some distinct biological and physico-chemical characteristics. Briefly, Sagres present colder and less salty waters than the Ria as a consequence of the different typology, hydrodynamics and depth of the water column. Nitrate and nitrite are generally higher in Sagres due to the nitrogen-rich upwelled waters, in contrast with ammonium values, which are similar in both locations. Inversely, phosphate and silicate are higher in the lagoon waters because of the influence of sewage discharges, sediment flux remineralization processes and freshwater inputs. The N:P ratios are as a consequence higher in Sagres. Also, the organic loadings within the lagoon waters and its consequent decomposition processes induce lower oxygen values in this area.

The levels of volumetric net production are similar in the two systems, although chl *a* concentration together with total microplankton abundance and carbon biomass are higher in Sagres. This implies a higher chl *a*-specific rate of net production (P^{chl}= P/chl) in the Ria, which is probably connected to the higher numbers of nanoflagellates in the lagoon. The taxa contributing most to the assemblage dissimilarities between the locations include

Leptocylindrus spp., *Chaetoceros* spp. and *Pseudo-Nitzschia* spp. taxa, more abundant in Sagres, and unidentified nanoflagellates, cryptomonads and *Thalassiosira* spp. more abundant in the Ria. Biotic dissimilarities seem to be associated with the distinct gradients in physical factors (temperature and salinity) and nutrient concentration (essentially related to nitrogen forms), according to the statistical assemblage analysis. It should be noted that other factors not included in this study, such as turbulence, mixing, grazing pressure, etc should be important for the observed biotic dissimilarities.

Results regarding nutrient enrichment bioassays suggest potential nitrogen limitation of algal production and/or biomass during certain periods in both systems. Fast growing diatoms were the group more stimulated by nitrogen addition. Diatoms are generally regarded as a beneficial group because they form an important link in the food chain to zooplankton and fish. Stimulation of potentially HABs taxa included *Pseudo-Nitzschia* spp., *Gymnodinium* spp. and *Scripsiella* spp. in the two locations.

Both systems generally presented mesotrophic conditions during the samplings. Mixing dynamics and transport processes decrease the impacts of eutrophication so that the critical level for the integrity of ecosystems is not usually surpassed.

Overall, although some differences were found between the two systems regarding the selected parameters, these seem not sufficient to distinguish between Natural and Anthropogenic Eutrophication and as such, not sufficient for the management of Anthropogenic Eutrophication on the studied areas. The elements selected could be complemented with other tools such as: other quality elements refered on the WFD (e.g. macroalgae, benthic fauna), the ratio between microphytoplankton and bacteria, isotopic studies to determine the source of nutrients, etc. Future studies could include isotopic preference experiments to test which nitrogen form is limiting the systems. The interactions between the benthic-pelagic-atmospheric domains should also be considered to reach a better understanding on the ecosystem level. A good management and preservation of ecosystems can only rely in a global understanding of their structure and functioning. This investigation contributes to create a general base of knowledge of the two productive systems under study: Sagres and the Ria Formosa, and to the on-going process of implementation of the WFD.

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ANNEX A: MATERIAL & METHODS

A.1. DETERMINATION OF DISSOLVED OXYGEN IN SEAWATER

This method is an adaptation from Winkler (1888), as modified by (Strickland & Parsons, 1972) and described in detail in Grasshoff *et al.* (1983).

Reactions

A divalent manganese solution and an alkali are added to the water sample, which results in the precipitation of maganese (II) as hydroxide:

$$Mn^{2+} + 2OH^{-} \rightarrow Mn(OH)_2 \quad (1)$$

manganese (II) hydroxide is oxidized to manganese (III) hydroxide:

$$2Mn(OH)_2 + 1/2O_2 + H_2O \rightarrow 2Mn(OH)_3 \quad (2)$$

Subsequent acidification ($1 < pH < 2.5$) dissolves the precipitated hydroxide; the iodide ions (part of the fixation reagents) are oxidized to iodine in stoichiometric proportion to the original oxygen concentration:

$$2Mn(OH)_3 + 2I^{-} + 6H^{+} \rightarrow 2Mn^{2+} + I_2 + 6H_2O \quad (3)$$

Iodine complexes with surplus iodine:

$$I_2 + I^{-} \leftrightarrow I_3^{-} \quad (4)$$

Iodine is titrated with a standardised thiosulfate solution. The endpoint of the redox titration is detected by means of a starch indicator.

$$I_3^- + 2S_2O_3^{2-} \rightarrow 3I^- + S_4O_6^{2-} \quad (5)$$

Reagents

Manganese (II) chloride reagent: 60g of $MnCl_2.4H_2O$ was dissolved and made upto 100 cm^3 with deionised water.

Potassium hydroxide solution: 32g of KOH was dissolved in deionised water in a 100 cm^3 volumetric flask.

Potassium iodide solution: 60g of KI was dissolved and made upto 100 cm^3 with deionised water.

Alkaline iodide reagent: 50cm^3 of NaI solution was mixed with 50cm^3 of NaOH solution.

Sulphuric acid: 50cm^3 of concentrated sulphuric acid was added to 50cm^3 of deionised water and the volume made up to 100cm^3 with deionised water.

*Sodium thiosulphate solution***:** 4.95g of $Na_2S_2O_3.5H_2O$ was dissolved and made upto 1dm^3 with deionised water.

Potassium iodate standard solution: Potassium iodate was dried for 1hour at 100º C and 0.3567g are dissolved and made upto 1dm^3 with deionised water.

Starch indicator: 1g of soluble starch is boiled in 100cm^3 of water.

Standardisation of thiosulphate

A volume of 50 cm^3 of deionised water was placed into a sample bottle to which it was added 1 cm^3 of acid sulphuric solution, 1 cm^3 of alkaline iodide reagent and 1 cm^3 of manganese(II) chloride reagent. A 10 cm^3 of the potassium iodate standard solution was added, followed by titration with the thiosuphate solution using a starch indicator. The solution was mixed after each addition. The concentration of the thiosulphate solution was calculated as:

$$C_t = (6 \times V_{std} \times C_{std})/V_t \quad (6)$$

where V_{std} = Volume (cm^3) of the iodate standard, C_{std} = molar concentration of the iodate standard, and V_t = added thiosulphate volume.

Blank

A volume of 50cm^3 of deionised water was placed in a sample bottle. A 1cm^3 of sulphuric acid solution was added followed by the addition of 1cm^3 of the alkaline iodide reagent and 1cm^3 of the manganese (II) chloride reagent. After each addition the sample is thoroughly mixed. A 1cm^3 of the iodate standard solution was added and the blank was titrated with the sodium thiosulphate solution and the starch indicator. Another 1cm^3 of iodate standard was added and titrated again. The reagent blank is the difference between the first and second volumes of the thiosulfate titrations.

Sample titration

The bottle samples were filled with seawater to which it was added the fixing reagents: 1 cm^3 of manganese(II) chloride reagent, followed by 1 cm^3 of alkaline iodide reagent. The bottle was stoppered and shaken. After the settlement of the precipitate, the sample bottle was unstoppered and 1 cm^3 of sulphuric acid was added immediately. The bottle was re-stoppered, without trapping air bubbles, and shaken to dissolve the precipitated hydroxides. A 50 cm^3 aliquot was poured into a 250 cm^3 conical flask and starch titrated with sodium thiosulphate solution.

