Influence of turbulence variability on osmotrophic plankton dynamics in a coastal area

Influència de la variabilitat de la turbulència en la dinàmica del plàncton osmotròfic en una zona costanera

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Cover Illustration: "The Sea God Neptune Commanding His Quadriga of Sea Horses".
Leonardo da Vinci, c1502-1504

都是大家有益之事，虽说不济，却也放屁添风。

From the novel “Journey to the west”, Chinese anonymous from the XVI century

A la mare,
a la Mariona.

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Introduction / Introducció

"L'aigua és la matèria i la matriu de la vida, mare i medi. No hi ha vida sense aigua"

(Albert Szent-Gyorgyi, bioquímic hongarès i premi Nobel de medicina)

"No hi ha vida sense aigua, ni vida a l'aigua sense turbulència"

(Ambühl 1960)

Per què la turbulència és tan important per al plàncton?

El plàncton es troba a la base de la xarxa tròfica marina. Des d'un punt de vista hidrodinàmic es pot definir com el conjunt d'organismes que viuen en suspensió en una massa d'aigua amb una capacitat limitada per regular la seva posició. Per tant, el plàncton es desplaça seguint tot tipus de moviments de l'aigua, com ara les corrents o les marees, que esdevenen propietats ambientals inherents a la vida planctònica. La mida d'aquests organismes abasta varis ordres de magnitud, des d'unes dècimes de micròmetres fins a metres, encara que la majoria són microscòpics. Poden alimentar-se d'altres organismes, i llavors parlem de fagòtrofs, o prendre substàncies dissoltes del medi, i llavors ho fem d'osmòtrofs. El plàncton osmotròfic domina el flux de carboni a l'ecosistema pelàgic, a través de la fixació de CO_2 (la producció primària) i la remineralització de matèria orgànica (respiració). De fet, la majoria de la producció primària en els sistemes aquàtics, que representa aproximadament la meitat de la producció primària global (Field *et al.* 1998), és duta a terme pel plàncton osmòtrofic a través de la fotosíntesi.

Els organismes planctònics capaços de realitzar la fotosíntesi, els equivalents aquàtics de les plantes terrestres, s'anomenen fitoplàncton. En general el creixement de qualsevol organisme fotosintètic, des d'una alga unicel·lular fins a un arbre, està limitat, entre altres coses, per la disponibilitat de llum i/o de nutrients, els elements químics necessaris per al seu creixement. Això estreny la distribució de la producció primària, perquè aquestes dues variables tenen distribucions verticals oposades. La llum és màxima a la superfície, mentre que els nutrients es troben pràcticament esgotats prop de la superfície i l'abastament prové del fons. Això és particularment dramàtic en els sistemes aquàtics, perquè la llum s'absorbeix molt més ràpidament a l'aigua que a l'aire, i decau exponencialment amb la profunditat seguint la llei de Beer-Lambert.

Per tant la fotosíntesi en sistemes aquàtics només pot tenir lloc en una capa relativament petita, anomenada capa eufòtica, en què aquestes dues variables coexisteixen. Per acció de la gravetat, hi ha un flux continu de nutrients en forma particulada cap a baix, a zones fosques i en darrer terme als sediments. Les dimensions verticals de mars i oceans fan impossible que els organismes fototròfics en general, i sobretot els planctònics, desenvolupin estratègies actives per tornar els nutrients a la zona eufòtica. Com que el plàncton no pot controlar completament la seva posició a la columna d'aigua, tot l'ecosistema planctònic depèn per al seu manteniment de l'entrada d'energia externa o auxiliar (Margalef 1991). L'energia mecànica introduïda per vents, onades, corrents, i marees provoca la mescla de la columna d'aigua, retornant d'aquesta manera nutrients des de les capes fondes o des dels sediments a la superfície, on poden ser utilitzats de nou.

La turbulència és el fenomen pel qual la majoria d'aquesta energia mecànica, que entra al sistema a escales grans, es transfereix a escales petites rellevants per als organismes planctònics. L'estudi dels efectes de la turbulència en el plàncton és enormement complex, i per tant científicament estimulant. Combina la complexitat dels sistemes biològics amb el què ha estat descrit pel premi Nobel Richard Feynman (1918-1988) com "el problema irresolt més important de la física clàssica". La turbulència és un procés ubic, amb un rang d'escales espacials i temporals més ampli encara que el del plàncton. Per tant els seus efectes sobre els organismes planctònics van més enllà de la redistribució de nutrients i fitoplàncton de què hem parlat, i afecta tots els seus processos (fig.1). Després de vàries dècades d'estudi ara comencem a tenir una noció conjunta

"Water is life's mater and matrix, mother and medium. There is no life without water."

(Albert Szent-Gyorgyi, Hungarian biochemist and Nobel Prize Winner for Medicine)

“There is no life without water, and no life in water without turbulence in water.”

(Ambühl 1960)

Why is turbulence so important for plankton?

Plankton is at the base of the marine trophic web. It can be defined from a hydrodynamic point of view, as the collection of organisms that live suspended in a body of water with a limited ability to regulate its position. Therefore plankton drifts with currents, tides and other water motions, which become environmental properties inherent to planktonic life. Planktonic organisms range several orders of magnitude in size, from tenths of microns to meters, although most are microscopic. They can feed on other particles (phagotrophs) or acquire dissolved substances (osmotrophs), incorporating them into particulate form. Osmotrophic plankton dominates the flux of carbon within the pelagic ecosystem, through CO_2 fixation (primary production) and re-mineralization (respiration). In fact, the majority of primary production in aquatic systems, which accounts for roughly half of the global primary production (Field *et al.* 1998), is performed by osmotrophic plankton through photosynthesis. Parallely, bacterioplankton is responsible for the majority of organic carbon respired in the ocean (e.g. Rivkin and Legendre 2001).

The planktonic organisms capable of photosynthesizing, the aquatic equivalents of terrestrial plants, are called phytoplankton. Generally speaking, the growth of any photosynthetic organism is limited by the availability of light and/or of nutrients (the chemical elements needed for growth). This constraints the spatial distribution of primary production, because these two variables have opposite vertical distributions. Whereas light is maximal close to the surface, nutrients are almost depleted near the surface and resupply comes from the bottom. This is particularly dramatic in aquatic systems, because light is absorbed much more rapidly in water than in air, and decays exponentially with depth, following the Beer-Lambert law.

Thus, photosynthesis in aquatic systems can take place only in a relatively thin layer, where these two oppositely distributed variables, namely light and nutrients, can coexist. The layer at which light is sufficient for photosynthesis to occur is called the euphotic zone. By the action of gravity there is a continuous flux of particulate nutrients out of the euphotic zone into the deeper layers of the aquatic systems and/or ultimately into the sediments. The vertical dimensions of oceans and seas make it impossible for phototrophic organisms, and particularly for plankton, to develop strategies for actively bringing nutrients upwards to the euphotic zone. Because planktonic organisms can not fully control their position in the water column, the whole planktonic ecosystem relies on the input of external or auxiliary energy into the euphotic zone for its maintenance (Mar-

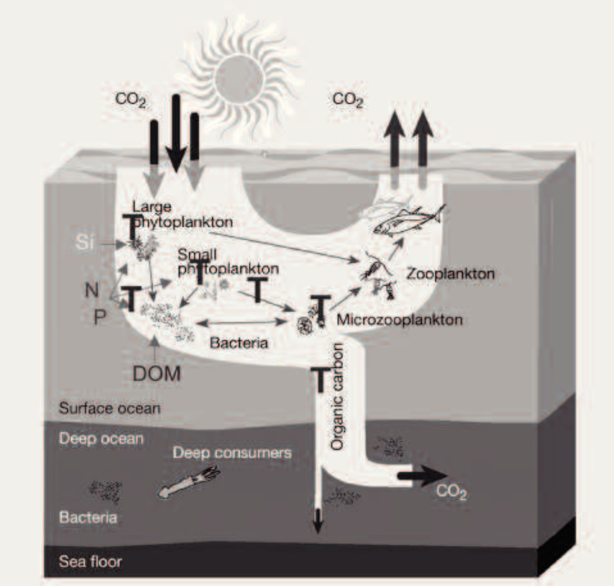

Figure 1: *Schematic representation showing the main planktonic processes and fluxes influenced by small-scale turbulence in a global biogeochemical framework. Modified after Chisholm (2000).*

Representació esquemàtica mostrant els principals processos i fluxos planctònics influenciats per la turbulència de petita escala en un marc biogeoquímicglobal. Adaptat de Chishlom (2000).

d'aquests efectes. Parlant en general, la turbulència pot augmentar la productivitat del sistema en incrementar l'eficiència de captació de nutrients per part dels osmòtrofs. Pot accelerar i optimitzar tant l'entrada de carboni a la xarxa tròfica planctònica, com el seu flux a través d'aquesta i la remineralització per part dels bacteris, i pot tenir un profund impacte en la composició i l'estructura de la comunitat planctònica. Entendre la dinàmica del plàncton osmotròfic i com la turbulència pot modular-la és important no només des d'un punt de vista científic, sinó que té implicacions socio-econòmiques, especialment in zones costaneres subjectes a escenaris de canvi global.

Com que les fonts i els mecanismes de generació de turbulència són tan diversos, la turbulència té una gran variabilitat tan espacial com temporal. No es dóna de manera contínua, sinó que és molt episòdica, intermitent a totes les escales d'observació. Els efectes d'aquests forçaments en el plàncton depenen fortament d'aquests patrons de variabilitat espacial i temporal, i de l'habilitat dels organismes per reaccionar-hi. En aquesta tesi tractarem aquesta variabilitat, i la influència que pot tenir en la dinàmica de l'ecosistema planctònic costaner, posant èmfasi en els organismes osmotròfics, és a dir, en el fitoplàncton i els bacteris.

Un tastet de turbulència

En paraules de Tennekes i Lumley (1972), "en dinàmica de fluids el flux laminar és l'excepció i no pas la norma". Però tot i que la turbulència és un fenomen quotidià, present en qualsevol fluid, és molt difícil d'explicar. El seu estudi començà fa mes d'un segle i tot i així encara no tenim una definició simple. Per tal d'entendre què és la turbulència, potser el millor és posar un exemple. La figura 2A mostra una sèrie temporal de velocitat de l'aigua, presa al davant del Port Olímpic de Barcelona. El senyal pot ser desglossat en tres components: la velocitat mitjana (fig. 2B), l'onatge (fig. 2C), i un senyal aleatori i imprevisible que inclou totes les oscil·lacions d'alta freqüència (fig. 2D). Aquesta variabilitat sobreposada a la velocitat mitjana i a l'onatge és de fet una mesura de la intensitat de la turbulència.

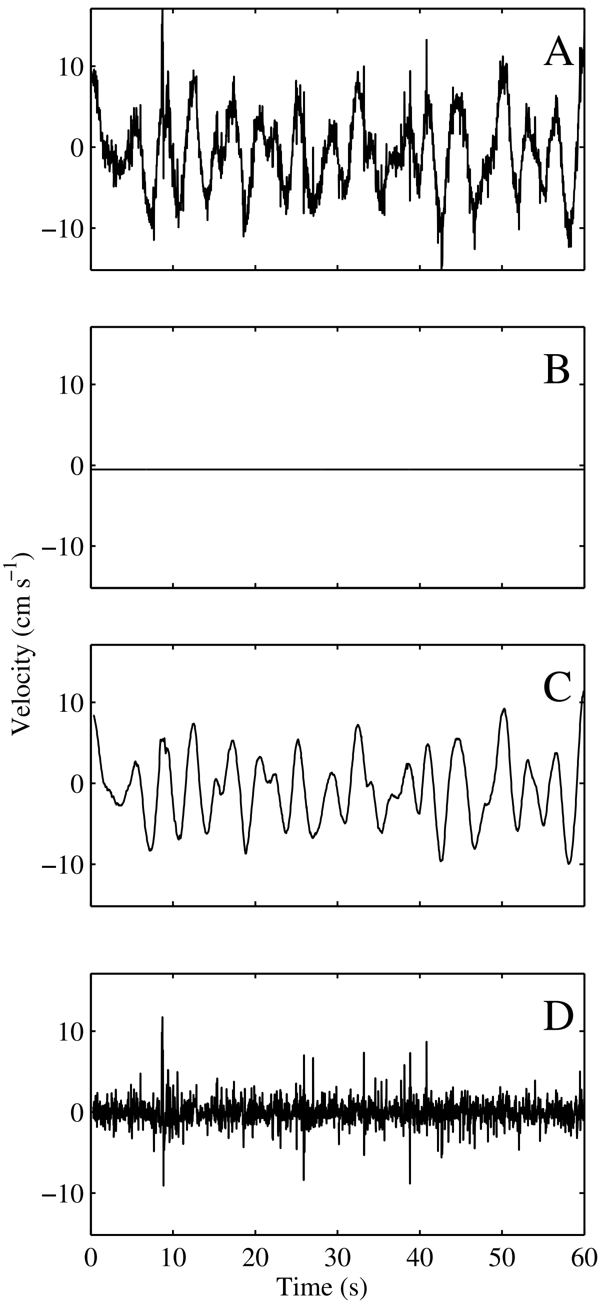

Figure 2: *Decomposition of the north component water velocity signal in front of the Olympic Port in Barcelona. A, original signal, B mean intensity, C wave component, D the high frequency turbulent signal. Notice that the sum of the three signals in B, C and D results the original signal.*

Descomposició de la component nort del senyal de velocitat de l'aigua davant del Port Olímpic de Barcelona. A, senyal original, B intensitat mitja, C, component de l'onatge, D, senyal turbulent d'alta freqüència. La suma dels tres senyals a B, C i D dóna el senyal orginal.

galef 1991). Mechanical energy input from winds, waves, tides and currents induces the mixing of the water column, and in this way brings nutrients from the deep layers or from the sediment back to the surface, where they can be utilised anew.

Turbulence is the phenomenon by which most of this mechanical energy, entered into the systems at large scales, is transferred down to scales relevant for the planktonic organisms. The study of the effects of turbulence on plankton is enormously complex, and therefore scientifically exciting. It combines the complexity of biological systems with what has been described as “the most important unsolved problem of classical physics” by Nobel prize-winner physicist Richard Feynman (1918-1988). Turbulence is an ubiquitous process with a range of temporal and spatial scales even broader than that of plankton. Consequently, its effects on planktonic organisms go well beyond the redistribution of nutrients and phytoplankton outlined above, and it affects all their processes (fig. 1). After several decades of study, we now begin to have put together pieces of the puzzle. Generally speaking, turbulence may increase the productivity of the system by increasing the efficiency of nutrient uptake by osmotrophs. It may accelerate and optimize both the input and the flux of carbon to and through the planktonic food web, and the re-mineralization of nutrients by bacteria, and can have a profound impact in the composition of the planktonic community and on its size structure. Understanding the dynamics of osmotrophic plankton and how turbulence may modulate it is important not only from a purely scientific point of view but has socio-economic implications, especially in coastal areas subjected to changing global scenarios.

Because the sources and mechanisms of turbulence generation are so diverse, turbulence is extremely variable both in space and time. It does not occur in a continuous way, but it is highly episodic, intermittent at all scales of observation. The effects of these forcings on plankton are strongly dependent on their temporal and spatial patterns, and on the ability of organisms to react to them. In the present thesis, we will deal with this variability, and with the influence it may have on coastal planktonic ecosystem dynamics, with emphasis in osmotrophic organisms, namely phytoplankton and bacteria.

A primer in turbulence

As stated by Tennekes and Lumley (1972), “in fluid dynamics laminar flow is the exception, not the rule”. Although turbulence is such a commonplace phenomenon, ubiquitous in any fluid, it is hard to explain. Its study roots more than a century ago, but is still escaping a simple definition. To understand what turbulence is maybe the best way is to look at an example. Figure 2A shows a time series of water velocity taken in front of the Olympic Port in Barcelona. The signal can be decomposed in three different components: the trend or mean velocity (fig. 2B), the waves oscillation (fig. 2C), and an unpredictable random signal, comprising all the high-frequency oscillations (fig. 2D). The variability superimposed to the mean velocity and the wavy motions, is in fact a measure of the turbulent intensity.

According to Tennekes and Lumley (1972) a turbulent flow can be identified by being:

- Irregular: a turbulent flow consists of random velocity fluctuations that make a deterministic approach to its study impossible. Statistical approaches are basic tools for describing it.

- Diffusive: turbulence enhances the diffusion of scalar properties, such as moment, mass, heat, salinity or nutrient concentration, by several orders of magnitude. This means that it favours the flux of unequally distributed properties.

- Dissipative: the mechanical energy which generates turbulence is rapidly dissipated as heat at the scale of the smallest eddies. In order of a turbulent flow to be maintained, there must be a continuous supply of energy.

- Isotropic: turbulence properties are equal in the three dimensions of space. Strictly speaking, therefore, the large 2-dimensional geostrophic

Segons Tennekes i Lumley (1972) un flux turbulent es caracteritza per ser:

- Irregular: consisteix en fluctuacions aleatòries de la velocitat que impedeixen una aproximació determinista al seu estudi. L'estadística és una eina bàsica per a la seva descripció.

- Difusiu: incrementa en varis ordres de magnitud la difusió de magnituds escalars, com ara el moment, la massa, la calor, la salinitat, o la concentració de nutrients. Per tant afavoreix el flux de propietats que es distribueixen heterogèniament.

- Dissipatiu: l'energia mecànica que genera és ràpidament dissipada en forma de calor a les escales dels remolins més petits. Perquè un flux turbulent es mantingui és necessària una entrada contínua d'energia.

- Isotròpic: les seves propietats són iguals en les tres dimensions de l'espai. Estrictament parlant doncs, els grans remolins geostròfics bidimensionals no es poden considerar turbulents.

Un flux turbulent es pot identificar a partir del número adimensional de Reynolds (Re):

$$\mathrm{Re} = \rho l u / \mu \tag{1}$$

en què ρ [ML^{-3}] és la densitat del fluid, l és la longitud característica del moviment, u [LT^{-1}] és la seva velocitat, i μ [$ML^{-1}T^{-1}$] és la viscositat dinàmica del fluid. El número de Reynolds és de fet la relació entre les forces inercials i les viscoses. Pot ser aplicat tant a un líquid fluint a través d'una canonada com a un organisme movent-se a través d'un fluid. La turbulència apareix a Re elevats, quan l'entrada de energia mecànica es gran i apareixen forts gradients de velocitat.

La cascada d'energia i les escales turbulentes

La transferència de l'energia cinètica turbulenta (TKE per "turbulent kinetic energy" en anglès), des de les escales grans en què entra en el sistema fins a les petites, les més rellevants per als organismes planctònics, es produeix a través d'una cascada de remolins de mida decreixent (fig. 3). A mesura que els remolins es van fent més i més petits, i per tant a mesura que el seu Re disminueix, la viscositat comença a jugar un paper important. Arriba un punt, a l'escala espacial de Kolmogorov (η), en què les forces viscoses igualen a les inercials. Per sota d'aquesta escala, els remolins deixen de formar-se, i queden només uns gradients de velocitat laminar, el què anomenem cisallaments, que canvien aleatòriament de direcció i que dissipen la calor. El rang que va des de les escales d'entrada d'energia fins a les de dissipació s'anomena subrang inercial, i l'energia s'hi transfereix amb una taxa constant i sense pèrdues per dissipació considerables. Per tant, en condicions ideals, és a dir quan la turbulència és isotròpica, homogènia i estacionària, la taxa de dissipació per unitat de massa de l'energia cinètica turbulenta que es produeix a les escales més petites (ε, [L^2T^{-3}]), ha de ser igual a l'entrada d'energia que es dóna en les més grans (Taylor 1935).

La longitud de Kolmogorov es pot calcular com:

$$\eta = 2\pi v^{3/4} \varepsilon^{-1/4} \tag{2}$$

(Mann i Lazier 1989) on ν [L^2T^{-1}] és la viscositat cinemàtica del fluid. Valors típics d'ε mesurats a la superfície de l'oceà es troben entre 10^{-7} i 10^{-1} cm^2s^{-3} (Mackenzie i Leggett 1993, i referències incloses). Les η derivades d'aquests rangs d'ε, utilitzant l'equació 2 i assumint ν=1.047 10^{-3} cm^2s^{-1} es troben aproximadament entre 1 mm i un parell de cm, longituds molt més grans que la majoria d'organismes del plàncton. Probablement la longitud de Batchelor (η_b) és més rellevant en el context del plàncton osmotròfic (Peters i Marrasé 2000). η_b està relacionada amb l'escala més petita de fluctuació deguda a la turbulència de magnituds escalars, com són la temperatura o la concentració de nutrients. Es defineix com:

$$\eta_b = 2\pi(vD^2\varepsilon^{-1})^{1/4} \tag{3}$$

eddies, can not be considered as turbulent.

One way to identify a turbulent flow is by calculating the dimensionless Reynolds number (Re):

$$\mathrm{Re} = \rho l u / \mu \tag{1}$$

where ρ [ML^{-3}] is the density of the fluid, l is a characteristic length scale of the motion, u [LT^{-1}] is the velocity, and μ [ML^{-1}T^{-1}] is the dynamic viscosity of the fluid. The Reynolds number is in fact the ratio of inertial to viscous forces. This number may be applied as well to a flow moving into a pipe and to an organism moving within the fluid. Turbulence appears at high Re, when the input of mechanical energy is large and there are strong gradients of velocity.

The cascade of energy and the turbulent scales

The transfer of turbulent kinetic energy (TKE), entered into the system at large scales, down to scales relevant for the organisms is produced through a cascade of decreasing sized eddies (fig. 3). As eddies get smaller and their Re decrease, viscosity starts to play an important role. The inertial forces are equalled by the viscosity forces at the Kolmogorov length scale (η). Below this scale eddies are no longer formed, and there remains only a laminar shear, changing randomly in direction, that dissipates heat. In the inertial subrange (from the scales of input of kinetic energy to the Kolmogorov length scale), energy is transferred at a constant rate without dissipation. Therefore, under ideal conditions, namely in isotropic, homogeneous and stationary turbulence, the rate of dissipation of turbulent kinetic energy per unit mass at the smallest scales (ε, [L^{2}T^{-3}]), must equal its rate of input at the largest scales (Taylor 1935).

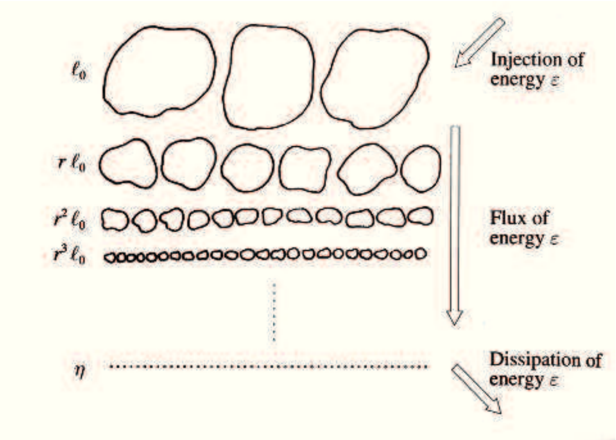

Figure 3: *Conceptual sketch of the cascade of turbulence eddies according to Kolmogorov (1941). Taken from Frisch 1995.*

Esquema conceptual de la cascada de remolins turbulents segons Kolmogorov (1941). Tret de Frisch (1995).

The Kolmogorov length scale can be calculated as:

$$\eta = 2\pi \nu^{3/4} \varepsilon^{-1/4} \tag{2}$$

(Mann and Lazier 1989) where ν [L^{2}T^{-1}] is the kinematic viscosity. Typical reported values of ε for the upper ocean range between 10^{-7} and 10^{-1} cm^{2}s^{-3} (Mackenzie and Leggett 1993, and references therein). The η derived from these ε using equation 2 and assuming ν=1.047 10^{-3} cm^{2}s^{-1} range between ca. 1 mm and a couple of cm, which are scales much larger than many of the organisms of the plankton. The Batchelor length scale (η_b) is probably more relevant in the context of osmotrophic plankton (Peters and Marrasé 2000). It refers to the smallest scale of fluctuation of scalar properties within the water, such as temperature or nutrient concentrations, due to turbulence:

$$\eta_\mathrm{b} = 2\pi(\nu D^2 \varepsilon^{-1})^{1/4} \tag{3}$$

(Mann and Lazier 1996) where D [L^{2}T^{-1}] is the molecular diffusion of the scalar. η_b is lower than η, which means that although velocity fluctuations may be not perceived by most planktonic organisms, at least some will perceive the fluctuations owing to turbulence in the nutrient fields.

The maximum length scale for the largest isotropic eddies in stable stratified turbulence is known as the Ozmidov length or the buoyancy length scale:

$$l_0 = \varepsilon^{1/2} \mathrm{N}^{-3/2} \tag{4}$$

(Mann i Lazier 1996) on D [L^2T^{-1}] és la difusió molecular de l'escalar. La longitud de Batchelor és més petita que la de Kolmogorov, cosa que significa que encara que la majoria d'organismes poden no percebre les fluctuacions en el camp de velocitats degudes a la turbulència, si que poden percebre en canvi fluctuacions en el camp de nutrients.

La mida màxima de remolins isotròpics en turbulència estable estratificada es coneix com l'escala d'Ozmidov o de flotabilitat:

$$l_0 = \varepsilon^{1/2} N^{-3/2} \qquad (4)$$

(Denman i Gargett 1983), en què N [T^{-1}] és la freqüència de flotabilitat, també anomenada de Brunt-Väisälä, una mesura de l'estabilitat de la columna d'aigua. A l'oceà, l_o pren valors entre 10cm i 100m (Franks 2005).

Fonts de turbulència a l'oceà superficial

Hi ha molts processos diferents que introdueixen energia mecànica al sistema, amb un rang d'escales enormement ample (fig. 4). Entre ells podem anomenar la fricció del vent sobre la superfície (Oakey i Elliot 1982, Oakey 1985), les circulacions de Langmuir (D'Asaro i Dairiki 1997; McWilliams *et al.* 1997), els afloraments (Csanady 1989, Dewey i Moum 1990), les marees (St. Laurent *et al.* 2002), la convecció (Shay i Gregg 1986), i les ones internes (Sun i Kunze 1999). A les escales més grans, l'energia es concentra en ciclons i anticiclons de varis milers de km d'amplada, que són quasi estacionaris i anisotròpics.

A la capa superficial, i especialment en aquelles àrees en què les marees són febles, com per exemple en el Mar Mediterrani, el vent és el mecanisme més important d'entrada d'energia mecànica. La generació de turbulència per part del vent es produeix en primer lloc per la fricció que aquest exerceix sobre la superfície de l'aigua. Segons la teoria de la capa límit (e.g. Turner 1973, Gargett 1989) la dissipació de l'energia introduïda depèn de la profunditat i de la intensitat del vent:

$$\varepsilon = (\rho_a \rho_w C_d^{-1})^{3/2} u_{10}{}^3 k^{-1} z^{-1} \qquad (5)$$

(Oakey 1985), en què ρ_a i ρ_w són la densitat de l'aire i de l'aigua respectivament, C_d és el coeficient de fricció, u_{10} és la velocitat del vent a una alçada de 10 m, k és la constant de von Kàrmàn, que se sol assumir que es troba al voltant de 0.4 (Tennekes i Lumley 1972), i z és la profunditat.

Sovint les taxes de dissipació a la capa superficial són més altes del què prediu aquest model. Aquesta anomalia s'atribueix a dos processos originats pel vent (Gargett 1989, Thorpe 2004): les circulacions de Langmuir (Thorpe *et al.* 2003) i el trencament de les ones superficials (Kitaigorodskii *et al.* 1983, Agrawal *et al.* 1992, Terray *et al.* 1996, Gemmrich i Farmer 2004, Stips *et al.* 2005). Aquest últim mecanisme de generació de turbulència és particularment important en la part més soma, la capa superficial afectada per l'onatge (WASL, per "wave-affected-surface-layer" en anglès, Stips *et al.* 2005). En aquesta capa, i sobretot en la subcapa d'onatge turbulent, on es produeix la dissipació de la meitat de l'energia implicada en el trencament d'ones (Terray *et al.* 1996), els valors d' ε poden estar un o dos ordres de magnitud per sobre del què preveu el model de la capa límit (Stips *et al.* 2005 i referències incloses). La profunditat d'aquesta subcapa s'estima que és similar o més petita que l'alçada d'ona significant (Terray *et al.* 1996, Gemmrich i Farmer 1999), això és, que l'alçada mitja del terç d'ones més altes.

Distribució de la turbulència

La turbulència se sol quantificar utilitzant ε. L'estimació d'ε al mar es fa amb sensors especialitzats, com ara perfiladors de caiguda lliure (Osborn 1974), i més recentment amb sistemes Doppler. Tot i que els velocímetres acústics de tipus Doppler són cada cop més assequibles per al seu ancoratge per a mesures a llarg termini de turbulència de petita escala (e.g. Gargett i Donaghay 2003), encara manquen dades com per identificar els patrons de distribució tant temporal com espacial de la turbulència en el mar. Les mesures fetes amb sensors de caiguda lliure són disperses, i a més com

(Denman and Gargett 1983), where N [T^{-1}] is the buoyancy (or Brunt-Väisälä) frequency, a measure of the water column stability. In the ocean, l_o ranges between 10 cm and 100 m (Franks 2005).

Sources of turbulence in the upper ocean

Mechanical energy is entered into the system at an enormously wide range of scales (fig. 4) and by many different processes. Among these processes there is wind stress over the surface (Oakey and Elliot 1982, Oakey 1985), Langmuir circulations (D'Asaro and Dairiki 1997; McWilliams *et al.* 1997), upwelling (Csanady 1989, Dewey and Moum 1990), tides (St. Laurent *et al.* 2002), convection (Shay and Gregg 1986) and internal waves (Sun and Kunze 1999). At the largest possible scales, energy is concentrated in quasi-stationary anisotropic cyclones and anticyclones of the order of thousands of km wide.

In the upper layer, and especially in those areas where tides are relatively unimportant (e.g. the Mediterranean Sea), wind is the dominant mechanism of energy input. The generation of turbulence by wind occurs in the first place by the stress over the water surface. According to boundary layer theory (e.g. Turner 1973, Gargett 1989), under conditions of boundary constant stress, the dissipation of energy should scale with wind speed and depth, following:

$$\varepsilon = (\rho_a \rho_w \mathrm{C_d}^{-1})^{3/2} u_{10}{}^3 k^{-1} z^{-1} \qquad (5)$$

(Oakey 1985), where ρ_a and ρ_w are the density of air and water respectively, C_d is the drag coefficient, u_{10} is the wind velocity at 10 m over the sea surface, k is the von Kàrmàn's constant, typically assumed to be around 0.4 (Tennekes and Lumley 1972), and z is the depth.

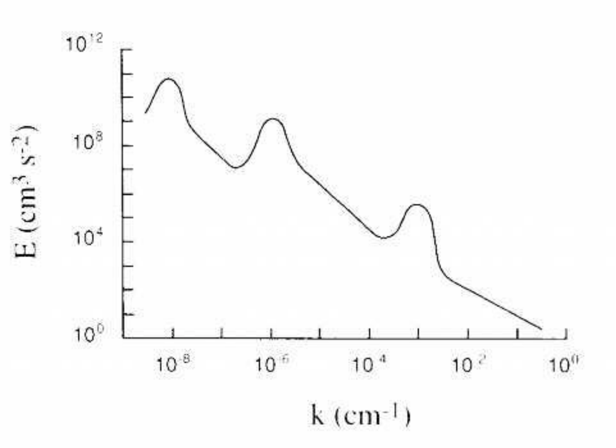

Figure 4: *Distribution of energy input in the ocean for a large range of wavenumbers. Taken from Peters and Redondo 1997.*

Distribució de l'entrada d'energia a l'oceà per a un ample rang de números d'ona. Modificat de Peters i Redondo 1997.

