

Convocatoria de ayudas de Proyectos de Investigación Fundamental no orientada

TECHNICAL ANNEX FOR TYPE A or B PROJECTS

1. SUMMARY OF THE PROPOSAL (the summary must be also filled in Spanish)

PROJECT TITLE: Control of the structure of marine phytoplankton communities by turbulence and nutrient supply dynamics (CHAOS)

PRINCIPAL INVESTIGATOR: Beatriz Mouriño Carballido

SUMMARY

(brief and precise, outlining only the most relevant topics and the proposed objectives)

The taxonomic composition and the size structure of phytoplankton communities control the synthesis of organic carbon that can be exported either to the deep ocean or to higher trophic levels, and therefore play a crucial role in the efficiency of the biological pump and the productivity of marine fisheries. Turbulent mixing through nutrient supply to the upper layers of the ocean is often the primary control on the size and taxonomic structure of phytoplankton communities. The Margalef's mandala represented one of the first attempts to describe the role of turbulence and nutrient supply in the selection of different taxonomic/functional groups of phytoplankton. Since then a number of limitations have been noted when applying this approach to the field. The difficulties to measure ocean turbulence have limited the research to lab experiments, where phytoplankton communities are subjected to unrealistic levels of turbulence. Versus the traditional succession of phytoplankton groups described by the Margalef model, picoplankton (which due to methodological limitations at the time was not included in the original diagram) has been also observed to respond to the onset of favorable growth conditions for diatom-dominated blooms. Finally, recent experiments in the lab using continuous culture systems have shown that the frequency of nitrate pulses, and not only the magnitude of the pulses, controls the temporal progression of phytoplankton succession. For the first time, we proposed to combine data base analysis, observations in the field and chemostat experiments in the lab in order to verify that turbulence controls the rate and frequency of nutrient supply, which in turns determine the phytoplankton size-structure and community composition. Our specific objectives are: 1) to quantify the supply of nutrients into the euphotic zone driven by enhanced mixing associated with the internal wave activity during spring and neap tides in the shelf edge of NW Spain, 2) to determine potential relationships between a multidimensional environmental control matrix related to nutrient supply and phytoplankton size-structure and community composition, 3) to analyze the relationship between small-scale turbulence and diatom and dinoflagellate abundance, 4) to determine the relationship between small-scale turbulence and the vertical distribution of cell abundance, and 5) to determine the effect of changes in the rate of nutrient supply and frequency of nutrient pulses in the outcome of competitive interaction between species belonging to different taxonomic groups. Climate-driven processes that influence turbulent mixing in the ocean seem to have strongly influenced the diversity and relative abundance of the major eukaryotic phytoplankton groups over the past 200 million years. Therefore, understanding the link between turbulent mixing, nutrient supply and phytoplankton size and taxonomic structure is crucial to predict how phytoplankton communities will change in the future.

TÍTULO DEL PROYECTO: El papel de la turbulencia y la dinámica del suministro de nutrientes en la estructura de las comunidades de fitoplancton marino

RESUMEN

(breve y preciso, exponiendo solo los aspectos más relevantes y los objetivos propuestos)

La composición taxonómica y la estructura de tamaños de las comunidades fitoplanctónicas determinan la cantidad de carbono orgánico sintetizado disponible para la exportación hacia las capas profundas del océano o hacia niveles tróficos superiores. Estos factores desempeñan, por lo tanto, un papel crucial en la eficiencia de la bomba biológica y la producción pesquera. Mediante su influencia en el aporte de nutrientes hacia las capas superiores del océano, la mezcla turbulenta es con frecuencia el principal factor que determina la estructura de tamaños y la composición taxonómica del fitoplancton. El mandala de Margalef representó una de las primeras aproximaciones que describieron el papel de la turbulencia y los aportes de nutrientes en la selección de diferentes grupos funcionales de fitoplancton. Desde entonces, varios estudios han descrito una serie de limitaciones en la aplicación de este modelo al medio natural. En primer lugar las dificultades metodológicas para obtener medidas de turbulencia en el océano han limitado las investigaciones en este campo, en su mayor parte, a experimentos de laboratorio, en los que las comunidades de fitoplancton experimentan niveles no realistas de turbulencia. Frente a la visión tradicional de sucesión fitoplanctónica incluida en el modelo de Margalef, se ha observado que el picoplancton (que debido a las limitaciones metodológicas de la época no se incluyó en el diagrama original) también responde a las condiciones favorables de crecimiento que estimulan la formación de proliferaciones de diatomeas. Por último, experimentos recientes en el laboratorio utilizando cultivos en continuo mostraron que la frecuencia en las entradas de nitrato, y no sólo la magnitud de los pulsos, controla la progresión temporal de la sucesión fitoplanctónica. Por primera vez, este proyecto propone combinar análisis de bases de datos, observaciones en el campo y experimentos de laboratorio con quimiostatos con el fin de verificar que la turbulencia controla la magnitud y frecuencia de las entradas de nutrientes, que a su vez determinan la estructura de tamaños y la composición de las comunidades de fitoplancton. Nuestros objetivos específicos incluyen: 1) cuantificar la entrada de nutrientes hacia la capa fótica originada por procesos de mezcla asociados a la actividad de ondas internas durante periodos de mareas vivas y mareas muertas en el borde de la plataforma NO de la Península Ibérica, 2) determinar la existencia de posibles relaciones entre una matriz multidimensional de factores ambientales y la estructura de tamaños y composición de la comunidad fitoplanctónica, 3) analizar la relación entre turbulencia de pequeña escala y la abundancia de diatomeas y dinoflagelados, 4) determinar la relación entre turbulencia de pequeñas escala y la distribución vertical de abundancia celular, y 5) determinar el efecto de las tasa de entrada de nutrientes y la magnitud de los pulsos en la interacción competitiva entre diferentes grupos funcionales de fitoplancton. Las variaciones en la intensidad de los procesos de mezcla turbulenta en el océano asociadas a cambios en el clima parecen haber sido determinantes en la diversidad y abundancia relativa de los principales grupos de fitoplancton eucariota a lo largo de los últimos 200 millones de años. Por lo tanto, entender las relaciones existentes entre mezcla turbulenta, aportes de nutrientes y estructura de tamaños y composición taxonómica del fitoplancton es crucial para predecir los cambios que las comunidades fitoplanctónicas experimentarán en el futuro.

2. INTRODUCTION

(maximum 5 pages)

- The introduction should include: the aims of the project; the background and the state of the art of the scientific knowledge, including the essential references; the most relevant national and international groups working in the same or related topics

Environmental control of phytoplankton community structure

Carbon fixation by marine phytoplankton and its export into the deep ocean (the biological pump), promotes the diffusive drawdown of atmospheric carbon dioxide, and hence contributes to the modulation of Earth's climate (Volk and Hoffert 1985). The efficiency of the biological pump depends on two critical factors: the taxonomic composition and the size structure of phytoplankton communities (Falkowski et al. 2004; Legendre and Le Fevre 1989). For instance, some groups of phytoplankton such as diatoms and coccolithophores possess mineral skeletons of silica and calcium carbonate. These mineral compounds are denser than seawater and facilitate their gravitational sinking (Smayda 1970; Smetacek 1985). Likewise, according to Stoke's law, larger cells, like large particles in a fluid, sink faster and further contribute to massive export. Understanding the mechanisms that control these two fundamental features of marine phytoplankton communities has increasingly become the focus of marine microbial ecologists and biogeochemists (Cermeño et al. 2008; Follows et al. 2007; Litchman 2006).

The dynamics of nutrient supply controls the structure of phytoplankton communities through interspecific competition and species selection (Tilman 1982). Classical competition theory predicts that the success of phytoplankton taxa reflects their intracellular nutrient quota and uptake rate, where the organism with the lower requirement for the limiting nutrient, which is defined as R^* , becomes at a competitive advantage (Grover 1990; Tilman 1977). Following this logic, Tilman (1977) demonstrated that the outcome of competition for silicate among planktonic diatoms closely follows the R^* rule at steady state. However, the prediction given by R^* may change if the system does not reach the equilibrium, as in the case of variable nutrient supply regimes typical of dynamic coastal ocean ecosystems (Grover 1990; Miller et al. 2005).

On this theoretical basis, Ramón Margalef proposed in his mandala that different combinations of nutrient concentration and turbulence levels select for phytoplankton taxa according to species-specific functional traits and survival strategies (Margalef 1978; Smayda and Reynolds 2001). Based on the conceptual foundations of the mandala, resource-based competition models suggested that, owing to the rapid uptake of nutrients by diatoms, increased ocean turbulence (i.e. higher frequency of nutrient pulses) potentially would increase their ecological success at the expense of slow-growing phytoplankton.

The Margalef's mandala (1978) represented one of the first attempts to describe the role of turbulence and nutrient supply in the selection of different taxonomic/functional groups of phytoplankton (Figure 1). High turbulence levels and massive/intermittent nutrient supplies favour large-sized phytoplankton, primarily diatoms, which possess high maximum uptake rates and the ability to store nutrients in large, intracellular vacuoles. Conversely, motile species and those with high affinity for nutrients such as dinoflagellates and coccolithophores dominate community structure in nutrient-impooverished, stratified systems where nutrients enter the system near the steady-state.

For Margalef the available external energy, on which advection and turbulence depend, is the best predictor of primary production and dominant life-forms of phytoplankton. However, the input of nutrients is usually associated with turbulence. Thus, species adapted to high nutrient concentrations are adapted, as well, to higher turbulence levels, and viceversa. In the original diagram of the mandala different life-forms are placed in an ecological space defined by nutrient concentration and the vertical diffusivity (K_z), which is considered as an indicator of turbulence level (figure 1). Margalef (1978) suggested that a mean K_z of $0.4 \text{ cm}^2 \text{ s}^{-1}$ represents the transition state between diatom- and dinoflagellate-dominated assemblages, with diatoms occurring in regions of high turbulence ($2\text{--}100 \text{ cm}^2 \text{ s}^{-1}$) and dinoflagellates at lower turbulence levels ($0.02\text{--}1 \text{ cm}^2 \text{ s}^{-1}$). This conceptual framework has

served for decades to qualitatively explain phytoplankton distribution across space and through time, yet a quantitative estimate in the field of the effect of turbulence and nutrient supply dynamics on species selection remains elusive.

Revisiting the mandala of Margalef

Several studies carried out in the last decade noted that a number of limitations exist when applying this conceptual model to the field (Barber and Hiscock 2006; Li 2002). Due to methodological limitations at that time, the smaller-size picoplankton groups (*Prochlorococcus*, *Synechococcus* and picoeukaryotes) were not included in the original Margalef diagram (Figure 1). Li (2002) used a set of flow cytometric measurements carried out in the North Atlantic to show distinct trends of abundance of pico- and nano-phytoplankton size classes. His analysis showed that as phytoplankton communities arrange from low to high biomass, there is an increment in large nanoplankton, a decrease in picoplankton, and no apparent variation in small nanoplankton, which constitutes a uniform background. Li (2002) also investigated the abiotic control on the assemblage of the phytoplankton community by analyzing the degree of stratification. Picoplankton was favoured under conditions of high stratification, whereas as stratification diminished, the balance shifted towards large nanoplankton species.

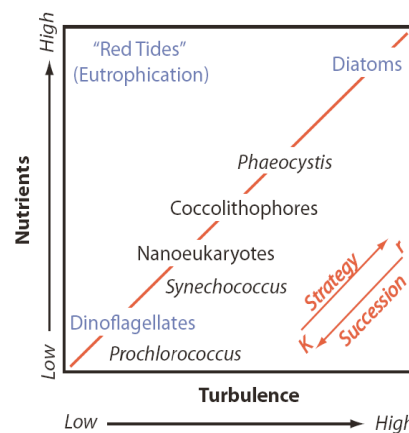


Figure 1. Adaptation of the Margalef's Mandala (1978) by Kudela (2010), where blue color indicate the functional types included in the original conceptual diagram.