Calculation of oxygen concentration:

The oxygen concentration of the sample is:

$$C_{ox}\ (\mu mol/dm^3) = (Vt - Vb) \times C_t \times V_f\, 10^6 / (Vf - Vr)Vs \times 4 \qquad (7)$$

Vt: vol. of sodium thiosulphate used in titration of the sample (cm^3)

Vb: volume of sodium thiosulphate used in titration of blank (cm^3)

C_t: concentration of the thiosulphate solution

V_f: volume of sample bottle (cm^3)

Vr: volume of fixing reagents (cm^3)

Vs: volume of sample titrated (cm^3)

A.2. DETERMINATION OF NUTRIENTS IN SEAWATER

Nutrients determination followed the methods described in Grasshoff *et al*. (1983), modified according to Newton (1995):

Determination of Ammonium-Nitrogen Dissolved in Seawater

Scope

The molar absorptivity of the final product is about 20 000. Both the standard deviation and the standard error are of about 5%. The sum of NH_3 and the NH_4^+ ion is recorded, however the NH_4^+ ion is the dominant species in natural seawater with a pH of 8.2 or less.

References

The method is described in detail by Koroleff in Grasshoff *et al* (1983).

Principle

The ammonia dissolved in the seawater reacts with the hypochlorite, donated by the dichloroisocyanuric acid, to form monochloramine, which, in the presence of phenol, gives indophenol blue. The tri-sodium citrate solution acts as a buffer. The reaction is catalysed by the di-sodium nitroprusside dihydrate.

Reagents

Sodium hydroxide solution: 0.5M: 2g of analar sodium hydroxide was dissolved and made upto 100 cm^3 with freshly deionized water.

Tri-sodium citrate buffer solution: 24g of tri-sodium citrate dihydrate was dissolved in about 50cm^3 of freshly deionised water. 2cm^3 of 0.5M sodium hydroxide solution was added and made upto 100cm^3. The solution was stored in a polyethylene bottle.

Phenol reagent with nitroprusside catalyst: 3.8g of analar phenol and 40mg of analar di-sodium nitroprusside dihydrate were dissolved and made upto 100 cm^3 in freshly deionised water. The solution was stored in a dark glass bottle in a refrigerator.

Dichloroisocyanuric acid, hypochlorite reagent: 24mg of analar dichloroisocyanuric acid was dissolved in 10 cm^3 of 0.5M sodium hydroxide solution. The solution was used within 24 hours.

Standard Solutions

Stock Solution, (NH_4^+) = 100mM**:** Some NH_4Cl p.a. was dried at 100°C for 1 hour. 535mg was dissolved and made upto 100cm^3 in freshly deionised water. One drop of chloroform was added and the solution stored in a dark glass bottle, where it was stable for several months.

Working Solution, (NH_4^+) = 500µM: 0.5cm^3 of the Stock Solution was diluted to 100cm^3 with freshly deionised water and one drop of chloroform was added. The solution was stable for several weeks when kept refrigerated in a dark glass bottle.

Standard Solution, (NH_4^+) = 5µM: 1 cm^3 of the Working Solution was diluted to 100cm^3 with freshly deionised water.

Apparatus

A Shimadzu UV-Visible spectrophotometer B was used to determine the molecular absorbance. The absorbance was read at 630nm using 1cm glass cells and a deionised water blank.

Test

A dilution series was prepared from the standard solution by pipetting different volumes of the standard and deionised water into glass test tubes with teflon screw tops. These volumes are given in the table below:

	STANDARD VOLUME (cm^3)	WATER VOLUME(cm^3)	FINAL VOLUME (cm^3)	FINAL CONC. µM
Blank	0	5	5	0
	0.5	4.5	5	0.5
	1	4	5	1
	2	3	5	2
Standard	5	0	5	5

150µl of the tri-sodium citrate buffer, 150µl of the phenol - nitroprusside reagent and 150µl of the hypochlorite oxydizing agent were added to each tube. The tubes were shaken after each

addition, closed and kept in the dark for more than 6 hours before determining the molecular absorbance by spectrophotometry. The absorbance was determined at 630 nm.

Blank

5 cm^3 of freshly deionised water was pipetted into 3 glass test tubes with teflon screw-tops. The same procedure as detailed in TEST above was followed.

Determination

Triplicate 5cm^3 seawater subsamples were pipetted into screw top test tubes. The same procedure was followed as for the TEST.

Calculation of Results

A correction was applied to compensate for the salt-effect, which reduces the indophenol blue produced by ammonia as salinity increases. Corrections are given in the table below.

Salinity	0 - 8	11	14	17	20	23	27	30	33	36
Salt effect correction	1.00	1.01	1.02	1.03	1.04	1.05	1.06	1.07	1.08	1.09

$[NH_4\text{-}N]\mu M = S \times F \times (A_S - A_c)$

where: S = salt correction

F = calibration factor = [standard] $\mu M / A_{st} - A_b$

where: A_{st} = mean absorbance of standard

A_b = mean absorbance of blank

A_S = mean absorbance of sample

A_c = cell to cell absorbance of blank

Precision

The standard deviation calculated for all the analyses of the study was equal to 5% of the range. The detection limit is 0.1μM using a dilution series of the standard solution of concentrations 1.0, 0.5,0.25 and 0.1μM.

Bibliography

Koroleff in Grasshoff *et al.* (1983).

Determination of Nitrite-Nitrogen Dissolved in Seawater

Scope

The method is very sensitive and gives excellent reproducibility, with a detection limit of 0.02μM (Grasshoff *et al.* 1983).

References

The method is described in detail by Grasshoff *et al.* (1983).

Principle

The nitrite ions react with an aromatic amine, sulphanilamide, to form a diazonium compound. This couples with a second aromatic amine, N-(1-naphthyl)-ethylenediamine dihydrochloride, to form an azo dye.

Reagents

Sulphanilamide hydrochloride reagent: 1g of analar sulphanilamide was dissolved in about 50cm^3 of deionised water. 10cm3 of concentrated hydrochloric acid was added and the volume made up to 100cm^3 with deionised water. The reagent was stored in an amber glass bottle in the refrigerator.

N-(1-naphthyl)-ethylenediamine dihydrochloride reagent: 100mg of analar n-(1-naphthyl)-ethylenediamine dihydrochloride was dissolved and made upto 100cm^3 with deionised water. The reagent was stored in an amber glass bottle in the refrigerator.

Standard Solutions

Stock Solution, (NO_2^-) = 100mM**:** Sodium nitrite was dried at 100°C for 1 hour. 690mg was dissolved and made upto 100cm^3 in deionised water.

Working Solution, (NO_2^-) = 500μM: 0.5cm^3 of Stock Solution was diluted to 100cm^3 with deionised water.

Standard Solution, (NO_2) = 5μM: 1cm^3 of the Working Solution was diluted to 100cm^3 with deionised water.

Apparatus

A Shimadzu UV-Visible spectrophotometer was used to determine the molecular absorbance. The absorbance was read at 540nm using 1cm glass cells and a deionised water blank.

Test

A dilution series was prepared from the standard solution by pipetting different volumes of the standard and deionised water into test tubes. These volumes are given in the table below:

	STAND. VOLUME (cm^3)	WATER VOLUME. (cm^3)	FINAL VOLUME. (cm^3)	FINAL CONC. µM
Blank	0	5	5	0
	0.5	4.5	5	0.5
	1	4	5	1
	2	3	5	2
Standard	5	0	5	5

100µl of sulphanilamide hydrochloride solution was added to each tube, which was shaken. 100µl of N-(1-naphthyl)-ethylenediamine dihydrochloride was then added to each tube that was then shaken. The molecular absorbance was determined by spectrophotometry at 540 nm.

Blank

5 cm^3 of freshly deionised water was pipetted into 3 glass test tube. The same procedure as detailed in TEST above was followed.

Determination

Triplicate 5cm^3 seawater subsamples were pipetted into test tubes. The same procedure as detailed in TEST above was followed.

Calculation of Results

$$[NO_2^- - N]\mu M = A_S \times F$$

where A_S = mean absorbance of sample

F = calibration Factor

= [standard]µM/$A_{st} - A_b$

where A_{st} = mean absorbance of standard

A_b = mean absorbance of blank

(reagent blank and cell to cell blank combined)

Precision

The standard deviation calculated for all the analyses of the study was less than 5% of the range. The detection limit was 0.02 μM with a test dilution series of concentrations 0.1, 0.05, 0.25, 0.01 prepared from a standard solution.