In the upper layer measured dissipation rates are often higher than predicted by this model. This anomaly is attributed mainly to two wind-driven processes (Gargett 1989, Thorpe 2004): Langmuir circulations (Thorpe *et al.* 2003) and surface wave breaking (Kitaigorodskii *et al.* 1983, Agrawal *et al.* 1992, Terray *et al.* 1996, Gemmrich and Farmer 2004, Stips *et al.* 2005). This last mechanism of turbulence generation is particularly important in the uppermost layer, the wave-affected-surface-layer (WASL, Stips *et al.* 2005). In this layer, and especially in the wave-turbulent sublayer, where around half of the energy dissipation of wave breaking occurs (Terray *et al.* 1996), values of ε can be one or two orders of magnitude higher than those predicted by the boundary layer model (Stips *et al.* 2005 and references therein). The depth of this sublayer is estimated to be similar or lower than the significant wave height (Terray *et al.* 1996, Gemmrich and Farmer 1999), that is, than the mean height of the highest 1/3rd of the waves.

Distribution of turbulence

Turbulence is usually quantified by ε. Estimations of ε in the sea are done by means of specialized sensors, such as free-falling airfoil shear probes (Osborn 1974), and more recently by Doppler systems. Although acoustic Doppler velocimeters are becoming increasingly available to be moored for long term small-scale turbulence measurements (e.g. Gargett and Donaghay 2003) there is still a lack of data that prevents from identifying the patterns of spatial and temporal distribution of turbulence in the sea. Measurements from free-falling microstructure profilers are scattered and que es requereix una mar relativament calmada per efectuar-les, també estan esbiaixades cap a valors baixos (Peters i Marrasé 2000).

Mentre no disposem de prou mesures directes de turbulència, podem utilitzar dades de vent per tal d'estimar la distribució de la turbulència a la zona superficial. Tal i com hem vist la turbulència en la capa superficial, i sobretot quan les marees són poc importants, és generada principalment per processos que tenen la seva força motora en el vent, és a dir, la fricció del vent sobre la superfície, les circulacions de Langmuir, i el trencament d'ones,. Fent servir dades de la literatura, Mackenzie i Legget (1993) van desenvolupar models estadístics que relacionaven ε amb la profunditat i la velocitat del vent a una alçada de 10 m. Aquest models són capaços de predir les taxes de dissipació fins i tot en zones amb marees considerables. Per tant, avaluant la distribució espacial i temporal del vent a partir de sèries temporals meteorològiques podem tenir una estima de la distribució d'episodis de turbulència a l'oceà superficial.

Efectes de la turbulència en el plàncton

Escales d'interacció

Com que la turbulència és un fenomen amb un rang d'escales tan ampli, la seva influència en els organismes planctònics es produeix a escales temporals i espacials molt diferents. Essencialment, els seus efectes es poden classificar entre els de gran i els de petita escala (Thomas *et al.* 1997). A les escales més grans afecta la distribució relativa del plàncton i dels seus recursos, com ara la llum, els nutrients o les preses. A les més petites la turbulència tendeix a accelerar els processos de transferència a través de la xarxa tròfica (Peters i Marrasé 2000).

Escales grans: la redistribució del plàncton i els seus recursos

En climes temperats com el nostre, l'energia mecànica que entra al sistema té una component estacional molt important. Normalment és màxima a la tardor i la primavera i mínima a l'estiu. Això determina uns patrons estacionals en la successió fitoplanctònica, que Margalef va sintetitzar elegantment en el seu famós mandala (Margalef 1978, Margalef *et al.* 1979). En aquest espai ecològic bidimensional (fig. 5), definit per l'entrada de nutrients i per la intensitat de la turbulència, les diferents "formes vitals" del fitoplàncton es distribueixen seguint la típica successió estacional d'aigües temperades, des de condicions amb poca turbulència i pocs nutrients a l'estiu, fins a condicions oposades a l'hivern. Les diatomees, unes algues unicel·lulars sense mobilitat, són les dominants a l'hivern, quan la columna d'aigua està ben barrejada i les concentracions de nutrients són més altes. A mesura que la radiació solar incrementa i els dies es fan més llargs i calorosos, la columna d'aigua es va estabilitzant per estratificació tèrmica. Durant un breu període de temps les concentracions de nutrients a la capa superficials són encara força altes i la llum no és limitant. És en aquestes condicions a finals d'hivern o començaments de primavera, que es produeix el màxim de concentració de biomassa fitoplanctònica, degut sobretot a les diatomees. És l'anomenada proliferació hivernal (Sverdrup 1953). Els nutrients però s'acaben esgotant en la capa eufòtica. A l'estiu la comunitat està dominada per formes mòbils com ara les dinoflagel·lades, i per algues molt petites, com ara les cianobactèries. El cicle estacional comença de nou a la tardor quan l'estratificació tèrmica es debilita en disminuir la radiació solar i augmentar la força del vent i l'onatge.

En aquest context, situacions amb poca turbulència i molts nutrients són anòmales, i poden conduir a proliferacions massives de dinoflagel·lades, popularment anomenades marees roges. Diversos formes vitals (tal i com les defineix Smayda 2000) de dinoflagel·lades poden donar lloc a proliferacions també en condicions turbulentes, com per exemple en fronts i afloraments, perquè s'aprofiten de les seves capacitats natatòries per oposar-se a la dispersió i l'enfonsament (Smayda 2000).

Així doncs, la turbulència s'associa molt sovint a augments de la concentració de nutrients (Margalef *et al.*

since they require relatively calm sea conditions, they are also biased to low values (Peters and Marrasé 2000).

Meanwhile, in order to assess the spatial and temporal distribution of turbulence in the upper zone, it is possible to use wind data as a proxy. As we have seen, turbulence in the surface layer is mainly generated by wind driven processes, namely velocity shear, Langmuir circulations and surface wave breaking, especially in those zones where tidal flows are weak. Using data from the literature, Mackenzie and Leggett (1993) developed statistical models relating ε with depth and wind speed at 10 m height. These models were successfully predicting dissipation rates even in zones with tidal flows. Thus, by evaluating the temporal and spatial distribution of wind from easily available meteorological time series, it is possible to assess the distribution of turbulence events in the upper ocean.

Effects of turbulence on plankton

Scales of interaction

Since turbulence is a multi-scaled process, its influence on planktonic organisms operates at very different spatial and temporal scales. Essentially, effects of turbulence can be classified between those at the large scales and those at the small scales (Thomas *et al.* 1997). At the large scales turbulence affects the relative distribution of plankton and its resources, namely light, nutrients and prey. At the small scales, turbulence tends to accelerate the energy and matter transfer processes through the planktonic food webs (Peters and Marrasé 2000).

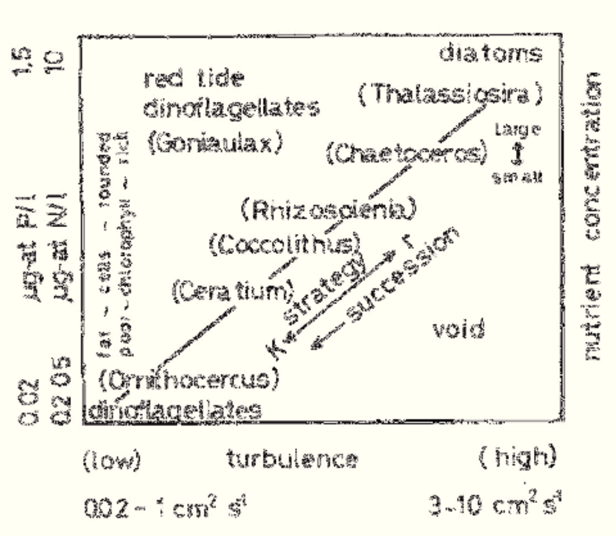

Figure 5: *Original Mandala of Margalef (1978). Planktonic organism placement in a two-dimensional space of nutrient cocentration and external energy*

Mandala original de Margalef (1978). Els organismes planctònics se situen en un espai bidimensional de concentració de nutrients i energia

Large scales: redistribution of plankton and resources

The external mechanical energy entered into the system has an important seasonal component in temperate waters. It is usually maximum in autumn and winter and minimum in summer. This determines seasonal patterns in the phytoplanktonic succession, elegantly summarized in "Margalef's mandala" (Margalef 1978, Margalef *et al.* 1979). In this two-dimensional ecological space (fig. 5), defined by the input of nutrients and by turbulence intensity, different phytoplanktonic life-forms are distributed following the common seasonal succession in temperate waters, from low turbulence-low nutrients to high turbulence-high nutrients conditions. Diatoms, a group of non-motile unicellular algae, are dominant in winter, when the water column is well mixed and nutrient concentrations are relatively high. In late winter or early spring, as solar irradiance increases, water column is stabilized by thermal stratification. During a short period, nutrient concentrations in the surface layer are still high as a result of the winter mixing, and light is non-limiting. In these conditions the annual maximum in biomass is achieved, due mainly to diatoms. It is the spring or late-winter bloom (Sverdrup 1953). Ultimately, nutrients are depleted from the euphotic zone. In summer the phytoplanktonic community is dominated by motile forms like dinoflagellates, and small algae like cyanobacteria. This seasonal cycle is restarted again in autumn, when low solar irradiance weakens the thermocline, and strong winds and waves deepen the mixed layer.

Situations with low-turbulence and high-nutrient concentrations are anomalous, and may conduct to

1979). Els nutrients són els elements químics dels quals es compon la matèria orgànica, i són per tant essencials per al creixement dels organismes. Els nutrients ecològicament més importants en sistemes aquàtics són el nitrogen el fòsfor, el silici i el ferro, perquè són els que es troben més sovint en condicions limitants per al creixement del fitoplàncton. Redfield (1934) va proposar que el quocient entre les concentracions dels diferents elements en aigües profundes, era un reflex de les proporcions amb què es troben en els organismes planctònics en la capa eufòtica. El quocient atòmic de Redfield és C:Si:N:P= 106:15:16:1 (Redfield *et al.* 1963). Desviacions respecte aquest quocient tant en la matèria orgànica com en les concentracions ambientals es consideren sovint simptomàtiques d'una limitació per al creixement del plàncton.

Un cop els nutrients han estat incorporats a la fracció particulada tendeixen a enfonsar-se. La velocitat d'aquest flux dependrà de l'estructura de mides de la comunitat i dels processos de reciclatge dins de la columna d'aigua, però és una tendència generalitzada fins i tot entre els organismes més petits (Richardson i Jackson 2007). En última instància hi ha d'haver una reposició de nutrients inorgànics a la zona eufòtica per tal de mantenir la producció primària. L'entrada de nutrients a la superfície es pot produir per deposició atmosfèrica, per escorrentia terrestre i per barreja vertical.

El paradigma de mar obert

En mar obert la turbulència pot disminuir l'estabilitat de la columna d'aigua, trencant en últim terme la termoclina, la capa en què el gradient vertical de temperatura (i per tant sovint de densitat).és més pronunciat. En aprofundir la capa de barreja i/o incrementar el flux turbulent a través de la termoclina, pot fer pujar aigües riques en nutrients o en diatomees (Smetacek 1985). A més la turbulència canvia el clima lumínic que experimenta el fitoplàncton en dos sentits. En primer lloc incrementa la variabilitat de la llum a què les algues es troben sotmeses (Denman i Gargett 1983), cosa que novament pot afavorir les diatomees, perquè aquestes estan ben adaptades a condicions fluctuants de llum (Litchman 2000). En segon lloc, en el cas que aprofundeixi la capa de mescla, disminueix la llum mitjana que rep cada cèl·lula. Quan la capa de mescla es fa massa fonda, la fotosíntesi, que es du a terme a la zona eufòtica, pot no ser suficient per compensar la respiració, que es dóna en tota la capa de mescla. La profunditat a la qual la producció i la respiració integrades són iguals s'anomena fondària crítica (Sverdrup 1953, Mann i Lazier 1996, Huisman *et al.* 1999). Si la profunditat de la capa de mescla està per sota d'aquest valor crític, la respiració integrada per tota la capa supera a la producció, i per tant no hi pot haver un creixement net del fitoplàncton.

Tanmateix l'existència d'una capa de mescla no és una condició imprescindible per al desenvolupament de floracions de fitoplàncton. Si la turbulència està per sota d'un valor crític màxim la taxa de creixement del fitoplàncton a la zona eufòtica pot excedir la taxa de mescla vertical fins i tot en el cas que tota la columna estigui completament barrejada (Huisman *et al.* 1999, 2002). A més, la turbulència ha d'estar per sobre d'un llindar mínim d'intensitat per tal de neutralitzar la pèrdua deguda a l'enfonsament de cèl·lules (Huisman *et al.* 2002).

Zones costaneres

En aigües somes, per exemple en zones costaneres o en llacs petits, la turbulència pot retornar els nutrients de capes fondes incrementant el flux de nutrients des del sediment (Dade 1993, Grémare *et al.* 2003). Un altra font de nutrients específica d'aquestes zones és l'escorrentia superficial. En les últimes dècades hi ha hagut una tendència global d'origen antròpic a incrementar les concentracions de nutrients en les entrades d'aigua dolça (Cloern 2001). La dinàmica temporal d'aquestes entrades, especialment en un clima sec com és el mediterrani, està molt lligada a la variabilitat meteorològica (fig. 6). Així l'entrada de nutrients associada a rius i rieres en les costes mediterrànies té un component episòdic important, que es relaciona amb el patró temporal de fenòmens de turbulència.

blooms of dinoflagellates, the so called red tides. Several dinoflagellates life-form species (as defined by Smayda 2002) may however bloom also under turbulent conditions, namely in fronts or upwellings, taking advantage of their swimming capability to counteract dispersion and sinking (Smayda 2002).

Thus, the occurrence of turbulence is often associated to increased nutrient concentrations (Margalef *et al.* 1979). Nutrients are the chemical elements of which organic matter is made of, and are thereby essential for the growth of organisms. The most ecologically important nutrients in aquatic systems are N, P, Si and Fe, because they are the ones most frequently found in limiting concentrations for phytoplankton growth. Redfield (1934) proposed that the ratios between the concentrations of these elements in deep waters were reflecting the ratios within the planktonic organisms in the euphotic zone. The so called Redfield atomic ratio is C:Si:N:P= 106:15:16:1 (Redfield *et al.* 1963). Deviations from this ratio in the organic matter or in the ambient water are generally thought to be symptomatic of limiting growth conditions for plankton.

Once nutrients have been incorporated into the particulate fraction they tend to sink downwards. The velocity of this flux will depend on the size structure of the planktonic community and on the recycling processes within the water column, but it is a general trend, even for the smallest organisms (Richardson and Jackson 2007). Ultimately, in order to sustain new production there has to be a replenishment of inorganic nutrients to the euphotic zone. The supply of nutrients to the surface is due to atmospheric deposition, terrestrial runoff and the input from the bottom.

The open sea template

In deep waters, turbulence can decrease water column stability and eventually disrupt the thermocline. By deepening the mixed layer and/or increasing the turbulent flux through the thermocline, it can bring up bottom waters rich in nutrients or in seeding diatoms (Smetacek 1985). Besides, turbulence changes the light climate of the phytoplankton in two senses. In the first place it increases the variability of light at which cells are subjected (Denman and Gargett 1983), which may favour again the dominance of diatoms, because these are well adapted to fluctuating light regimes (Litchman 2000). In the second place, whenever it causes the deepening of the mixed layer it decreases the average amount of light each cell receives. If the mixing layer is too deep, the photosynthesis performed in the euphotic zone may not compensate for the respiration occurring throughout the mixed layer. The depth at which integrated production

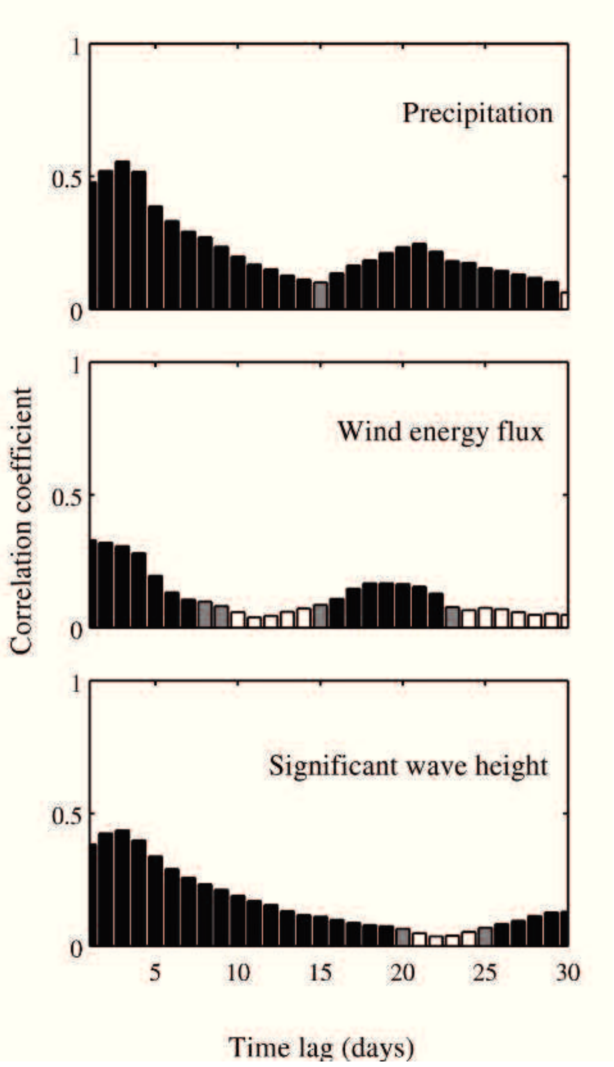

Figure 6: *Cross-correlograms between La Tordera river discharge, and precipitation, wind energy flux and significant wave height. Details of calculation are given in chapter 3.*

Correlacions creuades entre la descàrrega del riu Tordera, i la precipitació, el flux de energia eòlica i l'alçada d'ona significant. Els detalls del càlcul es donen al capítol 3.

La ressuspensió dels sediments produïda per la turbulència a més d'incrementar la concentració de nutrients a la columna d'aigua, pot portar a un increment de la terbolesa, i per tant a una limitació de la llum disponible per a la fotosíntesi (Cloern 1987, Cotner *et al.* 2000). Així doncs la resposta del fitoplàncton costaner a episodis de barreja pot no ser immediat. Hi pot haver un retard, relacionat amb el temps necessari per que el material suspès més gran torni a sedimentar, entre el començament de la pertorbació i l'increment observat en producció.

Efectes en la distribució espacial dels escalars

La distribució dels organismes planctònics no és uniforme, sinó que depèn de la distribució dels aliment, dels nutrients i de la llum, de processos biològics com ara el creixement o la depredació, i per suposat, del moviment de l'aigua. La influència de la turbulència en la distribució espacial del plàncton es pot veure mitjançant tècniques d'anàlisi espectral. En un anàlisi espectral o de Fourier, una sèrie temporal de dades és descomposta en un seguit de funcions de sinus i cosinus amb números d'ona creixents. L'amplitud de cada una d'aquestes funcions depèn de la variabilitat (o energia) associada al seu número d'ona. En un flux turbulent, l'espectre d'energia (E) en el subrang inercial segueix:

$$E(k) \propto \varepsilon^{2/3} k^{-5/3} \qquad (6)$$

on k és el número d'ona. Les propietats escalars, com ara la temperatura o la salinitat, tenen un espectre de variances amb la mateixa pendent, -5/3, perquè es comporten gairebé com a traçadors passius del moviment de l'aigua (Batchelor 1959).

Les concentracions de nutrients i la fluorescència (un indicador de la concentració de clorofil·la, i per tant de la quantitat de fitoplàncton), també s'ha vist que segueixen la llei dels -5 terços en el rang inercial en nombroses situacions (Platt 1972, Yamazaki *et al.* 2006, Mountain i Taylor 1996). A les escales grans, per sobre de l'escala d'Ozmidov, la distribució del plàncton està dominat per processos biològics, com ara el creixement i la depredació, i la pendent en teoria és més pròxima a -1 (Franks 2005). Per sota de l'escala de Batchelor, en el domini de la viscositat, la pendent torna a ser teòricament més propera a -1. Però en el subrang inercial la pendent es pot aproximar a -5/3. Recentment Franks (2005) ha senyalat els severs artefactes metodològics que poden patir aquest tipus d'estudis. Entre ells, mostrejar amb una freqüència fora del subrang inercial i assumint que el plàncton és una variable contínua, i no quàntica. Tot i això, l'espectre del plàncton s'ha vist que segueix la pendent de -5/3 en condicions de turbulència molt vigorosa, quan el subrang inercial és més ample, cosa que apunta la importància que la turbulència pot tenir en la distribució de plàncton i nutrients (e.g. Mountain i Taylor 1996). Les desviacions de la pendent teòrica en el subrang inercial es poden deure a interaccions entre poblacions planctòniques (Powell i Okubo 1994), i a que els organismes no es comporten com a traçadors perfectes, perquè tenen mobilitat, o bé perquè la seva densitat és diferent a la de l'aigua (Ghosal *et al.* 2000), i per tant el seu moviment no és completament solidari.

La turbulència de gran escala a més també pot dissipar taques o capes d'elevada concentració d'aliment, i per tant afecten la seva disponibilitat per part dels depredadors planctívors (Kiørboe 1997). Finalment, la turbulència, en destorbar el seu comportament natatori, pot afectar la distribució d'organismes que migrin verticalment (Kamykowski 1995).

Petita escala: efectes sobre els individus

Els efectes de la turbulència a les escales més petites es relaciona amb el moviment turbulent estricte, però també amb el camp de cisallament laminar que se'n deriva i que es troba per sota de la microescala de Kolmogorov. En aquestes escales, els efectes directes sobre els individus estan causats bàsicament per un increment del flux de soluts o de partícules cap a, o lluny de, els organismes. Aquest enunciat senzill es tradueix en efectes diferents, depenent del comportament tròfic dels organismes, i de la seva mida. En cèl·lules osmotròfiques la turbulència de petita escala afavoreix l'adquisició de nutrients (Karp-Boss *et al.* 1996). En organismes and respiration are equal is called the critical depth (Sverdrup 1953, Mann and Lazier 1996, Huisman *et al.* 1999). If mixed layer depth is below this critical value, the integrated respiration through the mixed layer is higher than the production, and therefore phytoplankton can not have a net growth.

However, the existence of a mixed layer is not a *sine qua non* for the development of phytoplankton blooms in deep waters. If turbulence is below a critical maximal value, even in the absence of a mixed layer or when this is deeper than the critical depth, the growth rate of phytoplankton in the euphotic zone may exceed the vertical mixing rates (Huisman *et al.* 1999, 2002). In addition, turbulence must be above a minimal turbulence intensity to neutralize the loss due to sinking (Huisman *et al.* 2002).

Coastal areas

In shallow systems, as for example in coastal waters or small lakes, turbulence may resupply nutrients from bottom layers by directly increasing the flux of nutrients from the sediment (Dade 1993, Grémare *et al.* 2003). Another major source of nutrients in coastal zones is the terrestrial runoff. In the past decades there has been an anthropogenic worldwide tendency to increase the concentrations of nutrients in freshwater inputs (Cloern 2001). The temporal dynamics of terrestrial input, especially in a dry climate as the Mediterranean, is linked to the meteorological forcing variability (fig. 6). Thus, the nutrient input from rivers and ephemeral water courses has a strong episodic component in the Mediterranean coasts, which is also related to the pattern of turbulent events.

The turbulence-driven resuspension of sediments, besides increasing the concentration of nutrients in the water column, can lead to increased turbidities and consequently to a limitation of the light available for photosynthesis (Cloern 1987, Cotner *et al.* 2000). Thus, the response of coastal phytoplankton to mixing episodes may be not immediate. A lag, related to the time for the large suspended material to sink again, may exist between the beginning of the perturbation and the observed increase in production.

Effects on the spatial distribution of scalars

Planktonic organisms do not distribute uniformly. Their distribution depends on food, nutrient and light fields, on biological processes, such as growth or predation, and on water motion. The influence of turbulent motions in the spatial distribution of planktonic organisms may be seen from spectral analysis. In Fourier or spectral analyses a data series is decomposed in sines and cosines functions with increasing wave numbers. The amplitude of a given function depends on the variability (or energy) associated to its wave number. In turbulence, the energy (E) spectrum in the inertial subrange follows:

$$E(k) \propto \varepsilon^{2/3} k^{-5/3} \qquad (6)$$

(Kolmogorov 1941), where k is the wave number. Scalar properties, such as temperature or salinity, show a variance spectrum with the same slope than turbulence, -5/3, because they behave almost as passive tracers of the water motion (Batchelor 1959).

Nutrient concentrations and fluorescence, a proxy for the chlorophyll concentration and therefore for the phytoplankton biomass, have also been reported to follow the -5/3 law within the inertial subrange in numerous situations (Platt 1972, Yamazaki *et al.* 2006, Mountain and Taylor 1996). This suggests that to some extend they behave also like passive tracers (Denman and Platt 1976). At the largest scales, above the Ozmidov length scale, the distribution of plankton is dominated by biological processes, like growth or grazing, and slope is theoretically closer to -1 (Franks 2005). Below the Batchelor scale, at the smallest viscous scales the slope is also close to -1. But at the inertial subrange the slope may approach to -5/3. Recently Franks (2005) has pointed out the severe methodological artefacts that this kind of studies often suffers from. Among them, sampling with frequency out of the inertial subrange and assuming plankton to be a continuum variable, rather than quanta. Still, the spectrum of plankton has been seen fagotròfics, incrementa les taxes d'encontre entre partícules (Rothschild i Osborn 1988), cosa que té implicacions no només per a la depredació, sinó també per a l'aparellament i l'agregació. També s'ha vist que augmenta la velocitat de sedimentació (Ruiz *et al.* 2004). Per últim, al nivell ecofisiològic, la turbulència pot inhibir determinades processos cel·lulars, sobretot en algues dinoflagel·lades (Berdalet 1992).

Absorció de nutrient i mida cel·lular

El flux de soluts cap a la cèl·lula, o lluny d'ella, està en última instància controlat per la difusió molecular. A l'estat estacionari, i en absència de moviment en l'aigua, el flux difusiu Q d'un solut amb un coeficient de difusió molecular D $[L^2T^{-1}]$ cap a una cèl·lula esfèrica de radi r_0 es pot descriure com:

$$Q = -4\pi D r_0 (C_\infty - C_0) \qquad (7)$$

on C_∞ i C_0 són respectivament la concentració ambiental i la de la superfície de la cèl·lula.

Segons Kiørboe (1993) la taxa d'absorció per unitat de volum Q' és proporcional al radi de la cèl·lula al quadrat:

$$Q' = \frac{Q}{4/3\pi r_0^3} = -3Dr_0^{-2}(C_\infty - C_0) \qquad (8)$$

Això vol dir que les cèl·lules petites són molt més eficients en absorbir o alliberar soluts que les cèl·lules grans. Aquestes últimes fàcilment es troben limitades per difusió, i estan en desavantatge en aigües oligotròfiques.

Per altra banda la mida òptima de la cèl·lula incrementa amb les concentracions de nutrients (Jumars 1993, Jumars *et al.* 1993, Karp-Boss *et al.* 1996). Segons aquest autors, la pèrdua bruta de substrat és proporcional a $\mathrm{m}r_0^2$, on m és un coeficient per al catabolisme de la cèl·lula. La mida òptima de la cèl·lula r_{opt} es dóna quan la diferència entre l'absorció i la pèrdua és màxima, és a dir:

$$\frac{\mathrm{d}(4\pi r_0 D(C_\infty - C_0) - \mathrm{m}r_0^2)}{\mathrm{d}r_0} = 0 \qquad (9)$$

que dóna:

$$r_{opt} = \frac{2\pi D(C_\infty - C_0)}{\mathrm{m}} \qquad (10)$$

Així doncs, la mida òptima de la cèl·lula es relaciona amb el coeficient de difusió i amb la concentració ambiental, cosa que pot explicar perquè les cèl·lules grans són relativament més abundants en condicions eutròfiques. La disponibilitat de nutrients, per tant, determina en part l'estructura de mides de la comunitat osmotròfica. Les diatomees poden desviar-se lleugerament d'aquesta relació, perquè tenen un gran vacúol intracel·lular que fa que els seus requeriments nutricionals siguin menors del que esperaríem pel seu volum extern.

Tot i que, tal i com hem mencionat, la difusió molecular controla l'absorció de nutrients, el cisallament derivat de la turbulència o del moviment relatiu del fluid que envolta una cèl·lula que s'enfonsa o que neda activament, pot incrementar indirectament el flux de soluts en fer més acusat el gradient de concentració i per tan en aprimar la capa límit difusiva (fig. 7). En general, l'increment en l'absorció de nutrients degut al moviment del fluid depèn de tres variables: el règim del flux (cisallament laminar, turbulència o moviment laminar), de la mida i forma de les cèl·lules, i del coeficient de difusió D propi del solut (Karp-Boss *et al.* 1996). Teòricament, donat el rang de nivells de turbulència que trobem a la superfície del mar, l'increment relatiu en l'absorció de nutrients degut al moviment turbulent només comença a ser important per al microfitoplàncton, és a dir, per cèl·lules entre 20 i 200 µm.

Resumint, altes concentracions de nutrients i turbulència vigorosa afavoreixen en general i de forma relativa les cèl·lules grans, o les cadenes, tot i que aquest avantatge probablement és insuficient per contrarestar els avantatges en l'absorció de nutrients derivats del fet de ser petit, i per tant, per si soles no poden explicar la dominància en la comunitat fitoplanctònica de cèl·lules to follow a -5/3 slope under strong turbulent conditions, when inertial subrange is broader, suggesting the importance of turbulence in the distribution of plankton and nutrients (e.g. Mountain and Taylor 1996). Deviations from the -5/3 theoretical slope within this range may occur because of interactions between plankton populations (Powell and Okubo 1994). Besides, many organisms have motility, or at least have densities higher than seawater (Ghosal *et al.* 2000), and therefore their movement is not completely solidary with water motion, departing from being perfect tracers.

Large scale turbulence can also dissipate patches or layers of elevated food concentration, hence affecting food availability to planktivorous predators (Kiørboe 1997). In addition, turbulence may affect the distribution of vertical migrating organisms by disturbing their swimming behaviours or patterns (Kamykowski 1995).

Small scales: effects on individuals

Effects of turbulence at the small scales are related to the turbulent motion *per se*, but also to the laminar shear field which derives from this motion and which is found below the Kolmogorov microscale. At these small scales and below, the direct effects on individuals are basically caused by an increased flux of solutes or particles to the organisms. This simple statement translates into different effects, depending on the trophic behaviour of the organism, and on its size. For osmotrophic cells small-scale turbulence leads to an enhancement of uptake rates of nutrients (Karp-Boss *et al.* 1996). For phagotrophic organisms, it means an increase in encounter rates between particles (Rothschild and Osborn 1988), which has implications in grazing, mating and aggregation. At these small scales turbulence has also been seen to increase sedimentation velocities (Ruiz *et al.* 2004). Finally, turbulence has been reported to inhibit cell processes at the ecophysiological level (Berdalet 1992), particularly in dinoflagellates.