According to Margalef's mandala, diatom assemblages are replaced by dinoflagellate species as nutrients deplete and water column stabilizes. However, contrary to this view, Barber and Hiscock (2002) showed that both picoplankton groups and diatoms increase growth rates and absolute abundance during favourable bloom conditions, yet, the huge increase in diatom biomass has led to the erroneous believe that, as the bloom matures, diatoms replace the nondiatom taxa by succession. So far it is unknown whether or not the Margalef's conceptual model holds for marine picoplankton, as the spatial and temporal variability of small taxa and their response to ocean turbulence and nutrient supply dynamics has not been yet well characterized.

The role of the degree of perturbation on species diversity

The "paradox of the plankton" formalized by G. Evelyn Hutchinson (1961) has puzzled and challenged oceanographers for decades. The raised problem was how it is possible for a number of plankton species to coexist in a relatively homogeneous environment all competing for the same sorts of materials. Today we now know that, at least in part, this paradoxical diversity is supported by spatial and temporal heterogeneity of factors influencing plankton (e.g., light, nutrients and turbulence). However, debate is still going about the degree of heterogeneity and perturbation that held the maximum diversity. Several studies have analyzed the relationship between nutrient supply, through the level of mixing or stratification, and phytoplankton diversity. Li (2002) analyzed the relationship between the degree of stratification and cytometric diversity. His work revealed maximum phytoplankton diversity at intermediate levels of biomass and degree of stratification, what Li interpreted as a support for the intermediate perturbation hypothesis (Connell 1978). According to this hypothesis, diversity is maximum

at intermediate levels of disturbance, as strong perturbations exclude all but a few robust species, whereas weak disturbance allows strong competition. Huisman et al (2005), by using a modelling approach, showed that reduced vertical mixing, parameterized as vertical diffusivity (K_z), can generate oscillations and chaos in phytoplankton biomass and species composition in the deep chlorophyll maxima. Their simulations also showed that different species persist in a non-equilibrium environment, confirming earlier notions that oscillations and chaos promote phytoplankton biodiversity (Huisman et al. 2005).

Using a classical physiological model based on nutrient uptake kinetics, Tozzi et al (2004) explored the effect of turbulence and nutrient supply dynamics on resource competition between diatoms and coccolithophorids. Their results showed that the concentration of nutrients regulated the overall carrying capacity of the system, whereas the frequency of the nutrient pulses was the key factor regulating the relative abundance of the two phytoplankton functional groups. More recently, Cermeño et al (2011) used continuous culture systems to study competition between the diatom *Thalassiosira pseudonana* and the coccolithophore *Coccolithus braarudii*. Their results showed an increasing linear relationship between the frequency of nitrate pulses and the rate of competitive exclusion, implying a longer coexistence of the two species at low perturbation frequencies.

Turbulence and its effects on phytoplankton

Heat exchanges, wind and tides, operating at various spatio-temporal scales, introduces eddy kinetic energy into the ocean. This energy is channelled from the large scales (10-1000s km, meso and macroscale) where generation occurs, towards to the smaller scales (1-10s cm or microscale) until the motion is resisted by molecular viscosity and the energy dissipated as heat. The amount of energy channelled down the cascade is equivalent to the energy dissipated by viscosity, which can be expressed by the dissipation rate of turbulent kinetic energy (ε). The relationship between ε and the buoyancy forces determine the vertical diffusivity (K_z), which plays a crucial role in the marine biosphere through their influence in the transport of dissolved gasses and nutrients (Rahmstorf 2003). A common assumption is that at the larger scales no energy is dissipated, and only when eddies reach a small enough size, the Kolmogorov length scale, the viscous forces becomes dominant.

Turbulence interacts with microorganisms through different temporal and spatial scales. At larger scales turbulence influences a range of biological processes through bringing nutrient-rich water into the euphotic zone, creating and disturbing high density patches of organisms, and transporting plankton and detritus away from their sites of production (Thomas et al. 1997). At the Kolmogorov scale, and below, the direct effects of turbulence on planktonic organisms include the alteration of a variety of physiological processes (Berdalet and Estrada 2005) and the influence on the settling velocities (Ross 2006; Ruiz et al. 2001).

At least in theory, the potential effects of turbulence on phytoplankton physiology increases when there is a significant mismatch between the size of the cells and the scale and velocity of the turbulent eddies (Margalef 1978). Those cells whose dimensions exceed the Kolmogorov scale will be more sensitive than smaller cells. However, experimental data show that organisms with lengthscales 1000 times smaller than the Kolmogorov scale can be also under the influence of turbulence (Peterson et al. 2000).

A number of revisions about the interaction of small-scale turbulence and planktonic organisms have been published so far (Davis 1996; Estrada and Berdalet 1998; Gregg et al. 2003; Karpboss et al. 1996; Kiorboe 1989; Peters and Redondo 1997). Peters and Marrasé (2000) reviewed a critical amount of experiments about the interaction of small-scale turbulence and planktonic organisms in order to find general trends. According to these authors, depending on the taxa growth rates exhibit a different response to turbulence; while in general there is no trend, dinoflagellates are negatively affected by turbulence (Peterson et al. 2000). Understanding the effect of turbulence, at small and larger scales, on dinoflagellates has an immediate application in the study of the proliferation of harmful algae blooms (HAB), as some dinoflagellate species produce toxic substances that can be accumulated in shellfish and ultimately poison humans and other vertebrates (Figueiras et al. 2006).

Intuitively, stirring keeps particles suspended in a fluid longer than when the fluid is at rest. However, recent studies propose that this assumption has long been used without any empirical support. By using a laboratory approach, Ruiz et al. (2001) described that phytoplankton settling velocity increases when

turbulence intensifies. More recently, Ross (2006) used a modelling setting to demonstrate that turbulence can either retard or accelerate particle settling depending on the experimental setup of the turbulent parameterization. The residence time of planktonic particles in the surface mixed layer influences the rates of carbon export to the deep ocean and so it is a crucial parameter for climate prediction modelling.

Turbulence is generated externally at sea and internally in experimental containers and therefore it is virtually impossible to simulate oceanic turbulence in the laboratory. Other problems associated with the study of turbulence in the lab are that the size of the experimental containers generates differences in the integral length scales, and that artificially generated turbulence does not reproduce the temporal intermittency found that characterize oceanic waters (Gregg et al. 2003; Peters and Redondo 1997). Often, the experimentally generated turbulence levels are higher than in situ values (Peterson et al. 2000). Moreover, the terms turbulence and stratification are very often used interchangeably when studying the connexion between mixing and phytoplankton dynamics (Huisman et al. 2005; Li 2002). However, both terms are not equivalent from a physical perspective neither in their effects on phytoplankton. As an example, high levels of dissipation rates of turbulent kinetic energy occur within the pycnocline associated with the passage of internal waves during the spring tides (Sharples et al. 2007) (Figure 2).

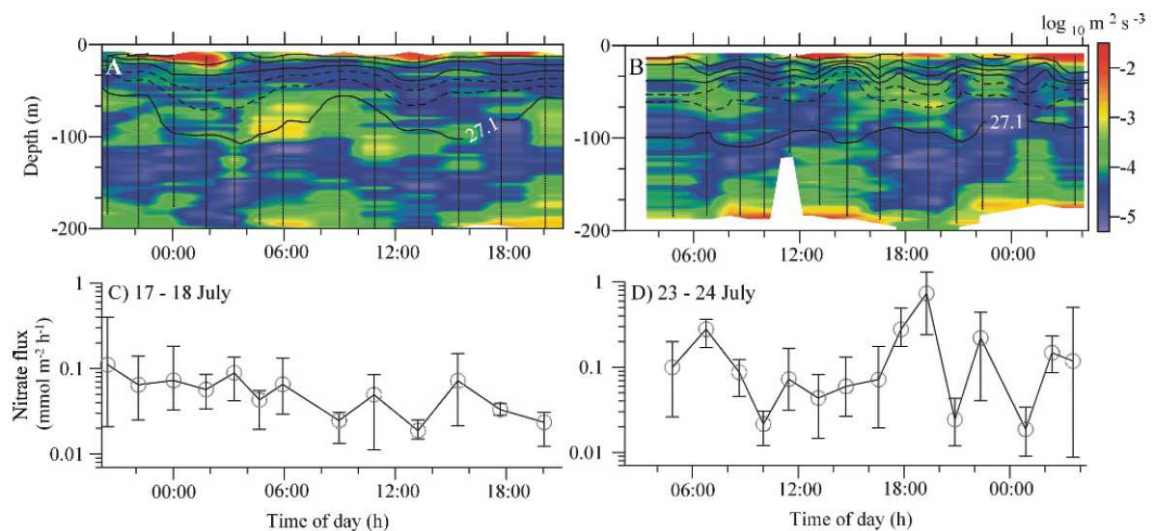


Figure 2. Time series of turbulent dissipation (colors) and sigma-t (lines) for (A) neap and (B) spring tide periods sampled in the shelf-break of the Celtic Sea. (C) and (D) are the time series of the vertical nitrate fluxes through the base of the thermocline during the neap and spring tide sampling periods respectively. Vertical bars in panels C and D are 95% confidence intervals (Sharples et al. 2007).

Sometimes turbulent dissipation rates in the ocean are derived from theoretical models which use wind velocity and sample depth as inputs (Soloviev et al. 1988). These models consider wind induced shear as the only turbulence-generating mechanism and neglect the potential contribution of other physical (Csanady 1989; Kitaigorodskii et al. 1983; Oakey and Elliott 1982) and biological processes (Katija and Dabiri 2009). The response of plankton communities to turbulence may differ from the sum of the effects on the different taxa composing the community (Dalsgaard et al. 2003; Estrada et al. 1987; Peters and Redondo 1997). Therefore in order to understand the effects of turbulence at the community level, there is a great need for experiments at sea including different members of the plankton community.

Microstructure turbulence measurements and the study of biological processes in the ocean

Due to methodological limitations, the dissipation rate of the turbulent kinetic energy has been extremely difficult to estimate in the past. Microstructure measurements started in the fifties impelled by militar research during the Cold War (Baumert et al. 2005). Today several companies commercialize different instruments suitable to obtain measurements of ε at sea: i.e. MSS (Prandke and Stips 1998), TurboMap (Wolk et al. 2002) and PME (Stevens et al. 1999). The information gathered during six decades of observations indicated that vertical diffusivity in the ocean is one order of magnitude smaller than estimates from mathematical models (Wunsch and Ferrari 2004). Oceanic models are very sensitive to the value of vertical diffusivity and so most of the studies using microstructure profilers have been

focussed on characterizing the distribution of K_z in different parts of the ocean (Gregg et al. 2003; Mori et al. 2008; Rainville and Winsor 2008; Rudnick et al. 2003; Sriver and Huber 2007). More recently these equipments have also been used to calculate the contribution of the vertical flux of nitrate to new production at the shelf edge of the Celtic Sea (Sharples et al. 2007) (figure 2), in the Mauritanian upwelling region (Schafstall et al. 2010), and across long distances in the Atlantic Ocean (Mourino-Carballido et al. 2011) and the Mediterranean Sea (Cuypers et al. 2011).

Nutrient supply to the euphotic layer occurs through a variety of mechanisms including diffusion across the thermocline, mesoscale, and submesoscale turbulence (Mourino-Carballido and McGillicuddy 2006; Mourino et al. 2005; Mouriño-Carballido 2009; Oschlies and Garcon 1998), lateral transport of organic and inorganic nutrients from the surrounding productive regions (Torres-Valdes et al. 2009; Williams and Follows 1998), atmospheric deposition (Duce et al. 2008; Mouriño-Carballido et al. 2012) and nitrogen fixation (Fernández et al. 2010; Moore et al. 2009). Vertical diffusion represents one of the main process over large extensions of the open ocean (Mourino-Carballido et al. 2011) and during seasonal stratified periods in coastal regions.