Bibliography

Grasshoff *et al.* (1983).

Determination of Nitrate-Nitrogen Dissolved in Seawater

Scope

The reproducibility of the method is 0.2μM with the same cadmium-reducing column (Grasshoff *et al.*, 1983). It is important to calculate the efficiency of the column, which should be more than 90%. (95% is very good).

References

The method is described in detail by Grasshoff *et al.* (1983).

Principle

Nitrate ions are reduced to nitrite ions by passing through a cadmium-reducing column. The reaction is buffered with ammonium chloride to ensure that the reduction is complete, but does not proceed beyond the first reduction product. The nitrite ions react with an aromatic amine, sulphanilamide, to form a diazonium compound. This couples with a second aromatic amine, N-(1- naphthyl)-ethylenediamine dihydrochloride, to form an azo dye. The original concentration of Nitrite-N in the samples must be known and subtracted from the concentration obtained after reduction of Nitrate-N to Nitrite-N.

Reagents

Sulphanilamide hydrochloride reagent: 1g of sulphanilamide p.a. was dissolved in about 50cm^3 of deionised water. 10cm^3 of concentrated hydrochloric acid were added. The volume was made up to 100cm^3 with deionised water. The reagent was stored in an amber glass bottle in the refrigerator.

N-(1-naphthyl)-ethylenediamine dihydrochloride reagent: 100mg of N-(1-naphthyl)-ethylenediamine dihydrochloride p.a. was dissolved in deionised water in a 100 cm^3 volumetric flask. The reagent was stored in an amber glass bottle in the refrigerator.

Hydrochloric acid: 5 cm^3 of concentrated hydrochloric acid was added to 95 cm^3 of deionised water and stored in a dark glass bottle.

Copper sulphate solution: 10g of copper sulphate pentahydrate p.a. was dissolved in deionised water in a 1dm^3 volumetric flask.

Ammonium chloride buffer: 10g of ammonium chloride p.a. was dissolved in deionised water in a 1dm^3 volumetric flask. The pH was adjusted to 8.5 with concentrated ammonia.

Standard Solutions

Stock Solution, (NO_3) = 100mM): Some potassium nitrate p.a. was dried at 100°C for 1 hour. 1.011g was dissolved in deionised water in a 100cm^3 volumetric flask.

Working Solution, (NO_3) = 10uM: 1cm^3 of Stock Solution was diluted to 100cm^3 with deionised water. Standard Solution, (NO_3) = 10μM: 1cm^3 of the Working Solution was diluted to 100cm^3 with deionised water.

Apparatus

A Shimadzu UV-Visible spectrophotometer was used to determine the molecular absorbance. The absorbance was read at 540 nm using 1cm glass cells and a deionised water blank.

Preparation of Cadmium reducing column

The reducing column was prepared for small sample volumes. Originally a 50cm^3 syringe was adapted so that a fine bore plastic tube was fitted onto the end of the syringe and a small tube clamp used as a tap. However, this was eventually abandoned in favour of a more satisfactory adaptation of a 50cm^3 glass burette. A small ball of fine copper wire, from

stripped electrical wire, was dropped into the burette and gently pushed to its bottom (using a piece of thick wire). Fine cadmium granules (not powder) were washed in hydrochloric acid and then swirled in copper sulphate solution. They were then rinsed in deionised water and kept in a tightly closed bottle with the ammonium chloride buffer when not in use. The granules and the ammonium chloride buffer were poured into the burette, which was tapped gently to pack the granules tightly. The column was packed with granules so that the integranular volume was $5cm^3$, which was determined empirically. A small, flat mesh of the fine copper wire was placed above the cadmium granules.

Activation of Cadmium reducing column

$1cm^3$ of the Stock Solution and $10cm^3$ of the ammonium chloride buffer was passed through the burette to activate the column. Several volumes of $10cm^3$ of the ammonium chloride buffer were passed through the column and collected. $0.5cm^3$ of the sulphanilamide hydrochloride reagent and $0.5cm^3$ of the N-(1-naphthyl)-ethylenediamine dihydrochloride reagent were added to the collected buffer. This procedure was repeated until $10cm^3$ of the ammonium chloride buffer collected no longer turned pink on addition of the reagents.

Test

$5\ cm^3$ of the standard solution of 10 µM, was pipetted into each of 3 glass test tube. $5cm^3$ of the ammonium chloride buffer was added to each tube, which was shaken. The contents were poured into the reducing column and the first $5cm^3$ passing through were discarded. The next $5cm^3$ were collected in the original tube. 200µl of the sulphanilamide hydrochloride solution and 200µl of the N-(1-naphthyl)-ethylenediamine dihydrochloride were added to each tube. The molecular absorbance at 540nm was determined spectrophotometrically in glass 1cm cells with a deionised water blank.

Efficiency of the Reducing Column

The efficiency of the reducing column was determined as follows. The absorbance of the diluted nitrate standard solution (5µM) was compared to the absorbance of the nitrite standard solution (5µM) used in the nitrite determination described above. The efficiency of the reducing column was calculated as follows:

Eff = mean absorbance of diluted , reduced nitrate standard/

X 100 mean absorbance of nitrate standard

The efficiency of the column was checked by passing a standard through the column after every batch of 10 samples. If the calculated efficiency was below 90%, the column was reactivated with 1cm3 of the nitrate stock solution and washed as described above.

Blank

5 cm^3 of freshly deionised water was pipetted into each of 3 glass test tube. 5cm^3 of the ammonium chloride buffer was added to each tube that was shaken. The same procedure as detailed in TEST above was followed.

Determination

Triplicate 5cm^3 seawater subsamples were pipetted into test tubes. 5cm^3 of the ammonium chloride buffer was added to each tube, which were shaken, and the mixture was poured into the burette. The same procedure as detailed in TEST above was followed.

Calculation of results

$[NO_3 - N]\ \mu m = F \times A_c - ([NO2\text{-}N]\ \mu M \times D)$

Where : $F = [standard]\ \mu M\ / A_{st} - A_b$

where: A_{st} = mean absorbance of standard

Ab = mean absorbance of blank

(combines reagent blank and cell to cell blank)

A_c = corrected Absorbance = $A_s - A_d$ x % efficiency

where: As = mean absorbance of samples

D = dilution factor with NH_4Cl buffer solution

of sample (half in this case).

Precision

The standard deviation calculated for all the analyses of the study was less than 8% of the range. The detection limit was 0.1μM determined from a test with a dilution series of 1.0, 0.5, 0.25, 0.1 μM prepared from a standard solution.

Bibliography

Grasshoff *et al.* (1983).

Determination of Orthophosphate-Phosphorus Dissolved in Seawater

Scope

The molar absorptivity of the final product is about 22 700. Both the standard deviation and the standard error are approximately 10%.

References

The method is described in detail by Koroleff in Grasshoff *et al.* (1983).

Principle

The inorganic phosphate dissolved in seawater reacts with a mixture of acidified molybdate and antimony tartrate yielding a phosphomolybdate complex. This is then reduced, by adding ascorbic acid, to form a blue complex containing antimony. There may be some interference from any dissolved silicate if the final pH is greater than 1 or after more than 30 minutes. The absorbance should therefore be read soon after the addition of the reagents.

Reagents

Sulphuric acid, 4.5M: 25cm^3 of concentrated sulphuric acid was added to 75cm^3 of deionised water.

Molybdate solution: 0.95g of ammonium heptamolybdate tetrahydrate p.a. was dissolved and made upto 10cm^3 in deionised water .

Tartrate solution: 0.325g of potassium antimony tartrate p.a. was dissolved and made upto 10cm^3 in deionised water .