Uptake of nutrients and cell size

The flow of solutes to and from a cell is ultimately controlled by molecular diffusion. At the steady state, and in the absence of relative fluid motion, the diffusional flux Q of a solute with molecular diffusion coefficient D [L^2T^{-1}] into an ideal spherical cell of radius r_0 can be described by:

$$Q = -4\pi D r_0 (C_\infty - C_0) \tag{7}$$

where C_∞ and C_0 are the ambient and cell surface concentration of the solute respectively.

According to Kiørboe (1993) the volume specific uptake rate Q' scales with the minus 2nd power of radius:

$$Q' = \frac{Q}{4/3\pi r_0^3} = -3Dr_0^{-2}(C_\infty - C_0) \tag{8}$$

This means that small cells are more efficient in taking up or releasing solutes than large cells. The latter can easily become diffusion limited, and are at disadvantage in oligotrophic waters.

On the other hand the optimal cell size increases with increasing nutrient concentration (Jumars 1993, Jumars *et al.* 1993, Karp-Boss *et al.* 1996). According to these authors, the gross loss of substrate scales with $\mathrm{m}r_0^2$, where m is a coefficient for catabolism of the cell. The optimal cell size r_{opt} is achieved when the difference between uptake and loss is maximum, i.e., when:

$$\frac{\mathrm{d}(4\pi r_0 D(C_\infty - C_0) - \mathrm{m}r_0^2)}{\mathrm{d}r_0} = 0 \tag{9}$$

which gives:

$$r_{opt} = \frac{2\pi D(C_\infty - C_0)}{\mathrm{m}} \tag{10}$$

Thus, optimal cell size is directly related to the diffusion coefficient and to the ambient concentration, which may explain why large cells are more common under eutrophic conditions. The availability of nutrients is therefore partly determining the size structure of the osmotrophic community. Diatoms may partly escape this general relationship, because they have a big vacuole grans que es dóna en determinades èpoques de l'any (Kiørboe 1993).

Taxes d'encontre

La turbulència de petita escala incrementa la probabilitat d'encontre entre partícules perquè augmenta la velocitat relativa entre elles. Això fou formulat per primer cop per Rothschild i Osborn (1988) tractant amb taxes d'encontre entre depredadors i preses. Amb nivells moderats de turbulència això es pot traduir en un augment de la taxa de depredació, tot i que el resultat final dependrà de la mida i velocitat tant del depredador com de les preses, i sobretot, dels seus comportaments. Quan la turbulència és massa intensa, aquest efectes positius es veuen contrarestats per un consum més gran d'energia, degut per exemple a un increment de les reaccions d'evasió (Marrasé *et al.* 1990), i per una disminució de l'eficiència de captura (Saiz i Kiørboe 1995, Mackenzie i Kiørboe 2000). Per tant, en el cas de taxes d'alimentació l'efecte de la turbulència segueix una campana de Gauss, i és màxim a nivells intermitjos de dissipació.

Aquest efecte teòric positiu és més important en depredadors de mides properes a l'escala de Kolmogorov, com són els copèpodes i les larves de peixos (Kiørboe i Saiz 1995, Kiørboe 1997). De fet, la turbulència s'ha vist experimentalment que incrementa les taxes d'encontre i de depredació tan en copèpodes (Marrasé *et al.* 1990, Saiz *et al.* 1992, Saiz i Kiørboe 1995) com en larves de peixos (Sundby i Fossum 1990, Sundby *et al.* 1994). Per organismes més grans, comparada amb la seva velocitat de natació, la velocitat turbulenta ambiental és massa baixa per tenir cap rellevància. Per sota de l'escala de Kolmogorov el cisallament laminar també pot generar un moviment relatiu entre els depredadors protozous i les seves preses.. Experimentalment s'ha observat diverses vegades un increment de les taxes de depredació de protozous sobre bacteris (Peters i Gross 1994, Shimeta *et al.* 1995). Així la turbulència pot accelerar la transferència de carboni a través de la xarxa tròfica microbiana.

Una altra conseqüència de l'augment en les taxes d'encontre és un increment de la coagulació de partícules (Jackson 1990, Kiørboe 1994, Kiørboe 1997). L'agregació és la causant de la formació de l'anomenada neu marina. Un increment en la coagulació porta a un augment en les velocitats de sedimentació de les partícules (Kiørboe 1997).

Efectes fisiològics : el cas de les dinoflagel·lades

La resposta dels diferents grups del plàncton a la turbulència de petita escala és molt diversa, no només a causa de diferències en les mides, la morfologia i l'estil de vida, sinó també per efectes ecofisiològics. Thomas i col·laboradors. (1997) van predir que la sensibilitat a la turbulència en els diferents grups de fitoplàncton s'ordenava de la següent manera: cloròfits < cianòfits < diatomees < dinoflagel·lades.

Les dinoflagel·lades constitueixen doncs el grup d'algues unicel·lulars més sensible a la turbulència, i potser per això són també les més extensament estudiades des d'aquesta òptica. Aquesta conclusió es deriva tan d'observacions de camp com d'estudis experimentals. Al camp s'ha vist que l'aparició de proliferacions massives de dinoflagel·lades es veu afavorida per condicions de calma meteorològica i d'estabilitat de la columna d'aigua (Wyatt i Horwood 1973, Margalef 1978, Berman i Shteiman 1998). Per altra banda, nombrosos estudis experimentals han tractat el tema de les respostes particulars de les dinoflagel·lades a la turbulència (per una revisió del tema, vegeu per exemple Estrada i Berdalet 1997, 1998, Thomas *et al.* 1997, Berdalet i Estrada 2005), que sembla que són molt específiques (Berdalet i Estrada 1993, Sullivan i Swift 2003, Berdalet *et al.* en premsa). En general, tot i que el creixement d'algunes espècies no es veu afectat per la turbulència, o ho és d'una manera positiva, la majoria de respostes descrites són negatives. Aquestes inclouen inhibició o fins i tot supressió del creixement net (Berdalet 1992, Thomas i Gibson 1992, Berdalet i Estrada 1993), inhibició de la divisió cel·lular (Berdalet 1992, Thomas *et al.* 1995), canvis en la morfologia cel·lular (Zirbel *et al.* 2000), pèrdua de flagells (Thomas i

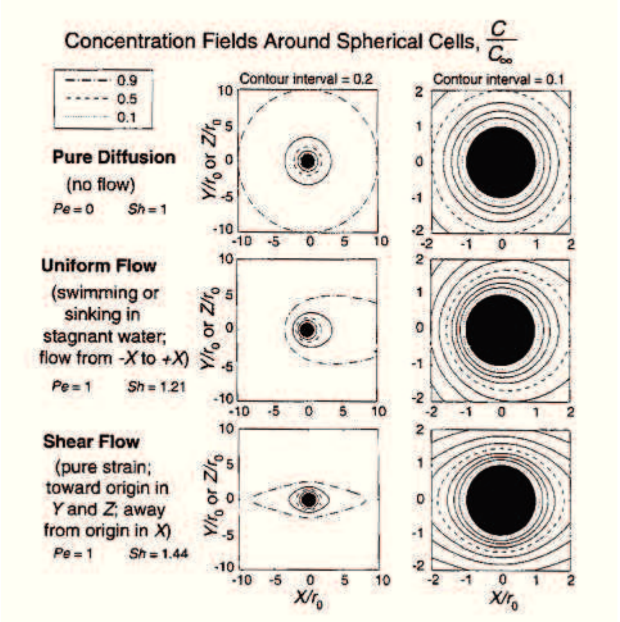

Figure 7: *Solute concentration fields around idealized spherical cells, standardized to surface concentration, under three different flow regimes: still water, laminar flow derived from sinking or swimming and small scale shear occurring under turbulent conditions. Taken from Karp-Boss et al. 1996.*

Concentracions de soluts al voltant d'una cèl·lula esfèrica ideal, normalitzades per la concentració superficial, sota differents règims hidrodinàmics: aigua quieta, flux laminar derivat de la natació o l'enfoonsament, i cisallament produit pen condicions turbulentes. Tret de Karp.-Boss et al. 1996.

inside, which lower their requirement in nutrients with respect of what would be expected for their size.

Although, as stated above, molecular diffusion controls the uptake of nutrients, turbulence derived laminar shear, as well as relative fluid motion in sinking and swimming cells, can indirectly increase the flux of solutes by steeping their concentration gradient and thus thinning the boundary diffusional layer (fig. 7). The increase in nutrient uptake due to fluid motion will depend in general of three variables: the flow regime (laminar shear, turbulent or laminar motion), the size and shape of the cells, and the diffusion coefficient *D* of the solute (Karp-Boss *et al.* 1996). For the range of turbulence levels encountered in the ocean surface, the relative increase in uptake rate of dissolved inorganic nutrients due to fluid motion is important only for microphytoplankton (cell sizes between 20 and 200 µm).

Thus both high nutrient concentration and vigorous turbulence flows are in general relatively favouring large cells or chains, although this is probably not enough to counterbalance the advantage in nutrient uptake of being small, and can not explain by itself the dominance of large cells in the phytoplanktonic community at certain times of the year (Kiørboe 1993).

Encounter rates

Small scale turbulence increases the encounter probability rates between particles because it increases the relative velocity between them. This was first envisioned by Rothschild and Osborn (1988) dealing with predator-prey contact rates. Under moderate turbulence levels this enhancement may translate into higher grazing rates, but this will depend on the size (and velocity) of both predator and prey, and especially on their behaviour. Under strong turbulence these positive effects can be in part counterbalanced by higher energy expenditure, related for example with escape responses (Marrasé *et al.* 1990), and by lower capture efficiency (Saiz and Kiørboe 1995, Mackenzie and Kiørboe 2000). In the case of feeding rates therefore, the effect of turbulence is dome-shaped, being maximal at intermediate levels of dissipation.

The theoretical positive effect is most important for predators of sizes near the Kolmogorov length scale, such as copepods and fish larvae (Kiørboe and Saiz 1995, Kiørboe 1997). Turbulence has been experimentally seen to enhance encounter and feeding rates for both copepods (Marrasé *et al.* 1990, Saiz *et al.* 1992, Saiz and Kiørboe 1995) and fish larvae (Sundby and Fossum 1990, Sundby *et al.* 1994). For much larger organisms the turbulent velocity is too slow to be of any significance when compared with the organism velocity. Below the Kolmogorov scale the laminar shear can also create relative motion between protozoan predators and their prey,. Several experimental responses of protozoan grazing rates to small-scale turbulence or laminar shear have been reported (Peters and Gross 1994, Shimeta *et al.*

Gibson 1990), o alteració del moviment natatori (Pollingher i Zemel 1981, Thomas i Gibson 1990, Zirbel *et al.* 2000, Berdalet *et al.* en premsa)

La rellevància ecològica d'aquesta especial sensibilitat encara ha de ser estudiada en alguns aspectes. Al mar les diatomees es troben en general en avantatge competitiu respecte les dinoflagel·lades quan hi ha turbulència. Aquests dos grups d'organismes representen probablement dos estratègies evolutives oposades per resoldre el problema de la limitació per difusió de l'absorció de nutrients en cèl·lules grans. Les dinoflagel·lades han optat per incrementar l'absorció nedant activament, les diatomees aprofiten el moviment turbulent, així com velocitats de sedimentació altes i flotabilitats variables.

Efectes a nivell d'ecosistema

L'estudi del efectes de la turbulència a nivell d'ecosistema s'han basat sobretot en experiments en què la comunitat planctònica natural era reclosa en condicions controlades (e.g. Reynolds *et al.* 1983, Oviatt *et al.* 1981, Estrada *et al.* 1987, 1988, Howarth *et al.* 1993, Peters *et al.* 1998, 2002, Petersen *et al.* 1998, Svensen *et al.* 2001). Això permet separar més fàcilment els efectes de la turbulència d'altres deguts a altres factors ambientals. Diverses tendències, força robustes, emergeixen d'aquests estudis, i confirmen la majoria d'efectes teòrics i ecofisiològics que hem resumit en seccions prèvies.

Canvis en la composició específica de la comunitat

El mandala de Margalef (fig. 5), que està basat principalment en observacions de camp, ha estat confirmat per nombrosos estudis de microcosmos o mesocosmos. Diverses investigacions han demostrat que la turbulència, especialment si es dóna juntament amb un enriquiment de nutrients, incrementa la proporció de diatomees (Oviatt 1981, Reynolds *et al.* 1983, Estrada *et al.* 1988, Petersen *et al.* 1998, Arin *et al.* 2002). En canvi els organismes més petits i els flagel·lats són dominants en condicions de calma o en estadis madurs de la successió planctònica (Estrada *et al.* 1987). Sens dubte part de la variabilitat en la resposta de la comunitat a les pertorbacions està causada per diferències en la composició de la comunitat inicial (Estrada *et al.* 1987).

Encara que la turbulència incrementa les taxes d'encontre, això no es tradueix necessàriament en taxes de creixements de consumidors més altes. De fet, en microcosmos turbulents amb la comunitat natural, els copèpodes sovint es troben en abundàncies més baixes que en els corresponents tractaments quiets (Oviatt 1981, Alcaraz *et al.* 1988, Petersen *et al.* 1998). A nivells molt alts, la turbulència augmenta les taxes metabòliques i redueix l'eficiència de captura en esbullar els corrents de captació i estimular les reaccions d'evasió.

Estructura de mides

Tant la turbulència (Arin *et al.* 2002, Cózar i Echevarría 2005) com l'enriquiment en nutrients (e.g. Duarte *et al.* 2000, Samuelsson *et al.* 2002, Malits *et al.* 2004) poden suavitzar la pendent de l'espectre de biomassa-mida. Això vol dir que fan que augmenti la concentració d'organismes grans en relació als petits. L'entrada episòdica de nutrients i de turbulència es prediu que han de causar la dominància del microfitoplàncton per dues raons: perquè aquestes condicions afavoreixen l'absorció de nutrients en cèl·lules grans (efecte ascendent, de baix a dalt), i perquè les cèl·lules petites no poden respondre plenament a aquestes entrades perquè estan estretament controlades pels seus depredadors (efecte descendent, o de dalt a baix).

Aquest efecte de dalt a baix mereix una mica de desenvolupament. Totes les taxes específiques metabòliques, com ara la de creixement, són proporcionals a la mida:

$$M \propto V^{b} \tag{11}$$

on V és el volum de l'organisme i b és un exponent molt proper a -1/4 tant per organismes unicel·lulars com pluricel·lulars (Banavar *et al.* 2002). Així doncs els

1995). Thus, turbulence may accelerate carbon transfer rates within the microbial food web.

Another consequence of the increase in contact rates is the enhancement of the coagulation of particles (Jackson 1990, Kiørboe 1994, Kiørboe 1997). Aggregation is responsible for the formation of the marine snow. The increased coagulation leads to higher settling velocities (Kiørboe 1997).

Physiological effects: the case of dinoflagellates

The response of the different planktonic groups to small-scale turbulence is very diverse, not only because of size-dependent dynamics and differences in life styles and morphologies, but also because of physiological effects. Thomas *et al.* (1997) predicted the sensitivity to turbulence in phytoplankton groups to order as follows: chlorophytes< cyanophytes< diatoms< dinoflagellates.

Dinoflagellates constitute the group of algae most sensitive to turbulence, and which has been more extensively studied under this point of view. This conclusion derives from both field observations and experimental studies. Dinoflagellates blooms are most usually favoured by periods of calm weather conditions and high water column stability (Wyatt and Horwood 1973, Margalef 1978, Berman and Shteiman 1998). Numerous experimental studies have addressed the particular responses to turbulence of dinoflagellates (for a review see for example Estrada and Berdalet 1997, 1998, Thomas *et al.* 1997, Berdalet and Estrada 2005), which appear to be highly species-specific (Berdalet and Estrada 1993, Sullivan and Swift 2003, Berdalet *et al.* in press). Although the growth of some species is insensitive to or favoured by small-scale turbulence, a diversity of negative effects have reported including inhibition or even suppression of net growth (Berdalet 1992, Thomas and Gibson 1992, Berdalet and Estrada 1993), inhibition of cell division (Berdalet 1992, Thomas *et al.* 1995), induction of changes in cell morphology (Zirbel *et al.* 2000), loss of flagella (Thomas and Gibson 1990), or alteration of the swimming behaviour (Pollingher and Zemel 1981, Thomas and Gibson 1990, Zirbel *et al.* 2000, Berdalet *et al.* in press).

The ecological relevance of the sensitivity of dinoflagellates to turbulence remains to be tested in some aspects. In the field diatoms are generally in a competitive advantage over dinoflagellates under mixing conditions. Diatoms and dinoflagellates probably represent two opposite evolutionary strategies to the problem of diffusive limitation of the uptake of nutrients in large osmotrophic cells. While dinoflagellates increase the uptake rates by actively swimming, diatoms take advantage of turbulent motion as well as of high sinking speeds and changing buoyancy.

Ecosystem effects

The study of the effects of turbulence at an ecosystem scale has been based mainly on enclosure experiments with natural planktonic assemblages (e.g. Reynolds *et al.* 1983, Oviatt *et al.* 1981, Estrada *et al.* 1987, 1988, Howarth *et al.* 1993, Peters *et al.* 1998, 2002, Petersen *et al.* 1998, Svensen *et al.* 2001) because it is easier to separate the effects of turbulence from those of other environmental factors. Several robust trends arise from such studies, which confirm most of the theoretical and physiological effects outlined in previous sections.

Changes in community composition

Margalef's Mandala (fig. 5), which is mainly based on field observations, has been confirmed by numerous enclosure studies. Several investigations have shown that turbulence, especially when in conjunction with nutrient enrichment, increases the proportion of diatoms (Oviatt 1981, Reynolds *et al.* 1983, Estrada *et al.* 1988, Petersen *et al.* 1998, Arin *et al.* 2002). By contrast, flagellate and small forms are dominant under still conditions or at mature stages of the succession (Estrada *et al.* 1987). Some of the variability in the community response to perturbations is undoubtedly caused by differences in the initial community composition (Estrada *et al.* 1987).

Although turbulence increases encounter rates, this does not necessarily translate into higher growth rates of consumers. In fact, in turbulent microcosm experiments with the natural community, copepods are often in lower abundances (Oviatt 1981, Alcaraz *et al.* 1988, Petersen *et* organismes petits creixen més ràpidament. En canvi la relació de mides entre el depredador i la presa és força constant, entre 10:1 i 100:1 (Kiørboe 1993 i referències incloses). El temps de generació és inversament proporcional a la taxa específica de creixement. La diferència entre els temps de generació de preses i depredadors, i per tant el temps relatiu de resposta d'aquests últims, tendeix a ser més llarg com més grans són els organismes. En conseqüència, el control per part dels depredadors és més estret sobre cèl·lules osmotròfiques petites que sobre les grans (Kiørboe 1993, Franks 2001). Mentre que qualsevol increment en la taxa de creixement del picoplàncton serà ràpidament correspost pels seus depredadors, les cèl·lules grans poden escapar-se més fàcilment i eventualment donar lloc a una proliferació algal massiva.

L'entrada episòdica de nutrients i/o d'energia externa estimula el creixement del fitoplàncton, i especialment del més gran. Com que els episodis són limitats en el temps, és possible que els consumidors grans no siguin prou ràpids en respondre. Per tant aquests episodis tenen la capacitat potencial de desacoblar diferents parts de la xarxa tròfica planctònica, particularment els productors grans dels consumidors grans. Això pot dur per exemple a la formació de proliferacions de diatomees que poden sedimentar al fons, produint ocasionalment episodis d'exportació i segrest de carboni (Kiørboe 1993). La durada i freqüència d'aquests episodis determinaran al capdavall la possibilitat que el mesozooplàncton (de mida entre 200 µm i 2 cm) pugui respondre als increments en el microfitoplàncton. Si els episodis són prou llargs i intensos per produir una resposta del fitoplàncton, però massa curts per permetre al zooplàncton reaccionar-hi, és probable que es produeixi la proliferació. Aquesta situació es pot veure magnificada si els copèpodes, que constitueixen el principal grup de mesozooplàncton, es veuen negativament afectats per la turbulència en comptes de beneficiar-se de taxes més altes d'encontre.

Com més suau sigui l'espectre de mides, més curta serà la cadena tròfica, i més eficient serà la transferència d'energia i matèria als nivells tròfics superiors, inclosos els peixos. En altres paraules, la cadena tròfica clàssica hauria de ser relativament més important després d'episodis d'enriquiment per nutrients i de turbulència. Això és important almenys per dues raons: perquè la producció de peixos pelàgics pot ser major, i perquè es pot incrementar el segrest de carboni de l'atmosfera.

Canvis metabòlics i estequiomètrics

Tant el fitoplàncton com els bacteris necessiten nutrients inorgànics per al seu creixement. Això pot conduir a situacions de competència entre ells (Bratbak i Thingstad 1985, Thingstad *et al.* 1993, Thingstad i Rassoulzadegan 1995). Teòricament, degut a la forta dependència de la mida que tenen els efectes de la turbulència i de l'enriquiment per nutrients, s'espera que ambdós factors, sobretot quan es donen conjuntament, afavoriran relativament la dominància de fitoplàncton, particularment de diatomees, sobre els bacteris (Maar *et al.* 2002). D'aquesta manera la fracció de biomassa osmotròfica que és osmotròfica hauria de disminuir en condicions eutròfiques i turbulentes, és a dir, la importància del bucle microbià (Pomeroy 1974, Azam *et al.* 1983) s'espera que sigui menor. Es preveu que la turbulència sigui un potencial modulador del grau d'autotròfia de la comunitat planctònica. Encara més, els canvis observats en les poblacions de copèpodes (e.g. Alcaraz *et al.* 1988, Petersen *et al.*1998) poden incrementar aquesta tendència. De nou, això té implicacions en l'exportació i segrest de carboni (Legendre i Le Fèvre 1989, Thingstad i Rassoulzadegan 1995) així com en la producció de peixos (Kiørboe 1993).

Efectivament després d'un episodi important de fertilització la turbulència, en estimular el creixement del fitoplàncton, sobretot de diatomees (Reynolds *et al.* 1983, Estrada *et al.* 1987, Petersen *et al.* 1998, Oviatt *et al.* 1981, Arin *et al.* 2002), empeny el sistema cap a condicions relativament més autotròfiques (Alcaraz *et al.* 2002, Arin *et al.* 2002, Maar *et al.* 2002). Alcaraz i col·laboradors (2002) van trobar que el sistema es mantenia autotròfic durant més temps en tractaments *al.* 1998). At high levels, turbulence increases metabolic rates and reduces feeding efficiency by disturbing feeding currents or enhancing escape reactions.

Size structure

The slope of the biomass-size spectrum may be smoothed by the influence of turbulence (Arin *et al.* 2002, Cózar and Echevarría 2005) and of nutrient enrichment (e.g. Duarte *et al.* 2000, Samuelsson *et al.* 2002, Malits *et al.* 2004). Episodic nutrient input and turbulence are predicted to cause the dominance of microphytoplankton for two reasons: because these conditions favour the uptake of nutrients by large cells (bottom-up effect), and because small cells can not fully respond to these inputs because of a tighter control by their predators (top-down effect).

This top-down effect deserves some development. All specific metabolic rates, as for example growth, scale with body size following:

$$M \propto V^b \qquad (11)$$

where V is the organism volume and b is an exponent which is remarkably close to -1/4 for both unicellular and multicellular organisms (Banavar *et al.* 2002). That is, small organisms are more rapidly growing. The size ratio between predator and prey is fairly constant, between 10:1 and 100:1 (Kiørboe 1993 and references therein). The generation time scales with the inverse of the growth rate. The difference in generation times between prey and predator, and therefore the response time of grazers, tends to get longer the larger the organisms. As a consequence, the top-down control is stronger over small osmotrophic cells than over large ones (Kiørboe 1993, Franks 2001). Whereas any increase in growth of small cells will be quickly matched by growth in their predators, large cells are more likely to grow unchecked and to eventually result in a bloom.

Episodic inputs of nutrients and/or of external energy trigger phytoplankton growth, and especially large phytoplankton growth. As episodes are limited in time, it is possible that large consumers are not able to respond to them. Thus, episodes can potentially uncouple different parts of the planktonic food web, particularly large producers from large consumers. This may lead for example to the formation of diatom blooms which settle out to the bottom, eventually producing episodes of net carbon export and sequestration (Kiørboe 1993). The duration and frequency of such episodes will ultimately determine the possibility of mesozooplankton (zooplankton between 200 µm and 2 cm) to respond to the increases in microphytoplankton. If events are long and intense enough to produce phytoplankton response, but too short to allow zooplankton to react, likely there will be a bloom. This situation may be maximized if copepods, which constitute the main group of mesozooplankton, are negatively affected by turbulence rather than being favoured by higher encounter rates.

The smoother the size spectrum, the shorter the food chain, and the more efficient the transfer of energy and matter to the highest trophic levels, namely fishes. In other words, the classical food web should be relatively more important after events of nutrient enrichment and turbulence. This is important for two reasons: the production of pelagic fishes may be higher, and the sequestration of organic carbon may also be higher.

Metabolic and stoichiometric changes

Both phytoplankton and bacteria need inorganic nutrients for their growth. This can potentially lead to situations of competition between them (Bratbak and Thingstad 1985, Thingstad *et al.* 1993, Thingstad and Rassoulzadegan 1995). Theoretically, because of the strong size-dependence of the effects of turbulence and nutrient fertilization, it is expected that both factors, especially when occurring together, will relatively favour the dominance of phytoplankton, and particularly of diatoms, over bacteria (Maar *et al.* 2002). Thus, the fraction of osmotrophic biomass which is heterotrophic should decrease under eutrophic and turbulent conditions, that is, the importance of the microbial loop (Pomeroy 1974, Azam *et al.* 1983) is expected to be smaller. Turbulence is predicted to be a potential modulator of the degree of autotrophy of planktonic community. Furthermore, its observed effects on natural agitats, si bé tant la producció primària com la respiració eren més altes.

En condicions de forta limitació per nutrients, en canvi, la turbulència incrementa el percentatge de biomassa heterotròfica (Peters *et al.* 1998, 2002), bàsicament a causa d'una resposta positiva dels bacteris. Els bacteris són teòricament massa petits per beneficiar-se d'un increment en l'absorció de nutrients inorgànics (Karp-Boss *et al.* 1996) i normalment no responen a nivells naturals de turbulència ni en cultius ni en ambients lliures dels seus depredadors (Moeseneder i Herndl 1995, Peters *et al.* 1998, 2002, Malits *et al.* 2004). Tot i això el flux turbulent pot incrementar significativament l'absorció de substàncies dissoltes d'elevat pes molecular, induir canvis en la estructura de mides de la comunitat (Malits *et al.* 2004). La resposta positiva en bacteris sovint s'observa quan la xarxa tròfica microbiana és completa. S'ha hipotetitzat que la causa és un relaxament de la pressió de depredació sobre els bacteris per part dels flagel·lats heterotròfics, que seleccionen preses més grans i nutritives, això es, pico- i nanofitoplàncton (Peters *et al.* 1998, 2002). Els canvis descrits en la composició i estructura de mides de la comunitat condueixen a un increment en la quantitat total de carboni orgànic particulat (Peters *et al.* 2002) i a canvis en les relacions estequiomètriques (C:N:P) de la matèria orgànica particulada (Maar *et al.* 2002).

Així doncs els episodis de turbulència poden afavorir alternativament el component autotròfic de la comunitat o el bucle microbià i el reciclatge de nutrients, depenent del nivell d'enriquiment de nutrients que acompanya l'episodi. La situació d'elevada turbulència i baixes concentracions de nutrients, que és considerada com un espai buit en el mandala de fitoplàncton de Margalef (fig. 5), ¿podria ser de fet un espai heterotròfic dominat pels bacteris i pel fitoplàncton més petit?

Sedimentació

La impressió popular és que la turbulència manté les partícules en suspensió. Això sembla un fet en aigües somes (una badia, una tassa de cafè), on la barreja turbulenta ressuspèn partícules del fons (Garstecki *et al.* 2002). Tanmateix hi ha encara certa controvèrsia sobre els efectes de la turbulència en la sedimentació de partícules en aigües més fondes. Un treball experimental recent (Ruiz *et al.* 2004) indica, contràriament a la intuïció, que la turbulència de petita escala incrementa les velocitats de sedimentació de les partícules més denses que l'aigua. En canvi els models indiquen que quan es té en compte tota la capa de mescla, amb dissipacions decreixents amb la profunditat, la turbulència pot reduir la velocitat de sedimentació de les cèl·lules del fitoplàncton en alterar la seva distribució vertical (Ruiz 1996, Ruiz *et al.* 1996, Ross 2006). Aquest efecte serà més important per partícules que s'enfonsin ràpidament, i per tant la seva magnitud dependrà de l'estructura de mides de la comunitat i de l'estat fisiològic de les diferents poblacions (Ruiz *et al.* 1996). Tot i això, encara ens manca una comprensió completa de la interacció de la turbulència amb les partícules que cauen.

A més de la seva influència en les velocitats de sedimentació de partícules, la turbulència afecta indirectament el flux cap avall perquè altera l'estructura de mides del sèston (partícules en suspensió, tan vives com mortes). Tal i com hem comentat, la turbulència promou el creixement de cèl·lules més grans, sense mobilitat pròpia, i que poden enfonsar-se ràpidament, i la transferència de matèria amunt en la cadena tròfica. També incrementa l'agregació entre partícules (Kiørboe 1997). La velocitat de caiguda de les partícules es relaciona d'una forma directa amb la seva mida, segons la llei de Stokes. Per tant, les partícules més grans tenen, a priori, taxes de sedimentació més grans.

Resumint, la turbulència pot incrementar el flux de material dissolt a particulat, i al mateix temps l'enfonsament d'aquest fora de la capa de mescla. Per tant, fenòmens episòdics de turbulència poden no només estimular el creixement del fitoplàncton, sinó també provocar que una gran part d'aquesta nova matèria orgànica sigui exportada fora de la zona eufòtica per sedimentació. Com que la dinàmica dels episodis està lligada al patrons de canvi climàtic (IPCC 2007), la seva populations of copepods (e.g. Alcaraz *et al.* 1988, Petersen *et al.*1998) may increase this tendency. Again, this has implications on the export and sequestration of C (Legendre and Le Fèvre 1989, Thingstad and Rassoulzadegan 1995) as well as on the production of pelagic fishes (Kiørboe 1993).

After a strong nutrient input event turbulence pushes the system to relatively more autotrophic conditions (Alcaraz *et al.* 2002, Arin *et al.* 2002, Maar *et al.* 2002), by enhancing the growth of phytoplankton, particularly diatoms (Reynolds *et al.* 1983, Estrada *et al.* 1987, Petersen *et al.* 1998, Oviatt *et al.* 1981, Arin *et al.* 2002). Alcaraz *et al.* (2002) found the system to be kept net autotrophic longer in turbulent treatments, although both primary production and respiration were higher under turbulent conditions.