The application of microstructure turbulence measurements to the study of plankton distribution has been very rare. Kunze et al (2006) described that mixing elevated by a factor of 100 during the dusk ascent of a dense acoustic-scattering layer of krill. To our knowledge, only in one occasion microstructure turbulence and vertical distribution of plankton have been studied simultaneously at sea. Maar et al (2003) investigated microscale vertical distribution of zooplankton in relation to turbulent diffusion, and confirmed their hypothesis that the variability of the vertical distribution would be independent of turbulence up to a threshold where dispersion overwhelms the swimming ability of the organisms. As far as we know the influence of turbulent diffusion on the species composition and size-structure of the phytoplankton community has not been directly investigated in the ocean.

Internal wave activity in the shelf edge of NW Spain

The generation of internal tides involves the conversion of barotropic tide energy into energy of the internal motion and, in general, the efficiency of the conversion increases with the stratification, topographic gradient and amplitude of the barotropic current (Baines 1982). The edges of continental shelves are frequently sites of internal tides and elevated vertical mixing driving vertical nutrient fluxes (Sharples et al. 2007) (figure 2). Enhanced nutrient fluxes driven by the internal wave activity underpin horizontal and vertical gradients in the phytoplankton community structure with strong implications on fishing activity and the export of particulate organic carbon (Mourino et al. 2001; Sharples et al. 2009). Based on the distribution of eggs and larvae, the spawning habitat of several fishing commercial species is very tightly associated with the 200 metre isobath at the shelf edge (Ibaibarriaga et al. 2007). Rather than higher phytoplankton and zooplankton biomass or production, it appears that internal waves could influence the distribution of spawning fish via the impact of vertical mixing on the size structure of the phytoplankton and zooplankton community (Albaina and Irigoien 2004; Conway et al. 1999). Enhanced nutrient supply and subsequent large-celled phytoplankton could offer better quality food than a small-size dominated community (Muller-Karger et al. 2005). It has been also proposed that increased turbulent mixing at the shelf edge may play a role in prey capture rates by fish larvae (Kiorboe and Saiz 1995).

The shelf edge of NW Spain is very steep and propitious for the generation of internal tides (Garcia-Lafuente et al. 2006). In this region enhanced dissipation rates and vertical eddy diffusion coefficients have been observed within the thermocline associated with the breaking of internal waves (Barton et al., 2001). Their signature has been observed in Synthetic-aperture radar (SAR) imagery and their effect on near-surface chlorophyll distributions has been identified in ocean color satellite images (Relvas et al. 2007). However, characterization of nutrient supply dynamics associated with the internal wave activity in this region, and their role on phytoplankton species composition and size-structure, is an outstanding problem which remains unresolved.

3. OBJECTIVES

(maximum 2 pages)

3.1 Describe the reasons to present this proposal and the **initial hypothesis** which support its objectives (maximum 20 lines)

Previous studies suggest that turbulence through nutrient supply has an important role on controlling size-structure and phytoplankton species composition (Margalef 1978). However, the difficulties to measure ocean turbulence have restricted the research on this field to laboratory experiments and indirect estimates of nutrient supply at sea by using proxies and empirical parameterizations for turbulence. Here we propose to combine data base analysis, observations in the field and chemostat experiments in the lab in order to verify the following **hypotheses**:

1. Enhanced mixing driven by internal wave activity is an important factor controlling nutrient supply dynamics into the photic layer.
2. Turbulence through nutrient supply dynamics controls phytoplankton size structure, with an increase in the relative contribution of larger cells to total phytoplankton biomass.
3. Turbulence through nutrient supply promotes phytoplankton biodiversity with maximal diversity at intermediate levels of turbulence and nutrient fluxes.
4. The effects of turbulence on phytoplankton are highly species-specific: dinoflagellates have lower turbulence intensity threshold/optimum levels than diatoms.
5. Rate and frequency of nutrient supply determine the dynamics of competitive exclusion between diatoms, dinoflagellates and cyanobacteria.

Climate-driven processes that influence turbulent mixing in the ocean seem to have strongly influenced the diversity and relative abundance of the major eukaryotic phytoplankton groups over the past 200 million years (Falkowski and Oliver 2007). Therefore, understanding the link between turbulent mixing, nutrient supply and phytoplankton size and taxonomic structure is crucial to predict how phytoplankton communities will change in the future.

3.2. Indicate the **background and previous results** of your group or the results of other groups that support the initial hypothesis

The background and previous results that support the proposed hypothesis and objectives have been previously explained in detail in the introduction. A summary of the key points is presented:

- The Margalef's mandala (1978) represented one of the first attempts to describe the role of turbulence and nutrient supply in the selection of different taxonomic/functional groups of phytoplankton. Due to methodological limitations at that time, the smaller-size picoplankton groups were not included in the original Margalef diagram. So far it is unknown whether or not the Margalef's conceptual model holds for marine picoplankton.
- Margalef (1978) also suggested that a mean K_z of $0.4 \text{ cm}^2 \text{ s}^{-1}$ represents the transition state between the predominance of diatoms and dinoflagellates, with diatoms occurring in regions of high turbulence and dinoflagellates at lower turbulence levels. Due to the methodological difficulties to measure ocean turbulence, a quantitative estimate in the field of the effect of turbulence on species selection remains elusive.
- Today we now know that, at least in part, the paradoxical diversity of the plankton (Hutchinson 1961) is supported by spatial and temporal heterogeneity of environmental factors. However, debate is still going about the degree of heterogeneity and perturbation that held the maximum diversity. According to Li (2002) phytoplankton diversity is maximum at intermediate levels of stratification, what supports for the intermediate perturbation hypothesis (Connell 1978).
- Recent studies using classical physiological models (Tozzi et al. 2004) and continuous culture systems (Cermeno et al. 2011) emphasized that the frequency of nitrate pulses, and not only

the concentration of nutrients, are the key factors regulating the relative abundance of phytoplankton functional groups.

- Large differences in the vertical flux of nitrate have been observed between spring and neap tides at the shelf edge of the Celtic Sea, associated with the passage of internal solitons (Sharples et al. 2007). The shelf edge of NW Spain is very steep and propitious for the generation of internal tides (Garcia-Lafuente et al. 2006). However, characterization of nutrient supply dynamics associated with the internal wave activity in this region, and their role on phytoplankton species composition and size-structure, remains unresolved.

3.3. Describe briefly the **objectives** of the project

1. To quantify the supply of nutrients into the euphotic zone driven by enhanced mixing associated with the internal wave activity during spring and neap tides in the shelf edge of NW Spain.
2. To determine potential relationships between a multidimensional environmental control matrix related to nutrient supply (stratification, small-scale turbulence (represented by ε), nutrient gradient, nutrient fluxes) and phytoplankton size-structure and community composition.
3. To analyze the relationship between small-scale turbulence and diatom and dinoflagellate abundance.
4. To determine the relationship between small-scale turbulence and the vertical distribution of cell abundance.
5. To determine the effect of changes in the rate of nutrient supply and frequency of nutrient pulses in the outcome of competitive interaction between species belonging to different taxonomic groups (diatoms, dinoflagellates and cyanobacteria).

3.4. For coordinated projects only, the **coordinator** must indicate (maximum 2 pages):

- the global objectives of the coordinated project, the need for coordination, and the added value provided by this coordination
- the specific objectives of each subproject
- the interaction among the objectives, activities and subprojects
- the mechanisms of coordination for an effective execution of the project.

4. METHODOLOGY AND WORKING PLAN

(in the case of coordinated projects this title must include all the subprojects)

Detail and justify precisely the methodology and the working plan. Describe the working chronogram.

- ◆ The working plan should contain the tasks, milestones and deliverables. The projects carried out in oceanographic ships or in the Antarctic zone must include the operation plan.
 - ◆ For each task, it must be indicated the centre and the researchers involved in it.
 - ◆ If personnel costs are requested, the tasks to be developed by the personnel to be hired must be detailed and justified. Remember that personnel costs are eligible only when personnel is contracted, **fellowships are not eligible** as personnel costs.
-

Dra. Beatriz Mouriño (PI) will lead the research planned in this proposal including the data base analysis, the observations at sea and the experiments in the laboratory. Dra. Belén Martín will be in charge of the design and data processing of experiments in the field resolving diurnal cycles of internal waves. Dra. Renate Scharek will be responsible for describing microphytoplankton composition. A hired specialist technician will be responsible for the data base analysis and the maintenance of the chemostat experiments during the second year of the project.

The working activities of this proposal can be grouped in three different work packages: data base analysis, observations in the field and chemostat experiments.

1. Data base analysis

Since 2006 the Biological Oceanographic research group at the University of Vigo has been in charge of measuring dissipation rates of turbulent kinetic energy by using microstructure profilers in twelve oceanographic cruises carried out in different coastal and oceanic environments in the global ocean (see figure 3 and Table 1). Measurements of microstructure turbulence were taken during the CARPOS and FAMOSO cruises in order to characterize vertical diffusivity and to compute geochemical estimates of net production. In TRYNITROP2 and Malaspina cruises vertical diffusivity was used to estimate vertical diffusive fluxes of nitrate. PERFIL cruises were planned to study the influence of turbulence in the small-scale vertical distribution of zooplankton. COUPLING and HERCULES mapped the temporal and spatial variability of turbulence in the Shetland Islands (Antartic) and the Golfo Ártabro (NW Spain), respectively. Finally the DISTRAL project (PI Pedro Cermeño, UVIGO) will use the microstructure data to investigate the plankton community dynamics and diversity through a combination of neutral modelling and experimental approaches. The data set includes a total of 975 profiles conducted in 179 stations parallel to the collection of samples for the determination of nitrate concentration, pico and microphytoplankton abundance, size-fractionated phytoplankton biomass and phytoplankton species composition. This data base will be extended with the data set that we plan to collect during the observations in the field (see below).

Task 1.1. Multidimensional environmental control matrix related to turbulence, nutrient supply and phytoplankton size-structure and species composition.

The large data set constitutes a very valuable tool in order to explore the influence of turbulent and nutrient supply on phytoplankton species composition and size-structure. We will study potential relationships between stratification, small-scale turbulence (represented by ε), nutrient gradient, nutrient diffusive fluxes, size-fractionated chlorophyll, and pico and microphytoplankton abundance and composition.

Responsible: Beatriz Mouriño (UVIGO), hired technician

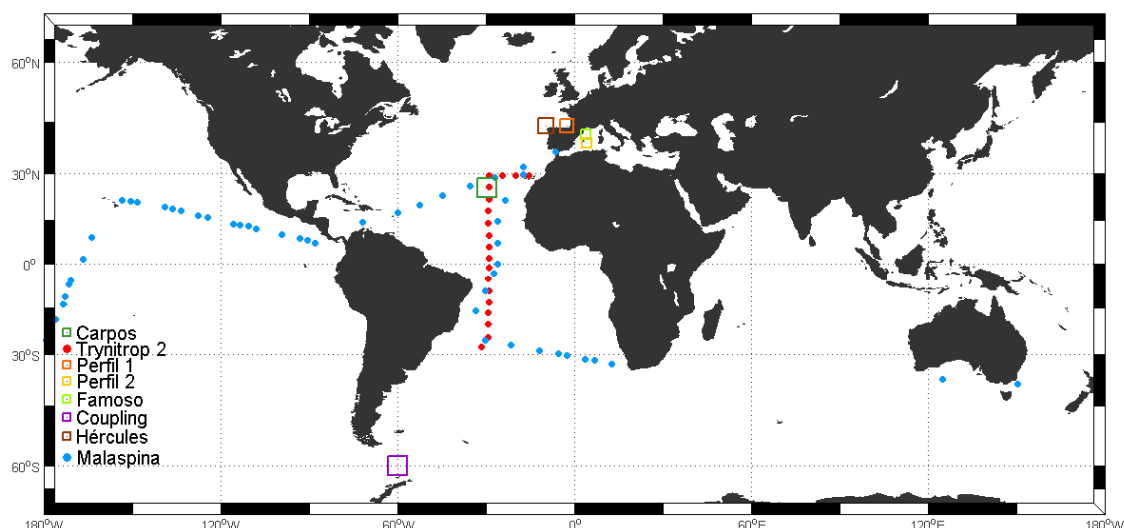


Figure 3. Measurements of microstructure turbulence carried by the Biological Oceanographic group at the University of Vigo by using a microstructure turbulence profiler since 2006.