Mixed reagent : 4.5cm^3 of the molybdate solution was added to 20cm^3 of the sulphuric acid. 0.5cm^3 of the tartrate solution was then added. The mixed reagent was stored in an amber glass bottle.

Ascorbic Acid: 0.7g of ascorbic acid p.a. was dissolved in 10cm^3 of deionised water. The acid was kept in an amber glass bottle in the refrigerator and discarded when it turned yellow.

Standard Solutions

Stock Solution, $(PO_4)^{3-}$ = 100mM: Some potassium dihydrogen sulphate p.a. was dried at 100°C for 1 hour. 1.361g was dissolved in about 50cm^3 of deionised water. 1cm^3 of the sulphuric acid was added and the volume adjusted to 100cm^3.

Working Solution, $(P0_4)^{3-}$ = 500µM: 0.5cm^3 of the Stock solution was diluted and made up to 100cm3 with deionised water.

Standard Solution, $(PO_4)^{3-}$ = 5µM: 1cm^3 of the Working solution was diluted to 100cm^3 with deionised water.

Apparatus

A Shimadzu UV-Visible spectrophotometer was used to determine the molecular absorbance. The absorbance was read at 880 nm using 1cm glass cells and a deionised water blank.

Test

A dilution series was prepared from the standard solution by pipetting different volumes of the standard and deionised water into the test tubes. These volumes are given in the table below:

	STAND. VOLUME (cm^3)	WATER VOLUME. (cm^3)	FINAL VOLUME. (cm^3)	FINAL CONC. µM
Blank	0	5	5	0
	0.5	4.5	5	0.5
	1	4	5	1
	2	3	5	2
Standard	5	0	5	5

150µl of the mixed reagent and 150µl of the ascorbic acid were added to each tube that was shaken after each addition. The absorbance was read at 880nm using 1cm glass cells and a deionised water blank.

Blank

5 cm^3 of freshly deionised water was pipetted into 3 glass test tubes. The same procedure as detailed in TEST above was followed.

Determination

Triplicate 5cm^3 seawater subsamples were pipetted into test tubes. The same procedure was followed as for the TEST.

Calculation of Results

$$[PO_4 - P]\ \mu M = A_S \times F$$

Where: As = mean absorbance of sample

$$F = [\text{standard}]\ \mu M / A_{st} - A_b$$

where: Ast = mean absorbance of standard

Ab = mean absorbance of blank

(combines blank and cell to cell blank)

Precision

The standard deviation calculated for all the analyses of the study was less than 5% of the range. The detection limit was 0.07µM determined from a test with a dilution series of 1.0, 0.5, 0.25, 0.1 µM prepared from a standard solution.

Bibliography

Koroleff in Grasshoff *et al.* (1983).

Determination of Silicate-Silicon Dissolved in Seawater

Scope

The molar absorptivity of the method is about 22 000 (standards prepared with deionised water), but lower in seawater, about 19 000.

References

The method is described in detail by Koroleff in Grasshoff *et al*. (1983).

Principle

The inorganic silicate dissolved in the seawater reacts with an acidified molybdate reagent yielding a silicomolybdate complex. This is then reduced, by adding the ascorbic acid, to form a blue silicomolybdic complex. The reaction is pH dependent (pH 3-4) and there may be some interference from any dissolved phosphate if the final pH is less than 3. This interference is eliminated by the addition of oxalic acid.

Reagents

Sulphuric acid, 4.5M: 250cm^3 of concentrated sulphuric acid were carefully added to 750cm^3 of deionised water in a plastic beaker. The acid was stored in an opaque polyethylene bottle.

Molybdate solution: 20g of ammonium heptamolybdate tetrahydrate p.a. was dissolved and made upto 100cm^{3}in deionised water.

Mixed reagent: 25cm^3 of the molybdate 10cm^3 solution was added to 25 cm^3 of the sulphuric acid. The mixed reagent was stored in an opaque plastic bottle.

Ascorbic acid: 175mg of ascorbic acid p.a. was dissolved in10cm^3 of deionised water. The solution was stored in an opaque plastic bottle in the refrigerator and discarded when a yellow colour developed.

Oxalic acid: 1g of oxalic acid p.a. was dissolved and made upto 10 cm^3 in deionised water in a volumetric flask. The acid was stored in a plastic bottle.

Standard Solutions

Stock Solution, $(SiO_4)^{4+}$= 10mM: Some disodium hexafluoro silicate p.a. was dried at 100°C for 1 hour. 188.1mg was dissolved and made upto 100cm^3 in deionised water.

Working Solution, $(SiO_4)^{4+}$= 1mM: 10cm^3 of the Stock solution were diluted to 100cm3 with deionised water.

Standard Solution, $(SiO_4)^{4+}$= 10µM: 1cm^3 of the Working solution was diluted to 100cm^3 with deionised water.

Apparatus

Polyethene laboratory ware was used instead of glassware. A Shimadzu UV-Visible spectrophotometer was used to determine the molecular absorbance. The absorbance was read at 810 nm using 1cm plastic cells and a deionised water blank.

Test

A dilution series was prepared from the standard solution by pipetting different volumes of the standard and deionised water into test tubes. These volumes are given in the table below:

	STAND. VOLUME (cm^3)	WATER VOLUME (cm^3)	FINAL VOLUME (cm^3)	FINAL CONC. µM
Blank	0	10	10	0
	2.5	7.5	10	2.5
	2.5	7.5	10	2.5
	5	5	10	5
Standard	7.5	2.5	10	7.5

150µl of the mixed reagent was added to each tube. After 15 minutes, 100µl of the oxalic and then 100µl of the ascorbic acid were added to each tube that were shaken after each addition. The molecular absorbance was determined spectrophotometrically at 810nm using 1cm plastic cells and a deionised water blank.

Blank

5 cm^3 of freshly deionised water was pipetted into 3 polyethelene test tubes. The same procedure as detailed in TEST above was followed.

Determination

Triplicate 5cm^3 seawater subsamples were pipetted into plastic test tubes. The same procedure was followed as for the TEST.

Calculation of Results

A correction was applied to compensate for the salt-effect, which reduces the colour of the silicomolybdic acid as the salinity increases. The corrections are given in the table (after Koroleff, in Grasshoff *et al*., 1983).

Salinity	5	9	14	20	25	30	35
Salt effect correction	1.02	1.04	1.06	1.09	1.11	1.13	1.15

$$[SiO_4^{4+} - Si]\ \mu M = A_S \times F \times S$$

Where: As = mean absorbance of samples

F = calibration factor

= [standard] μM / $A_{st} - A_b$

where Ast = mean absorbance of standard

Ab = mean absorbance of blank

(combines reagent blank and cell to cell blank)

S = salt correction

Precision

The standard deviation calculated for all the analyses of the study was less than 5% of the range. The detection limit was 0.1μM determined from a test with a dilution series of 1.0, 0.5, 0.25, 0.1 μM prepared from a standard solution.

Bibliography

Grasshoff *et al.* (1983)

A.3. MICROSCOPIC IDENTIFICATION

Microplankton samples were preserved with acidified Lugol Iodine solution to ~1% final concentration and stored in cool, dark conditions until analysis by inverted microscopy (Utermöhl, 1958) using a Zeiss Axiovert 25. Samples were placed in sedimentation cylinders for concentration purposes. Natural samples were sedimented using cylinders of 100 ml, whereas 24 h incubated samples (control and nutrient enriched) settled in cylinders of 10-50 ml, according to sample concentration. Prior to counting, the samples were checked with low-magnification objective for even distribution of the cells through the chamber. Larger and less

abundant microplankton cells were counted at 100x magnification through the entire bottom of the chamber. The number of individuals counted during this study ranged from 14 to 326, with a mean of 71, which correspond to a counting error (based on 95% confidence limits) of 53%, 11% and 24%, respectively. Smaller cells were enumerated at 400x magnification until a total of 100 optical fields. The number of cells counted varied between 247-2772 with an average of 1079; these values correspond to 12%, 4% and 6% counting errors (HELCOM, 2002). Abundance values are expressed in $x10^3$ cell l^{-1}. Biovolume was estimated by measuring linear cells dimension, by means of a calibrated ocular micrometer, and using approximations to the nearest geometric shape (Hillebrand *et al.*, 1999). Conversion to biomass carbon units (mg C m^{-3}) was made according to Verity and Langdon (1984) and Verity et al. (1992).