In stronger nutrient limiting situations, by contrast, turbulence relatively increases the percentage of heterotrophic biomass (Peters *et al.* 1998, 2002). This increase is due mainly to positive responses of bacteria. Bacteria are theoretically too small to benefit from increased inorganic nutrient uptake rates (Karp-Boss *et al.* 1996), and generally do not respond to natural levels of turbulence neither in cultures nor in predator-free environments (Moeseneder and Herndl 1995, Peters *et al.* 1998, 2002, Malits *et al.* 2004). However, turbulent flow may significantly increase the uptake of high-molecular-weight dissolved molecules and directly induce changes in the size-structure of the bacterioplankton community (Malits *et al*. 2004). The increase in bacterial abundance under natural turbulent conditions often happens only when the whole microbial food web is present. It has been hypothesized that the cause is a release in the grazing pressure over bacteria as heterotrophic flagellates can change to larger and more nutritious prey, namely pico- and nanophytoplankton (Peters *et al.* 1998, 2002). The described changes in the composition and size structure of the community lead to an increase in the total amount of particulate organic carbon (Peters *et al.* 2002) and to changes of the stoichiometric ratios (C:N:P) of the particulate organic matter (Maar *et al.* 2002).

Thus, turbulence episodes would at first either enhance the autotrophic component of the community or favour the microbial loop and accelerate the recycling of nutrients, depending on the nutrient input associated to the episode. The situation of high-turbulence-low nutrients is considered as void space in Margalef's Mandala of phytoplankton (fig. 5). ¿Could it be a heterotrophic space dominated by bacteria and small phytoplankton?

Sedimentation

The common impression is that turbulence keeps particles in suspension. This seems to be a fact in shallow waters (a coastal bay, a cup of coffee), where turbulent mixing resuspends particles from the bottom (Garstecki *et al.* 2002). However there is still some controversy about the effects of turbulence on sedimentation of particles in deep waters. Recent experimental work (Ruiz *et al.* 2004) points that, contrary to intuition, small-scale turbulence increases the sedimentation velocities of particles denser than water. Models suggest that when taking into account the whole mixed layer, with decreasing dissipations with depth, turbulence may decrease the sedimentation velocity of phytoplankton cells by altering their vertical distribution (Ruiz 1996, Ruiz *et al.* 1996, Ross 2006). This effect will be more important for fast sinking particles, and will depend therefore on the size structure of the community and on the physiological state of the different populations (Ruiz *et al.* 1996). However, a thorough understanding of the interaction of turbulence with settling particles is still lacking.

Besides its direct influence on sedimentation velocities of particles, turbulence is going to indirectly affect the downward flux by changing the size structure of seston (particles in suspension, both life and dead). As seen above, turbulence promotes the growth of larger and non-motile fast sinking cells and the transfer of matter up in the food chain through enhanced grazing rates. It also increases aggregation between particles (Kiørboe 1997). The sinking velocity of particles is in direct relation with the radius of the particles according to the Stokes' law.

comprensió és crucial per entendre els cicles biogeoquímics.

Variabilitat de la turbulència

La variabilitat espacial i temporal de la turbulència és clau per entendre els efectes de la mescla en els ecosistemes planctònics, així com en la distribució dels organismes planctònics. En el domini temporal, per exemple, s'han de tenir en compte la freqüència, intensitat i persistència dels episodis de turbulència. La freqüència dels episodis determinarà l'habilitat de la comunitat planctònica per adaptar-s'hi, i per tant pot modificar la comunitat a llarg termini. La seva intensitat determinarà quins organismes i processos seran afectats, en funció de la mida. Finalment la seva persistència determinarà el destí final del carboni produït, perquè pot explicar desacoblaments entre diferents grups de la xarxa tròfica, per exemple entre diatomees i copèpodes.

Hem repassat superficialment l'amplíssim rang d'escales a les quals la turbulència pot afectar els processos planctònics. És important entendre que la turbulència està distribuïda intermitentment a totes aquestes escales, tant en el domini temporal com en l'espacial (e.g. Jiménez 1997, Jou 1997). Fins i tot a les escales més petites (s, cm) està distribuïda en esclats i taques molt petits. La variabilitat temporal en aquestes escales, de l'ordre de segons, s'ha de comparar amb processos fisiològics i comportamentals, com ara l'absorció de nutrients, la producció i la captura de preses. Des del punt de vista dels organismes planctònics, s'ha d'entendre com un "oratge hidrodinàmic" (Catalan 1999). El tema de la distribució de la turbulència en aquestes escales, i dels seus efectes sobre el plàncton, tot i ser molt interessant està fora de l'àmbit d'aquesta tesi.

La nostra recerca es basa en el tema de la variabilitat a unes escales temporals més llargues (d'hores a dies, de metres a decàmetres), el que podem anomenar "clima hidrodinàmic" (Catalan 1999). A aquestes escales la distribució de la turbulència està lligada a la variabilitat en els seus forçaments, per exemple en el vent. La majoria de l'energia que entra en el sistema es concentra en el rang entre hores i dies (fig. 8), que és també el rang de les taxes demogràfiques, com ara les de creixement o les de depredació, i que pot ser estudiat avaluant els canvis en biomassa. Els episodis de turbulència i les entrades de nutrients que s'hi associen es poden veure com pertorbacions de l'ecosistema planctònic. Com que aquests rangs temporals són propers als temps de generació dels organismes, pot ser que la turbulència allunyi constantment el sistema de l'equilibri, cosa que pot ajudar a explicar l'alta diversitat d'organismes planctònics (Hutchinson 1961, Kemp i Mitsch 1979).

Hi ha pocs estudis que hagin investigat experimentalment el tema de la variabilitat temporal en la turbulència i en l'entrada de nutrients. Eppley *et al.* (1978) barrejant diàriament grans contenidors experimentals (1700m^3) van trobar que les cadenes de diatomees més grans de 100µm eren dominants durant tot l'experiment, mentre que en els tractaments quiets els flagel·lats esdevenien més importants cap al final. Estrada et al. (1988) van sotmetre comunitats planctòniques naturals a una barreja vigorosa amb una periodicitat de 2-3 dies. Van trobar que el sistema responia fluctuant a freqüències més baixes que les del forçament, cosa que, segons els autors, és un tret típic de sistemes no-lineals. Alcaraz *et al.* (2002) van sotmetre microcosmos de 15 litres a tres nivells de turbulència: quiet, agitació continua (ε= 0.05 cm^{2}s^{-3}), i intermitent (2 dies quiet, dos dies a ε= 0.10 cm^{2}s^{-3}). Trobaren canvis en el metabolisme i en l'estat tròfic dels contenidors, deguts al patró d'entrada d'energia. Es va veure que els tractaments intermitents es mantenien autotròfics durant més temps.

Altres estudis ecofisiològics, centrats principalment en dinoflagel·lades formadores de marees roges, han estudiat les respostes de diverses espècies a cisallaments intermitents en el temps mitjançant cilindres de Couette (Gibson i Thomas 1995, Juhl *et al.* 2000, 2001, Juhl i Latz 2002). Gibson i Thomas (1995) van observar que una breu exposició diària a un cisallament alt pot inhibir el creixement d'aquests organismes. Altres estudis (Juhl *et al.* 2000, 2001, Juhl i Latz 2002) han demostrat que la

Therefore, *a priori* larger particles means higher sedimentation rates.

In summary, turbulence may increase the flux of dissolved to particulate material, and at the same time the velocity with which this material sinks out of the mixed layer. Episodic turbulence events may therefore not only stimulate the growth of phytoplankton, but also cause a large part of the newly produced organic matter to be exported out of the euphotic zone by sedimentation. As dynamics of episodes is strongly linked to global change patterns (IPCC 2007), their understanding is crucial to comprehend biogeochemical cycle.

Variability of turbulence

The spatial and temporal variability of turbulence is key to understand the effects of mixing on planktonic ecosystems, as well as on the distribution of planktonic organisms. In the temporal domain, for example, frequency, intensity and persistence of turbulence episodes (above a certain background level) must be taken into account. The frequency of episodes is going to determine the ability of the planktonic community to adapt to them, and may thus shape the community in the long run. The intensity will determine which organisms and processes will be affected by the episode, according to size and specific sensitivity. Finally the persistence of the episode will determine the fate of the carbon produced, because it may explain uncouplings between different groups of the trophic web, for example between diatoms and copepods.

We have had a quick look at the wide range of scales at which turbulence may affect planktonic processes. It is important to understand that turbulence is intermittently distributed at all these scales both in the spatial and temporal domains (e.g. Jiménez 1997, Jou 1997). At the smallest scales (s, cm), it is also intermittently distributed into tiny bursts or patches. The temporal variability at the smallest scales (in the order of seconds) must be compared with physiological and behavioural processes, such as nutrient uptake, production or prey capture rates. From the point of view of planktonic organisms, it may be understood as a "hydrodynamic weather" (Catalan 1999). The topic of the distribution of turbulence at these smallest scales, and of its effects on plankton, is most interesting but out of the scope of the present thesis.

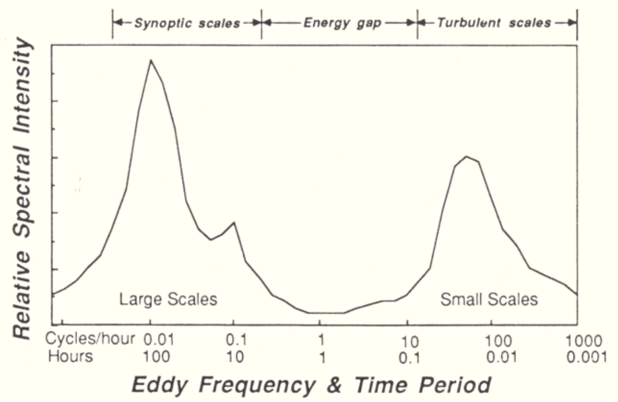

Figure 7: *Schematic spectrum of wind speed near the ground. Note that most of the wind energy is concentrated in the synoptic scales, in the range of hours and days. Taken from Stull 1988.*

Esquema de l'espectre de velocitat del vent prop del terra. La majoria de l'energia eòlica es concentra en les escales sinòptiques, en el rang d'hores a dies. Tret de Stull 1988.

We have based our research on the topic of larger scale variability (hours to days, metres to decametres), the "hydrodynamic climate" (Catalan 1999). At these scales, the distribution of turbulence is linked to the variability of the forcing parameters, such as wind (fig. 8). Most of the energy entered into the system from winds is concentrated in the range between hours to days, which is also in the range of demographic rates, such as growth and grazing that can be studied through changes in biomass. Episodic events of turbulence and the related nutrients inputs are perturbations of the planktonic ecosystem. As their time ranges are so close to the generation times of planktonic organisms, they may continually keep the ecosystem from reaching an equilibrium and may help explain the high diversity of planktonic organisms (Hutchinson 1961, Kemp and Mitsch 1979).

Few studies have approached experimentally the topic of temporal variability in turbulence and nutrient input. Eppley *et al.* (1978) mixed large experimental enclosures (1700m^3) daily and found that diatom chains >100µm were dominant throughout the experiment, sensibilitat de les dinoflagel·lades al flux turbulent depèn en gran mesura de la fase de creixement en què es trobi el cultiu, és a dir, dels seu estat fisiològic, i de la freqüència i persistència de l'exposició. D'aquesta manera la durada i freqüència dels episodis de turbulència en el cas de les dinoflagel·lades sembla ser molt important. Sovint les cèl·lules no poden dividir-se fins que hi torna a haver condicions de calma (Berdalet 1992, Berdalet *et al.* 2007).

L'estudi dels efectes de la turbulència en el plàncton: treball experimental o de camp?

L'estudi de la influència de la turbulència en organismes planctònics s'enfronta a diverses limitacions metodològiques. La majoria d'hipòtesis, sobretot les que fan referència a la turbulència de petita escala, són molt difícils de contrastar amb treballs de camp. En primer lloc, perquè hi ha altres paràmetres, com ara la llum, la temperatura o els nutrients, amb un fort efecte sobre el plàncton i que a més poden covariar amb la turbulència emmascarant el seu efecte net. A més els organismes es mouen amb l'aigua i és difícil de seguir els canvis en una massa d'aigua particular. Finalment, la turbulència és molt intermitent tant a l'espai com en el temps i a diferents escales d'observació. En resum, el sistema té massa graus de llibertat. Els estudis de camp s'han de basar en anàlisis estadístics conjunts de determinats paràmetres biològics sota un rang de situacions hidrodinàmiques (Pollingher i Zemel 1981, Haury *et al.* 1990, 1992, Sundby i Fossum 1990, Irigoien *et al.* 2000). Aquests estudis tenen l'avantatge de mirar a l'efecte integrat de la turbulència en l'ecosistema, tot i que pot ser complicat extreure conclusions prou robustes a causa de la quantitat de paràmetres que hi prenen part.

Tradicionalment els efectes de la turbulència s'han estudiat en recipients tancats. Això elimina el problema dels canvis advectius, i permet un control més estret de les condicions ambientals, com la temperatura o la llum. Aquests sistemes incrementen les possibilitats de contrastar hipòtesis, de trobar relacions de causa- efecte i de realitzar rèpliques per augmentar la potència estadística. L'inconvenient és que els resultats no són fàcilment extrapolables al mar. A més dels artefactes metodològics associats a posar organismes en sistemes tancats (canvis en el comportament, en la composició de la comunitat, en la dinàmica de creixement) hi ha el problema de generar turbulència realista, tant des del punt de vista quantitatiu com qualitatiu. Estudis més profunds sobre el tema de la generació de turbulència en sistemes experimentals es poden trobar a Peters et al. (1997) i Sanford (1997).

Un dels problemes principals és que les escales espacials d'entrada d'energia i generació de moviments turbulents són molt més grans en el mar que en el sistema experimental més gros. Això limita les possibilitats d'assajar els efectes de la mescla a gran escala, perquè no es pot tenir una cascada turbulenta completa. Tanmateix, diversos sistemes experimentals han reproduït amb èxit alguns dels aspectes de l'estructura vertical de la columna d'aigua, com ara la picnoclina (e.g. Donaghay i Klos 1985, Estrada *et al.* 1987). Altres estudis s'han centrat en simular les condicions fluctuants de llum a què es veu sotmès el fitoplàncton en diferents capes de mescla i intensitats de turbulència (e.g. Marra, 1978, Litchman 1998).

Els contenidors amb agitació són més adequats per a l'estudi dels efectes de la turbulència de petita escala. A aquestes escales les característiques de la turbulència són més independents de la seva generació (Sanford 1997), i tendeixen a la isotropia. Tot i això, que sapiguem ningú ha comprovat el grau d'isotropia en la majoria dels sistemes experimentals. La quantificació dels nivells de turbulència ha estat un problema important en els experiments de microcosmos. En primer lloc, no està clar quin paràmetre, si és que n'hi ha algun, és el millor descriptor de la turbulència tal i com l'experimenten els organismes. Els paràmetres que més s'utilitzen són la taxa de dissipació de l'energia cinètica turbulenta, la taxa de cisalla o de tensió i la difusivitat turbulenta. En segon lloc, la mesura de les velocitats del flux en els contenidors, sobretot quan aquests són petits, és difícil perquè requereix tecnologia especialitzada i sovint feta a mida. Com a conseqüència la determinació de la turbulència en els sistemes, quan s'ha fet, ha estat whereas in unstirred treatments small flagellates became more important at the end of the experiment. Estrada *et al.* (1988) subjected natural planktonic assemblages to nutrient fertilization and vigorous mixing with a periodicity of 2-3 days. They found that the system responded with fluctuations at frequencies lower than those of forcing. This is, according to these authors, a typical feature of non-linear systems. Alcaraz *et al.* (2002) subjected 15 L enclosures to three levels of turbulence: non-turbulent, continuously turbulent ($\varepsilon = 0.05$ cm^2s^{-3}), and intermittent (2 days still, 2 days at $\varepsilon = 0.10$ cm^2s^{-3}). They found changes in the metabolism and trophic status of enclosures due to the mode of input. Intermittent turbulent enclosures were found to keep autotrophy for a longer time.

Other ecophysiological studies, focused mainly on red-tide forming dinoflagellates, have studied the responses of several species to temporal intermittent laminar shear flows using Couette devices (Gibson and Thomas 1995, Juhl *et al.* 2000, 2001, Juhl and Latz 2002). Gibson and Thomas (1995) reported that a brief daily exposure to high turbulent shear is enough to inhibit the growth of these organisms. Other studies (Juhl *et al.* 2000, 2001, Juhl and Latz 2002) have shown that the sensitivy of dinoflagellates to turbulent flow depends on the growth phase of the culture, i.e. on its physiological state, and on the frequency and persistence of exposure. Thus, the duration and frequency of the turbulence events in the case of dinoflagellates seems to be important. Often times, cells will not divide until calm conditions are re-established (Berdalet 1992, Berdalet *et al.* 2007).

Study of turbulence effects on plankton: experimental vs. field work?

The study of the influence of turbulence on planktonic organisms faces several methodological constraints. Most hypotheses, especially those concerning small-scale turbulence, are very difficult to test in the field. In the first place, because there are many other co-varying parameters, some of them with a strong effect in plankton (e.g. light or temperature or nutrients), which can obscure the effects of turbulence. Besides, organisms are advected with the water and it is difficult to track the changes of a particular water mass. Finally, turbulence is highly intermittent in time and space and at very different scales of observation. In summary, the system has many degrees of freedom. Field studies therefore must rely on joint statistical analyses of biological parameters under a range of different hydrodynamic situations (Pollingher and Zemel 1981, Haury *et al.* 1990, 1992, Sundby and Fossum 1990, Irigoien *et al.* 2000). These studies have the advantage of looking at the integrated effect of turbulence on the ecosystem, although robust conclusions may be difficult to draw because of the number of parameters playing a part.

Traditionally, the effects of turbulence have been studied in controlled enclosures. This eliminates the problem of advective changes, and allows a tighter control of environmental conditions, like temperature light or other. The possibilities to test hypotheses, to find cause-effect relationships and to take advantage of replication to increase statistical power, are all characteristics of enclosure experiments. The drawback is that the results obtained can not be easily extrapolated to the field. In addition to the methodological artifacts associated with placing organisms in closed systems, such as uncontrolled changes in behaviour, in community composition, or in growth dynamics, we face the challenge of generating realistic turbulence, both quantitatively and qualitatively. More in depth treatments of the topic of generating turbulence in experimental containers can be found in Peters *et al.* (1997) and Sanford (1997).

One of the main problems is that the spatial scales of energy input and generation of turbulent motions are much larger in the sea than in the largest experimental enclosures. This limits the possibility to assay the effects of large scale mixing, because a whole turbulent cascade is not possible. However, several experimental systems have successfully reproduced some aspects of the vertical structure of the water column, like the pycnocline (e.g. Donaghay and Klos 1985, Estrada *et al.* 1987). Other studies have focused on the simulation of fluctuating light conditions as those experienced by phytoplankton realitzada utilitzant metodologies i sensors molt diversos, o bé diferents models teòrics. Això encara afegeix una nova dificultat a l'hora de comparar els estudis entre si o amb dades de camp.

Són molts els aparells emprats per a la generació de flux turbulent. Entre els més populars en recerca ecològica hi ha els de reixes o barres oscil·lants (e.g. Hopfinger i Toly 1975, Estrada *et al.* 1987, Peters *et al.* 2002), els agitadors (e.g. Berdalet 1992, Saiz i Alcaraz 1992, Colomer *et al.* 2005, NRC 2005), els sistemes de bombolleig (e.g. Eppley *et al.* 1978, Estrada *et al.* 1988), i els de pales (e.g. Donaghay i Klos 1985). Els cilindres de Couette han estat àmpliament utilitzats en l'estudi de la resposta dels organismes, sobretot de dinoflagel·lades, al cisallament laminar (e.g. Thomas i Gibson 1990, 1992, Thomas *et al.* 1995, Juhl *et al.* 2000, 2001, Karp-boss *et al.* 2000, Juhl i Latz 2002).

Sovint els nivells de turbulència utilitzats en aquests estudis han estat molt alts, fins al punt de no ser realistes. En els últims anys la tendència ha estat de reduir-los per tal d'aproximar les condicions experimentals a les naturals. Un nou pas en aquesta direcció ha de ser escurçar la durada dels experiments, o reproduir el patró temporal d'intensitat d'energia, perquè en el camp els episodis turbulents estan limitats en l'espai i en el temps. Per tant els efectes observats en la comunitat natural reclosa en un contenidor són difícilment generalitzables al camp després de diversos dies de turbulència intensa i contínua. El problema rau en el fet que tot i que la base de dades de mesures de turbulència al mar incrementa contínuament, no hi ha encara, que sapiguem, cap estudi sistemàtic de la seva distribució temporal i espacial. A més, tal i com ja hem mencionat, aquest conjunt de dades està probablement esbiaixat cap a nivells baixos (Peters i Redondo 1997) perquè el mostreig i la mesura sota condicions de molta agitació són complicats des d'un vaixell. Amb l'ancoratge de sensors físics i biològics d'alta freqüència aquesta manca de dades anirà desapareixent, al menys en zones costaneres. Això permetrà extreure conclusions robustes de les dades de camp i fer experiments ecològicament més rellevants.

Objectius i esquema d'aquesta tesi

Els fenòmens episòdics de turbulència, sobretot quan van acompanyats d'entrades de nutrients, poden estimular el creixement de cèl·lules fitoplanctòniques grans i sense mobilitat pròpia (bàsicament diatomees), i la seva agregació, cosa que deriva en episodis d'exportació de carboni. El nostre coneixement actual de la distribució espacial i temporal de la turbulència a la capa eufòtica a una escala sinòptica, i dels efectes d'aquesta variabilitat en els sistemes planctònics, és molt limitada. A més, tant la càrrega de nutrients en zones costaneres com la freqüència d'episodis meteorològics extrems estan sotmesos a canvis globals.

L'objectiu general d'aquesta tesi és:

Estudiar com la variabilitat en els forçaments, en concret en la turbulència, afecta la dinàmica de les comunitats planctòniques costaneres.

Hem abordat el problema des de diferents punts de vista i utilitzant tècniques diferents, sempre procurant lligar les dades de camp amb experiments de laboratori. Hem utilitzat series meteorològiques de vent per estimar la distribució espacial i temporal dels episodis de vent en les aigües superficials de la costa catalana (capítol 1). Per tal de millorar la interpretació de les dades generades al laboratori i aproximar-les a les condicions naturals, hem caracteritzat tant quantitativament com qualitativa la turbulència generada en dos dels aparells més utilitzats: reixes oscil·lants i agitadors orbitals (capítol 2). En el capítol 3 presentem els resultats d'un estudi de sèries temporals en què hem lligat l'estructura de mides i la composició de la comunitat osmotròfica planctònica de la Badia de Blanes a les entrades episòdiques de nutrients i d'energia externa. Al capítol 4 hem avaluat els canvis en la sensibilitat al llarg de l'any d'aquesta mateixa comunitat a episodis simulats de càrrega de nutrients i/o de turbulència a través d'experiments de microcosmos. Finalment, al capítol 5 discutim la significació d'aquests resultats com un primer pas en l'objectiu d'entendre els efectes de la turbulència en el plàncton en sistemes naturals.

under different mixed layer depths and turbulent intensities (e.g. Marra, 1978, Litchman 1998).

Enclosures with stirring are better suited for studying the effects of small-scale turbulence. At these scales characteristics of turbulence are more independent on the mechanism of generation (Sanford 1997), and they tend to be approximately isotropic, that is, equal in all spatial directions. However, to our knowledge no one has really tested the degree of isotropy in most experimental systems. The quantification of small-scale turbulence levels has been a major problem in microcosm experiments. In the first place, it is not clear what physical parameter (if any) is the best descriptor of the turbulence as experienced by planktonic organisms. The parameters used most often have been the turbulent kinetic energy dissipation rate, the shear rate (or the strain rate) and the turbulent diffusivity. Second, the measurement of flow velocities in the containers, especially when these are small, is difficult, because it requires of specialized, and often customized, technology. As a consequence the determination of turbulence in experimental enclosures, if done, has been performed with a diversity of sensors and methodologies, or using different theoretical models. This poses a difficulty when comparing different studies between them, or with field conditions.

The experimental devices used to study turbulence are many. Among them the most popular in ecological research are oscillating-grid or oscillating-rod systems (e.g. Hopfinger and Toly 1975, Estrada *et al.* 1987, Peters *et al.* 2002), shaker tables (e.g. Berdalet 1992, Saiz and Alcaraz 1992, Colomer *et al.* 2005, NRC 2005), bubbling (e.g. Eppley *et al.* 1978, Estrada *et al.* 1988), paddles (e.g. Donaghay and Klos 1985), etc... Couette cylinders have been used extensively to study the response of organisms, particularly of dinoflagellates, to laminar shear (e.g. Thomas and Gibson 1990, 1992, Thomas *et al.* 1995, Juhl *et al.* 2000, 2001, Karp-boss *et al.* 2000, Juhl and Latz 2002).

The turbulence levels used in these studies have often been high and even unrealistic. In recent years the trend in this kind of experiments has been to lower the turbulence levels in order to approach experimental conditions to nature. A next step in this direction must be to shorten the duration of experiments, or to reproduce a temporal pattern of energy intensity, because in the field turbulent events are episodic, limited in space and time. Therefore, effects on the enclosed natural community of strong turbulence after several days of treatment are hardly generalisable to the field. The problem lies in that, although the database of turbulence measurements in the field is continuously increasing, to our knowledge there has not been any systematic study addressing the temporal or spatial distribution of turbulence. In addition, as previously mentioned, this dataset is likely biased towards low levels (Peters and Redondo 1997), because sampling and measuring under strong turbulent conditions from a ship is difficult. With the mooring of high frequency physical and biological probes, this lack of data will disappear, at least for coastal areas. This will allow to derive robust conclusions from field data and keep refining ecologically relevant experiments.

Aims and outline of this thesis

Episodic inputs of turbulence, often associated to nutrient inputs, stimulate the growth of large non-motile phytoplankton cells (basically diatoms) and their aggregation, leading to events of carbon export. Our present knowledge of the temporal and spatial distribution of turbulence in the euphotic zone at a synoptic scale, and on the overall effects of this variability on planktonic systems is very scarce. In addition, both the nutrient load to coastal zones and the frequency and intensity of turbulence events are subject to global changes.

The overall aim of the present thesis is:

> To address how the variability in forcing factors, especially turbulence, affects the dynamics of coastal plankton communities.

We have approached the problem from different points of view and using different techniques, always trying to link field data with laboratory experiments. We have used meteorological time series of winds to assess the spatial and temporal distribution of wind events in the surface waters of the Catalan (NW Mediterranean) coast (chapter 1). As a mean to improve our interpretation of laboratory generated data we have we have characterized, both qualitatively and quantitatively, the turbulence generated in two of the most common devices used in the laboratory: oscillating grids and shaker tables (chapter 2). In chapter 3 we present the results of a time series study, in which we have linked the size structure and composition of the osmotrophic planktonic community in Blanes Bay to the episodic inputs of nutrients and external energy. In chapter 4 we evaluate the sensitivity of the osmotrophic planktonic community, throughout a whole year, to simulated nutrient load and turbulence episodes by means of microcosm experiments. Finally, in chapter 5 we discuss the significance of these results as a first step in our aim to understand the effects of turbulence on plankton in natural systems.

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Chapter 1: Analysis of wind events in a coastal area: a tool to assess turbulence variability for studies on plankton.

Anàlisi d'episodis de vent en una zona costanera: una eina per estimar la variabilitat de la turbulència per a estudis amb plàncton.

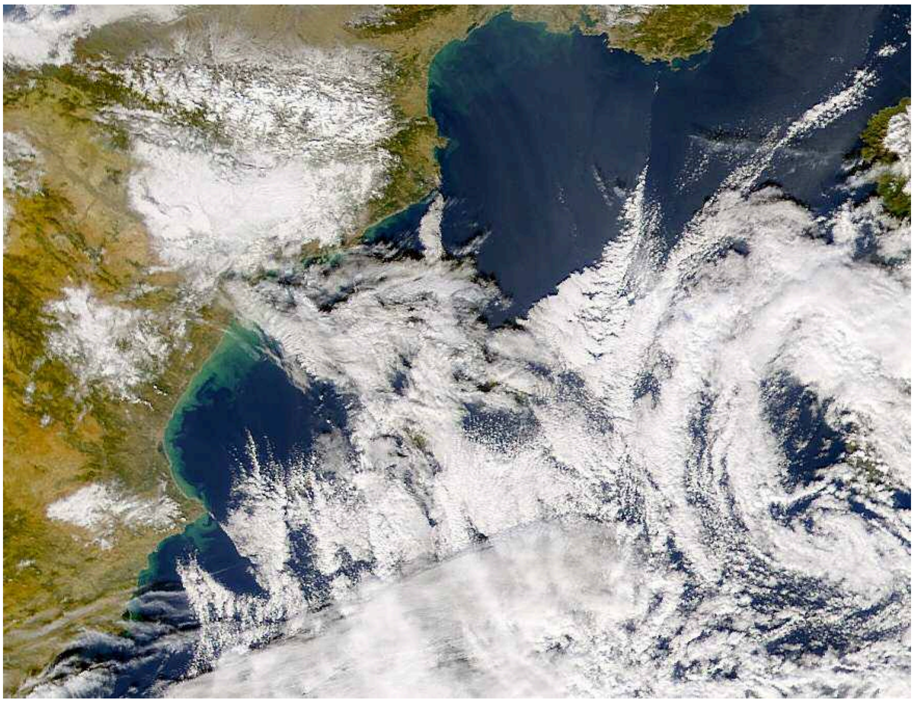

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Photo: Winter Storm sweeps across Europe in December 2001.

Visible Earth, NASA

Abstract

Turbulence at different scales, from generation to dissipation, influences planktonic communities. Many experimental studies have recently been done to determine the effects of small-scale turbulence on plankton, but it is difficult to state the relevance of the findings since there is little unbiased information on turbulence variability in the sea. In this study, we use wind velocity data series from several meteorological stations located along the Catalan coast to estimate the spatial and temporal variability of small-scale turbulence in the upper ocean. Using a peaks-over-threshold approach, we develop a statistical model to assess frequency of wind events, as a function of their persistence and intensity. Finally, the wind speed data series are converted into turbulent energy dissipation rate estimates at 1 m depth to determine the general distribution of turbulence in the Catalan coast. Geographical variability is larger than seasonal in frequency and persistence of wind events, owing to differences in local relief. These statistical models developed for wind events combined with empirical relationships between wind and turbulence, are tools to estimate the occurrence and persistence of turbulent events at a given location and season. They serve to put past, present and future studies of the effects of turbulence on coastal planktonic organisms and processes into context.

KEYWORDS: wind events, small-scale turbulence, plankton, peaks-over-threshold approach.

Resum

La turbulència a diferents escales, des de les de generació fins a les de dissipació, afecta a la comunitat planctònica. Recentment l'interès per els efectes de la turbulència d'escala petita s'ha desenvolupat molt, però és difícil establir la importància d'aquests estudis, donat que no existeix informació completa sobre la variabilitat de la turbulència al mar. En aquest treball utilitzem series temporals de dades de vent, de diverses estacions meteorològiques situades a la costa catalana, per a estudiar la variabilitat espacio-temporal de la turbulència d'escala petita. Mitjançant una aproximació POT ("peaks-over-theshold-analysis"), desenvolupem un model estadístic que permet estimar la freqüència d'episodis de vent en funció de la seva durada i intensitat. Amb l'objectiu de determinar la distribució de la turbulència a la costa catalana, les dades de velocitat de vent són utilitzades per estimar les taxes de dissipació de l'energia turbulent a 1 metre de profunditat. Pel que fa a la variabilitat en la freqüència i persistència d'episodis de vent, la geogràfica és més gran que l'estacional, degut al relleu local. El model estadístic desenvolupat, junt amb relacions empíriques entre vent i turbulència, poden ser utilitzats per a estimar la ocurrència i durada d'episodis en una localitat i estació donades. Serveix per posar en context estudis passats, presents i futurs sobre els efectes de la turbulència en el plàncton.