Table 1. Details of measurements of microstructure turbulence carried by the Biological Oceanographic group at the University of Vigo by using a MST profiler. A TURBOMAP profiler from the ULPG was used during the COUPLING cruise. N represents the number of stations where measurements of dissipation rates of turbulent kinetic energy were conducted, and n is the number of MST profiles. CARPOS (Flujos de CARbono mediados por el Plancton en ambientes Oligotróficos Subtropicales: una aproximación lagrangiana, PI: Ramiro Varela, MYCIT), TRYNITROP (Trichodesmium spp. y fijación de nitrógeno (N₂) en el Atlántico tropical, PI: Emilio Marañón, MYCIT), PERFIL (Pautas en la distribución vertical a pequeña escala del zooplancton marino: Control ambiental y consecuencias a nivel de consumidores superiores, PI: Miguel Alcaraz, MYCIT), FAMOSO (Fate of the northwestern Mediterranean open sea spring Bloom, PI: Mikel Latasa, MYCIT), COUPLING (Acoplamiento físico-biológico en rango de mesoscala alrededor de las South Shetland Islands (Antártida): distribución e interacción del fitoplancton y el meroplancton, PI: Elsa Vázquez, MYCIT), HERCULES (Variabilidad hidrodinámica e de plancto mariño a curta escala na plataforma galega: eventos de afloramento no Golfo Ártabro, PI: Manuel Ruiz, Xunta de Galicia), MALASPINA (Circumnavigation Expedition Malaspina 2010: Global Change and Biodiversity Exploration of the Global Ocean, PI: Carlos Duarte, MYCIT), DISTRAL (Dispersal, neutral models and the assembly of marine microbial plankton communities, PI: Pedro Cermeño), MYCIT.

Cruise	Vessel	Year	N (n)	Date	Region	Variables
CARPOS	Hespérides	2006	11 (99)	20 Oct-21 Nov	NASE	Cytometry, size-fractionated chlorophyll-a
TRYNITROP2	Hespérides	2008	19 (115)	14 Abr-2 May	Atlantic Ocean	Cytometry, size-fractionated chlorophyll-a
PERFIL1	García del Cid	2008	27 (181)	18-26 Jul	Biscay Bay	Size-fractionated chlorophyll-a
PERFIL2	García del Cid	2009	15 (94)	27 Jun-1 Jul	Mediterranean Sea	Size-fractionated chlorophyll-a
FAMOSO1	Sarmiento de Gamboa	2009	6 (26)	14-22 Mar	Mediterranean Sea	Cytometry, microphytoplankton taxonomy
FAMOSO2	Sarmiento de Gamboa	2009	10 (69)	30 Apr-19 May	Mediterranean Sea	Cytometry, microphytoplankton taxonomy
FAMOSO3	Sarmiento de Gamboa	2009	3 (21)	17-19 Sep	Mediterranean Sea	Cytometry, microphytoplankton taxonomy
COUPLING	Hesperides	2010	9 (27)	8-25 Jan	Shetland Islands (Antartic)	Cytometry, microphytoplankton taxonomy, size-fractionated chlorophyll-a
HERCULES1	Lura	2010	4 (28)	6 Jul	Artabro Gulf	Cytometry, microphytoplankton taxonomy
HERCULES2	Lura	2011	17 (69)	28-29 Sep	Artabro Gulf	Cytometry, microphytoplankton taxonomy
MALASPINA	Hesperides	2010-211	50 (166)		Global Ocean	Cytometry, microphytoplankton taxonomy, size-fractionated chlorophyll-a
DISTRAL	Mytilus	2012	8 (80)	12 Jun; 11,31 Jul; 7 Sep; 10 Oct; 13, 27 Nov; 13 Dec	Ría de Vigo	Cytometry, microphytoplankton taxonomy, size-fractionated chlorophyll-a

Task 1.2. Relationship between small-scale turbulence and cell abundance.

Picoplankton distribution, biomass and cellular characteristics measured by flow cytometry analysis and microphytoplankton abundance from microscope counts will be used to study potential relationships between vertical distribution of small-scale turbulence and cell abundance.

Responsible: Beatriz Mouriño (UVIGO), hired technician

Task 1.3. Relationship between small-scale turbulence and diatom and dinoflagellate abundance.

Diatom and dinoflagellate abundance determined from microscope counts and small-scale turbulence data will be used to determine turbulence intensity thresholds for both groups.

Responsible: Beatriz Mouriño (UVIGO), hired technician

Main deliverables of this work package are:

1. Description of the control mechanisms influencing phytoplankton size-structure and species composition.
2. Determination of the influence of small-scale turbulence in the vertical distribution of cell abundance.
3. Determination of turbulence intensity thresholds for diatom and dinoflagellate abundance.

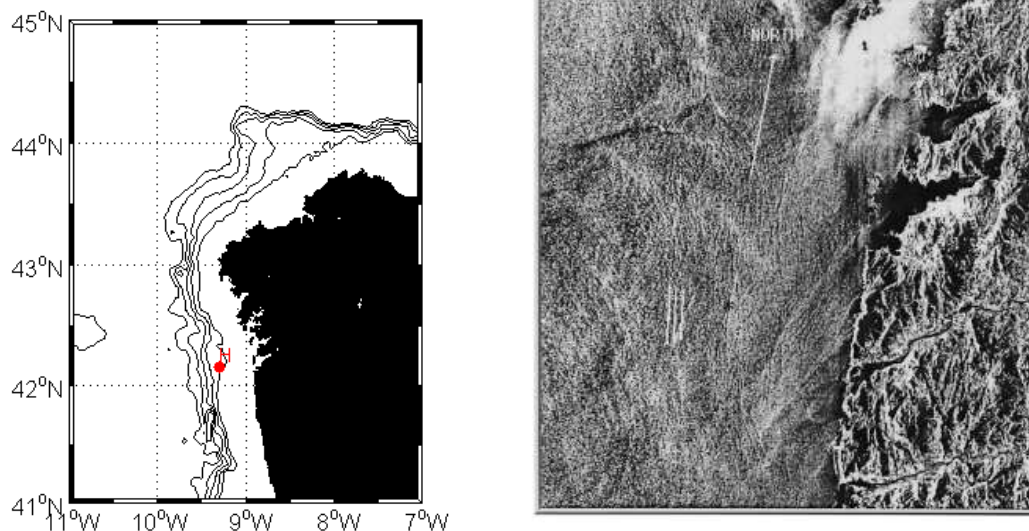


Figure 4. Map indicating the sampling site position planned for the diurnal cycles study in the NW Spain shelf-break (Left) and Synthetic-aperture radar (SAR) image showing internal wave activity in the region in summer 1998 (Barton et al., 2001) (Right).

2. Observations in the field

The goal of the experiments at sea is to have an in situ observation about the effects of a frequency gradient of nutrient pulses on the structure of marine phytoplankton. The sampling station will be located in the NW Spain shelf edge, where several studies and satellite images support that this region is under the influence of enhanced mixing events associated with internal waves (Barton et al., 2001) (see figure 4). Internal wave activity is stronger during the summer season as shallow pycnoclines present during this period act as waveguides along which internal waves propagate (Moum et al. 2008). Moreover, the strong stratification that characterized the upper layer during this period

forces low nutrient concentration at the surface. For this reason we plan our samplings to be conducted during the summer, when the nutrient pulses driven by internal waves are expected to be larger, and to represent the main fertilizing mechanism to the surface plankton communities. In order to cover a gradient in the frequency of nutrient pulses, intensive sampling of physical, chemical and biological variables will be carried out during two diurnal cycles under the influence of spring and neap tides. For those samplings we will try to avoid the influence of strong upwelling events that could reduce the surface stratification and introduce nutrients into the surface layers. We plan to conduct two three-days samplings on board R/V Mytilus centered around the dates of maximum spring and neap tides (21st August 2013 and 28th August 2013, respectively).

The sampling design will be as follows:

Day 1: Deployment of the AMP-C100 vertical profiler.

A Wetlabs® Autonomous Moored Profiler (AMP-100) will be deployed by Dr. César González-Pola who has shown his interest in the research project and the possibility of conducting these measurements (see enclosed a letter expressing his interest). The system is a recently developed autonomous profiler system acquired by the Spanish Institute of Oceanography (IEO) in the framework of the project “Análisis de viabilidad de dispositivos perfiladores autónomos permanentes como parte del sistema de monitorización oceánica del ICTS de Asturias” (CAC-2007-53, intended for feasibility of devices studies). The AMP includes a winch-driven profiling platform with an integrated control and power modulus, and a payload prepared to accommodate physical, optical and biological sensors. The system provides sub-meter scale vertical resolution of these properties from the sea floor to the surface air-water interface. Its current configuration includes conductivity, temperature and pressure (CTD, Sea-Bird Electronics SBE49) sensors, and fluorescence and turbidity sensors (WET Labs FLNTUS). After a series of successful cruises in 2010 in the framework of ‘Ecoanchora’ and ‘Hercules’ Projects, the system is currently stored at the Gijón IEO Center pending of a minor revision. It should be operational by the time of conducting the cruises proposed in the present proposal. Previous experiments have shown autonomy exceeding 100 profiles, therefore a sampling rate of ~10-15 minutes will be set for the 25-hour experiments.

Day 2: Tidal cycle (25 hours)

Samples for characterizing the size-structure and the composition of the phytoplankton community will be collected at 10 depths in the surface water column at the beginning and at the end of the tidal cycle. Continuous (yo-yo) CTD profiles will be conducted during the tidal cycle. Every hour the CTD yo-yo will be interrupted to collect samples for nutrient determination with the rosette sampler (where the CTD is attached) and measurements of dissipation rates of turbulent kinetic energy by using a microstructure profiler. Nutrient samples will be collected at 15 depths, with higher vertical resolution at the nutricline.

Day 3: Recovering of the AMP-C100 vertical profiler.

Furthermore, shipboard ADCP and Synthetic-aperture radar (SAR) images will be used to provide additional information about the internal wave activity in both periods. SAR wave mode provides two-dimensional spectra of ocean surface waves. For this function the SAR records regularly spaced samples within the image swath. The images are transformed into directional spectra providing information about wavelength and direction of wave systems. Automatic measurements of dominant wavelengths and directions show the effects of oceanographic phenomena, such as internal waves, slicks, small scale variations in wind and modulations due to surface currents and the presence of sea ice. SAR images are provided by the European Space Agency (<http://earth.esa.int/object/index.cfm?fobjectid=4001>).

Task 2.1. Hydrography, irradiance and fluorescence. On the second day of each survey, vertical casts with a SeaBird CTD probe, available on-board R/V Margalef, will be used to obtain the vertical variability

in temperature, salinity and fluorescence over a tidal cycle. The vertical distribution of incident irradiance will be determined with a Li-Cor spherical PAR sensor connected to a datalogger.