Cells were classified according to Tomas (1997) and Therriault *et al*. (1999), generally down to genus level with some exceptions down to species level. Whenever this classification was not possible, cells were included in wider groups (e.g. *Gymnodinium*+*Gyrodinium* spp., small unidentified dinoflagellates). The microplankton assemblage was divided into four major taxonomic components: diatoms, dinoflagellates, ciliates and nanoflagellates (including Crytophyceae, Dictyochophyceae, Euglenophyceae and nanoflagellates).

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ANNEX B: ADDITIONAL DATA

The data presented in this annex are not included in the manuscripts that have been published (chapters 2.1, 3.1 and 3.2). Nevertheless, they are discussed in Chapter 4 (COMPARISON BETWEEN THE TWO SYSTEMS: SAGRES & RIA).

B.1. RIA FORMOSA PRODUCTION AND RESPIRATION RATES

Oxygen production and respiration rates were measured in June (2001), September (2001) and July (2002) samplings (Fig. B.1). Gross production (GP) rates reached their peaks in June and September (2001) samplings (max: 65±2 μM O_2 d^{-1}, Ponte-LW, June 2001). Net community production (NCP) followed the same trend (max: 50±2 μM O_2 d^{-1}, Ponte-LW, June 2001). A period of net heterotrophy (NCP < 0) was observed in September at Ponte-HW.

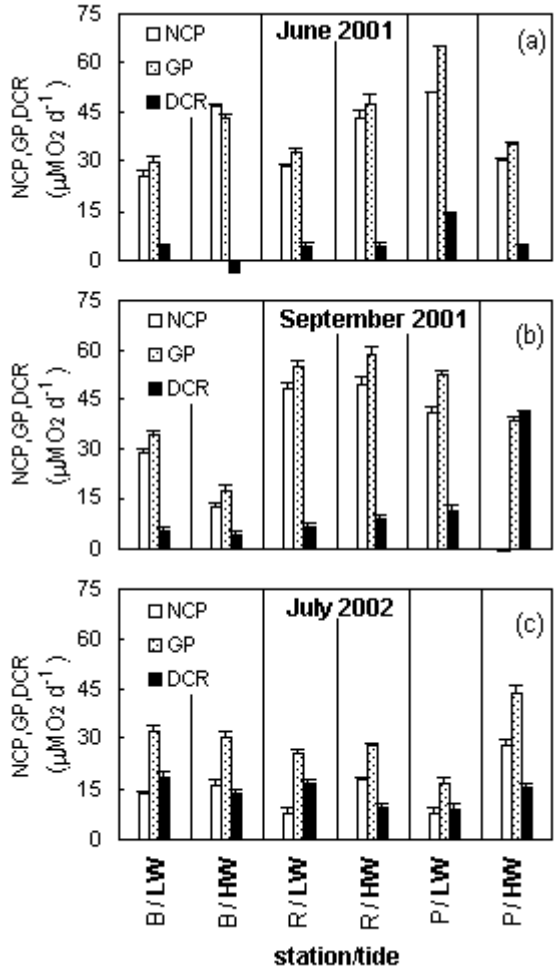

Fig. B.1. Net community production (NCP), gross production (GP) and dark community respiration (DCR) during (a) June 2001, (b) September 2001 and (c) July 2002, at the three stations (B = Barra, R = Ramalhete, P = Ponte) of the Ria Formosa lagoon, at both high (HW) and low water (LW) conditions (in bold). 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.

Dark community respiration (DCR) peaked in September (max: 45±1 μM O_2 d^{-1}, Ponte-HW), although mean values were higher in July (2002) sampling (14±4 μM O_2 d^{-1}). An anomaly in respiration rates (oxygen in "dark" incubated bottles was higher than in initial samples) was observed at Barra-HW in June (2001), probably due to light-associated mechanisms such as light-enhanced algae respiration (Moncoiffé *et al*., 2000).

Both production and respiration rates were within the ranges previously described for mesotrophic coastal lagoons (Meyercordt *et al*., 1999).

B.2. RIA FORMOSA CARBON BIOMASS

During the survey in the Ria Formosa lagoon, from June 2001 to July 2002, the highest biomass values (max: 226 μg C l^{-1}, Ramalhete-HW) were noted in June 2001 (Fig. B.2), with a major contribution from the nanoflagellate group (max: 68% at Ponte-LW), whereas the lowest values were registered during December 2001 (min: 6 μg C l^{-1}, Ramalhete-LW).

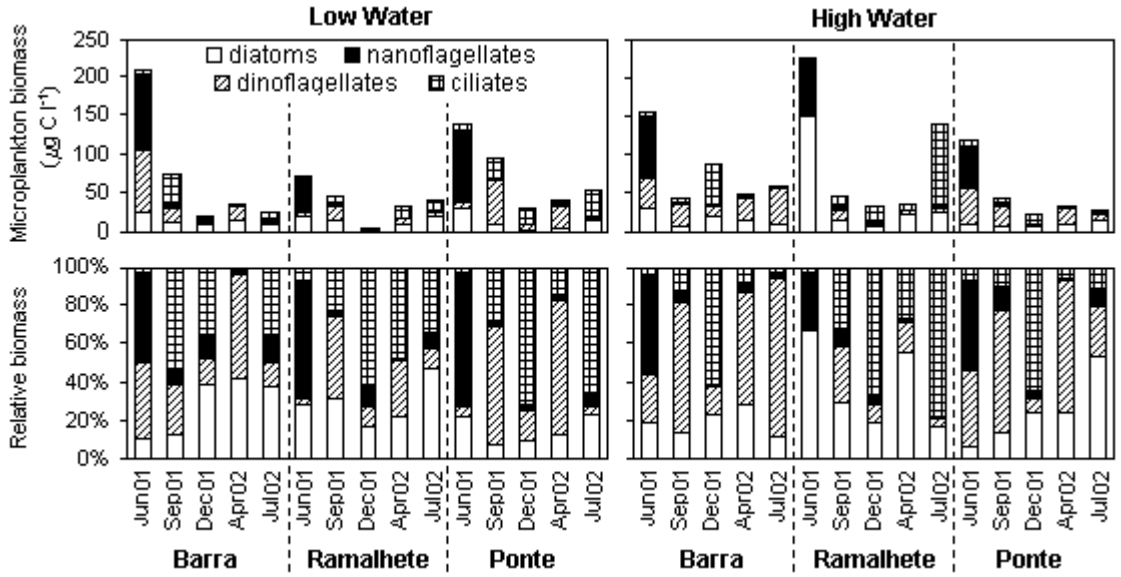

Fig. B.2. Biomass and relative biomass of the defined microplanktonic groups identified during the studied period (June 2001 to July 2002), at the three stations (B = Barra, R = Ramalhete, P = Ponte) of the Ria Formosa lagoon, at both high (HW) and low water (LW) conditions.