PARAULES CLAU: episodis de vent, turnulència de petita escala, plàncton, mètode "peaks-over-threshold".

Introduction

Marine plankton is generally small, below ca. 1 cm and has characteristic time scales for their life cycles and their energy and matter acquisition processes that range from minutes to weeks. During the past few decades, turbulence has been shown to influence planktonic organisms and processes at different temporal and spatial scales. At the larger scales, there is a redistribution of the populations and their resources. For example frontal zones (Holligan 1981), upwelling areas (Csanady 1989) and internal waves (Pingree *et al.* 1981; Dietze *et al.* 2004) increase the mixing of nutrients from nutrient rich water masses into waters where light is available to facilitate primary production. Turbulence also influences horizontal transport, sedimentation (Ruiz 1996; Ruiz *et al.* 2004) and resuspension of particles. At smaller scales, turbulence, and the shear derived from it, affects the flux of solutes to and away microscopic particles (Lazier and Mann 1989; Karp-Boss *et al.* 1996) and the encounter probability between particles (Rothschild and Osborn, 1988). Thus, it influences nutrient uptake (Karp-Boss *et al.* 1996; Maar *et al.* 2002), particle feeding processes (Marrasé *et al.*, 1990; Saiz and Kiørboe, 1995; Mackenzie and Kiørboe 2000), mating (Yen *et al.* 1998), and aggregation/ disaggregation processes (Kiørboe 1997).

Experimental efforts on this topic have been focused mainly on determining turbulence threshold values that trigger a response in plankton. Most of this empirical knowledge comes from laboratory studies under controlled and constant, on average, turbulence levels (Peters and Redondo 1996; Peters and Marrasé 2000). But turbulence is highly variable both in space and time. Thus, in order to better understand the roles of small-scale turbulence on plankton dynamics, it is necessary to know the frequency, intensity and duration of turbulence events. Direct measurements of turbulence in the ocean have been carried out with specialized sensors, such as free-falling airfoil probes (Osborn and Crawford 1980). However, measurements are scattered both in space and time and require relatively calm sea conditions, which may bias bulk turbulence estimations. More recently, acoustic Doppler velocimeters are becoming available to be moored for long term small-scale turbulence measurements (e.g. Gargett and Donaghay 2003). In order to extract annual variability in turbulence one needs at least several years of continuous data, which do not seem to be available yet.

The main mechanical energy input into the upper layer of the ocean is due to wind, mostly directly through shear induced by wind stress on the ocean surface. Turbulent kinetic energy dissipation in the upper layer of the ocean has been described by a boundary layer model, in which dissipation rates near the sea surface layer depend only on wind velocity and distance from the boundary (e.g. Turner 1973). In the uppermost layer -the so called wave-affected-surface-layer (WASL) - there is an enhancement of dissipation rates with respect to the boundary layer model due to wave-breaking (Terray *et al.* 1996; Gemmrich and Farmer 1999; Gemmrich and Farmer 2004; Stips *et al.* 2005) especially for very strong winds and high waves. Mechanisms such as Langmuir circulations (Gargett 1989; D'Asaro and Dairiki 1997; McWilliams *et al.* 1997), upwelling (Csanady 1989; Dewey and Moum 1990), tides (St. Laurent *et al.* 2002), internal waves (Sun and Kunze 1999) and other may participate in the energy input process. However, in areas with low tidal energy input (25 cm at maximum height in the Catalan coast), wind alone may be a reasonably good although conservative predictor of mean turbulence. Empirical models that relate wind speed over water with turbulence at a certain depth (Mackenzie and Leggett 1993) can successfully explain dissipation data found in the literature even in conditions where wind is not the only possible direct source of turbulence since the data used to generate the models is inherently affected by such processes. Such models can provide long records of the spatial and temporal variability of turbulence fast and easily, given that meteorological data is their input.

The aim of this study was to determine the bulk variability of turbulence in the upper layer of a coastal zone. We used wind velocity data series from several meteorological stations located along the Catalan coast (NW Mediterranean) to assess the spatial and temporal distribution of events in this area. It is not the purpose of this paper to predict specific turbulence events in a weather forecast-like fashion.

Methods

Data

Wind data series were obtained from 5 automatic meteorological stations of the Catalan Meteorological Service (SMC, http://www.meteocat.net) and of Puertos del Estado (http://www.puertos.es/) located on land along the Catalan coast, (fig. 1, additional info in Table 1). The temporal distribution and length of the series are shown in Table 2.

The stations recorded, among other meteorological parameters, wind speed (m·s^{-1}) and direction (degrees) as the vectorial average of the instantaneous values over an hour or a half hour intervals. When provided with a half hour frequency, we computed the hourly average. The minimum period of constant wind direction blowing in order to produce fully developed turbulence is still a matter of discussion. Nevertheless, there is some consensus that turbulence increases within one hour of increased wind speed (Dewey and Moum 1990). Oakey and Elliot (1982) also found a good correlation between turbulence measurements and the previous hourly averaged wind. Thus the use of one hour averages seems to be consistent with the mechanics of generation of wind-induced turbulence.

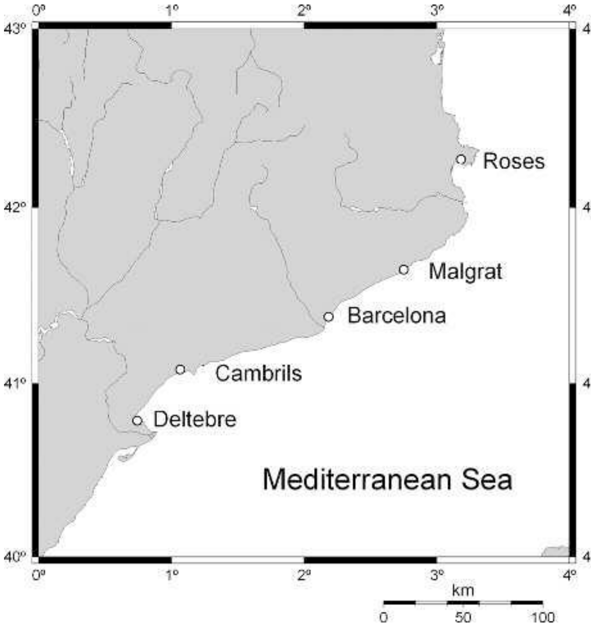

Figure 1: *Map of the Catalan coast showing the locations analyzed in this study.*

In order to detect long-term possible changes in anemometer conditions owing to malfunctioning or to changes in the surrounding terrain, moving averages of wind speed with a window of 90 days were plotted and examined. The series did not show any trend for any of the locations during the several years which the study covered.

Meteorological stations were chosen according to several criteria: 1) shortest distance to the coastline, 2) low height of location, 3) terrain as homogeneous and smooth as possible in order to have a low aerodynamic roughness length -the height where the wind speed

Table 1: *Information on the meteorological stations used in this study.*

Location	Latitude	Longitude	Distance to coastline	Station height	Anemometer height	Source
Roses	42º16’14”N	3º10’57”E	810 m	24 m	10 m	SMC
Malgrat de Mar	41º38’57” N	2º45’8” E	520 m	4 m	2 m	SMC
Barcelona Harbour	41º22’51” N	2º11’7”E	570 m	-	11 m	Puertos del Estado
Cambrils - Vinyols i els Arcs	41º4’51” N	1º3’58”E	1690 m	24 m	2 m	SMC
Deltebre-el Fangar	40º47’16” N	0º44’30”E	70 m	1 m	2 m	SMC

Table 2: Temporal distribution and length of series of wind data.

Location	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Roses	□□□□	□□□□	□□□□	□□□□	□□□□	□□□□	□□□■	■■■■	■■■■	■■■■	■■■□	□□□□	□□□□	□□□□
Malgrat	■■■■	■■■■	■■■■	■■■■	■■■■	■■■■	■■■■	■■■□	□□□□	□□□□	□□□□	□□□□	□□□□	□□□□
Barcelona	□□□□	□□□□	□□□□	□□□□	□□□□	□□□□	□■■■	■■■■	■■■■	■■■■	■■■■	■■■■	■■■■	■□□□
Cambrils	□□□□	□□□□	□□□□	□□□□	■■■■	■■■■	■■■■	■■■■	■■■■	■■■■	□□□□	□□□□	□□□□	□□□□
Deltebre	□□□□	□□□□	□□□□	■■■■	■■■■	■■■■	■■■■	■■■■	■■■■	■■■■	□□□□	□□□□	□□□□	□□□□

becomes zero (Stull 1988)-, and 4) a relatively even distribution along the studied coast.

Anemometers were positioned at 2 or at 10 meters over the ground (Table 1). When at 2 meters, wind speed data were rescaled to 10 m using the logarithmic wind profile (Stull 1988):

$$\overline{M_{10}} = \overline{M_n}\,\frac{\log\left(10/z_0\right)}{\log\left(n/z_0\right)} \qquad (1)$$

where the mean velocity at a height of n meters is $\overline{M_n}$ and the aerodynamic roughness length is z_0. The aerodynamic roughness length was assumed to be 0.03 m, corresponding to roughness class 1, i.e. open agricultural areas without fences and hedgerows and very scattered buildings and with only softly rounded hills (Trøen and Petersen 1989).

The integrity of time series was investigated and gaps of 2 hours or less were linearly interpolated. This longest acceptable interpolation was determined as follows. One year of data without any gaps was selected. Gaps of increasing length (from 1 to 12 hours) were introduced into the series and interpolated linearly. Coefficients of determination (r^2) were calculated between the interpolated values and the real values. For 2 hour long gaps, the r^2 was acceptably good (about 75%).

Wind events analysis

Wind data series were divided into four 3 months seasons, i.e. winter (January, February and March), spring (April, May and June), summer (July, August and September), and autumn (October, November and December).

To assess the number, intensity and persistence of wind events, a peaks-over-threshold (POT) approach was used. Events were defined as parts of the temporal series in which all the measurements of wind speed were over the given threshold. This kind of approach is common in statistics of extreme values, but here the aim was not to determine the occurrence of the extreme events but the general structure of all kinds of events. Therefore, a wide range of thresholds was used rather than only one.

For each geographical series and seasonal subseries an analysis was conducted to determine the number, intensity and persistence of wind events. A routine was designed to find events over thresholds which ranged from the mean value of the given series to 15 m s^{-1}, in intervals of 0.1 m s^{-1}, which was the resolution of the anemometers. In order to register an event it had to be complete, that is, it could not contain missing values, nor start or end with a gap. Otherwise, the event was rejected. When calculating the frequency of events, these rejections were taken into account and subtracted from the length of the series.

The arithmetic mean speed of each detected event, and its persistence expressed as number of hours the event lasted, was computed and recorded. For a given threshold, and for every persistence class p, frequency (f) of events was calculated as follows:

$$f = \frac{1}{L}\sum_{i=p}^{p_{\max}} N_i \qquad (2)$$

where L is length of series expressed in seasons or in years, p_{max} is maximum observed persistence for that particular threshold, and N_i is number of events of persistence i. Thus, frequency expresses number of events over a given threshold that persist at least p hours.

Turbulence data

Model 6 in Mackenzie and Leggett (1993) was used to convert wind speed into turbulent kinetic energy dissipation rate (ε):

$$\log_{10} \varepsilon = 2.688 \log_{10} U - 1.322 \log_{10} z - 4.812 \tag{3}$$

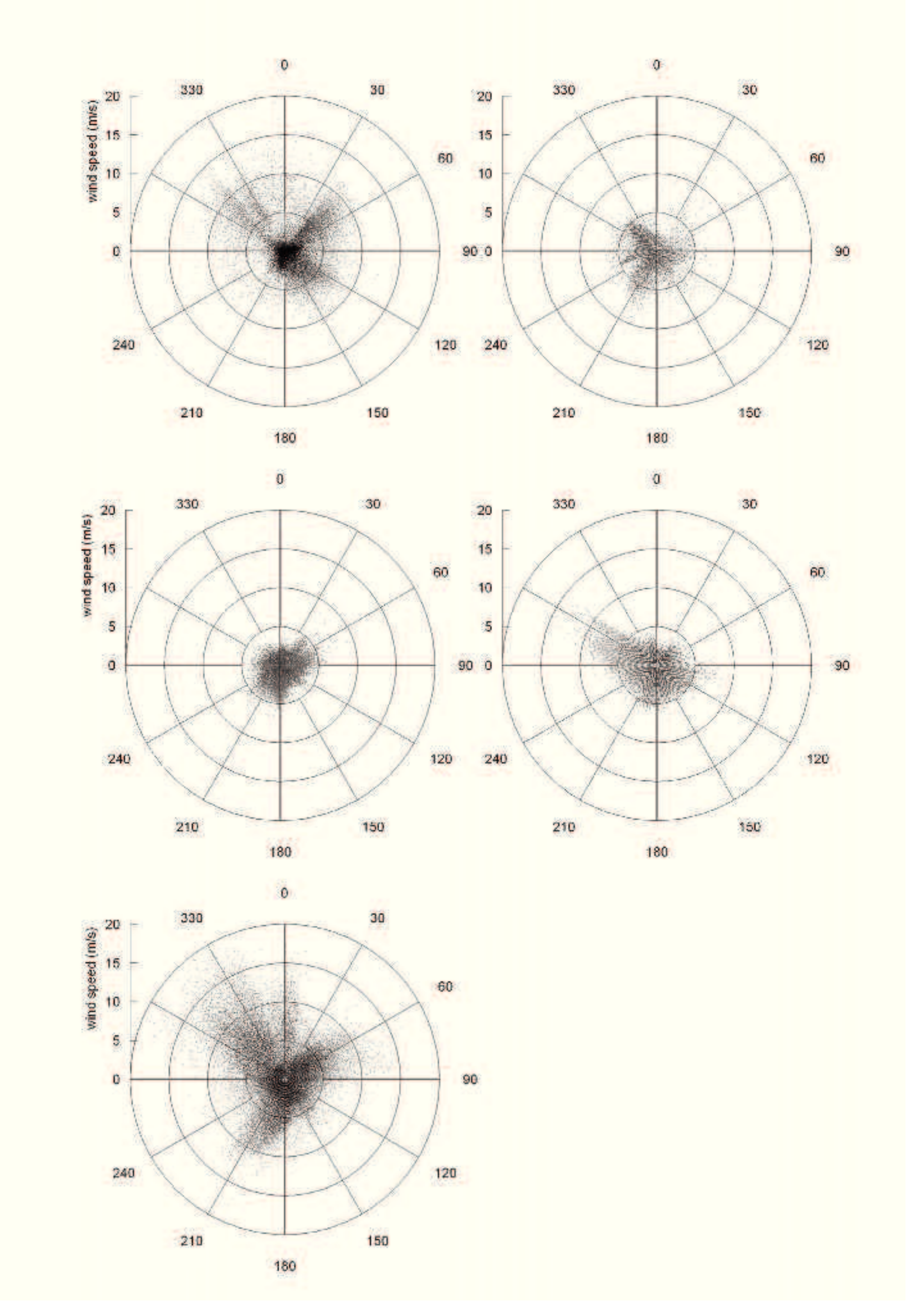

Figure 2: *Polar plots of wind intensity and direction for each location: Roses (A), Malgrat (B), Barcelona (C), Cambrils (D), and Deltebre (E).*

where ε is in $W \cdot m^{-3}$, U is wind velocity in m s^{-1} and z is depth in m.

We used model 6 in Mackenzie and Leggett (1993) since it is the most general among the ones put forth by these authors. It was developed using data from very different hydrographical situations, including coastal zones where the wind was not the only possible source of surface turbulence. This multiple regression model was originally generated from vertical ε profiles and daily averaged wind speed from the literature. Therefore in order to apply it, daily averages of wind speed were used, instead of the one hour frequency original data. Depth in this study was of 1 meter. We chose 1 m because many studies of turbulence effects on plankton have been carried out with surface-subsurface water and we deemed it most relevant. Other depths may be plugged in Eq. 3 at convenience. Statistical analyses were done using the Statistica 6 software package. From now on ε will be expressed in units of $cm^2 \cdot s^{-3}$.

Results

General wind conditions

There are several characteristic synoptic situations in the North Western Mediterranean (Reiter 1975). The more intense and persistent winds are land winds which blow from N or NW channelled through the coastal mountain ranges and are connected with the advance of an anticyclone from the west following a depression in the Mediterranean. The E and NE wind ("llevants") are very strong and characteristic in storm conditions ("llevantades") which are often associated with a depression in the South Western Mediterranean. "Garbins" are southwesterlies originated as strong SE sea breezes, modified by the Coriolis Effect. These southwesterlies are moderate winds typical from the summer that blow when a high pressure system embraces Southern Europe and the Mediterranean and also under similar calm-weather situations.

Polar plots for each location (fig. 2) show this general pattern to some degree, but there is geographical variability both in direction and speed along the Catalan

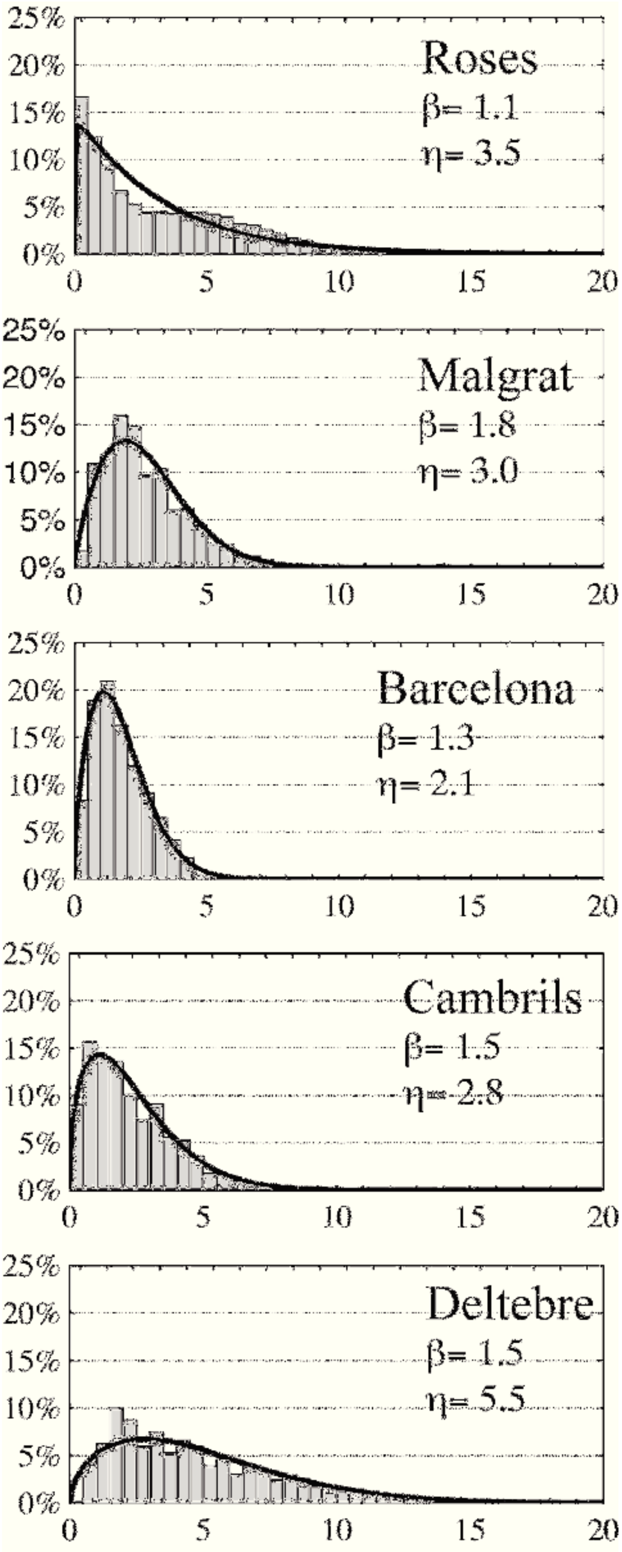

Figure 3: *Histograms of frequency of wind data for the five meteorological stations used in this study. The line corresponds to the fitted Weibull frequency distributions obtained using the maximum likelihood method. Scale (η) and shape (β) parameters are shown for each station.*

coast. This general pattern generated by synoptic situations is altered at each location because of local relief (García and Ballester 1984). It affects not only the dominant directions in each location but also the mean wind speed values, as winds can be channelled or blocked by the different topographical features. Geographical differences are, for this reason, much more conspicuous than seasonal differences. Local relief is marked by two ranges of mountains - the Serralada Litoral and the Serralada Prelitoral - that run parallel to the coast separated by a central depression. In the north and in the south the presence of the Pyrenees and of the Ebro Valley respectively have strong influence on wind patterns.

Inspection of Weibull probability plots of data for each different location showed that data fitted well to two-parameter Weibull distributions, except in the case of Roses, where the data are closer to an exponential distribution. The maximum likelihood parameter estimates for the Weibull distributions can be seen in Figure 3. Shape parameters are between 1 and 2. Scale parameters vary between 2 and 6. The highest wind speeds by far are found in Deltebre, and the lowest in Barcelona.

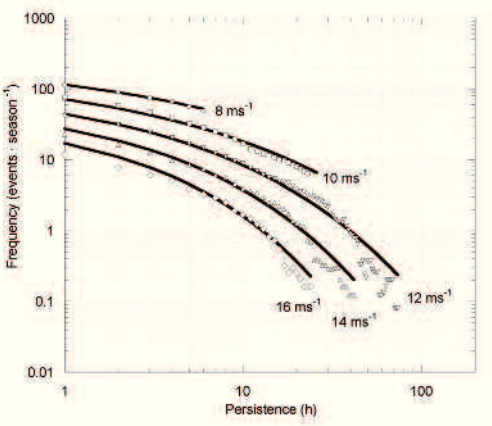

Figure 4: *Logarithmic plot of frequency expressed as events per season against their persistence in hours for Deltebre station. Points are measured frequencies of events with the following mean values: 8 m* s^{-1} *(circles), 10m* s^{-1} *(squares), 12 m* s^{-1} *(downward triangles), 14 m* s^{-1} *(upward triangles) and 16 m* s^{-1} *(diamonds). Lines are modelled frequencies using Eq.8.*

Distribution of wind events

The purpose of this part of the study was to establish the relationship between the frequency of an event (or its probability of occurrence) and its intensity and persistence. The relationship of frequency with persistence was examined first. For each threshold within the studied range, frequency (f) was found to be closely related to minimum persistence of events following a stretched exponential distribution of the form:

$$f = \mathrm{b}_1 \exp(-p^{\mathrm{b}_2}) \tag{4}$$

where p is minimum persistence of events, and b_1 and b_2 are parameters different for each threshold value. Stretched exponential probability density functions have been increasingly found in many fields (Frisch and Sornette 1997; Laherrère and Sornette 1998).

Then the relationship of the two parameters b_1 and b_2 in Eq. 4 with the threshold value (t) was investigated. The term b_1 fitted well to an exponential distribution, whereas b_2 was linearly related with threshold value:

$$\mathrm{b}_1 = \beta_1 \exp(-\beta_2 t) \tag{5}$$

$$\mathrm{b}_2 = \beta_3 + \beta_4 t \tag{6}$$

Combining Eq. 4, 5 and 6 we obtain:

$$f = \beta_1 \exp(-\beta_2 t) \exp(-p^{\beta_3 + \beta_4 t}) \tag{7}$$

This model allowed predicting the frequency of events that surpass a given threshold and a given persistence within the studied range.

Mean speed was calculated for each threshold and persistence class as the mean of all events within that class. This mean speed value ($\bar{U}$) had a very strong linear relationship with threshold value, so we can substitute threshold for mean wind velocity in Eq. 7:

$$f = \beta_1 \exp(-\beta_2 \bar{U}) \exp(-p^{\beta_3 + \beta_4 \bar{U}}) \tag{8}$$

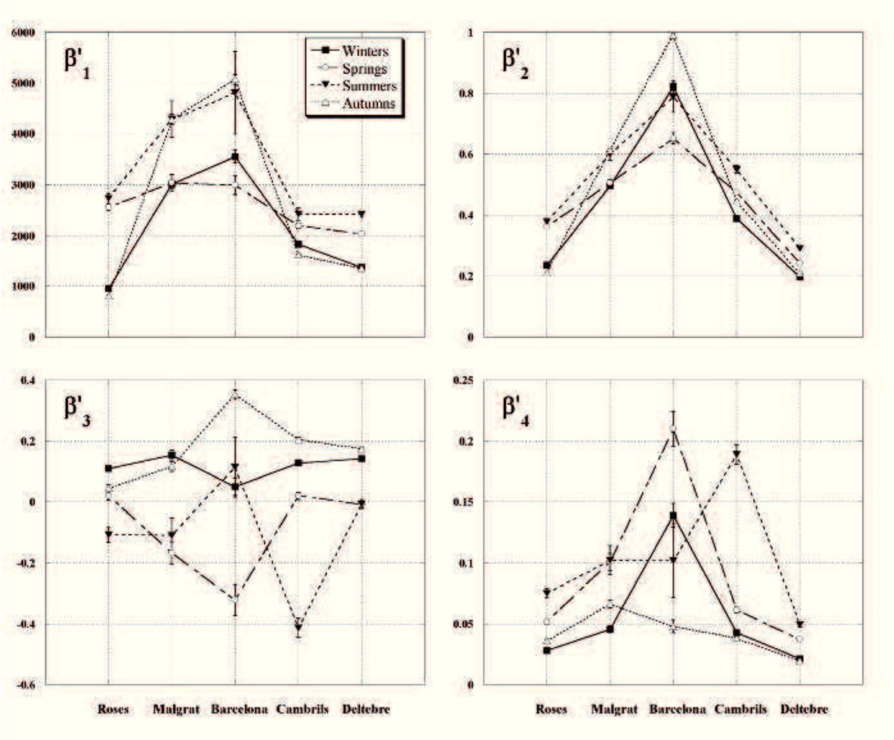

Figure 5: *Parameter estimations for the Eq. 7 model. Error bars are 95% confidence intervals. Squares: winter; circles: spring; diamonds: summer; triangles, autumn.*

Now, Eq. 8 relates the frequency of an event not with the threshold but with the mean value of the event. The best fit parameters of this model for the different series were found using nonlinear least-squares data fitting (Gauss-Newton method). Initial values for the β' parameters were: β'_1=1000; β'_2=-0.5; β'_3=0, β'_4=0. Found models fit very well to data, with R^2 always >90% and in most cases >98%. β'_1 ranged between 800 and 5000; β_2' from 0.20 to 1.00; β'_3 from -0.41 to 0.35, and β'_4 from 0.02 to 0.21. An example of the resulting distribution can be seen in figure 4.

These β' parameters are statistical tools and have no easy physical interpretation, because of the nonlinear nature of the distribution and cross-effects among the 4 parameters. β'_1 determines the maximum frequency, i.e., the frequency of events when persistence and mean tend to 0. β'_2 determines the steepness in relation with the mean, as the proportion of low to high threshold events increases with β'_2. The last two parameters, β'_3 and β'_4 determine frequency of events in relation to their persistence. Higher values of these parameters imply that frequency of events decreases faster with persistence. Note that for the range of thresholds studied in each series, the exponent ($\beta'_3 + \beta'_4\bar{U}$) is always positive. The larger the exponent the steeper the decrease of frequency against persistence. For weak events (that is, when $\bar{U}$ is small), β'_3 has a larger relative importance, whereas when $\bar{U}$ is high β'_4 determines the shape of the distribution. Particularly β'_4 parameterizes the effect of threshold in the persistence of events. When β'_4 is high, frequency of long events with respect to short events will decrease quickly as we consider higher and higher thresholds.

Parameter estimations for the final model (Eq. 8) can be seen in figure 5. The highest maximum frequencies (β'_1) are generally found in summer, but they decrease quickly with increasing thresholds (β'_2), so that intense events are less frequent in summer than in any other season. Autumn and winter have, in general, longer events than spring and summer (β'_3 and β'_4). In summer and spring, as the exponent β'_3 is low, the decrease of frequency against persistence is smoother for weak events. On the other hand, β'_4 is lower for autumn and winter, which means that for strong events it is in these seasons that frequency decays more slowly for increasing

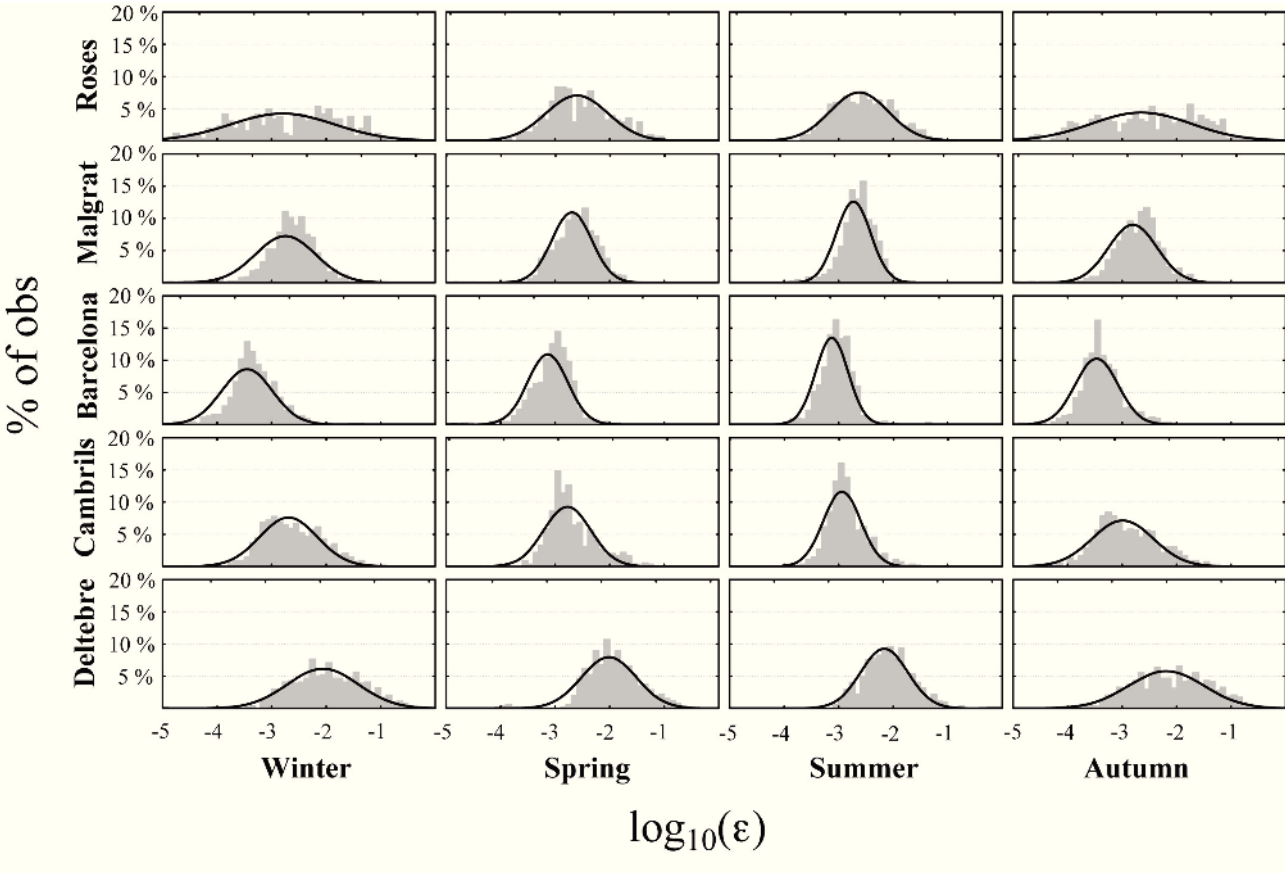

Figure 6: *Frequency distributions of log10(ε) estimated values at 1 m depth from wind velocity using model 6 in Mackenzie and Leggett (1993). Lines are fitted normal distributions obtained using the maximum likelihood method.*

persistences. What this means is that long weak events are more frequent in spring and summer and that strong events are not only more frequent in autumn and winter but also longer.