Responsibles: B. Martín (CETMAR), B. Fernández (UVIGO)

Task 2.2. Measurements of dissipation rates of turbulent kinetic energy (ε). Measurements of ε will be conducted by using a microstructure profiler (MSS, ISW Wassermesstechnik; (Prandke and Stips 1998)). The profiler is equipped with two velocity microstructure shear sensors (type PNS98), a microstructure temperature sensor, a high-precision CTD probe, and also a sensor to measure horizontal acceleration of the profiler. Vertical diffusive fluxes of nutrients will be calculated from the product of the vertical diffusion coefficient (K_z) and the vertical gradient of nutrient concentration, where K_z will be estimated from Osborn (1980) as:

$$K_z = e \frac{\varepsilon}{N^2}$$

Where ε is the dissipation rate of the turbulent kinetic energy, N the Brunt-Vaisala frequency, and e a mixing efficiency.

Responsibles: R. Graña (UVIGO), B. Fernández (UVIGO), B. Mouriño (UVIGO)

Task 2.3. Nutrient concentration. The concentration of nitrate, nitrite, ammonium, phosphate and silicate will be measured with a Technicon autoanalyser in the University of Vigo's Research Support Service, following the protocols described in Grasshoff et al (1983).

Responsibles: P. Chouciño (UVIGO), B. Mouriño

Task 2.4. Size-fractionated chlorophyll-a

Size-fractionated chlorophyll-a concentration will be measured after filtration of 250-ml samples onto 20, 5, 2 and 0.2- μ m pore size polycarbonate filters. Samples will be extracted for 12 h in 90% acetone at 4°C overnight. Fluorescence will be measured with a Turner TD-700 fluorometer previously calibrated with pure chlorophyll a.

Responsibles: P. Chouciño (UVIGO), B. Mouriño

Task 2.5. Microscopy analyses.

Abundance and cell volume of nanophytoplankton and microphytoplankton will be measured following Utermöhl's method (1958). One hundred ml samples will be preserved in acid Lugol's solution and 100 ml in buffered formaldehyde solution and, after sedimentation of sub-samples (10-50 ml), cells will be counted, identified to taxonomic group level (e.g. flagellates, coccolithophores, dinoflagellates, diatoms, etc.), counted and measured with an inverted microscope. The microscope is connected to a video-interactive, image analysis system that permits the discrimination and elimination of detritus and other non-living particles. Individual linear measurements will be used to compute cell volume by assigning each cell to the geometric shape that most closely resembles the real shape of each species.

Responsible: R. Shareck

Task 2.7. Flow cytometry

The abundance, biovolume and composition of picoplankton will be determined by using the flow cytometer service in the University of Vigo's Research Support Service. Water samples of 2 to 10 ml in volume will be preserved with 1% (v/v) glutaraldehyde and stored in liquid nitrogen until cytometry analysis. The size and abundance of all cells in the range 0.6 to 10 μ m of equivalent spherical diameter (ESD) will be determined by using their fluorescence and side light scatter (SLS) signals, which will be

calibrated with image-analysis measurements of the volume of several species cultured in the laboratory (Rodríguez et al. 1998).

Responsible: P. Chouciño (UVIGO), B. Mouriño

Task 2.8. Pigment composition

Phytoplankton pigments are now routinely used to map the chemotaxonomic composition of phytoplankton communities. 0.5-1.0 L of water will be filtered under low pressure through glass fiber filters (0.7 µm nominal pore). Filters will be frozen in liquid nitrogen and stored at -80°C until high performance liquid chromatography (HPLC) in the University of Vigo's Research Support Service, according to the method of Zapata et al [2000] and Latasa [2007].

Responsibles: P. Chouciño (UVIGO), B. Mouriño (UVIGO)

Main deliverables of this work package are:

1. Quantification of the amount of nutrients pumped into the euphotic zone by enhanced mixing associated with the internal wave activity during spring and neap tides.
2. Determination of the effect of the frequency of nutrient pulses in the structure of marine phytoplankton communities.
3. The data set collected during the experiments at sea will also contribute to the deliveries specified in the previous work package.

3. Experiments in the lab: chemostats approach

We will conduct competition experiments using two taxa and two cell sizes in mixed culture assemblages. One set of experiments will involve a mixed-population culture of a large and a small diatom (e.g. *Thalassiosira weissflogii* and *Thalassiosira pseudonana*, respectively). A second set of experiments will involve a diatom and a dinoflagellate (e.g. *T. weissflogii* versus *Alexandrium tamarensis*), and a third set of experiments will involve a diatom and the cyanobacterium *Synechococcus*. The basic experimental design utilizes chemostats in which the inflow medium is supplemented with pulses of nutrients at a predetermined frequency (e. g. 0.5-5 pulses/day). This will depart the system from the steady-state simulating the effect of internal waves in injecting nutrients into the photic layer. The experimental matrix yields a combination of taxa, sizes and nutrient supply dynamics that simulates both nitrate-limiting and -pulsing conditions. The experimental unit (Sartorius Biostat Plus) physically consists of two nutrient reservoirs connected to computer-controlled peristaltic pumps, where the desired nutrient combination can be directed to each of two bioreactors operating in parallel under identical light and temperature regimes.

The bioreactors will be sampled every two days to monitor nutrient concentration in the bulk medium, in vivo fluorescence, and the cell size and abundance of each species.

Our goal is to simulate nutrient supply dynamics (i.e., number of pulses and magnitude) observed in the field and quantify the competitive dynamics of mixed culture assemblages. We constrain the experiments to one of resource competition by maintaining cells at a single temperature under constant light/dark cycles at a single irradiance. By reducing experimental complexity, i.e., two taxa and one single limiting nutrient, we will be able to isolate and quantify the importance of nutrient supply dynamics on the selection of taxa and cell sizes. Key properties such as V_{max} and K_m for nitrate uptake of the species used in these experiments are already known since they have been experimentally determined in the project PERSEO (CTM2008-03699/MAR), thus will allowing us to make explicit, quantitative predictions as to the outcome of each competition experiment.

Task 3.1. Culture maintenance. Phytoplankton cultures will be maintained under continuous growth conditions (e.g. steady-state) in a Sartorius Biostat Plus system, which allows a high-precision control of temperature and dilution rate as well as the continuous monitoring of turbidity, pH and O₂ saturation.

Responsible: hired technician, Beatriz Mouriño (UVIGO)

Task 3.2. Every 2 days samples will be collected for the determination of nitrate concentration, cell abundance, total chlorophyll a and PSII photosynthetic efficiency (Fv/Fm). Nitrate concentration, cell abundance and total chlorophyll a will be measured following the protocols specified in the section 2. Fv/Fm will be measured with a Waltz PAM fluorometer.

Responsible: hired technician, Beatriz Mouriño (UVIGO)

Main deliverables of this work package are:

1. Empirical functions relating rate and frequency of nutrient supply with the relative abundance (and hence competitive ability) of different species belonging to different taxonomic groups and/or cell size classes.
2. An experimental verification of the ecophysiological mechanisms involved in phytoplankton succession that result from taxon- and cell size-related differences in nutrient acquisition and use.

This work package will be carried out in collaboration with Pedro Cermeño (UVIGO) and Emilio Marañón (UVIGO) who have shown their interest in the research project and have participated in the experiments design (see enclosed letters expressing their interest).

4.1 CHRONOGRAM MODEL (EXAMPLE)

This chronogram must indicate the persons involved in the project, including those contracted with project funds. Underline the name of the person responsible for each task.

Tasks	Centre	Persons	First Year (*) JFMAMJJASOND	Second Year (*) JFMAMJJASOND	Third Year (*) JFMAMJJASOND
1. Data base analysis					
Task 1.1. Multidimensional environmental control matrix related to turbulence, nutrient supply and phytoplankton size-structure and species composition	UVIGO	<u>B. Mouriño</u> , hired technician	XXXXXXXXXXXXX	XXXXXX	
Task 1.2. Relationship between small-scale turbulence and cell abundance	UVIGO	<u>B. Mouriño</u> , hired technician	XXXXXXXXXXXXX	XXXXXX	
Task 1.3. Relationship between small-scale turbulence and diatom and dinoflagellate abundance	UVIGO	<u>B. Mouriño</u> , hired technician	XXXXXXXXXXXXX	XXXXXX	
2. Observations in the field					
Task 2.1. Hydrography, irradiance and fluorescence	CETMAR	<u>B. Martín</u> , B. Fernández	X		
Task 2.2. Measurements of dissipation rates of turbulent kinetic energy	UVIGO	<u>R. Graña</u> , B. Fernández, B. Mouriño	X		
Task 2.3. Nutrient concentration	UVIGO	<u>P. Chouciño</u> , B. Mouriño	XX		
Task 2.4. Size-fractionated chlorophyll-a	UVIGO	<u>P. Chouciño</u> , B. Mouriño	X		
Task 2.5. Microscopy analyses	IEO-Gijón	<u>R. Scharek</u>	XXXXX	XXXXXX	
Task 2.7. Flow cytometry	UVIGO	<u>P. Chouciño</u> , B. Mouriño	XX		
Task 2.8. Pigment composition	UVIGO	<u>P. Chouciño</u> , B. Mouriño	XX		
3. Experiments in the lab					
Task 3.1. Culture maintenance	UVIGO	<u>Hired technician</u> , B. Mouriño		XXXXXXXXXXXXX	
Task 3.2. Measurements of nitrate concentration, cell abundance, total chlorophyll a and photosynthetic efficiency (Fv/Fm)	UVIGO	<u>Hired technician</u> , B. Mouriño		XXXXXXXXXXXXX	
4. Data processing, discussion of results, preparation of publications	All centers	All reaserchers		XXXXXX	XXXXXXXXXXXXX

(*) Mark an X inside the corresponding boxes (months)

5. BENEFITS DERIVED FROM THE PROJECT, DIFFUSION AND EXPLOTATION OF RESULTS

(maximum 1 page)

The following items must be described:

- ◆ Scientific and technical contributions expected from the project, potential application or transfer of the expected results in the short, medium or large term, benefits derived from the increase of knowledge and technology.
 - ◆ Diffusion plan and, if appropriate, exploitation plan of the results.
-

In 1978 the Margalef's Mandala first codified the role of the intensity of turbulence and nutrient supply in the selection of phytoplankton species. Since then a number of limitations have been note when applying this approach to the field. First at all, the difficulties to measure ocean turbulence have limited the research on this field to laboratory experiments, where phytoplankton communities are under the effect of unrealistic levels of turbulence, and experiments at sea where nutrient supply is computed by using proxies and empirical parameterizations for turbulence. Versus the traditional succession of phytoplankton groups described by the Margalef model, picoplankton (that due to the methodological limitations at that time were not included in the original diagram) has been also observed to respond to the onset of favorable growth conditions for diatom-dominated blooms (Barber and Hiscock 2006). It has also been noted that the Margalef's Mandala breaks down for many phytoplankton functional types because it has insufficient dimensions to take into account all the changes in the multiple limiting factors that control a phytoplankton group (Boyd et al.). Finally, recent experiments in the lab using continuous culture systems have shown that the frequency of nitrate pulses, and not only the magnitude of the pulses, controls the temporal progression of phytoplankton succession (Cermeno et al. 2011).

For the first time we proposed to analyze a large data set of small-scale turbulent measurements at sea and phytoplankton size-structure and species composition, covering different oceanographic regimes, to specifically determine:

1. The role of turbulence in the vertical distribution of phytoplankton cell abundance.
2. The role of turbulence and nutrient supply on phytoplankton size-structure and species composition.
3. The turbulence intensity threshold/optimum levels for diatoms and dinoflagellates.