Dinoflagellates and ciliates co-dominated the assemblage in terms of biomass in September 2001 (max: 58 μg C l^{-1} of dinoflagellates, 38 μg C l^{-1} of ciliates, at Ponte-LW and Barra-LW, respectively). In December 2001 the maximum biomass was reached at the Barra-HW (86 μg

C l^{-1}). Ciliates were the major community contributors (max: 72% at Ponte-LW). April 2001 followed a very similar distribution pattern as described for September 2001. During July 2002 the biomass maximum was at Ramalhete-HW (139 μg C l^{-1}). Dinoflagellates reached high values at Barra-HW (48 μg C l^{-1}, 83% of relative biomass), whereas ciliates peaked at Ramalhete-HW (108 μg C l^{-1}, 78% of relative biomass). Ponte and Ramalhete followed the same tidal variation as for abundance (see chapter 3.1, Fig. 5, pag. 100), (Ponte total biomass was higher at LW, whereas Ramalhete total biomass was higher at HW), unlike the Barra station which did not present any fixed trend. The differences found between the abundances of the distinct microplanktonic groups (diatoms, dinoflagellates, nanoflagellates and ciliates) and its correspondent biomass is probably due to variations in cell size and single species biomass (Sarno *et al*., 1993).

In the biomass dendrogram (Fig. B.3) three clusters were observed at 40% of the Bray-Curtis similarity index (December 2001; June 2001 & July 2002; September 2001 & April 2002) with an extra one formed by a single sample (Barra-HW, December). Some of the

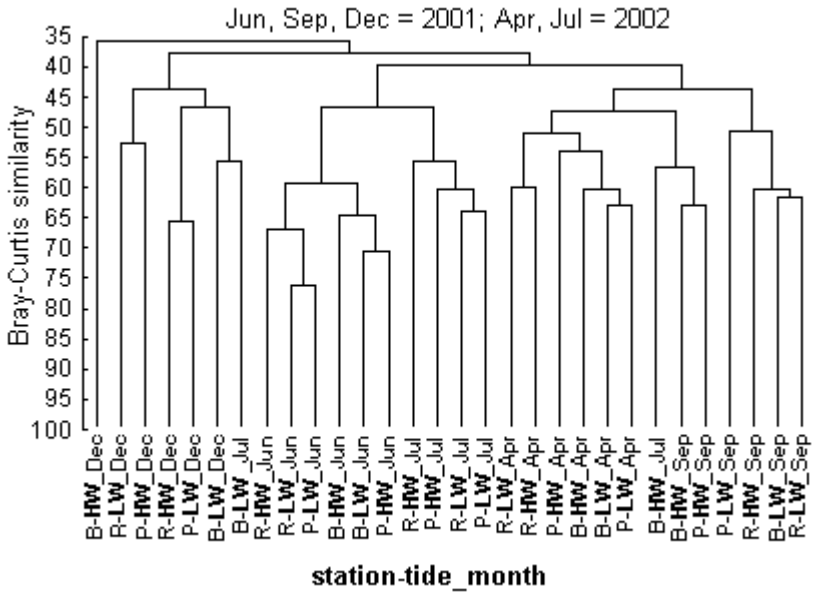

Fig. B.3. Cluster dendrogram of the Bray-Curtis similarity matrix of the square root transformed biomass. B = Barra station, the oceanic inlet; R = Ramalhete station, next to an urban waste water treatment plant; P = Ponte station, upstream channel used for touristic activities and bivalve culture. LW = low water, HW = high water.

samples from these three clusters separated from its seasonal grouping, namely: Barra-LW July, which grouped with December cluster; Barra-HW July, which grouped with Barra-HW and Ponte-HW September cluster. Thus, in terms of carbon biomass the Barra station assemblage differed from that of the lagoonal waters during December 2001 and July 2002.

ANOSIM tests (Table B.1) performed on the similarity matrices of the square root transformed biomass of the sampling groups (June 2001, September 2001, December 2001, April 2002 and July 2002) revealed that the community groups were statistically distinct (global R = 0.66). Specific R values for each pairwise comparison showed that the largest community separation was between June 2001 and April 2002 samples (R = 0.97), whereas the weakest community separation was between September 2001 and July 2002 (R = 0.33) samples.

Periods	R pairwise test	Number of Permuta.	Signifi. level
Jun - Sep	0.83	462	0.002
Jun - Dec	0.84	462	0.002
Jun - Apr	0.97	462	0.002
Jun - Jul	0.68	462	0.002
Sep - Dec	0.58	462	0.002
Sep - Apr	0.60	462	0.002
Sep - Jul	0.33	462	0.015
Dec - Apr	0.68	462	0.004
Dec - Jul	0.49	462	0.002
Apr - Jul	0.67	462	0.004
Global R	0.66	0.999	0.001

Table B.1. Results from the one-way ANOSIM test on the Bray-Curtis similarity matrices of the square-root transformed biomass of the *a priori* seasonal groups (2001: June, September and December; 2002: April and July).

The similarity percentage analysis (SIMPER) on the square root transformed biomass indicated the most discriminating taxa between the different groupings. As such, June 2001 was mainly characterized by *Thalassiosira* spp., Mesodiniidae, and unidentified ciliates; September 2001 was mainly characterized by *Alexandrium* spp. and big (> 20 μm) unidentified dinoflagellates; December 2001 was characterized by low microplanktonic biomass, higher values being represented by Tintinnina, Oligotrichida and unidentified ciliates; April 2002 was mainly characterized by *Leptocylindrus* spp., *Guinardia* spp. and *Scrippsiella* spp.; July 2002 was mainly characterized by *Rhizosolenia* spp., *Thalassiosira* spp. and *Dinophysis* spp.. The evolution of the main taxa is represented on Figure B.4.

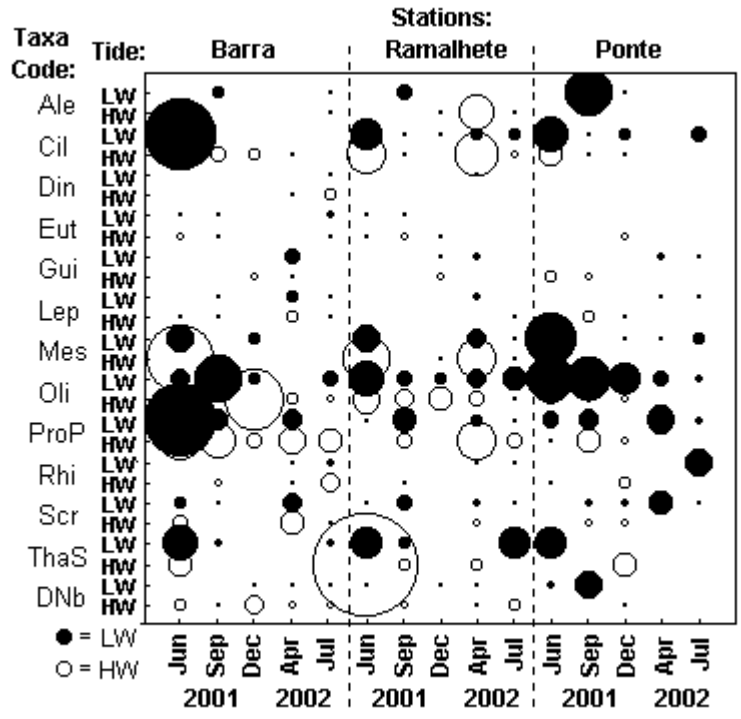

Fig. B.4. Biomass evolution of the main taxa contributing to the Bray-Curtis dissimilarities between the seasonal groups (2001: June, September and December; 2002: April and July). See Chapter 3.1, Table 2 for taxa codes. Circles are proportional to biomass (max: 150 μg C l^{-1}); to avoid overlapping of circles, they represent 50% of their original size, as such, the absence of a bubble does not necessarily mean no occurrence, but that the relative biomass is low. LW = low water; HW = high water.