The most pronounced differences are, however, geographical rather than seasonal. Both β'_1 and β'_2 have a maximum in Barcelona, where mean speed is lower. That means Barcelona is where frequency of intense events is lowest. Frequency and intensity of wind events seem to be positively related with a shape parameter (or with mean wind speed). The difference of Barcelona harbour compared to other locations is probably caused by a higher aerodynamic surface length owing to the proximity of the city.

Turbulence

Mean ε values at 1 m depth for the different locations estimated from wind ranged between ca. $3.5 \cdot 10^{-3}$ and $2.0 \cdot 10^{-2}$ $cm^2\ s^{-3}$. The data clearly followed a dome-shaped frequency distribution (fig. 6), albeit a Shapiro-Wilks normality test was not quite significant. An ANOVA was performed with season and location as categorical factors. The number of data points within each subpopulation was always large (more than 250) so even if they were not normally distributed the results are expected to be robust (Motulsky, 2003). Both factors and their interaction were significant ($p < 0.01$ in all cases). Differences in mean values are very conspicuous between the different locations, whereas seasonal patterns, even if significant, are not so clear (fig. 7A). Variances were non-homogeneous as determined with Barttlet's test. This could be an artefact due to the deviations from a Gaussian found in the subpopulations (Motulsky 2003). An examination of variances (fig. 7B) shows consistent differences between different locations, as seen already for mean values (fig. 7A), and also between seasons. Winter and autumn have consistently more dispersion than spring, and especially summer. This

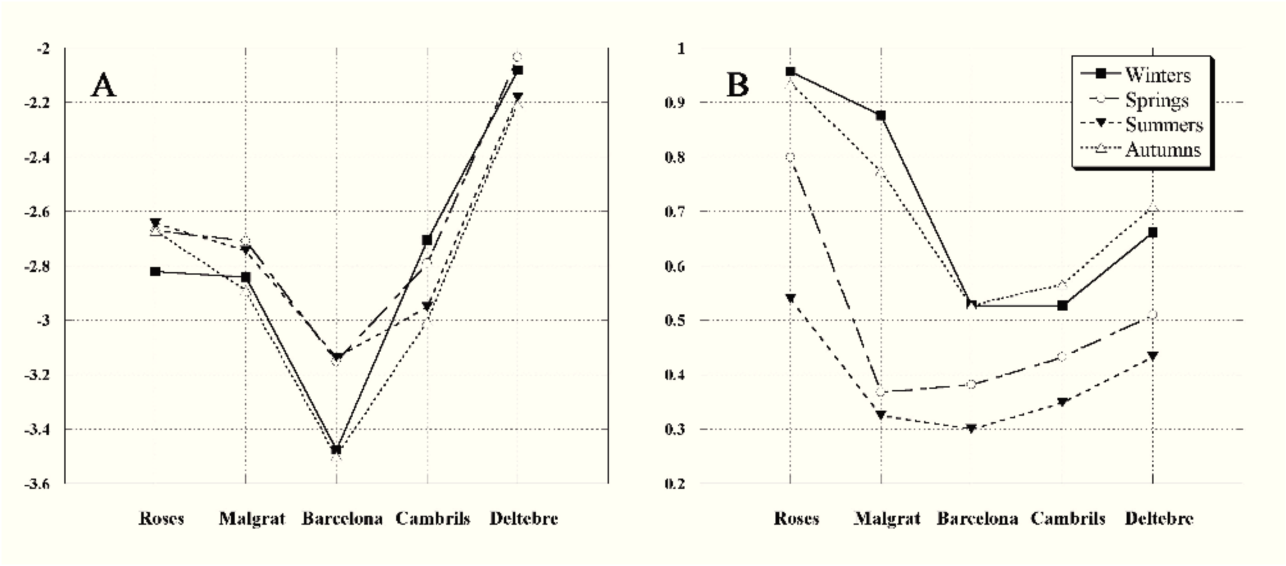

Figure 7: *Mean (A) and variance (B) of* $log_{10}(\varepsilon)$ *for the different locations and seasons. Symbols as in Fig. 5.*

means that even if differences in turbulence mean values are hard to distinguish between seasons, there still is a higher frequency of high turbulence levels in winter and autumn. This is consistent with the seasonal differences found in the β'_3 and β'_4 parameters from the wind events analysis.

Discussion

Applications of the model

Models describing the frequency of wind events can be used as estimators of the probability for a wind event with a given minimum mean value and a given minimum persistence to occur within a relatively long period (a season or a year). Despite of the large differences both in speed and direction of the wind between the different locations and seasons, the analysis conducted did not fail to fit a model to the data. It is therefore a powerful tool to assess the frequency and intensity of events of wind, and thus of turbulence variability. This descriptive capacity can be useful when dealing with wind-generated processes in which not only the threshold value is important but also the duration of the event, such as studies of gas transfer, including contaminants. This is the case of small-scale turbulence effects on plankton dynamics. Up to now most experimental work has been aimed at understanding how turbulence affects different communities and organisms, and at the determination of threshold values for these effects to appear. Therefore, in order to have detectable responses of the plankton community, experiments generally have been conducted under constant, relatively high turbulence conditions lasting for several days. But when the aim is to reproduce turbulence as found in the sea we have to consider time as an important factor. Even if relatively high levels of turbulence, such as those used in many laboratory experiments, are common in the sea, organisms may rarely experience them for a long time (compare fig. 6 with fig. 1 in Peters and Marrasé 2000).

Using models that relate wind and turbulence (such as those in Mackenzie and Leggett 1993), we can estimate the wind speed necessary to generate any given turbulence. The statistical models developed in the present study allow us to estimate the frequency of a wind event of given characteristics at a given location and see if it matches the time scales of planktonic organisms and their response to turbulence disturbance. Table 3 shows estimated frequency values for a range of turbulence levels normally used in experimental work.

Note that occurrence and intensity of turbulence events are very location-dependent, as is the wind distribution pattern. Extrapolations to other locations are difficult to make and parameters must be locally

***Table 3**: Estimated frequency values (number of events per year) for a range of turbulence levels usual in experimental work, for persistences of 1, 2, 4 and 8 days, for each location.*

ε (cm^{2}s^{-3})	$\bar{U}$ (m s^{-1})	Persistence (d)	Roses	Malgrat	Barcelona	Cambrils	Deltebre
$5\ 10^{-3}$	3.58	1	*	187.3 ± 66. 7	9.1 ± 5.5	110 ± 24.5	*
		2	*	113.9 ± 61.1	1.5 ± 1.5	63.2 ± 21.7	*
		4	*	62.5 ± 48.5	0.1 ± 0.2	32.1 ± 16.3	*
		8	*	30.2 ± 33.1	<0.1	14.1 ± 10.2	*
$1\cdot10^{-2}$	4.61	1	163.7 ± 32.2	53.3 ± 12.0	0.2 ± 0.4	41.6 ± 6.7	*
		2	101.4 ± 30.1	22.6 ± 8.2	<0.1	18.4 ± 4.8	*
		4	57.0 ± 24.5	7.6 ± 4.2	<0.1	6.6 ± 2.6	*
		8	28.5 ± 17.3	1.9 ± 1.6	<0.1	1.8 ± 11	*
$5\cdot10^{-2}$	8.30	1	15.4 ± 2.2	0.1 ± 0.2	<0.1	0.5 ± 0.6	65.5 ± 12.0
		2	5 ± 1.2	<0.1	<0.1	<0.1	31.1 ± 9.0
		4	1.1 ± 0.4	<0.01	<0.01	<0.01	12.2 ± 5.3
		8	0.2± 0.1	<0.01	<0.01	<0.01	<0.01
$1\cdot10^{-1}$	10.70	1	2.4 ± 1.1	<0.01	<0.01	<0.01	20.1 ± 2.8
		2	0.4 ± 0.3	<0.01	<0.01	<0.01	6.8 ± 1.6
		4	<0.01	<0.01	<0.01	<0.01	1.6 ± 0.6
		8	<0.01	<0.01	<0.01	<0.01	0.3 ± 0.1
$5\cdot10^{-1}$	19.27	1	<0.01	<0.01	<0.01	<0.01	0.1 ± 0.1
		2	<0.01	<0.01	<0.01	<0.01	<0.01
		4	<0.01	<0.01	<0.01	<0.01	<0.01
		8	<0.01	<0.01	<0.01	<0.01	<0.01

* *$\bar{U}$ is under the mean wind value for this location.*

estimated in order to apply the model. Data shown in Table 3 gives an idea of the extreme range of situations one can find near shore.

This information provides a tool to put previous biological work into an ecological relevance context and to address future studies on effects of turbulence, as they give us a range of situations an organism is likely to experience during its lifetime. In particular, these models can be used to design experiments dealing with effects of turbulence variability on plankton. This is especially interesting because most of the empirical knowledge on the topic comes from laboratory studies under controlled and constant, on average, turbulence levels (Peters and Redondo 1996; Peters and Marrasé 2000). The information of the frequency and intensity of events can help us address some pending questions. For example, is the mean turbulent field during a long time relevant for an organism's physiological response? or is the maximum level encountered (even if this maximum level is not sustained for a time period comparable to the processes of the organism) more important?

Another issue is how the time response of the different elements of the community can generate alternate scenarios. For example, usually, the first element to respond positively to turbulence in laboratory experiments is phytoplankton, particularly diatoms. Then bacteria increase because of the released DOM but also because of changes in the structure of the food web (Peters *et al.* 1998), and organic matter enters the microbial food web. The duration of the turbulent event in this situation is critical, since it can determine the fate of the organic matter produced, either sedimenting out of the system or going up the food web to higher trophic levels.

The observed large geographical differences in wind temporal patterns in a coastal area can also have implications in the distribution of different biological assemblages and particularly in the appearance of blooms of some phytoplankton groups, such as dinoflagellates, with a certain sensitivity to turbulence (Margalef 1978; Estrada and Berdalet 1998). It may be interesting to compare the rate of appearance of these blooms with the frequency and intensity of wind events in particular locations. This may be of special concern to the construction of harbours and breakers, the location of aquaculture facilities and the development of touristic areas, as they further alter the coastline and hence the local input of energy from wind into turbulence. Taking into account the distribution of wind events and their dominant directions when designing and managing coastal areas can help to minimize the appearance of harmful algal blooms or hypoxic zones.

Limits and improvements

These estimates of turbulence from wind speed are rather rough. Some improvements or refinements for further studies could include other possible sources of coastal turbulence. The model developed by Mackenzie and Leggett (1993) used here, included data from different hydrographic situations where it was known that wind was not the only source of turbulence. Even so, wind explained a significant amount of variability (54 %) of the data. But there are other possible sources of energy especially near shore (Holman 1995; St. Laurent *et al.* 2002), such as wave breaking, bottom shear stress or tidal energy. All these factors could increase our estimates of turbulence. On the other hand, the presence of a marked summer thermocline could reduce by an order of magnitude the level of dissipation down in the water column (Sherwin *et al.* 2002). Concerning the effect of wave breaking, the timescale of the model we used is of 24 hours, which is much longer than the observation scales of enhanced dissipation rates caused by the process. Thus, we are assessing a mean state rather than the particular effect of a local wave breaking phenomenon. The turbulence that we estimate has to be seen as a background turbulence, or a mean field, rather than as a precise instantaneous estimation. There is therefore a need for time series of direct turbulence measurements, at least at some locations, in order to validate and fine tune model results from wind.

Another important source of variation in wind generated turbulence can be distance from the coast. The meteorological stations used here are not located at sea but at some distance inland. In open water, wind speed tends to be higher as no obstacles are found. Therefore, we expect wind generated turbulence to be somewhat more important as we move away from the coastline. This hypothesis was tested using wind data from a meteorological station at the "Casablanca" oil drilling platform, located at about 35 km offshore from the southern Catalan coast. We applied the formula of Mackenzie and Leggett (1993) to assess the turbulence intensity at 1 m depth from daily averaged wind data. T-test for dependent samples between the estimated turbulence data from "Casablanca" and from Deltebre, the closest of our meteorological stations and the one with the highest wind intensities, revealed that wind generated turbulence was significantly higher in "Casablanca" ($N=283$, $p<0.001$).

The use of wind direction can be another element of refinement in models. When turbulence close to the coast is considered, winds blowing onshore will generate larger waves than winds blowing from land (García and Ballester 1984) since they have a longer fetch. This can translate into higher turbulence. In this study, all winds were considered equal.

Overcoming these uncertainties and fine-tuning the estimation of turbulence in water from wind data will require the use of long-time direct turbulence measurements. This contribution is a first approach to the study of the temporal and spatial variability of small-scale turbulence, particularly useful to the understanding of plankton dynamics that can also be used by other fields of biological oceanography, as well as in air-seawater interaction studies and in coastal engineering.

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Chapter 2: Evaluation of oscillating grids and orbital shakers as means to generate isotropic and homogeneous small-scale turbulence in laboratory enclosures commonly used in plankton studies.

Avaluació de reixes oscil·lants i agitadors orbitals com a eines per a generar turbulència de petita escala, isotròpica i homogènia, en contenidors de laboratori utilitzats freqüentment en estudis de plàncton.

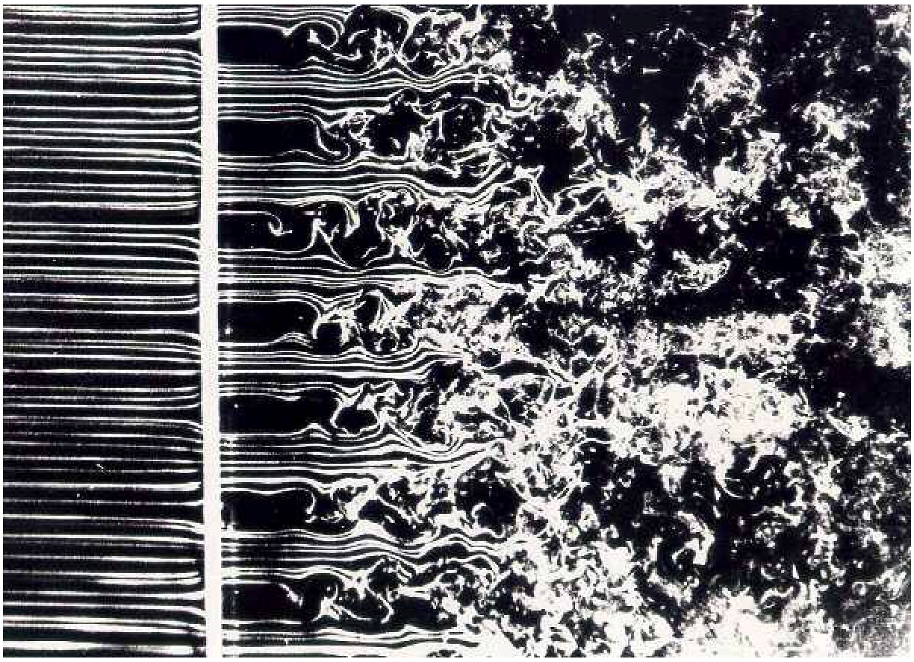

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Photo: Turbulence behind a grid

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Abstract

The effects of turbulent motion on planktonic organisms have mainly been studied in the laboratory with devices capable of generating controlled turbulent conditions. Assessments of hydrodynamics in such experiments are often inferred or simply not made. In this study we examine the suitability of two widely used systems to generate isotropic, homogeneous and stationary turbulence in laboratory containers: oscillating grid devices and orbital shaker tables. Turbulent kinetic energy dissipation rates were estimated from velocity measurements made with acoustic Doppler velocimeters. Both systems were shown to generate isotropic conditions in a relatively broad range of dissipation rates. Grid stirred tanks produce homogeneous turbulence as long as stroke length is comparable to the height of the container. Turbulence in orbital shakers is not completely homogeneous as it depends on the distance to the wall. Empirical models are derived as a tool for the calculation of dissipation rates in the two systems within the ranges and conditions examined in this study.

KEYWORDS: oscillating-grid, orbital shaker, turbulence, plankton, ADV.

Resum

Els efectes del moviment turbulent en organismes planctònics han estat estudiats sobretot en el laboratori, amb aparells capaços de generar condicions controlades de turbulència. Les avaluacions de la hidrodinàmica en aquests experiments han estat sovint inferides, o senzillament no s’han fet. En aquest estudi s’examina l’adequació de dos sistemes àmpliament utilitzats per generar turbulència isotròpica, homogènia i estacionària en contenidors de laboratori: aparells de reixes oscil·lants i agitadors orbitals. Les taxes de dissipació de l’energia turbulenta han estat estimades a partir de mesures de velocitat fetes amb velocímetres Doppler. Es demostra que ambdós sistemes generen condicions isotròpiques per a un rang relativament ampli de taxes de dissipació. Els contenidors agitats amb reixa produeixen turbulència homogènia sempre i quan l’amplitud del moviment sigui comparable a l’alçada de la columna d’aigua. La turbulència en agitadors orbitals no és completament homogènia perquè depèn de la distància a la paret del contenidor. Es deriven models empírics que, en el rang de condicions del present estudi, permeten calcular les taxes de dissipació en els dos sistemes.

KEYWORDS: reixes oscil·lants, agitadors orbitals, turbulència, plàncton, ADV.

Introduction

Turbulent flow is ubiquitous in aquatic systems and thus can potentially affect a wide range of planktonic organisms and processes. Turbulence is still often referred to as one of the unsolved problems in physics, and there has been a strong interest in its effects on plankton, especially during the last 20 years, resulting in a growingly active area of study (for a review see Peters and Marrasé 2000). Field studies on the effects of turbulence on plankton have been hindered by the lack of routine turbulence measurements and the difficulty to discriminate the effects of turbulence from those of other variables, such as temperature, light or nutrient concentration, which often co-vary. Therefore, much of the current knowledge has been derived from laboratory or enclosed systems, with configurations to generate controlled turbulence conditions.

Ideally the generation of small-scale turbulence in laboratory containers should conform to a few requirements in order to correctly assess the response of plankton to a certain level of turbulence in open water (i.e. not considering responses to turbulence close to bottom boundary layers). Firstly, turbulence should be constant, that is stationary in time and homogeneous in space. Although plankton experiences shifting turbulent conditions in nature, it is necessary to establish the responses to constant levels of turbulence before much more challenging non-stationary fields can be addressed. Secondly, the system should not induce changes in the behaviour or distribution of the organisms other than those directly triggered by water motion. And finally, organisms must perceive turbulence as "natural". This implies for example that all relevant scales influencing the investigated process should be contained in the fully developed cascade of turbulent eddies (i.e. within the inertial subrange of the turbulent energy spectrum). This is difficult since the scales of generation of turbulent motion in the field are much larger than in the containers used for experiments (Sanford 1997). One needs to be aware of the dimensions of the organisms and the process under study with respect to the dimensions of the container. Thus, a container used to study fish larvae-zooplankton contact rates must be much larger (relevant scales tens of centimetre to meters) than a container used to study nutrient uptake by phytoplankton (relevant scales millimetres to centimetre).

Turbulence at the small scales is generally isotropic, and much of the developed theory is based on isotropic, stationary, homogeneous turbulence. However there are a number of situations in which turbulence is anisotropic even at small scales; for example close to boundaries or in situations of strong stratification (Yamazaki 1990). The investigation of the influence of anisotropic turbulence on planktonic organisms has not yet been undertaken. As a first approximation, the experimental study of effects of small scale turbulence on plankton has been based on the assumption of isotropic conditions even if deviations from isotropy can also be found in containers, especially close to boundaries (having a preferred direction, i.e. the component normal to the border becomes restricted), with the size of the affected eddies decreasing towards the walls. Such situations are not considered in this paper.

As a consequence of this large set of requirements, the devices used to generate turbulence in enclosed systems are diverse, depending on the organisms or processes studied, on the technological and logistical limitations, and on the choice by the researchers. Some of these systems include Couette cylinders, shaker tables, oscillating grids, paddles, etc... (Peters and Redondo 1997, Sanford 1997). And this list is continuously increasing (e.g. Webster *et al.* 2004, Warnaars *et al.* 2006). Most of these devices have been used for decades in other research fields before they were adopted for studies on effects of turbulence on plankton. For example, shaker tables have traditionally been used to maximize the growth of cell cultures, but they are also routinely utilized in fields not related with biology such as dispersion of oil contaminants (NRC, 2005). Couette cylinders are used as viscosimeters in the study of fluid dynamics. Oscillating grids, a favourite of fluid dynamics experiments, have been extensively used in the study of sediment dynamics and mixing in stratified fluids (e.g. Rouse and Dodu 1955, Thompson and Turner 1975, Hopfinger and Toly 1976).

In order to try to meet ecologically realistic conditions, these devices are often used in biological experiments with settings outside the ranges defined in their original application and without a previous examination of hydrodynamics. A quantitative estimate of turbulence is rarely given, and when given, it is often based on theoretical estimations of the energy input, an approach that should be calibrated for each particular system with real measurements.

Turbulent kinetic energy dissipation rate (ε) is the parameter most widely used to characterize turbulence in biological experiments. It is defined as the rate at which the turbulent energy is dissipated to heat due to the molecular viscosity of the fluid. According to the Kolmogorov theory of isotropic homogeneous turbulence (Kolmogorov, 1941), turbulence intensity is uniquely determined by the energy dissipation rate in the inertial subrange, that is, in the range of scales between the input of mechanical energy and its dissipation as heat, in which energy is transferred from larger to smaller eddies at a constant rate. This parameter is usually estimated from measurements of flow velocity fluctuations. Velocity can be measured in an Eulerian way, as for example with laser or acoustic Doppler velocimeters (LDV or ADV), or in a Lagrangian way, as in particle tracking velocimetry (PTV) or particle image velocimetry (PIV).

In this paper we present turbulence measurements in two different systems commonly used in biological experiments: vertically oscillating grid systems and orbital shakers. We assess turbulence in an array of different container shapes and volumes using acoustic Doppler technology for direct measurements of turbulent velocity. The aim of this study is to examine the suitability of these two types of systems to generate homogeneously distributed, stationary and isotropic small-scale turbulence, in a wide range of container volumes and measurement conditions. Additionally, we have developed statistical models to easily estimate ε within the range of conditions examined in this study, without need for direct measurements of the turbulent velocity.

Materials and procedures

Data acquisition

In all experiments acoustic Doppler velocimeters (ADV, NDV from NORTEK) were used to measure all three Cartesian flow velocity components. ADV utilizes the Doppler effect for measuring the velocity. A beam of acoustic pulses is emitted by an acoustic transducer, and the pulses scatter back from particles moving with the flow to receiving acoustic transducers. The velocity can then be derived from the measured frequency phase shift of the sound wave in a cylindrical sampling volume that is about 9 mm in diameter and 3-9 mm in height. Each receiver measures the velocity along the direction of the axis between the transmitter and the receiver beam (the bistatic angle). By applying a conversion matrix it is possible to transform the along-beam velocities into two or three orthogonal velocities. Turbulence parameters are estimated by analysing the resulting velocity time series.

The data was acquired at a sampling rate of 25 Hz during at least 10 minutes for the oscillating grid systems and at least 5 minutes for the orbital shakers. There is no exact way of determining the adequate record length, as it depends on the size of the container (i.e. larger tanks need longer time series in order to resolve the larger eddies) and the current meter noise floor. Thus the choice of the record lengths was based on previous experience (e.g. Stiansen and Sundby 2001). Only time series with velocity correlations (directly output it by the sensor) consistently over 60%, and with signal-to-noise ratios consistently over 20 were used (McLelland and Nicholas 2000). The resolution for each of the three velocity components from the ADV is given to be 0.1 mm s^{-1} with a velocity bias of ±0.5% (instrument specification).

The sampling volume, the number of samples per pulse and the time lag between pulses were optimised for each measurement configuration, after visual inspection of the data, to avoid aliasing of the Doppler signal (Goring and Nikora 2002). Time lag is inversely proportional to the instrument noise for velocity measurements and was always set as high as the fluid velocity in the container allowed. Time lags that could lead to pulse-to-pulse interference were avoided.

Laboratory experiments were performed with unfiltered clean tap water at ca. 20ºC. To increase signal strength and reduce noise, water was seeded with hollow glass spheres with a density close to that of water and a size around 10 µm (Nortek A/S). The concentration of particles was ca. 50 mg·L^{-1}, i.e. ca. 7·10^{4} particles mL^{-1}.

For a precise determination of oscillation frequency, we used the autocorrelation function for each time series. Oscillation frequency was calculated as the inverse of the time lag of the first maximum in the function.

Turbulence estimation

The estimation of ε from single point velocity time series is usually done using two different approaches. In the first approach it is assumed that turbulence energy is mostly produced in the largest scales and transferred from large to small eddies until its dissipation occurs around the Kolmogorov microscale. We can then estimate the dissipation, that mainly occurs at small scales, from the rate of input of turbulent kinetic energy (TKE) in the largest eddies (Taylor 1935):

$$\varepsilon = \mathrm{A}u^3 / l \qquad (1)$$

where A is an universal constant assumed to be of order one (Tennekes and Lumley 1972), *u* is the root mean squared (rms) turbulent velocity, and *l* is the characteristic size of the largest eddies, also called integral length scale (Tennekes and Lumley 1972). The determination of *l* is not possible when measuring only at a single point. In oscillating grids systems, *l* is commonly assumed to be the mesh size (Peters and Redondo 1997), and in orbital shakers it can be assumed to be a length between the orbital diameter and the container diameter.

The second widely used approach consists in estimating ε from the energy spectrum, which is related to ε and to the wave number *k* in the inertial subrange:

$$E(k) = \alpha\varepsilon^{2/3}k^{-5/3} \qquad (2)$$

where α is a constant of ca. 1.5 in the three-dimensional case and 0.5 in the one-dimensional case (Tennekes and Lumley 1972). The problem with this approach is that spatial information, needed to compute the energy spectrum, can not be obtained from a single point velocity time series. However, when the mean flow velocity is larger than the turbulent fluctuations, it is possible to assume that the spatial structure of turbulence is not significantly changing as it is advected past the probe (Taylor's “frozen turbulence hypothesis”). Then, frequency can be converted from the temporal to the spatial domain in an energy spectrum. However, in laboratory systems, for example in oscillating grid systems, net mean flows are often insignificant and therefore this assumption is broken. There are different ways to overcome this problem. For example, the probe can be moved at a large and constant velocity to have a significant relative mean flow (e.g. Thompson and Turner 1975, Hopfinger and Toly 1976). In this study we used the linear regression method developed by Stiansen and Sundby (2001), which uses the energy spectrum approach. This method allows the calculation of turbulence independently of having a net flow, and therefore can be used both in different laboratory systems and in the field. Furthermore, it filters out the instrumental white noise due to the resolution of the ADV and electronic disturbances within the instrument. Dissipation was calculated for each velocity component of each whole time series. Then, the logarithmic mean of the three components was computed for an integrated estimate of ε.

In the case of oscillating grids systems, an alternative method for the estimation of ε from single point velocity time series may be used. ε may be determined as the mean rate of decay of TKE after the passage of the grid (Peters and Gross 1994). The drawback of this method is that it can only be used for oscillating grid systems and only in configurations where the grid is passing through the measurement point. An exercise of comparison between this method, the energy dissipation law method (eq. 1) and the method from Peters and Gross (1994) is presented in the “Assesment” section.

Measurements in oscillating grid systems

In physical studies turbulence is often generated through a grid oscillating rapidly with a small stroke. Turbulent velocities decay with the distance from the oscillating grid following a power law (Hopfinger and Toly 1976). The aim in these systems is to generate isotropic stationary turbulence at a certain distance from the grid rather than to have homogeneous conditions in the container. Similarly, in some biological experiments stroke length is small compared to the dimensions of the tank (e.g. Estrada *et al.* 1987, Howarth *et al.* 1993, Svensen *et al.* 2001). This method generates spatial gradients in turbulence and is useful for looking at effects on organisms localized in layers (Utne-Palm and Stiansen 2002), to study the effect of a gradient of turbulence (Seuront *et al.* 2004), or to reproduce the vertical mixing in a water column (Estrada *et al.* 1987).

An alternative approach is to use a relatively large grid stroke, comparable to container size, and a relatively low frequency of oscillation (Peters and Gross 1994). In this case the aim is to generate homogeneous and isotropic conditions throughout the mesocosm. We have focused our measurements on evaluating this last scenario. Three different vertically oscillating grid systems were used, one with 15 L cylindrical containers, another with 2 L cylindrical containers, and a third one with 2500L tanks (a two-grided system). In addition we used published data of 3 more systems: Peters and Gross 1994 (P&G94), Stiansen and Sundby 2001 (S&S01), and Utne-Palm and Stiansen 2002 (U&S02). Measurement conditions for all systems are listed in Table 1.

The 15 L system has been described in Peters *et al.* (2002). Containers were cylinders of 242 mm inner diameter and 345 mm of height. Grids were made of cylindrical bars with a thickness of 3.8 mm and had a mesh size of 14 mm. Grid diameter was 216 mm, which gave a distance of 13 mm to the wall of the container. Solidity, that is, the percentage of solid surface perpendicular to the direction of the movement, was 37.8%. This system allowed changing the frequency of oscillation (between 0.034 and 0.750 Hz) and the stroke length. The lowest grid position in the experiments was always 5 mm from the bottom of the container.

The 2 L system has been described in Colomer *et al.* (2005). Containers were cylinders of 129 mm inner diameter and 170 mm of height. Grids were as above but of 125 mm in diameter. This system allowed for the use of AC gear-head motors and a variable frequency controller, which could reduce speed to 1/20 of the nominal revolutions per minute (rpm). We used two motors, of 5 and 20 nominal maximal rpm, which gave a measured frequency range between 0.065 and 0.352 Hz. Again, the lowest grid position in all experiments was 5 mm from the bottom.