The experiments at sea designed to cover a gradient of internal wave activity will allow us to:

4. Quantify the nutrient supply into the euphotic zone driven by enhanced mixing associated with the internal wave activity during spring and neap tides.
5. Characterize the response of phytoplankton size-structure and species composition to a frequency gradient of nitrate pulses.

The chemostat experiments in the lab will allow us to:

6. Determine how changes in the rate of nutrient supply as well as changes in the frequency of nutrient pulses affect the competitive interactions of pair of species differing in taxonomic affiliation or cell size.
7. Obtain a mechanistic understanding of the way in which differences in functional traits related to nutrient acquisition and use contribute to observed patterns of phytoplankton succession driven by hydrodynamically-induced changes in the dynamics of nutrient supply to the euphotic layer.

Phytoplankton community composition and size-structure are key pieces in the functioning of aquatic ecosystems and global climate. For this reason it is crucial to understand the factors that control phytoplankton community assembly and dynamics. Global database of phytoplankton traits under

different environmental gradients would constitute a very valuable tool for predicting community reorganizations under global change scenarios (Kohfeld et al. 2005; Litchman et al. 2007).

Enhanced mixing events associated with internal wave activity are globally common processes over topographic features as shelf edges and seamounts (Baines 1982). Important fisheries of commercial species are frequently associated over the same topography (Ibaibarriaga et al. 2007). For this reason the impact of mixing on the phytoplankton community structure is an important, and so far unreported, topic that merits consideration.

We plan to focus the diffusion of the results during the last seven months of the project (see cronogram). As this research constitutes a topic of high relevance and impact, we intend to publish the results in leading journals in the field of Ecology and Oceanography such as *Limnology & Oceanography*, *Geophysical Research Letters*, *Marine Ecology Progress Series* or *Journal of Geophysical Research*. We will also make available the most outstanding scientific results to a wider audience through publication in our own website (<http://webs.uvigo.es/bmourino/>).

6. BACKGROUND OF THE GROUP

(In the case of a coordinated project the topics 6. and 6.1. must be filled by each partner)

(maximum 2 pages)

◆ **Indicate the previous activities and achievements of the group in the field of the project:**

If the project is related to other previously granted, you must indicate the objectives and the results achieved in the previous project.

If the project approaches a new research field, the background and previous contributions of the group in this field must be indicated in order to justify the capacity of the group to carry out the project.

The Biological Oceanography group at the University of Vigo is coordinated by Dr. Emilio Marañón and it has a long trajectory in the field of phytoplankton ecology and physiology. The main research lines of this group include: 1) Diversity, metabolism, biogeography and biogeochemical role of marine microbial plankton, 2) Physiological and ecological response of bacterio- and phyto-plankton to global change (UV radiation, CO₂, nutrient supply) and 3) Coupling between ocean turbulence and the distribution and metabolism of plankton. Currently the group is composed by three seniors, four tenure-track fellows, six PhD students and three research assistants.

Over the last 10 years, the Biological Oceanography group at the University of Vigo has participated in more than 15 research projects in regional, national and international programmes including Xunta de Galicia, Ministerio de Ciencia e Innovación, and European Union, has published over 90 papers in scientific journals (SCI), 8 doctoral thesis and 3 underway. Currently, the team participates in several research projects related to the field of marine phytoplankton ecology including the projects: Turbulence during the open ocean spring bloom in the NW Mediterranean Sea (Ministerio de Ciencia e Innovación/CTM2009-06712-E/MAR, PI: Beatriz Mouriño), Temporal and spatial variability in the synthesis, remineralization and export of carbon in the North Atlantic subtropical gyre (Xunta de Galicia/09MDS001312PR, PI: Beatriz Mouriño), and the project Malaspina 2010 (Consolider) for which is responsible for the measurements of small-scale turbulence.

Dra. Beatriz Mouriño, principal investigator of this proposal, is a tenure-track fellow (Ramón y Cajal) at the Biological Oceanography group at the University of Vigo. Previously, she had an appointment as postdoctoral fellow at the Woods Hole Oceanographic Institution (USA). Her research uses a combination of altimeter images, time series data analyses, physical and biological observations from specific cruises, and ocean model simulations to assess the mechanisms that control primary production in the upper ocean, and in particular the processes responsible for intermittent nutrients supply to plankton communities. Her skills cover experiments in the lab related to plankton ecology, hydrographical data processing, measurements of microstructure turbulence and numerical modelling. Over the last five years, she has developed two independent lines of research related to this proposal:

•Biogeochemical role of ocean (sub)mesoscale turbulence. Through a multidisciplinary approach we assess the role of intermittent nutrients supply associated with (sub)mesoscale turbulence to plankton communities. Our results showed that 1) in highly dynamic ecosystems influenced by (sub)mesoscale dynamics respiration can be as variable as photosynthesis (Mouriño-Carballido 2009), 2) the age and type of the mesoscale eddies are important factors responsible for this variability and the composition of the plankton community (Mourino-Carballido and McGillicuddy 2006), and 3) important regional differences exist in the role of eddy pumping in subtropical regions (Cianca et al. 2007; Mourino-Carballido and Neuer 2008; Mourino et al. 2005).

•Application of measurements of microstructure turbulence to the study of biological processes in the ocean. Since 2006 we have carried out measurements of small-scale turbulence in the ocean by using a microstructure profiler (Prandke et al. 2000) in order to: 1) quantify nutrient inputs into the euphotic zone through vertical diffusion, 2) study the relationship between small-scale turbulence and vertical distribution of plankton, and 3) study the sensitivity of ocean models to empirical parameterizations of turbulence. Our results showed that the variability in nitrate diffusion resulted in significant differences between the south and the north subtropical Atlantic in the contribution of N_2 fixation to the new N input (Mourino-Carballido et al. 2011). Moreover, measurements of small-scale carried out in the NE subtropical Atlantic showed that empirical parameterizations of dissipation rates of turbulent kinetic energy overestimated (underestimated) the mixing at high (low) values of wind speed (Grana et al. 2011).

Belén Martín Míguez obtained her PhD in Physical Oceanography in 2003. After that, she has been appointed as a postdoctoral researcher in several institutions both in Spain and France (Puertos del Estado, CSIC, University of La Rochelle, UNESCO/IOC and CNRS). She is currently working as a tenure-track fellow (Parga Pondal Programme) at the Marine Technologies Unit of the CETMAR (Marine Technological Centre) in Vigo. She has participated in several oceanographic cruises, including NIVMER07 on board of the French vessel “Marion Dufresne” as chief scientist. She has broad experience in the analysis of time series of oceanographic data, including hydrography and currents (Míguez et al. 2001; Míguez et al. 2005) and more recently sea level (Míguez et al. 2008; Martín-Míguez et al., 2012). She has also investigated the effect of hydrodynamics on the phytoplankton communities in coastal systems (Tilstone et al. 2000; Míguez et al., 2001b).

Renate Scharek is a biological oceanographer specialized in phytoplankton ecology and marine biogeochemistry. She obtained her PhD in Biological Oceanography at the AWI in Bremerhaven, Germany. After that she has been appointed as a postdoctoral researcher in several institutions in Germany, The Netherlands, the United States and Spain (AWI in Bremerhaven, NIOZ in Texel, SOEST at the University of Hawaii, ICM-CSIC in Barcelona). She is currently working as a tenured scientist at the IEO (Instituto Español de Oceanografía) in Gijón. She worked on her research topics in the Southern Ocean, the subtropical North Pacific, the North Sea, the Mediterranean, the Indian Ocean and the Gulf of Biscay (Scharek et al 1997, Scharek et al 1999, Klöpper et al. 2003, Scharek and Latasa 2007, Not et al. 2008, Latasa et al. 2010).

The participants in this research proposal have contributed notably to the field of phytoplankton ecology by publishing in top scientific journals: *Limnology and Oceanography*, *Journal of Geophysical Research*, *Marine Ecology Progress Series*, *Deep-Sea Research* and *Biogeosciences*. By combining data base analysis, observations in the field and chemostat experiments in the lab this proposal may significantly improve our ability to understand the role of turbulence and nutrient supply in the phytoplankton size-structure and specie composition.

6.2 PUBLIC AND PRIVATE GRANTED PROJECTS AND CONTRACTS OF THE RESEARCH GROUP

Indicate the project and contract grants during the last 5 years (2007-2011) (national, regional or international)

Include the grants for projects under evaluation

Title of the project or contract	Relationship with this proposal (1)	Principal investigator	Budget	Funding agency and project reference	Project period (2)
			EUROS		
Turbulencia a lo largo de un transecto latitudinal en el Atlántico tropical (TURBYTROP)	1	Beatriz Mouriño	25.000	Ministerio de Educación y Ciencia (CTM2007-28925-E/MAR)	01/11/2007 hasta 01/05/2009 C
Turbulencia durante la proliferación primaveral de mar abierto en el mediterráneo noroccidental (TURBIMOC)	1	Beatriz Mouriño	25.000	Ministerio de Educación y Ciencia (CTM2009-06712-E/MAR)	01/01/2009 hasta 31/12/2011 C
Variabilidade espazo-temporal na síntese, remineralización e exportación de carbono no xiro subtropical do atlántico norte (VARITROP)	2	Beatriz Mouriño	71.679,50	Xunta de Galicia (09MDS001312PR)	01/09/2009 hasta 31/12/2012 C
Temporal and spatial variability in the synthesis, remineralization and export of carbon in the North Atlantic subtropical gyre	2	Beatriz Mouriño	15.000	Ministerio de Educación y Ciencia (Programa Ramón y Cajal) (RyC-2010- 06305)	01/01/2011 hasta 31/12/2012 C
Destino de la floracion primaveral de mar abierto en el Mediterráneo Noroccidental: Transferencias Troficas (FAMOSO-TROPH).	2	Renate Scharek	6.000	Ministerio de Educacion y Ciencia CTM2007-066945-C03-03 / MAR	31/12/2007 hasta 31/12/2008 C
Destino de la floracion primaveral de mar abierto en el Mediterráneo Noroccidental: Transferencias Troficas (FAMOSO-TROPH).	2	Renate Scharek	229.900	Ministerio de Ciencia e Innovación CTM2008-06261-C03-02 / MAR	31/12/2008 hasta 31/12/2012 C
(FOFA) FondeoFamoso: Fondeo de trampas de sedimento y correntímetros en el marco del proyecto FAMOSO	2	Renate Scharek	40.000	Ministerio de Ciencia e Innovación CTM2010-11165-E / MAR	01/01/2010 hasta 31/12/2012 C

Sistema autónomo integrado para la monitorización de flujos biogeoquímicos	2	Renate Scharek	167.232	Viceconsejería de Ciencia y Tecnología. Gobierno del Principado de Asturias EQUIP11-19	24/10/2011 hasta 15/01/2012 C
(FOCA) FONDEO CAÑON GAVIERA: FONDEO BIOGEOQUIMICO EN EL AREA DEL CAÑON DE AVILES, MAR CANTABRICO	2	Renate Scharek	30.000	Ministerio de Ciencia e Innovación CTM2011-14096-E / MAR	01/01/2012 hasta 31/12/2013 C

(1) Write 0, 1, 2 or 3 according to: 0 = Similar project; 1 = Very related; 2 = Low related; 3 = Unrelated.

(2) Write C or S if the project has been funded or it is under evaluation, respectively.

7. TRAINING CAPACITY OF THE PROJECT AND THE GROUP

(In the case of coordinated projects this issue must be filled by each partner)

This title must be filled only in case of a positive answer to the corresponding question in the application form.

Justify that the group is able to receive fellow students (from the Suprograma de Formación de Investigadores) associated to this project and describe the training capacity of the group. In the case of coordinated projects, each subproject requesting a FPI fellowship must fill this issue.