Regarding Spearman rank-order correlations, the total microplankton biomass, expressed as carbon content, was highly correlated with abundance (Spearman, $r_s = 0.76$, $p<0.05$). (Table B.2). Temperature and solar radiation were significantly correlated to diatom, nanoflagellate

n = 30	BMdiat	BMdino	BMnano	BMcilia	BMtotal
T	**0.48**	0.11	**0.60**	0.19	**0.57**
S	**0.40**	0.26	0.10	-0.08	**0.39**
Light	**0.51**	0.09	**0.43**	-0.19	**0.39**
NO_3^-	-0.07	-0.11	0.33	0.14	0.26
NO_2^-	0.21	0.05	**0.37**	0.24	**0.44**
NH_4^+	0.09	0.17	**0.61**	0.15	**0.49**
PO_4^{3-}	0.11	-0.14	0.19	-0.07	0.13
SiO_4^{2-}	-0.17	-0.14	0.07	0.34	-0.08
O_2	**-0.43**	**-0.43**	-0.17	-0.19	-0.45
Chl *a*	0.16	0.34	**0.55**	0.00	**0.45**
[diat]	**0.62**	0.11	**0.53**	0.05	0.64
[dino]	-0.05	**0.84**	0.25	0.17	0.40
[nano]	0.12	0.13	**0.55**	0.1	0.28
[cilia]	**0.50**	-0.05	**0.47**	0.34	**0.58**
[Total]	**0.54**	0.20	**0.74**	0.08	**0.76**

Table B.2. Pairwise Spearman correlations between biological, chemical and physical parameters: temperature (T), salinity (S), solar radiation (light); nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), silicate (SiO_4^{2-}), dissolved oxygen (O_2) and chlorophyll *a* (Chl *a*) concentrations; diatom ([diat]), dinoflagellate ([dino]), nanoflagellate ([nano]), ciliate ([cilia]) and total microplankton ([total]) abundances; BM = biomass. Bold underlined figures are significant at $p<0.05$; *n* represents the number of samples.

and total microplankton, whereas salinity was related to diatom and total microplankton biomass. Also, nitrogen reduced forms were related to nanoflagellate and total microplankton biomass. Dissolved oxygen was inversely correlated with diatom and dinoflagellate biomass.

The generally similar trends of microplankton numbers and biomass (as carbon) found in this study, suggest that the use of abundance data could be sufficient to evaluate the microplankton dynamics in the Ria Formosa. Determination of carbon content is a time-consuming technique and is usually not included in coastal waters studies.

B.3. UNIVARIATE INDICES

Sagres: summer 2001

The total number of taxa of the distinct periods (P1, P2 and P3) was not significantly different from each other (ANOVA, p = 0.88; Fig. B.5a). The number of individuals during

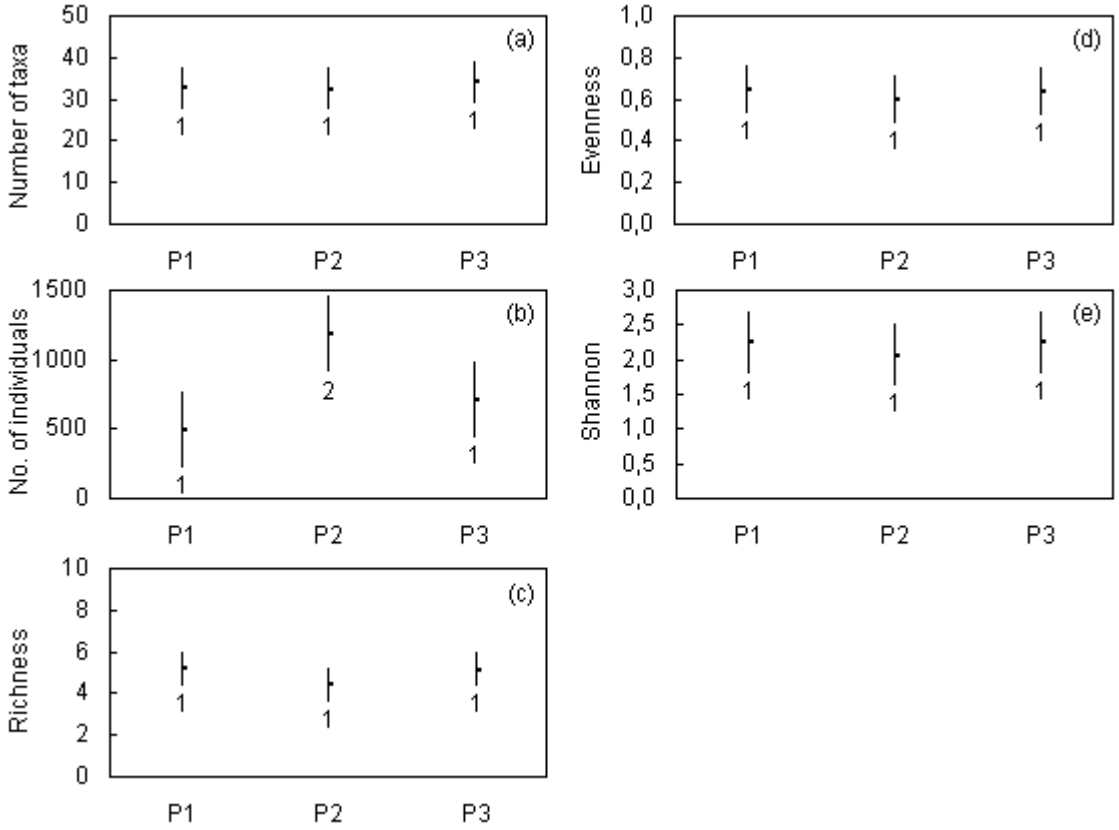

Fig. B.5. Mean and 95% confidence intervals, based on pooled standard deviations, of ecological indices for the three periods (P1, P2 and P3). Numbers under bars corresponds to a *post hoc* test (LSD Fisher test): bars labeled with different numbers have significant different means; bars with the same numbers do not have significant different means.

P2 was significantly different from P1 and P3 (ANOVA, p = 0.009, *post hoc* LSD Fisher test; Fig. B.5b). Although no significant differences were found for richness, diversity and equitability (Fig. 1c, d, and e), these indices were modestly lower during P2.

The slight higher cumulative abundance curve (Fig. B.6) for this period corroborates its lower diversity, in relation to P1 and P3 communities.

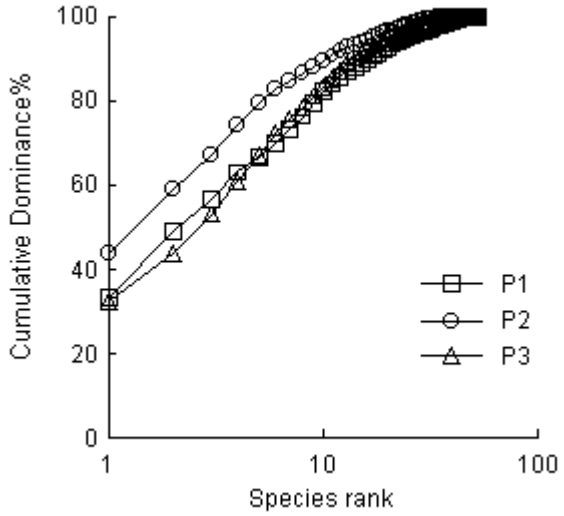

Fig. B.6. Abundance *k*-dominance curves of the different periods (P1, P2 and P3).

Ria Formosa: Enrichment Experiments

The microscopic identification of microplankton in N stimulated samples during September 2001 and July 2002 showed a general decrease of taxa numbers, together with a general increase of number of individuals, which led to a lower diversity and evenness indices (Table B.3).

B.4. POTENTIALLY HARMFUL ALGAL BLOOMS (HAB) TAXA

Ria Formosa: Natural Samples

Although identification was primarily done down to genus level, which does not allow the detection of harmful species within each taxa, a brief description of the main potentially

Table B.3. Uni-variate indices of control (24 hours) and N production stimulated samples.