In oscillating grid systems, measurement with intrusive probes is not possible unless the grid has a mesh large enough for the probe to pass through the mesh holes, which was not the case for ordinary ADVs with most of the grids used in this study. To overcome

***Table 1**. Range of settings used for the measurements in the oscillating grid systems.*

	15 L cylindrical container	***2 L cylindrical container***	***2500 L tank***	***P&G94***	***S&S01***	***U&S02***
Volume (L)	7.0-15.0	2.0	2500	0.8	1600	62
Stroke radius (cm) *	4.0-14.0	2.0-7.0	2.8-31.2	8.5	5-18.5	20
Frequency of oscillation (Hz)	0.03-0.75	0.07-0.34	0.02-0.14	0.18-0.91	0.03-0.68	0.06-0.51
Measurement points	3	5	~10	5	9	26
Number of valid measurements	358	117	137	39	92	131

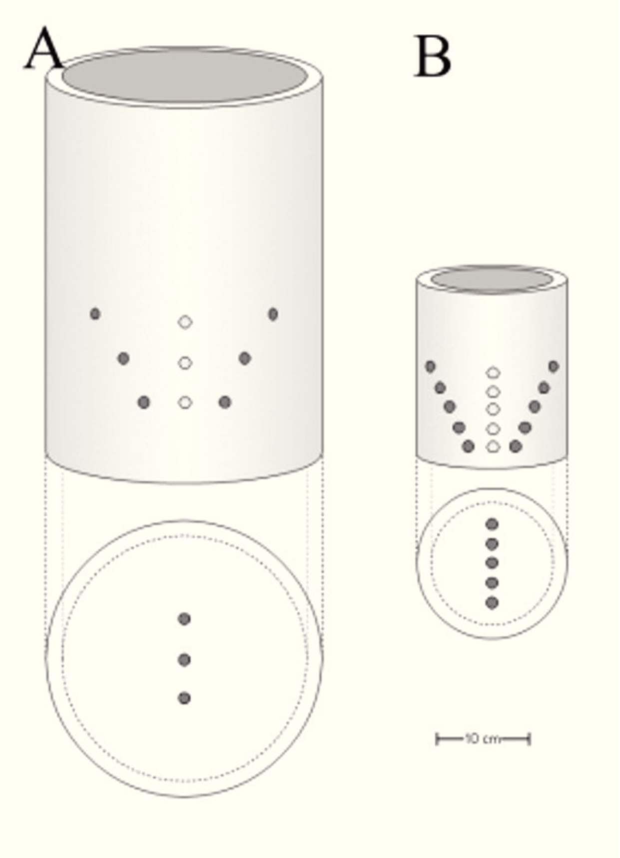

Figure 1: *Drawing of the containers used to measure oscillating grid generated turbulence. A 15 L container and B 2L container. Light circles are transmitter transducers and dark circles are receptors.*

this problem in the 2 L and 15 L cylinders, we used custom-made non-intrusive ADVs designed by Nortek. The acoustic transducers were embedded into the inner wall of the containers. For each measurement point, an array of 4 transducers was needed in order to have a three-dimensional velocity series. Each array consisted of one transmitter attached to the wall, and three receivers, two of them attached to the wall at the same height than the transmitter, and the third one located perpendicular to the measurement volume at the bottom of the container (fig. 1). All receivers were equidistant to the sampling volume so that the signal would reach them at the same time. This restricted the height of the sampling measurement points, since it had to be smaller than the diameter of the container. The transducers were similar to those found in the transmitter of conventional ADVs, and embedded in epoxy resin. Their acoustic frequency was 10 MHz. Containers were made of Delrin to minimize wall-reflected signals.

The grids were aligned to minimize grid bar blocking of the vertical sound beams. However, when the grid was at the same horizontal level as the sampling volume, both the transmitting signal and the two horizontal receiving signals could be partially blocked, and a drop in both velocity correlations and signal-to-noise ratios in the three beams could be detected. A complete blockage was not possible since the thickness of the grid (3.8 mm) was smaller than the width of the beam, which was similar to the 7-mm diameter of the transducer. The importance of this source of noise will depend on the time the probe was blocked, and therefore on the frequency of oscillation and on the velocity of the grid when passing through the sampling volume. This velocity will depend on the position of the sampling volume, on the stroke length and on the frequency of oscillation, so that the relative importance of the blocking will change with the settings. The noise will be more important for the smaller strokes and frequencies, that is, for the lower levels of turbulence. In general, this was a minor problem in the further analysis.

The 15 L containers had 3 sets of transducers (fig. 1A), resulting in three measurement points at a height of 70, 120 and 160 mm and at a horizontal distance to the nearest wall of 70, 120 and 82 mm, respectively. The 2 L system had 5 sets of transducers (fig. 1B) with measurement points at 23, 43, 64, 83 and 103 mm of height and 23, 43, 64, 46 and 25 mm distance to the nearest wall, respectively.

We also evaluated a system with 2500 L cylindrical tanks (diameter 1.44 m and height 1.48 m) that was used in mesocosm experiments which took place at the Biological station Espegrend (University of Bergen, Norway) in 2001 and 2002. In order to ensure constant temperature the tanks were further placed into larger tanks (5 m in diameter) filled with recirculating deep-water from the fjord. The purpose of the mesocosm experiments was to investigate the effect of turbulence on the lower levels of the food web. Biological results are not treated in this paper, but can be found elsewhere

(Metcalfe *et al.* 2004, Beauvais *et al.* 2006). Each system consisted of 2 vertically oscillating grids separated by 69 cm. The grids moved together at a given frequency powered by a pneumatic cylinder system. Changing of the stroke and the oscillation frequency controlled the generated turbulence levels. The distance from the bottom of the tank to the lowest position of the lowest grid was fixed at 25 cm in all tanks. Grid strokes ranged from 2 to 40 cm, oscillation frequencies ranged between 0.01 and 0.07 Hz. However, the oscillation of the grid was not a harmonic sinusoidal motion. The grids took between 1 and 2 seconds for the up or down movement (a little bit slower upwards than downwards due to gravity), and then were at rest until the next oscillation cycle. In this case, as mesh size was large enough for a conventional acoustic Doppler sensor to pass through the holes, the measurements were conducted with a standard Nortek NDV, which was placed in different positions within the tanks.

Measurements in orbital shaker tables

Shaker tables were some of the first devices employed in the study of turbulence effects on plankton mainly because they are common in many marine laboratories. They have been used with planktonic organisms ranging from bacteria (Moeseneder and Herndl 1995) to copepods (e.g. Saiz and Alcaraz 92), and have been especially important in studies with dinoflagellates (e.g. Pollingher and Zemel 1982, Berdalet 1992, Zirbel *et al.* 2000). They have also been used to study exopolymer particles (Stoderegger and Herndl 1999) and aggregation dynamics (Colomer *et al.* 2005). In most of these studies, turbulence was not determined, or estimates were based on theoretical approaches that have not been validated with data. The determination of turbulence in these systems will allow us to place historical data into an ecological context, and to evaluate the suitability of shaker tables for the study of small-scale turbulence effects on plankton.

Most measurements were done with an SBS AOS-5 orbital shaker with a range of frequencies of oscillation between 0.67 and 2.34 Hz and an orbit of 30 mm. Measurements were made in an array of containers commonly used in laboratories such as glass Florence and Erlenmeyer flasks and 2.5 l polycarbonate cylindrical Nalgene bottles. Table 2 lists the different containers, water volumes and number of measurement points. In order to test the effect of orbit diameter additional measurements were done in a FinePCR SH30, with and orbital diameter of 15 mm, and in a Heidolph Unimax 2010, with and orbit of 20 mm. The measurements with these two shakers were made only in the smallest container (the 1 L Florence flask) owing to weight constraints.

Turbulence was measured with a conventional side-looking 10 MHz ADV probe (Nortek NDV). The use of non-intrusive custom-made transducer devices was disregarded because of the irregular geometry and diversity of containers. The probe was mounted on a mechanical arm attached to the shaker table, so that the relative position between the sensor and the flask did not change. The number and the position of measurement points were constrained by the geometry of the container, the volume of water and the dimensions of the probe. Modifications were done to the mouths of the containers in order to allow the introduction of the probe. However, this should have no effect on the results since this modification was well above the water level in the containers. The probe was always positioned with the axis at 90 degrees with respect to the vertical. The sampling volume of the ADV was situated 50

Table 2. *Containers used in orbital shakers and conditions of measurement.*

	4 L Florence flask	***1 L Florence flask***	***4 L Erlenmeyer flask***	***2 L Nalgene bottle***
Volume (L)	3	0.75	3	2.6
Frequency of oscillation (Hz)	1.11-2.10	0.78-2.27	0.68-2.27	0.63-2.27
Nº measurement points	13	2	12	4
Nº of valid measurements	50	15	110	15

mm from the transmitter, and was positioned towards the center of the flask to minimize vortex shedding from the instrument. The sampling volume size was set at 9 mm.

Assessment

As a comparison exercise, velocity time series from the 15L cylindrical container in the oscillating grid system were processed using the three methods outlined in the turbulence estimations section, i.e. energy dissipation law method (equation 1), the linear regression method (Stiansen and Sundby 2001), and the TKE decay method (Peters and Gross 1994). For the dissipation law method the length scale used (l in equation 1) was the size of the mesh holes. In general ε obtained using linear regression method were highly correlated with those obtained with the other two methods, especially for dissipation rates higher than 10^{-4}cm^{2}s^{-3} (as assessed by linear regression method, fig. 2). The Pearson correlation coefficients between the logarithmically transformed series were in all cases higher than 0.92 (*p-value*<0.001). However, there were significant offset differences between methods. On average, Peters and Gross (1994) method yielded estimates 12.4 times higher than linear regression method, whereas energy dissipation law estimates were 2.5 times lower.

The range of turbulence levels that could be measured by linear regression method spanned over more than 8 orders of magnitude (from ε less than 10^{-6} to more than 10^{1} cm^{2}s^{-3} with oscillating grids and from ca. 10^{-7} to ca. 10^{2} cm^{2}s^{-3} with the shaker table). These ranges fit those previously measured using this method (Stiansen and Sundby 2001). For the less energetic situations, i.e. at lowest oscillation frequencies and, in the case of grids also at the shortest stroke lengths, an inertial subrange was often hard to identify. In these cases the number of data points valid to perform the regression was low, and the determination of the noise floor, uncertain. As a consequence the uncertainty of the estimates increases at the lowest values, and these must be treated with caution. On the other side, an upper limit to the measurable turbulence was not reached even if the peak of the dissipation spectrum can be smaller than the sampling volume (largest dimension 9mm), because the linear regression model extrapolates the highest frequencies, hidden by noise and lack of resolution, from the energy in the larger vortices (Stiansen and Sundby 2001). Yamazaki and Osborn (1988) gave the interval 10^{-6}–10^{2} cm^{2}s^{-3} for the possible energy dissipation rates in the ocean, with 10^{-4}–10^{-2} cm^{2}s^{-3} as typical values for the upper mixed layer (Veth 1983, and chapter 1). The range of ε obtained in this assessment therefore span the majority of turbulent situations encountered in nature.

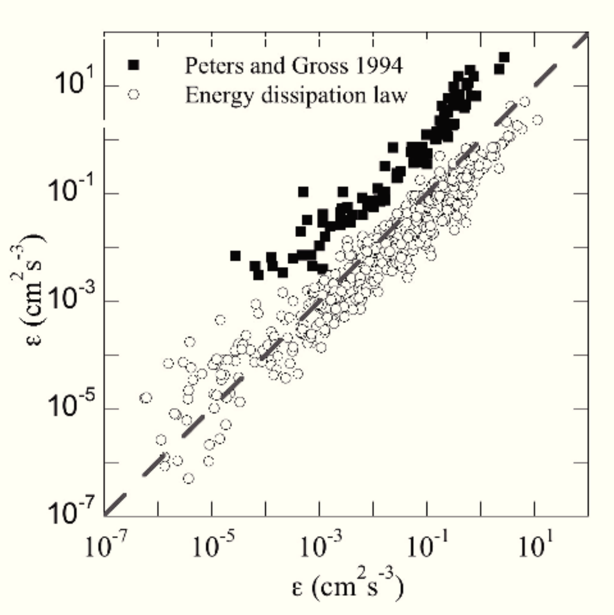

Figure 2: *Dissipation rates computed using the Peters and Gross (1994) method and the energy dissipation law against dissipations computed with the linear regression method. Data from velocity measurements in the 15 L cylinder oscillating grid system. Plotted lines are 1:1.*

The degree of isotropy is shown in fig. 3, where ε measured in one horizontal component (ε_u) is plotted against ε measured in the other horizontal component (ε_v) and in the vertical one (ε_w) for all the available systems. Differences between components in both systems were rarely greater than one order of magnitude and falling fairly close to the 1:1 slope. In order to assess the effect of grid solidity on the level of isotropy reached, we performed a series of additional measurements in the 15L grid system with three new grids (described in table 3). Remarkably, the different oscillating grid systems generated isotropic turbulence regardless of running settings, grid solidities or geometries. In the orbital

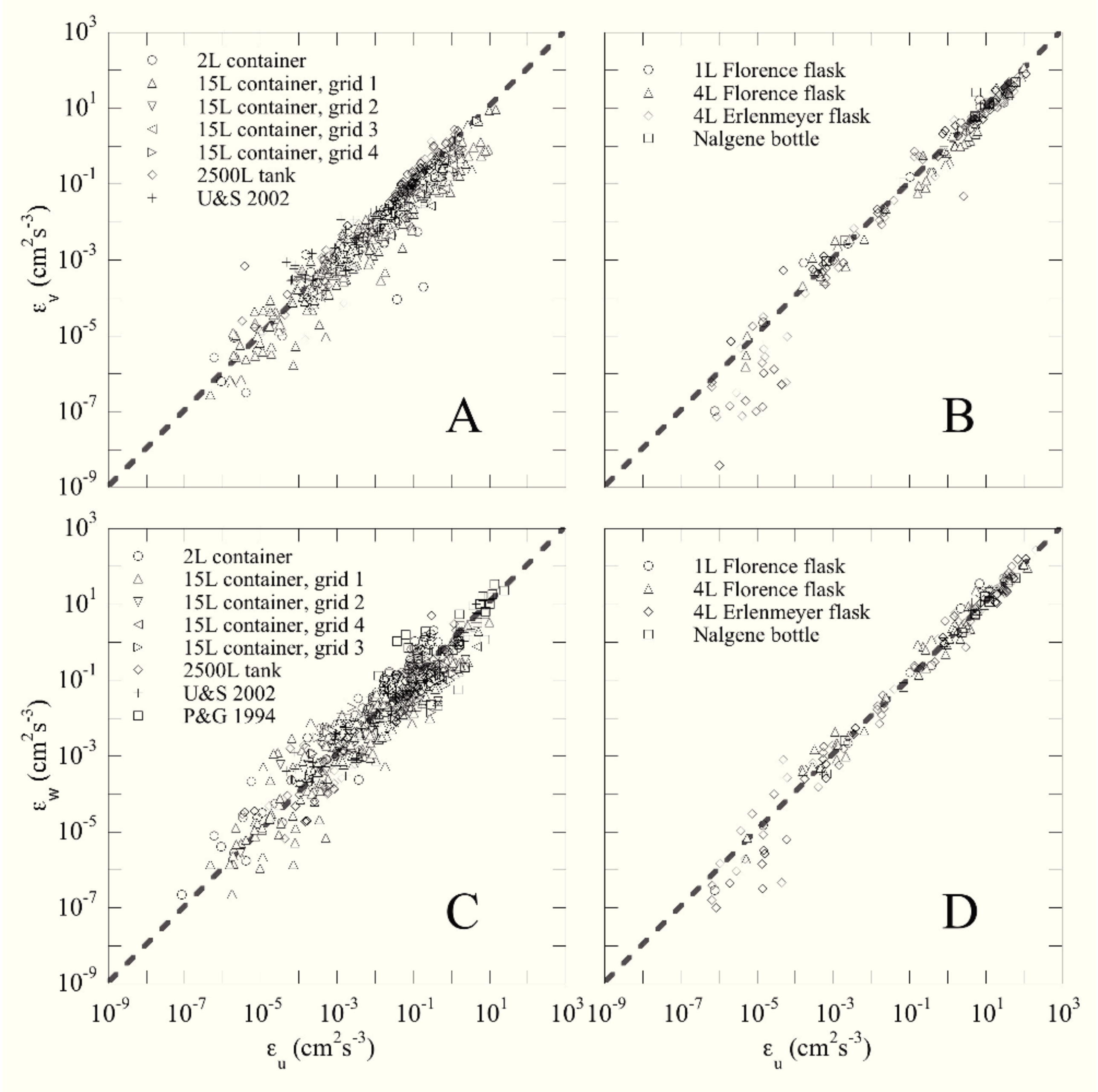

Figure 3: *Logarithmic scatterplots of ε computed from one of the horizontal velocity components and the vertical velocity component plotted against the ε computed from the other horizontal component. A and C correspond to measurements in the oscillating grid systems described in table 1 for the different grids defined in table 3. B and D correspond to measurements in the orbital shaker. Plotted lines are 1:1.*

shaker system, despite the fact that a mean circular horizontal flow is established within the container, turbulence was isotropic at least for the most energetic situations (fig. 3B and D). This isotropy is lost for $\varepsilon < 10^{-4}$ cm^{2}s^{-3} in our data. Below this value the inertial subrange was difficult to identify. This is due to lower signal-to-noise ratios and also to the motion possibly being in the transition zone to laminar flow.

The levels of turbulence achieved in both systems were strongly determined by the frequency of oscillation. Variability in turbulence levels was also influenced by differences in stroke length and grid geometry (for the oscillating grid systems), container volume and geometry and position of sampling measurement points relative to the walls and the bottom of the containers.

Table 3. *Estimated drag coefficients for the grids used in this study.*

Grid	**Container**	**Mesh pattern**	**Mesh size (cm)**	**Bar width (cm)**	**Bar section**	**S_a**	**Solidity**	**C_d**	**c.i.**
1	15 L cylinder	quadrangular	1.4	3.8	cylindrical	139	0.38	0.038	0.002
2	15 L cylinder	quadrangular	2.0	3.0	cylindrical	89	0.24	0.008	0.001
3	15 L cylinder	quadrangular	1.0	3.0	cylindrical	150	0.41	0.009	0.001
4	15 L cylinder	quadrangular	0.5	3.0	rectangular	223	0.61	0.020	0.002
1	2 L cylinder	quadrangular	1.4	3.8	cylindrical	46	0.38	0.009	0.001
5	Peters and Gross (1994)	rhomboidal	0.9	1.0	cylindrical	56	0.23	0.030	0.004
6	NTAP experiment	quadrangular			rectangular	5474	0.40	1.521	0.360
7	Utne-palm and Stiansen (2002)	cross	9.4	3.0	?	337	0.52	8.995	0.634

Deviations within one order of magnitude for the single components of ε can usually be neglected in practical biological applications and estimations. These deviations are due to a combination of measurements and analysis uncertainty, and also intermittency and unpredictability of turbulent motion. Therefore, the components should be averaged to reduce the deviation before used in a biological context.

Oscillating grid systems

Theoretical considerations

If the turbulent flow is steady and homogeneous the production of turbulent kinetic energy (*P*) must equal its dissipation (ε) (Tennekes and Lumley 1972). Following Peters and Gross (1994), in an oscillating grid system the energy input comes from the drag force *D* exerted by the grid in its movement:

$$D(t) = \frac{1}{2} C_d(t) \rho_w S_A v^2 \qquad (4)$$

where C_d is the drag coefficient, ρ_w is the density of water S_A is the solid area of the grid, and v is the velocity of the grid. If the grid follows a sinusoidal oscillation, velocity can be described as:

$$v(t) = s\pi f \sin(2\pi f t) \qquad (5)$$

where *f* is the frequency of oscillation and *s* is the stroke length, taken as the full amplitude of grid movement following Hopfinger and Toly (1976). The kinetic energy input in one oscillation is:

$$E = 2\int_0^{T/2} D(t) v(t) \mathrm{d}t \qquad (6)$$

where *T* is the period of oscillation. We can easily integrate this function to obtain the total energy input during one oscillation since we can assume that C_d is nearly constant with time (Higginson *et al.* 2003). Substituting equations 4 and 5 in the equation resulting after integration we obtain:

$$E = \frac{2}{3} C_d \rho_w S_A \pi^2 f^2 s^3 \qquad (7)$$

The rate of production of kinetic energy per unit volume is:

$$P = E T^{-1} V^{-1} \rho_w^{-1} \qquad (8)$$

where *V* is the volume of the container. Introducing equation 7 into equation 8 we obtain:

$$P = \frac{2}{3} C_d S_A \pi^2 f^3 s^3 V^{-1} \qquad (9)$$

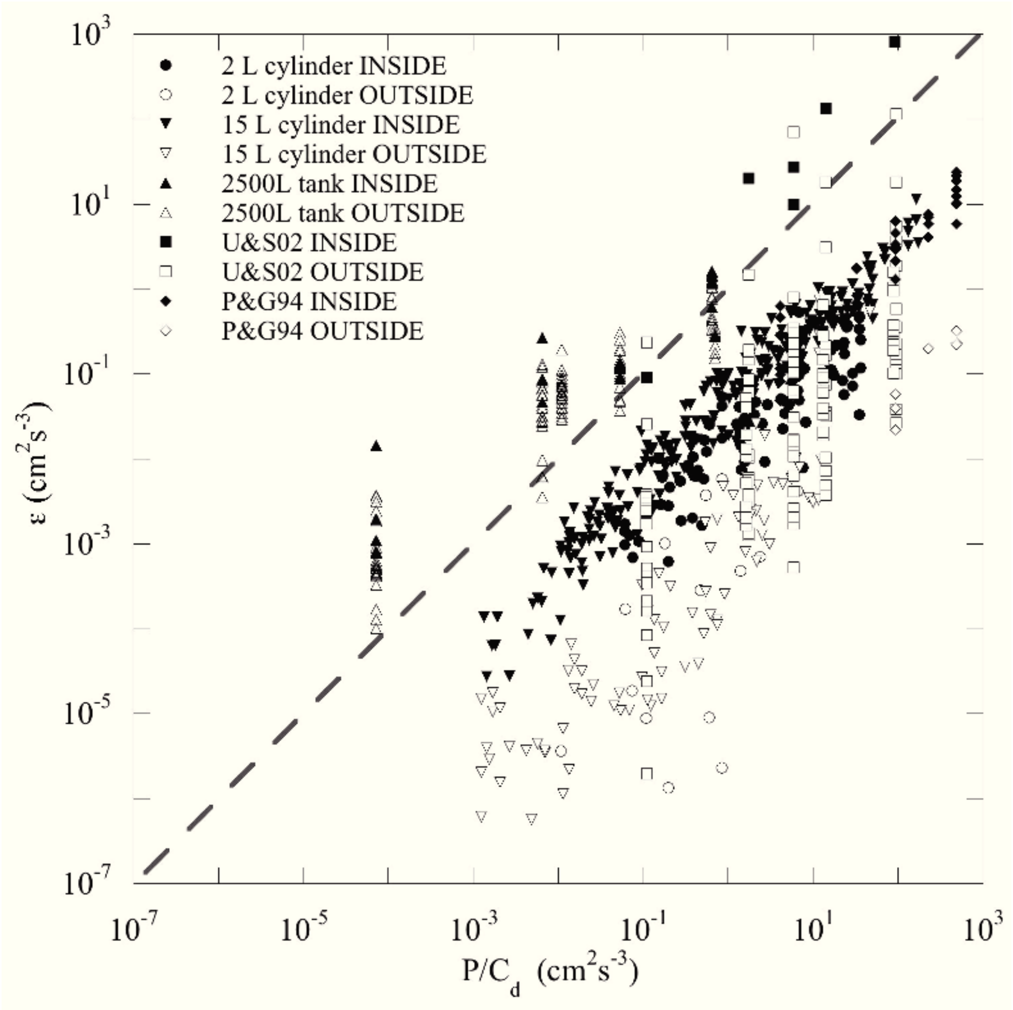

Figure 4: *Turbulent kinetic energy dissipation rate (ε) plotted against P/C_d for the oscillating grid systems, where P is the theoretical rate of kinetic energy production and Cd is the drag coefficient. Full points are measurements taken within the grid track whereas empty points are measurements taken outside. The plotted line is $\varepsilon = P/C_d$.*

This equation describes the theoretical production of turbulent kinetic energy into the system assuming a harmonic sinusoidal oscillation of the grid, and therefore should be applicable to most oscillating grid systems including the 2 L and the 15L systems.

In the 2500 L experimental system, which did not have a sinusoidal motion pattern, the grids moved up and down with a resting period between each half oscillation cycle. The velocity while the grid was moving can be considered constant:

$$\mathrm{v} = s / t \qquad (10)$$

where t was the time the grid took in each displacement, (t_1=1.7 seconds upwards and t_2=1.0 downwards). Therefore the energy introduced during the oscillation is:

$$E = \frac{1}{2}\mathrm{C_d}\rho_w \mathrm{S_A}\left(\frac{s}{t_1}\right)^3 t_1 + \frac{1}{2}\mathrm{C_d}\rho_w \mathrm{S_A}\left(\frac{s}{t_2}\right)^3 t_2 \qquad (11)$$

Substituting equation 11 into equation 8 we obtain an estimation of P:

$$P = \frac{1}{2}\mathrm{C_d S_A} s^3 V^{-1} f (t_1^{-2} + t_2^{-2}) \qquad (12)$$

where f refers to the frequency of the complete cycle.

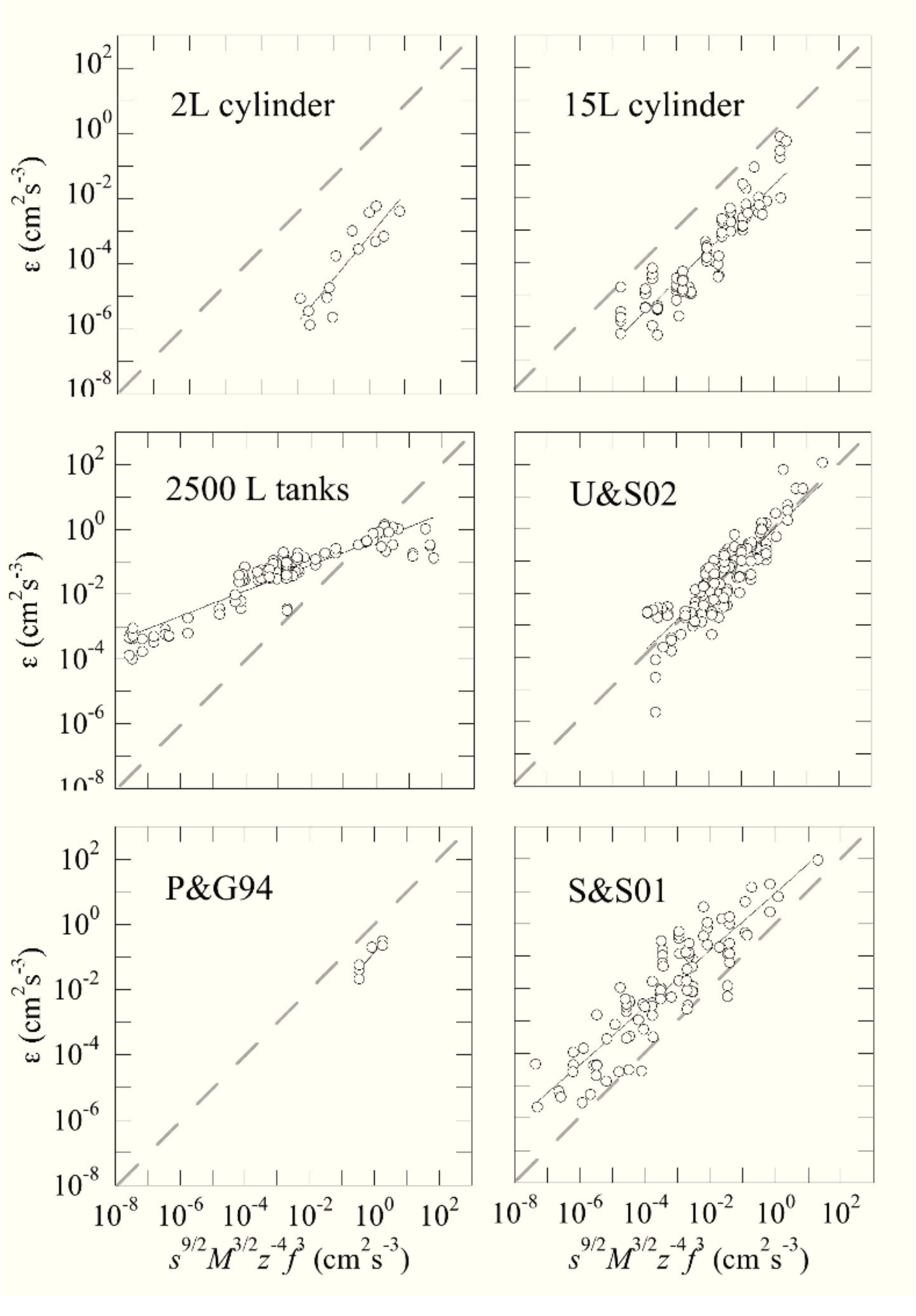

Figure 5: *Dissipation rates plotted against* $s^{9/2}M^{3/2}z^{-4}f^3$ *for the different oscillating grid systems examined in this study: A) 2L cylinder; B) 15L cylinder; C) 2500tanks; D) U&S02; E) S&S01. Plotted discontinuous lines are 1:1. Continuous lines are the best fit model.*

Distribution of ε within the experimental containers

For each oscillating grid system, all measurements of dissipation within the stroke of the grid are linearly related to the theoretical rate of energy production (fig. 4, solid symbols). This is evidence of the homogeneity of ε within the volume of water delimited by the movement of the grid. On the other hand, measurements done outside the movement are more variable. According to Hopfinger and Toly (1976) the root mean square

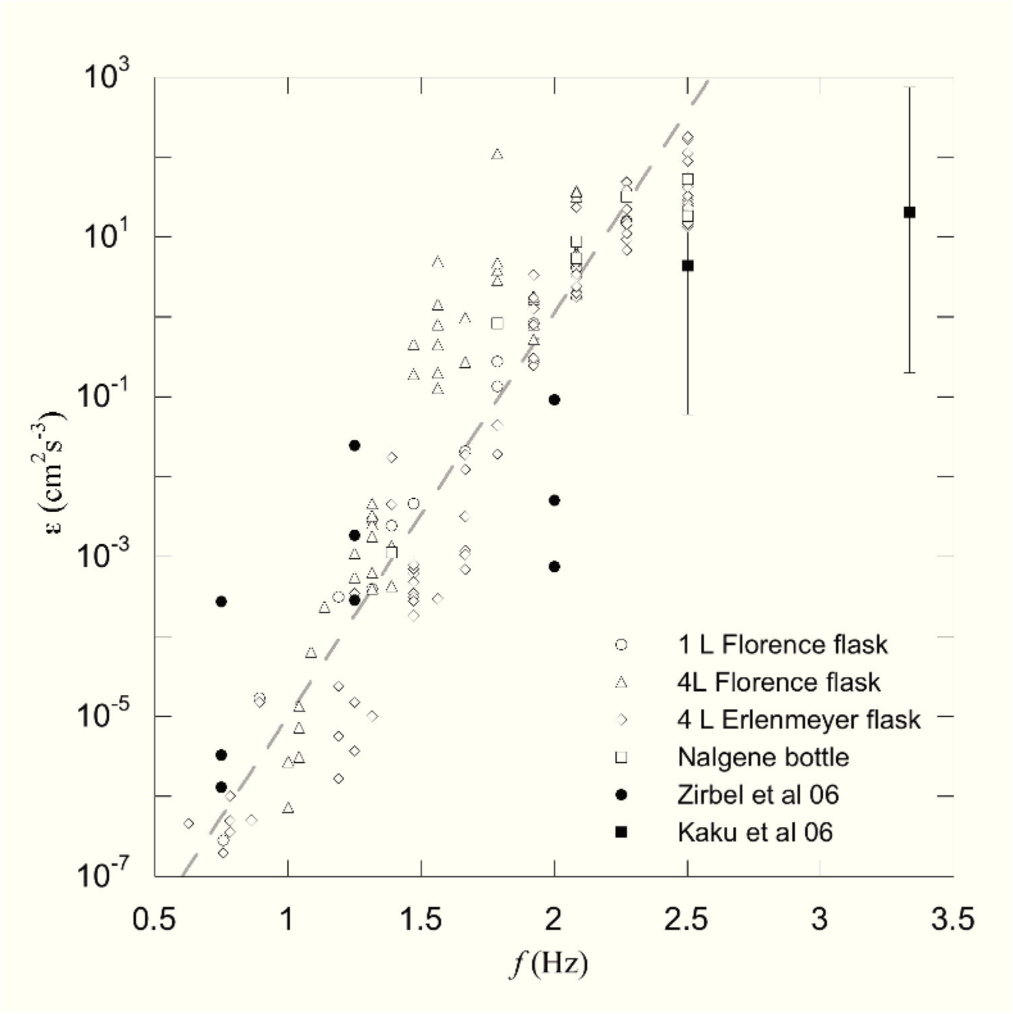

Figure 6: *Estimations of ε plotted against frequency of rotation in the orbital shaker systems. Empty points are measurements from this study; filled points are data from Zirbel et al. (2000) and Kaku et al. (2006). Plotted line is eq. (16) fitted with the maximum likelihood method using measurements in a SBS AOS-5 orbital shaker only. Error bars indicate minimum and maximum ε values.*

turbulent velocity (u) decreases with increasing distance from the wall following this equation:

$$u = \mathrm{C}s^{3/2}M^{1/2}z_0^{-1}f^1 \qquad (13)$$

where C is a constant which depends on grid geometry, M is the mesh size and z_0 is the virtual origin, the point at which the longitudinal integral length scale (l, in equation 1) becomes 0. The virtual origin can in practice be taken as the mean vertical position of the oscillating grid (Dohan and Sutherland 2002).