Note that all necessary personnel costs should be included in the total budget requested. The available number of FPI fellowships is limited, and they will be granted to selected projects as a function of their final qualification and the training capacity of the groups.

The research team maintains a steady output of scientific results, both in terms of scientific publications and completed PhD thesis (see attached CVs). During the last 8 years, the research group has published more than 90 articles in SCI journals, including leading journals such as Limnology and Oceanography, Journal of Geophysical Research, Marine Ecology Progress Series, Deep-Sea Research and Biogeosciences. 7 postgraduate students have completed their PhD thesis in our research groups during the last 8 years, and in all cases these results have resulted in the publication of several scientific articles in SCI journals. Most of these students have continued their scientific careers after completing their PhD thesis, and are now established researchers holding permanent or tenure-track positions (e.g. EvaTeira, Beatriz Mouriño and Pedro Cermeño at Universidad de Vigo). Beatriz Mouriño, the lead of this proposal, has been involved in student mentoring activities since 2007 (see attached CV). So far she has supervised three master and two undergraduate students. One of the master students, Bieito Fernández, was honored with a FPU fellowship and is currently carrying out his PhD thesis under her supervision.

The work we do is strongly multidisciplinary, as it includes modelling work, experimental observations conducted during oceanographic cruises and laboratory experiments both with cultures and natural assemblages. We are interested in the structure and functioning of the communities but also in the way organisms drive the cycling of elements, particularly carbon and nitrogen, in the upper ocean. Our studies therefore range in scope from ecophysiology, to community ecology and biogeochemistry. We also characterize the environmental forcings acting upon the plankton communities in order to understand the overall functioning of the pelagic ecosystem. We have strong links with many research groups in Spain (IEO Coruña, IIM-CSIC Vigo, CMIMA-CSIC Barcelona, IMEDEA-CSIC Palma de Mallorca, Universidad de Las Palmas de Gran Canaria) and abroad (IFM-GEOMAR Kiel, Woods Hole Oceanographic Center, Arizona State University). As far as the present proposal is concerned, we believe it will serve as a suitable framework for the training of a student, as it addresses major issues within the fields of biological oceanography and marine ecology, with implications in marine biogeochemistry and general ecology, and involves a wide range of observational, experimental and analytical approaches.

As a result of all the above, our research team and the present project offer an excellent prospect for a young researcher wishing to pursue a scientific career in marine ecology and oceanography.

References

- Albaina, A., and X. Irigoien. 2004. Relationships between frontal structures and zooplankton communities along a cross-shelf transect in the Bay of Biscay (1995 to 2003). *Marine Ecology-Progress Series* **284**: 65-75.
- Alvarez-Salgado, X. A., J. Aristegui, E. D. Barton, and D. A. Hansell. 2007. Contribution of upwelling filaments to offshore carbon export in the subtropical Northeast Atlantic Ocean. *Limnology and Oceanography* **52**: 1287-1292.
- Baines, P. G. 1982. On Internal Tide Generation Models. *Deep-Sea Research Part a-Oceanographic Research Papers* **29**: 307-338.
- Barber, R. T., and M. R. Hiscock. 2006. A rising tide lifts all phytoplankton: Growth response of other phytoplankton taxa in diatom-dominated blooms. *Global Biogeochemical Cycles* **20**.
- Barton, ED, Inall ME, Sherwin TJ, Torres R 2001. Vertical structure, turbulent mixing and fluxes during Lagrangian observations of an upwelling filament system off Northwest Iberia. *Progress in Oceanography* **51**, 249-267.
- Baumert, H., J. Simpson, and J. Sundermann. 2005. *Marine Turbulence: Theories, Observations, and Models*. Cambridge University Press.
- Berdalet, E., and M. Estrada. 2005. Effects of small-scale turbulence on the physiological functioning of marine algae, p. 459-500. *In* D. S. R. [ed.], *Algal Cultures, Analogues and Applications*. Science Publisher.
- Boyd, P. W., R. Strzepek, F. X. Fu, and D. A. Hutchins. Environmental control of open-ocean phytoplankton groups: Now and in the future. *Limnology and Oceanography* **55**: 1353-1376.
- Cermeno, P., S. Dutkiewicz, R. P. Harris, M. Follows, O. Schofield, and P. G. Falkowski. 2008. The role of nutricline depth in regulating the ocean carbon cycle. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 20344-20349.
- Cermeno, P., J. B. Lee, K. Wyman, O. Schofield, and P. G. Falkowski. 2011. Competitive dynamics in two species of marine phytoplankton under non-equilibrium conditions. *Marine Ecology-Progress Series* **429**: 19-28.
- Cianca, A., P. Helmke, B. Mourino, M. J. Rueda, O. Llinas, and S. Neuer. 2007. Decadal analysis of hydrography and in situ nutrient budgets in the western and eastern North Atlantic subtropical gyre. *Journal of Geophysical Research-Oceans* **112**.
- Connell, J. H. 1978. Diversity in Tropical Rain Forests and Coral Reefs - High Diversity of Trees and Corals Is Maintained Only in a Non-Equilibrium State. *Science* **199**: 1302-1310.
- Conway, D. V. P., S. H. Coombs, J. A. Lindley, and C. A. Llewellyn. 1999. Diet of mackerel (*Scomber scombrus*) larvae at the shelf-edge to the south-west of the British Isles and the incidence of piscivory and coprophagy. *Vie Et Milieu-Life and Environment* **49**: 213-220.
- Csanady, G. T. 1989. Energy-Dissipation and Upwelling in a Western Boundary Current. *Journal of Physical Oceanography* **19**: 462-473.
- Cuypers, Y., P. Bouruet-Aubertot, C. Marec, and J.-L. Fuda. 2011. Characterization of turbulence and validation of fine-scale parametrization in the Mediterranean Sea during BOUM experiment. *Biogeosciences Discuss.* **8**: 8961-8998.
- Dalsgaard, T., D. E. Canfield, J. Petersen, B. Thamdrup, and J. Acuna-Gonzalez. 2003. N₂ production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. *Nature* **422**: 606-608.
- Davis, R. E. 1996. Sampling turbulent dissipation. *Journal of Physical Oceanography* **26**: 341-358.
- Duce, R. A. and others 2008. Impacts of atmospheric anthropogenic nitrogen on the open ocean. *Science* **320**: 893-897.
- Estrada, M., M. Alcaraz, and C. Marrase. 1987. Effects of Turbulence on the Composition of Phytoplankton Assemblages in Marine Microcosms. *Marine Ecology-Progress Series* **38**: 267-281.

- Estrada, M., and E. Berdalet. 1998. Effects of turbulence on phytoplankton. *In* D. M. Anderson, A. D. Cembella and G. M. Hallegraeff [eds.], *Physiological ecology of harmful algal blooms*. NATO ASI Series. Springer-Verlag.
- Falkowski, P. G. and others 2004. The evolution of modern eukaryotic phytoplankton. *Science* **305**: 354-360.
- Falkowski, P. G., and M. J. Oliver. 2007. Mix and match: how climate selects phytoplankton. *Nature Reviews Microbiology* **5**: 813-819.
- Fernández, A., B. Mouriño-Carballido, A. Bode, M. Varela, and E. Marañón. 2010. Latitudinal distribution of *Trichodesmium* spp. and N₂ fixation in the Atlantic Ocean. *Biogeosciences* **7**: 3167–3176.
- Figueiras, F. G., G. C. Pitcher, and M. Estrada. 2006. Harmful Algal Bloom Dynamics in Relation to Physical Processes. *In* E. Graneli and J. T. Turner [eds.], *Ecology of Harmful Algae*. Springer-Verlag Berlin.
- Follows, M. J., S. Dutkiewicz, S. Grant, and S. W. Chisholm. 2007. Emergent biogeography of microbial communities in a model ocean. *Science* **315**: 1843-1846.
- García-Lafuente, J., G. D. Del Río, and C. S. Berrocal. 2006. Vertical structure and bottom-intensification of tidal currents off Northwestern Spain. *Journal of Marine Systems* **62**: 55-70.
- Grana, R., P. Chouciño, R. Varela, and B. Mourino-Carballido. 2011. Medidas de microturbulencia en el océano, p. 304-314. *Métodos y técnicas en investigación marina*. Editorial Tecnos (Grupo Anaya).
- Grasshoff, K., M. Ehrhardt, and K. Kremling. 1983. *Methods of seawater analysis*, 2nd Ed. Verlag Chemie, Weinheim.
- Gregg, M. C., T. B. Sanford, and D. P. Winkel. 2003. Reduced mixing from the breaking of internal waves in equatorial waters. *Nature* **422**: 513-515.
- Grover, J. P. 1990. Resource Competition in a Variable Environment - Phytoplankton Growing According to Monod's Model. *American Naturalist* **136**: 771-789.
- Huisman, J., H. C. P. Matthijs, and P. M. Visser. 2005. *Harmful Cyanobacteria*. Springer.
- Hutchinson, G. E. 1961. The Paradox of the Plankton. *American Naturalist* **95**: 137-145.
- Ibaibarriaga, L. and others 2007. Egg and larval distributions of seven fish species in north-east Atlantic waters. *Fisheries Oceanography* **16**: 284-293.
- Karpposs, L., E. Boss, and P. A. Jumars. 1996. Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. *Oceanography and Marine Biology*, Vol 34 **34**: 71-107.
- Katija, K., and J. O. Dabiri. 2009. A viscosity-enhanced mechanism for biogenic ocean mixing. *Nature* **460**: 624-U687.
- Kiorboe, T. 1989. Phytoplankton Growth-Rate and Nitrogen-Content - Implications for Feeding and Fecundity in a Herbivorous Copepod. *Marine Ecology-Progress Series* **55**: 229-234.
- Kiorboe, T., and E. Saiz. 1995. Planktivorous Feeding in Calm and Turbulent Environments, with Emphasis on Copepods. *Marine Ecology-Progress Series* **122**: 135-145.
- Kitaigorodskii, S. A., M. A. Donelan, J. L. Lumley, and E. A. Terray. 1983. Wave Turbulence Interactions in the Upper Ocean .2. Statistical Characteristics of Wave and Turbulent Components of the Random Velocity-Field in the Marine Surface-Layer. *Journal of Physical Oceanography* **13**: 1988-1999.
- Klöpper, S., R. Scharek and G. Gerdts (2003). Diarrhetic shellfish toxicity in relation to the abundance of *Dinophysis* spp. Ehrenberg in the German Bight near Helgoland. *Marine Ecology Progress Series*, 259: 93-102.
- Kohfeld, K. E., C. Le Quere, S. P. Harrison, and R. F. Anderson. 2005. Role of marine biology in glacial-interglacial CO₂ cycles. *Science* **308**: 74-78.
- Kudela, R. M. 2010. Does horizontal mixing explain phytoplankton dynamics? *Proceedings of the National Academy of Sciences* **107**: 18235-18236.