Date	Station/Tide/ Incubation	No Taxa	No Individuals	Richness (Margalef)	Evenness (Pielou)	Shannon
September 2001	B/HW-Control	43	208	7.87	0.73	2.74
	B/HW,+N	37	610	5.61	0.70	2.51
July 2002	B/LW-Control	31	423	4.96	0.51	1.75
	B/LW,+N	31	952	4.37	0.56	1.92
	R/LW-Control	33	354	5.45	0.54	1.90
	R/LW,+N	24	1357	3.19	0.39	1.25
	R/HW-Control	30	753	4.38	0.55	1.87
	R/HW,+N	22	2982	2.62	0.50	1.55
	P/LW-Control	26	1649	3.37	0.27	0.87
	P/LW,+N	21	6846	2.26	0.19	0.56
	P/HW-Control	32	1210	4.37	0.49	1.70
	P/HW,+N	26	953	3.64	0.48	1.56

harmful taxa (Table B.4) is presented (for more detailed description about potentially HAB's see chapter 2.1, pag. 49). *Pseudo-nitzshia* spp. had higher abundances in April 2002 at Barra-HW (max: 36 x10^3 cell. l^{-1}), suggesting an import from external waters. *Alexandrium* spp. reached its maximum at Ponte-LW (101 x 10^3 cell l^{-1}) during September 2001. *Ceratium* spp. and *Dinophysis* spp. were present at low numbers during the survey (max: <2 x 10^3 cell l^{-1} and <0.5 x 10^3 cell l^{-1}, respectively). *Gymnodinium* spp, peaked during September 2001, and July 2002 at the Barra station during HW (max: 35 x 10^3 cell l^{-1} in July 2002), whereas *Gonyaulax* spp. peaked in September 2001 (max: 37 x 10^3 cell l^{-1} at Ponte station). *Prorocentrum* spp. was numerically more abundant in September 2001 and April 2002 at Barra-HW (max: 13 x 10^3 cell l^{-1}, April 2002), and *Scrippsiella* spp. reached the maximum in April 2002 (30 x 10^3 cell l^{-1} at Ponte-LW).

The high exposure to tidal currents, wind and wave action (Vila-Concejo *et al*., 2002) decreases the chances of negative effects of the potentially HAB taxa in this area. Transport mechanisms may, nevertheless, carry these organisms into areas of restricted renewal within the lagoon. The occurrence in high numbers of such organisms could affect the bivalve culture present in these areas.

Table B.4. Abundance of potentially HAB organisms observed at the Ria Formosa lagoon (stations: B = Barra, R = Ramalhete, P = Ponte; tide: LW = low water; HW = high water) during sampling campaigns. See Chapter 3.1, Table 2, pag. 101, for taxa codes.

Date	Samples	Taxa Codes							
		Psn	Ale	Cer	Din	Gon	Gym	ProC	Scr
		10^3 cell. l^{-1}							
Jun01	B/LW	-	0.08	0.08	0.02	-	2.89	4.75	6.17
	B/HW	2.56	0.08	0.56	0.10	-	2.09	0.64	5.75
	R/LW	-	-	0.02	-	-	1.70	1.70	2.56
	R/HW	-	-	-	0.04	-	0.80	0.80	1.60
	P/LW	-	-	-	-	-	4.47	3.20	0.64
	P/HW	0.66	9.31	0.20	0.08	-	1.74	0.33	4.99
Sep01	B/LW	8.33	5.33	-	-	0.67	12.99	1.00	4.00
	B/HW	5.77	-	0.64	-	-	13.45	10.89	-
	R/LW	2.57	11.55	-	-	-	-	1.28	6.42
	R/HW	12.83	-	-	-	-	15.39	5.13	5.77
	P/LW	0.64	101.21	-	-	-	-	1.28	4.48
	P/HW	4.08	3.34	0.37	-	36.70	0.37	10.75	-
Dec01	B/LW	1.00	-	0.33	-	0.33	0.67	-	-
	B/HW	2.33	-	0.67	-	0.33	0.67	-	-
	R/LW	1.02	0.24	-	-	-	0.53	-	0.35
	R/HW	3.33	0.33	0.33	-	-	-	-	-
	P/LW	-	0.33	0.33	-	-	-	2.00	1.67
	P/HW	0.16	0.04	0.03	-	-	2.85	0.02	0.14
Apr02	B/LW	16.76	0.19	-	-	4.34	11.27	10.02	12.43
	B/HW	36.29	-	0.33	0.02	1.66	32.63	12.98	14.98
	R/LW	20.44	-	-	0.33	3.99	1.00	4.33	3.66
	R/HW	4.33	0.33	0.04	-	-	1.00	-	2.00
	P/LW	12.40	-	-	-	2.94	-	3.59	29.38
	P/HW	20.42	-	1.67	0.33	-	4.69	3.01	3.68
Jul02	B/LW	2.33	1.67	-	0.05	2.00	11.66	0.01	-
	B/HW	7.99	0.33	0.28	0.43	1.00	34.92	1.68	2.33
	R/LW	2.34	-	-	0.01	5.00	1.67	0.33	1.00
	R/HW	2.85	-	0.48	-	1.43	0.95	-	0.48
	P/LW	-	-	-	0.02	0.48	8.56	-	0.95
	P/HW	2.33	-	0.04	0.03	3.33	5.66	0.33	4.33

Ria Formosa: Enriched samples

Table B.5 show the results obtained regarding the response of potentially HAB organisms to nitrogen enrichment during September 2001 and July 2002 in N production-stimulated samples. A general enhancement in numbers of *Pseudo-nitzschia* spp. was noted both in September and July experiments. Additionally, during September *Gymnodinium* spp. and *Scripsiella* spp. had a modest increase in abundance in enriched samples.

Table B.5. Potentially HAB organisms found at initial (time zero), control and N stimulated samples, during September 2001 and July 2002 experiments.

Date	Sample	Taxa Codes							
		PSN	Ale	Cer	Din	Gon	Gym	ProC	Scr
		10^3 cell. l^{-1}							
September 2001	B/HW-24H	7.9	0.3	-	-	4.5	6.5	4.5	4.1
	B/HW,+N	48.6	-	-	-	-	26.6	6.7	20.6
July 2002	B/LW-24H	4.0	-	-	0.1	0.3	14.4	-	1.7
	B/LW,+N	103.6	-	-	-	-	16.6	2.6	3.6
	R/LW-24H	2.7	-	-	0.1	-	1.4	0.7	1.0
	R/LW,+N	28.8	-	0.5	-	-	-	-	2.4
	R/HW-24H	12.8	-	-	0.1	-	0.6	-	4.5
	R/HW,+N	97.6	-	-	-	0.5	-	-	-
	P/LW-24H	2.2	-	-	-	-	0.8	0.7	2.2
	P/LW,+N	11.1	-	-	-	-	3.7	3.7	-
	P/HW-24H	30.4	-	-	0.1	-	19.4	-	4.8
	P/HW,+N	64.8	-	0.1	0.1	2.6	11.1	0.9	11.9

Overall, *Pseudo-nitzschia* spp. had a high response to N enrichment, reflecting its higher growth rates under such conditions. Negative impacts of such blooms may include human intoxication following ingestion of bivalves contaminated with ASP toxin (domoic acid) produced by some *Pseudo-nitzschia* spp.. High levels of ASP toxins were recorded on sentinel mollusc mainly during late winter at the south Portuguese coast (Vale & Sampayo, 2002). Stimulation of potentially toxic dinoflagellates during September bioassays included *Scripsiella* spp. (for which no harmful effects have been reported for this area) and *Gymnodinium* spp. The latter is associated with PSP toxins, especifically produced by *Gymnodinium catenatum*. Highest levels of such toxins are generally found in Portuguese comercial shellfish from August to December (Sampayo *et al*., 1997).

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