- Kunze, E., J. F. Dower, I. Beveridge, R. Dewey, and K. P. Bartlett. 2006. Observations of biologically generated turbulence in a coastal inlet. *Science* **313**: 1768-1770.
- Latasa M, R. Scharek, M. Vidal, G. Vila Reixach, A. Gutiérrez Rodríguez, M. Emeliano, J. M. Gasol (2010). Preferences of phytoplankton groups for waters of different trophic status in the Northwestern Mediterranean Sea. *Marine Ecology Progress Series*, 407: 27-42
- Legendre, L., and J. Le Fevre. 1989. Hydrodynamic singularities as controls of recycled versus export production in the oceans, p. 49-63. *In* W. Berger, V. Smetacek and G. Wefer [eds.], *Productivity of the Ocean: Present and Past*.
- Li, W. K. W. 2002. Macroecological patterns of phytoplankton in the northwestern North Atlantic Ocean. *Nature* **419**: 154-157.
- Litchman, E. 2006. Resource competition and ecological success of phytoplankton. *In* P. G. Falkowski and A. H. Knoll [eds.], *Evolution of Aquatic Photoautotrophs*. Academic Press.
- Litchman, E., C. A. Klausmeier, O. M. Schofield, and P. G. Falkowski. 2007. The role of functional traits and trade-offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. *Ecology Letters* **10**: 1170-1181.
- Maar, M., T. G. Nielsen, A. Stips, and A. W. Visser. 2003. Microscale distribution of zooplankton in relation to turbulent diffusion. *Limnology and Oceanography* **48**: 1312-1325.
- Margalef, R. 1978. Life-Forms of Phytoplankton as Survival Alternatives in an Unstable Environment. *Oceanologica Acta* **1**: 493-509.
- Martín Míguez B, Testut L, Wöppelmann G, 2012. Performance of modern tide gauges: towards mm-level accuracy. *Scientia Marina* (in press).
- Míguez BM, Souto C, Fariña-Busto L, Pérez FF, 2001b. Flujos residuales de intercambio entre la Ría de Vigo y la plataforma continental. *Física de la Tierra*, 13: 119-137.
- Miguez, B. M., L. Farina-Busto, F. G. Figueiras, and F. F. Perez. 2001. Succession of phytoplankton assemblages in relation to estuarine hydrodynamics in the Ría de Vigo: A box model approach. *Scientia Marina* **65**: 65-76.
- Miguez, B. M., R. Le Roy, and G. Wöppelmann. 2008. The use of radar tide gauges to measure variations in sea level along the French coast. *Journal of Coastal Research* **24**: 61-68.
- Miguez, B. M., R. A. Varela, G. Roson, C. Souto, J. M. Cabanas, and L. Farina-Busto. 2005. Physical and biogeochemical fluxes in shelf waters of the NW Iberian upwelling system. *Hydrography and dynamics. Journal of Marine Systems* **54**: 127-138.
- Miller, T. E. and others 2005. A critical review of twenty years' use of the resource-ratio theory. *American Naturalist* **165**: 439-448.
- Moore, C. M. and others 2009. Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability. *Nature Geoscience* **2**: 867-871.
- Mori, K., K. Uehara, T. Kameda, and S. Kakehi. 2008. Direct measurements of dissipation rate of turbulent kinetic energy of North Pacific subtropical mode water. *Geophysical Research Letters* **35**.
- Moum, J. N., J. D. Nash, and J. M. Klymak. 2008. Small-Scale Processes in the Coastal Ocean. *Oceanography* **21**: 22-33.
- Mourino-Carballido, B. and others 2011. Importance of N₂ fixation vs. nitrate eddy diffusion along a latitudinal transect in the Atlantic Ocean. *Limnology and Oceanography* **56**: 999-1007.
- Mourino-Carballido, B., and D. J. McGillicuddy. 2006. Mesoscale variability in the metabolic balance of the Sargasso Sea. *Limnology and Oceanography* **51**: 2675-2689.
- Mourino-Carballido, B., and S. Neuer. 2008. Regional Differences in the Role of Eddy Pumping in the North Atlantic Subtropical Gyre HISTORICAL CONUNDRUMS REVISITED. *Oceanography* **21**: 52-61.

- Mourino, B., E. Fernandez, R. Pingree, B. Sinha, J. Escanez, and D. De Armas. 2005. Constraining effect of mesoscale features on carbon budget of photic layer in the NE subtropical Atlantic. *Marine Ecology-Progress Series* **287**: 45-52.
- Mourino, B., E. Fernandez, P. Serret, D. Harbour, B. Sinha, and R. Pingree. 2001. Variability and seasonality of physical and biological fields at the Great Meteor Tablemount (subtropical NE Atlantic). *Oceanologica Acta* **24**: 167-185.
- Mouriño-Carballido, B. 2009. Eddy-driven pulses of respiration in the Sargasso Sea. *Deep Sea Research Part I: Oceanographic Research Papers* **56**: 1242-1250.
- Mouriño-Carballido, B., M. Pahlow, and A. Oschlies. 2012. High sensitivity of ultra-oligotrophic marine ecosystems to atmospheric nitrogen deposition. *Geophysical Research Letters*, in press.
- Muller-Karger, F. E., R. Varela, R. Thunell, R. Luerssen, C. M. Hu, and J. J. Walsh. 2005. The importance of continental margins in the global carbon cycle. *Geophysical Research Letters* **32**.
- Not F., M. Latasa, R. Scharek, M. Viprey, P. Karleskind, V. Balagué, I. Ontoria, A. Cumino, E. Goetze, D. Vaultot, and Massana R. (2008) Phytoplankton diversity across the Indian Ocean: a focus on picoeukaryotes. *Deep-Sea Research I*, 55, 1456-1473.
- Oakey, N. S., and J. A. Elliott. 1982. Dissipation within the Surface Mixed Layer. *Journal of Physical Oceanography* **12**: 171-185.
- Osborn, T. R. 1980. Estimates of the local rate of vertical diffusion from dissipation measurements. *Journal of Physical Oceanography* **10**: 83-89.
- Oschlies, A., and V. Garcon. 1998. Eddy-induced enhancement of primary production in a model of the north Atlantic Ocean. *Nature* **394**: 266-269.
- Peters, F., and J. M. Redondo. 1997. Turbulence generation and measurement: Application to studies on plankton. *Scientia Marina* **61**: 205-228.
- Peterson, L. C., G. H. Haug, K. A. Hughen, and U. Rohl. 2000. Rapid changes in the hydrologic cycle of the tropical Atlantic during the last glacial. *Science* **290**: 1947-1951.
- Prandke, H., K. Holtsch, and A. Stips. 2000. MITEC technology development: The microstructure/turbulence measuring system MSS: Technical report EUR 19733 EN. European Commission.
- Prandke, H., and A. Stips. 1998. Test measurements with an operational microstructure-turbulence profiler: Detection limit of dissipation rates. *Aquatic Sciences* **60**: 191-209.
- Rahmstorf, S. 2003. The current climate. *Nature* **421**: 699-699.
- Rainville, L., and P. Winsor. 2008. Mixing across the Arctic ocean: Microstructure observations during the Beringia 2005 expedition. *Geophysical Research Letters* **35**.
- Relvas, P. and others 2007. Physical oceanography of the western Iberia ecosystem: Latest views and challenges. *Progress in Oceanography* **74**: 149-173.
- Rodriguez, J. and others 1998. Patterns in the size structure of the phytoplankton community in the deep fluorescence maximum of the Alboran Sea (southwestern Mediterranean). *Deep-Sea Research Part I-Oceanographic Research Papers* **45**: 1577-1593.
- Ross, O. N. 2006. Particles in motion: How turbulence affects plankton sedimentation from an oceanic mixed layer. *Geophys. Res. Lett.* **33**: L10609.
- Rudnick, D. L. and others 2003. From tides to mixing along the Hawaiian ridge. *Science* **301**: 355-357.
- Ruiz, J. and others 2001. Surface distribution of chlorophyll, particles and gelbstoff in the Atlantic jet of the Alboran Sea: from submesoscale to subinertial scales of variability. *Journal of Marine Systems* **29**: 277-292.
- Schafstall, J., M. Dengler, P. Brandt, and H. Bange. 2010. Tidal-induced mixing and diapycnal nutrient fluxes in the Mauritanian upwelling region. *Journal of Geophysical Research-Oceans* **115**.
- Scharek R. and M. Latasa (2007) Growth, mortality and carbon flux of high- and low-nucleic-acid-bacteria differ in surface and deep chlorophyll maximum layers in the north-western Mediterranean. *Aquatic Microbial Ecology*, 46: 153-161.

- Scharek, R., M. A. van Leeuwe and H. J. W. de Baar (1997): Responses of Southern Ocean Phytoplankton to the Addition of Trace Metals. *Deep-Sea Research*, 44: 209 – 227.
- Scharek, R., L. Tupas and D. M. Karl (1999): Deep sea diatom fluxes in the oligotrophic North Pacific gyre at Station 'ALOHA'. *Marine Ecology Progress Series*, 182: 55-67.
- Sharples, J. and others 2009. Internal tidal mixing as a control on continental margin ecosystems. *Geophysical Research Letters* **36**.
- . 2007. Spring-neap modulation of internal tide mixing and vertical nitrate fluxes at a shelf edge in summer. *Limnology and Oceanography* **52**: 1735-1747.
- Smayda, T. J. 1970. The suspension and sinking of phytoplankton in the sea. *Oceanogr. Mar. Bio. Ann. Rev.* **8**: 353-414.
- Smayda, T. J., and C. S. Reynolds. 2001. Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. *Journal of Plankton Research* **23**: 447-461.
- Smetacek, V. S. 1985. Role of Sinking in Diatom Life-History Cycles - Ecological, Evolutionary and Geological Significance. *Marine Biology* **84**: 239-251.
- Soloviev, A. V., N. V. Vershinsky, and V. A. Bezverchnii. 1988. Small-Scale Turbulence Measurements in the Thin Surface-Layer of the Ocean. *Deep-Sea Research Part a-Oceanographic Research Papers* **35**: 1859-1874.
- Sriver, R. L., and M. Huber. 2007. Observational evidence for an ocean heat pump induced by tropical cyclones. *Nature* **447**: 577-580.
- Stevens, C., M. Smith, and A. Ross. 1999. SCAMP: measuring turbulence in estuaries, lakes, and coastal waters. *NIWA - Water and Atmosphere* **7**: 20-21.
- Thomas, W. H., C. T. Tynan, and C. H. Gibson. 1997. Turbulence-phytoplankton interrelationships, p. 283-324. *In* F. E. Round and D. J. Chapman [eds.], *Progress in Phycological Research*.
- Tilman, D. 1977. Resource Competition between Planktonic Algae - Experimental and Theoretical Approach. *Ecology* **58**: 338-348.
- . 1982. *Resource Competition and Community Structure*. Princeton University Press.
- Tilstone, G. H., B. M. Miguez, F. G. Figueiras, and E. G. Fermin. 2000. Diatom dynamics in a coastal ecosystem affected by upwelling: coupling between species succession, circulation and biogeochemical processes. *Marine Ecology-Progress Series* **205**: 23-41.
- Torres-Valdes, S. and others 2009. Distribution of dissolved organic nutrients and their effect on export production over the Atlantic Ocean. *Global Biogeochemical Cycles* **23**.
- Tozzi, S., O. Schofield, and P. Falkowski. 2004. Historical climate change and ocean turbulence as selective agents for two key phytoplankton functional groups. *Marine Ecology-Progress Series* **274**: 123-132.
- Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Internat Verein Limnol* 9:1-38.
- Volk, T., and M. I. Hoffert. 1985. Ocean carbon pumps: Analysis of relative strengths and efficiencies in ocean-driven atmospheric CO₂ exchanges, p. 99-110. *In* E. T. Sunquist and W. S. Broecker [eds.], *The Carbon Cycle and Atmospheric CO₂: Natural Variations Archean to Present*. American Geophysical Union.
- Williams, R. G., and M. J. Follows. 1998. The Ekman transfer of nutrients and maintenance of new production over the North Atlantic. *Deep-Sea Res. I* **45**: 461-489.
- Wolk, F., H. Yamazaki, L. Seuront, and R. G. Lueck. 2002. A new free-fall profiler for measuring biophysical microstructure. *Journal of Atmospheric and Oceanic Technology* **19**: 780-793.
- Wunsch, C., and R. Ferrari. 2004. Vertical mixing, energy and the general circulation of the oceans. *Annual Review of Fluid Mechanics* **36**: 281-314.