Taking equation 1, and assuming that l increases with z linearly (Thompson and Turner 1975), then:

$$\varepsilon \sim s^{9/2}\mathrm{M}^{3/2}z^{-4}f^3 \qquad (14)$$

According to this, dissipation should decay as z^4 (Brumley and Jirka 1987, Bache and Rasool 1996). Figure 5 shows the measured dissipation rates against $s^{9/2}\mathrm{M}^{3/2}z^{-4}f^3$ for those points away from the actual path of the grid. Although variability is relatively high, and even if the range of oscillating frequencies in our measurements is well below those commonly used in these kinds of experiments (e.g. Thompson and Turner 1975, Hopfinger and Toly 1976, Brumley and Jirka 1987), our data are in agreement with equation 14 except for the 2500L double-grided system (fig. 5C). In this case the dissipation rates were more homogeneously distributed and differences between measurements inside and outside the track of the grids were lower than in the 1-grided system (fig. 4). In all systems, the measurements inside the track of the grid did not follow equation 14, derived from Hopfinger and Toly (1976).

Assessment of drag coefficient C_d

As shown in equations 9 and 12, the drag coefficient is important in our estimates of turbulence production. C_d is usually determined experimentally. Theoretically, C_d is a function of the Reynolds number (Re). Typically the C_d of a simple cylinder is maximum at very low Re, and decreases logarithmically until it reaches a plateau at Re~1000 (Vogel 1994). Therefore the C_d changes with the velocity of the grid and its solid area. It is also dependent on the geometry of the grid (e.g. the pattern of the mesh, or the section of the bars) and on its surface roughness. To have precise estimates of dissipation rates using a particular grid, this should be calibrated for the range of Re implied in the system to find its particular $C_d(t)$, where t is time. Our data suggest however that the assumption of a constant drag coefficient is adequate, at least for these time-integrated dissipation rates, since the ratio between ε and P/C_d is constant within the grid movement (fig. 4). A bulk estimate of C_d for each system can be obtained from the slopes in figure 4. In table 3 the drag coefficients obtained in this way for the different grids assayed in this study are listed. We include in this list several grids, similar in their mesh pattern and in the section of their bars but different by their solidities, used in the 15 L containers to evaluate the effect of grid solidity on isotropy.

Orbital Shaker

Turbulence within containers in orbital shakers is much less studied. There are very few published measurements, and no theoretical background has yet been developed. In a shaker turbulence is produced mainly by the friction between the water and the wall, producing instabilities of the Tollmien-Schlichting type (Peters and Redondo 1997). Therefore the velocity of the container itself, which is function of the frequency of oscillation and the orbit diameter, will determine the level of turbulence. Also turbulence should be somewhat higher close to the walls, where the velocity shear is stronger. From our qualitative observations, water motion within the container can be divided into to two general horizontal motions: i) eddies of the same diameter and frequency as the orbital oscillation of the shaker, and ii) a lower frequency motion that follows the curvature of the container. There is also a wave of vertical displacement at the frequency of the shaker.

In order to explain the variation of ε we fitted a general regression model (GRM, Statistica 6) to the orbital shakers dataset. GRM is a statistical tool that allows the inclusion of both categorical predictor variables (e.g. type of container) and continuous predictor variables (e.g. frequency of oscillation) simultaneously within the same model. We accepted the model that explained more variability with a minimum number of predictors. Forward stepwise regressions were performed with *p-values* of 0.01 to enter/remove a given variable. Both backward and forward models yielded the same results.

Continuous predictor variables considered for inclusion into the GRM were frequency of oscillation (F), horizontal distance to the wall from the measuring point (D), vertical distance height from the bottom to the measurement point (H), and vertical distance to the surface from the measurement point (S). The type of container (C) was introduced as a sigma-restricted categorical factor, that is, the different levels (“4L Florence flask”, “1L Florence flask”, etc…) were given arbitrarily values which sum to zero (StatSoft, Inc. 2006). Continuous predictors were tested with and without logarithmic linearisation and in different combinations to find the best fit model. The dependent variable, ε, was also linearised. The final model (table 4), with N=183 valid measurements gave an adjusted $R^2= 0.91$.

Predictors introduced into the final model were F, D, S and C. Frequency was found to explain more than 88% of the variance in $\log_{10}(\varepsilon)$, whereas the other predictors, even when statistically significant, explained less than 5% of the remaining variation. As expected (Peters and Redondo 1997) ε increased with decreasing distances to

***Table 4**. Summary of forward stepwise analysis for predicting $\log_{10}(\varepsilon)$ using data of orbital shaker system. N=183 Adjusted $R^2= 0.91$, $F_{6,177}=310.65$, $p<0.0001$. The variables entered into the model where F (frequency of oscillation), C (type of container), D (horizontal distance to wall) and S (vertical distance to surface). The levels of categorical variable C were designed vs. the treatment "2 L plastic bottle". B are the coefficients for each variable, and standard (Std) errors refer to these coefficients.*

Parameter	***Step***	***Level of effect***	***B***	***Std Error***	***p***
Intercept	0	-	-8.84	0.31	*
F	1	-	5.05	0.12	*
C	2	4 L Florence flask	1.13	0.14	*
C	2	1 L Florence flask	-0.49	0.18	0.008
C	2	4 L Erlenmeyer flask	-0.71	0.12	*
D	3	-	-0.13	0.03	*
S	4	-	-0.10	0.03	0.003

the wall, at least until D~1.5 cm, which is the minimum distance sampled in this study. Also, turbulence levels increased with decreasing S. The final empirical model was:

$$\log_{10}(\varepsilon) = -8.84 + 5.05F - 0.13D - 0.10S + B \qquad (15)$$

where B is a parameter related to the type of container (table 4). To obtain estimates of the average dissipation rates within a given container, one can numerically integrate equation 15 for the volume of the container. We have done so for the range of S and D covered by the measurements. The resulting equation simplifies the GRM to the following expression:

$$\log_{10}(\varepsilon) = -a + 5.05F \qquad (16)$$

where a is a parameter different for each container. Values of a are given in table 5. The differences between containers are remarkably low. An average fitted parameter for all the data from all the containers gives a=10.1±0.1 (value ± 95 % confidence levels).

***Table 5**. Estimations of the intercept (a) in equation 17 ($\log_{10}(\varepsilon)=-a+5.05F$), for each kind of container after numerically integrating the general regression model obtained with data from orbital shaker.*

Type of container	***a***
1L Florence flask	9.9
4L Florence flask	8.7
4L Erlenmeyer flask	10.4
2 L bottle	10.8
All containers	10.1

In order to test the effect of orbit diameter we performed additional measurements in the 1L Florence flask with two additional orbital shakers, one with an orbit of 20 mm and the other with an orbit of 15 mm. Dissipation rates measured in the three shakers are plotted against frequency of oscillation in figure 7A. As expected shakers with lower orbital diameters generated lower turbulence dissipation rates. Both parameters in equation 16 relate linearly with the orbital diameter. From the linear regressions between orbital diameter and parameters of equation 16 (fig. 7B), it is possible to derive a general expression of $\log_{10}(\varepsilon)$ as a function of both the frequency of oscillation and the orbital diameter:

$$\log_{10}(\varepsilon) = -4.97 - 1.62Ø + (1.64 + 1.13Ø)F \qquad (17)$$

where $Ø$ is the orbital diameter in cm. Since we could not use the whole set of containers in the three different shaker tables, this expression do not account for the

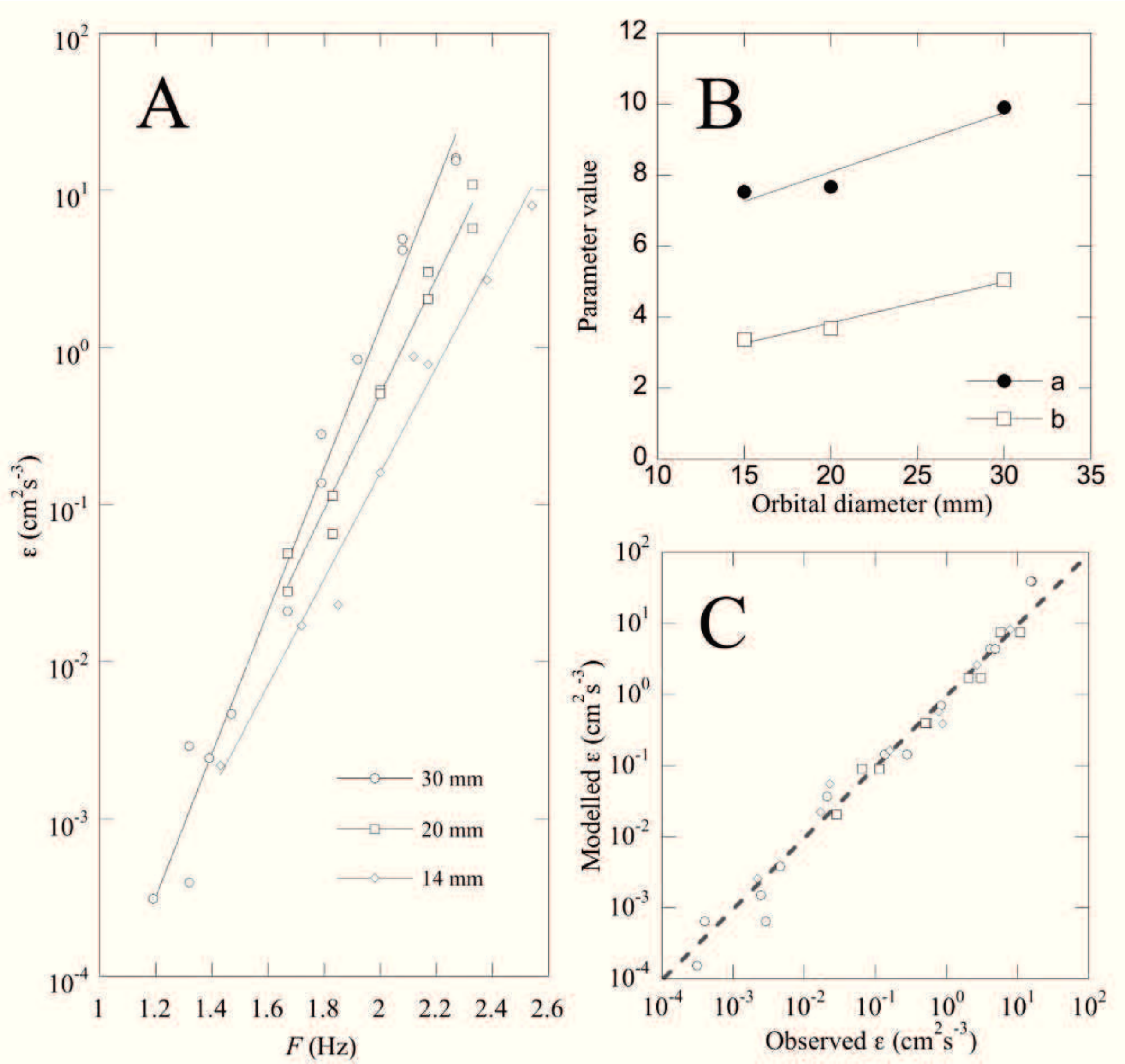

Figure 7: *Study of the effect of orbital diameter in 1 L Florence flask. A) Measured dissipation rates against frequency of oscillation. Plotted lines are best fitted regression lines ($\log_{10}\varepsilon=a+bf$) for each orbital diameter tested (14, 20 and 30 mm). B) Parameters a and b from the regression models plotted in A, against orbital diameter. Lines are best fitted linear regressions. C) Measured against modeled ε using equation 17.*

variability due to the type of containers and the distance from the wall.

Discussion

Although the three outlined methods for calculating dissipation rates were well correlated, they gave differences in estimates that can be as large as two orders of magnitude (between energy dissipation law and Peters and Gross methods, fig. 2). Differences between the methods are due to miss-estimation of constants and appropriate length scales implied in their calculations (Stiansen and Sundby 2001). This stresses the importance of future comparison studies between different approaches, and of the setting of related parameters. The estimates obtained following the linear regression method were considered most adequate because they gave intermediate values among the three methods tested. However, this implies that although the main conclusions of this study are solid given that the method is precise enough, the estimates of dissipation can be biased by as much as one order of magnitude, depending on estimation method. Turbulence levels given by different estimation methods in experimental studies of turbulence effects on plankton should therefore be interpreted within one order of magnitude.

Oscillating grid systems

Empirical data from different oscillating grid systems conforms to equations 9 and 12, derived from theoretical considerations about the energy input in the system. The most relevant parameter in these equations is the drag coefficient (C_d), because it is the most difficult to assess. The drag coefficient is a function of Reynolds number, and therefore it changes with the grid velocity and geometry. The fact that empirical dissipation rates collapse into a line when plotted against P/C_d (fig. 4) implies that, at least practically, C_d can be considered constant for the range of relatively low frequency oscillations explored in this study. Higginson *et al.* (2003) found that C_d was constant for a large range of Reynolds numbers (1000-3000). According to these authors, this is an expectable result for objects of sharp edges moving in a fluid. They explained this as a consequence of boundary layer separation occurring at the edges for Reynolds numbers greater than 100.

The empirical determination of C_d is difficult. Drag coefficient depends on several factors, such as the solidity, the shape of the bars forming the grid or the rugosity of the material. In order to calculate the C_d of a given grid one must measure the drag force necessary to maintain the grid moving at a range of constant velocities.

With our approximation it is not possible to resolve fluid dynamics very close to the walls in the oscillating grid systems, but we have shown that in most of the containers, small-scale turbulence is homogeneous throughout the volume covered by the grid movement. Outside this volume it decays with distance from the virtual origin following a power law (Hopfinger and Toly, 1976). A smaller scale inhomogeneity associated with the geometry of the grid can not be dismissed. The double-grided system does not adjust to Hopfinger and Toly prediction. Instead, dissipation rates decrease with distance more slowly than in the single grid systems. The reason is that dissipation rates in a given point are the result of the joined effect of the two grids, which individually are likely to follow Hopfinger and Toly's model. Because decrease of ε with distance is smoother in the double-grided system than in the single-grided one, all other settings being equal, turbulence was more homogeneously distributed, The difference between the highest and lowest dissipation rates within the tank in the double-grided tank were in the order of one magnitude even when the joint stroke of the two grids was not covering the whole container.

The average values of ε in these systems are stationary if we consider time intervals larger than the oscillation period. However, within each oscillation period there is a sinusoidal variation in the TKE. When the grid passes through the measurement point ε is maximum, and then decreases following a power law (Peters and Gross 1994). The estimations given are the average values that an organism will experience over time in these systems, but there is a range in turbulence within each stroke, which is expected to be wider as we increase the stroke and decrease the frequency, and which will change with the position inside the container, since the velocity of the grid varies with the distance from the center of oscillation. This sinusoidal oscillation, generally of a period of several seconds, could potentially have a significant influence on some processes with short time scales, particularly nutrient uptake processes and especially for small organisms (Peters and Marrasé 2000).

Orbital Shaker

The study was designed to look at differences between the containers. However, it was possible to detect a significant influence of wall proximity. Other variables, such as the type of motion (e.g. orbital vs. reciprocal) or the fluid volume within a given container should be the subject of future studies. Moreover, all containers had a circular base. Results will be different in containers with corners or irregular shapes at the base (Kaku *et al.* 2006).

There are several possible sources for turbulence within a flask in an orbital shaker. The main is friction of water with the wall. Therefore the angular velocity of the walls, which is related both with the frequency of oscillation and with the orbit diameter, is expected to determine the level of turbulence achieved. Coherently, our results show that turbulence intensity is mostly explained by the frequency when orbit diameter is held constant. Another possible source could be instabilities due to non-linearity in the wave motion.

Geometrical parameters, such as the height of the container or its horizontal shape, are also affecting the mean dissipation rates, but far less than we had previously expected. Dissipation rates estimated from equation 16 give an average value of turbulence within the tanks, i.e. the mean turbulence level an organism subjected to a given experimental condition is likely to experience. But turbulence is not completely homogeneous, since it depends on the distance to the side and bottom walls. When integrating the dissipation rates for the full container, this dependence on the distance to the wall may be critical, because the volume increases with the radius. This means that for the same orbital diameter and frequency of oscillations, average ε will increase non-linearly with the container diameter. It means also that the larger this diameter, the wider the range of ε reached within the container. From the empirical model (table 4) it is possible to infer a 10-fold difference in dissipation between points separated 10 cm.

Measurements of fluid motion in tanks on orbital shaker tables are very scarce. In figure 6 we have plotted the datasets of Zirbel *et al.* (2000) and of Kaku *et al.* (2006), together with our measurements. Zirbel *et al.* (2000) measured shear stress in 125 ml flasks with 60 ml of water for a shaker table with an orbit of 2.54 cm. The horizontal velocity field was measured with a two component DPIV (digital particle image velocimetry) system. They measured the velocity field at three different rotation frequencies (0.75, 1.25 and 2 Hz). Their results also show a decrease in turbulence with distance to the wall and a strong dependence with the frequency of rotation. However, their measurements depart from the empirical relationship found in this study, at least at the highest frequency. Measurement conditions were quite different from ours since they used a much smaller container and fluid volume than ours. According to Zirbel *et al.* (2000) there were some limitations associated with the fact that the measurement plane is horizontal and with interactions between the illumination and the glass of the flask. Differences form Zirbel *et al.* measurements (2000) can also come from a hypothetical bias in our data due to vortex shedding from the intrusive probe. This was tested by placing the 15 L cylindrical container with the wall-mounted velocity sensors used for the oscillating grid system on the orbital shaker. No change was detectable in measured ε from the wall-mounted sensors when the side-looking ADV probe was introduced in the tank. However this test is not entirely conclusive, since effect of the intrusive probe could be very different in containers of different shapes and sizes. Another factor that could explain the difference is the orbit, which was 5 mm larger in our case.

Kaku *et al.* (2006) measured turbulence in a 150 ml Erlenmeyer flask with 120 ml of water in a shaker with an orbital diameter of 19 mm oscillating at 2.5 Hz and at 3.3 Hz. Measurements were conducted by a hot wire anemometer with a sampling frequency of 1000 Hz during 10 s at 2 mm intervals. Their data fall below what should be expected from extrapolation of the empirical model to higher frequencies (fig. 5), which is reasonable since orbital diameter was lower than ours. This bias may also be related to the determination of dissipation rates, because they used the energy dissipation model, which gives lowest estimations (fig. 2).

The use of containers with different and relatively complex shapes makes it difficult to relate empirical results with theoretical estimations based on energy input in orbital shakers. The relationship found between ε and F is much steeper than what has been previously predicted by Colomer *et al.* (2005) by theoretical considerations. These authors also postulated that ε was inversely proportional to V. The effect of water volume on turbulence generated by an orbital shaker has not been systematically addressed here. It would require a set of measurements with a range of different water volumes for the same tank. However, this effort seems partly irrelevant, as frequency alone can explain so much variability despite the use of different types of containers, each with different water volumes.

Other parameters, such as orbit diameters or free surface could affect the results. Duertz and Whitholt (2001) observed large differences in fluid motion in a container subjected to two different orbit diameters (2.5 and 5 cm) but to our knowledge no one has quantitatively addressed this aspect. We have extended our set of measurements to test for the possible effect of orbital diameter. As expected, dissipation rates increase with the diameter of the orbital oscillation. Equation 18 offers a good first approximation to this problem. However this relationship must be taken with care because it is based in only three different orbital diameters and on only one type of container. Subsequent studies must extend the range of orbital diameters and of experimental containers which this study could not cover, in order to confirm and develop such relationship. In general frequency alone remains the best predictor of dissipation rate regardless of fluid volume and container shape.

Comments and recommendations

There are few evaluations of turbulence in agitated tanks used in biological experimentation. More measurements are necessary, especially in orbital shaker systems. Our results show that oscillating grid and shaker table systems attain a wide range of turbulence levels, covering well the turbulence values found in the field (Peters and Marrasé 2000). Despite that there is a dominant direction in the generation of movement in both kinds of systems, conditions are fairly isotropic in all tested container types and energy input conditions, with the exception of low energetic situations in orbital shakers where isotropy can be lost. It is also a fine line between laminar and turbulent conditions. Therefore we do not recommend frequencies below ca. 1Hz for studies on effects of turbulence on plankton in orbital shakers.

Oscillating grids produce more homogeneous conditions than orbital shakers, as long as the grid is moving throughout the container. When designing experiments with plankton it is therefore especially important to use maximum stroke possible in these systems, unless the objective is to study the effect of non-homogeneous turbulence (i.e. gradient). One constraint lays at low oscillation frequencies, since organisms will experience intermittent pulses of turbulence followed by periods of calmer conditions. In addition, organisms with enough swimming capability (e.g. fish larvae, copepods or dinoflagellates) could escape the movement of the grid. Consequently this system should be a first choice mainly for the smallest plankton, unless the systems are large and the plankton stays in a limited space in the tank. The decay of turbulence away from the zone of the grid is predicted using Hopfinger and Toly (1976).

Two main conclusions that can be derived from the grid oscillation systems analysis are: i) that C_d can be considered constant for practical biological applications, and ii) that the dissipation rate can be assessed from theoretical considerations. It is still not possible to predict the C_d from geometrical considerations, since it depends not only on solidity, but also on the size and shape of the bars which conform the grid, on its geometry, and on its smoothness. We have presented in table 3 a set of different C_d obtained from different grids as a guide for future studies. We strongly recommend using similar grids in future studies, or other models previously calibrated. More effort should be put into calibrating different grids under the oscillation conditions used in biological experiments.

In orbital shakers, as turbulence depends on geometrical parameters such as the distance from the wall and the bottom, there is always a gradient of dissipation. The effect is rather small, but it can account for an order of magnitude of difference in ε between the wall and the centre of the flask. Moreover, superimposed to the turbulent eddies, there is always a strong dominant flow that could affect the distribution and behaviour of the organisms. Small and narrow containers should minimize these inhomogeneities. To obtain an integrated average value for a given container on an orbital shaker, the best way is to integrate numerically the empirical model presented in table 4. If the type of container used is different from the ones of this study, one can use equation 16 using *a*=10.1 (table 5) to estimate a bulk value of the dissipation. For shaker tables with a different orbit, equation 17 gives the dissipation rates in a 1L container depending on the frequency and the orbital diameter.

With the cautions and limitations mentioned here, both systems are adequate for the study of effects of small-scale turbulence on plankton organisms and communities, and especially for organisms with relatively limited mobility and small size. Equations 9 and 16 can easily be used to assess average dissipation rates in oscillating grid systems and orbital shakers within the ranges of conditions explored in this paper.

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Chapter 3: Episodic meteorological and nutrient load events as drivers of coastal ecosystem dynamics: a time series analysis.

Episodis meteorològics i d'entrada de nutrients
com a conductors de la dinàmica de l'ecosistema
costaner: un anàlisi de sèries temporals.

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Josep-Maria Gasol, Cristina Roldán, Elisa Berdalet,
Ramon Massana, Anna Sabata

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Photo: Storm in Sa Palomera beach, in Blanes Bay

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Abstract

In temperate coastal zones, episodic meteorological forcing can have a strong impact in the classical seasonal phytoplankton succession. Episodes of continental runoff and wind storms involve nutrient enrichment and turbulence, two factors that can promote primary production and alter the planktonic community composition and structure. The aim of this study was to determine the influence of these variables in the osmotrophic planktonic community of Blanes Bay, Spain (NW Mediterranean). We used an 8 years long time series of monthly data of water column parameters, and we looked for correlations between these and several meteorological and physical high-frequency time series through cross correlation analyses. Influence of river runoff in this particular location was found to be very important for phytoplankton dynamics. Resuspension events caused by waves had a secondary impact on phytoplankton. Cross correlations allowed defining a sequence of responses to these forcings, from turbidity, to nutrient concentration, phytoplankton and bacteria. The maximum response of the ecosystem in terms of chlorophyll concentration lagged about one week nutrient enrichment events. A more detailed analysis was performed between June 2003 and June 2004. While the system was generally limited by phosphorus, diatoms were found to be strongly related with both phosphate concentration and the Si:N ratio. Easily available high frequency time series of meteorological and physical data are shown to be potentially good predictors of the state of coastal ecosystems.

KEYWORDS: river runoff, waves, turbulence, nutrients, osmotrophic plankton, Blanes Bay.

Resum

En zones costaneres de climes temperats, els forçaments meteorològics de caire episòdic poden tenir un fort impacte en la successió fitoplanctònica clàssica. Els episodis d‘escorrentia continental i les ventades impliquen enriquiment en nutrients i turbulència, dos factors que poden promoure al mateix temps la producció primària i canvis en la composició i l’estructura de la comunitat. L’objectiu d’aquest estudi era determinar la influència d’aquestes variables en la canviant comunitat osmotròfica planctònica de la Badia de Blanes (Mediterrani nordoccidental). Per tal d’aconseguir-ho vam utilitzar una sèrie temporal de 8 anys amb freqüència mensual, de diversos paràmetres de la columna d’aigua. Mitjançant anàlisis de correlacions creuades, vam buscar relacions entre aquests i diverses sèries temporals meteorològiques i hidrogràfiques d’alta freqüència. La influència de la descàrrega dels rius va resultar ser molt important per a la dinàmica del fitoplàncton. Episodis de ressuspensió causats per les onades tenien un impacte secundari. Les correlacions creuades van permetre establir una seqüència de respostes a aquests forçaments, des d’un increment de la terbolesa, a incements en la concentració de nutrients, de fitoplàncton i de bacteris. La resposta màxima de l’ecosistema a l’enriquiment per nutrients, en termes de concentració de clorofil·la, es retardava una setmana aproximadament. Entre juny de 2003 i juny de 2004 es va dur a terme un anàlisi més detallat. Encara que el sistema estava limitat en general per fòsfor, les diatomees van resultar estar fortament relacionades no només amb la concentració de fosfat sinó també amb el quocient Si:N. Es demostra que sèries temporals d’alta freqüència fàcilment disponibles poden ser bons predictors de l’estat d’un ecosistema costaner.

PARAULES CLAU: descàrrega de riu, onatge, turbulència, nutrients, plàncton osmotròfic, badia de Blanes.

Introduction

The successional pattern of phytoplankton populations in temperate coastal zones is strongly determined by the seasonal nature of physical forcing (Margalef 1978). Non motile microphytoplankton (i.e. diatoms) tends to be dominant in winter, when the water column is well mixed, nutrient concentrations are relatively high and light may be limiting. Thermal stratification becomes stronger as radiation increases. A diatom bloom can appear in late winter or spring, when nutrients in the euphotic zone are still high and water column stability prevents mixing of phytoplankton below the euphotic zone. Nutrients in this situation are rapidly depleted and eventually exported as sedimented particulate organic material. In summer the stability of the water column and the low nutrient concentrations in the upper layer favour the dominance of flagellated and/or small phytoplankton forms.

The littoral constitutes a boundary between three environments: land, sea and atmosphere. It is therefore a highly variable ecosystem both in space and time because it is subjected to the variability of continental, atmospheric and oceanic forcing. The North-Western Mediterranean coast is a good example of this. Its climate is characterized by sporadic episodes of strong rainfall, which derive in catastrophic continental runoffs and increased freshwater input (e.g. Llasat and Puigcerver 1997), and by wind storms, particularly of East component, which are accompanied by relatively high waves and promote sediment resuspension (Guillén *et al.* 2002, Ferré *et al.* 2005). Sea water turbidity has been found to be well correlated with both wave height and river runoff in NW Mediterranean coastal waters (Rossi and Gili 2005). In addition, occasional intrusions of nutrient rich cold and saline open ocean waters, favoured by the presence of submarine canyons (Masó and Tintoré, 1991), may also alter the seasonal pattern. Thus, episodic disturbances of the system can be of the same order of magnitude as seasonal variability (e.g. Duarte *et al.* 1999), and can therefore induce large changes in the osmotrophic planktonic community, namely bacteria and phytoplankton (e.g. Nogueira *et al.* 2000, Grémare *et al.* 2003).

The occurrence of episodic heavy rainfalls, especially in autumn, is a characteristic of the Mediterranean climate (Llasat and Puigcerver 1997). Precipitation increases surface runoff and consequently land input, producing unpredictable pulses in the terrestrial inputs of both inorganic and organic particulate and dissolved nutrients (Cebrián *et al.* 1996, Lucea *et al.* 2005). These inputs can have a strong impact on the generally oligotrophic Mediterranean waters, either by affecting water clarity (Cloern 1987) or by increasing production through nutrient enrichment. In recent decades, the development of large-scale industrial farming and of mass tourism in the area, as well as the construction of river dams that retain sediments, has altered the composition and amounts of the nutrient load derived from freshwater inputs, increasing the importance of this source of variation.

Direct mechanical energy is a forcing to be considered in the coastal zone as well. In the NW Mediterranean Sea tides are relatively unimportant and wind is the main source of mechanical energy input to the upper mixed layer. This input is produced directly by the wind stress on the ocean surface or indirectly through the generation of wind waves. Turbulence generated by winds or waves is a multi-scaled process, and as such influences the osmotrophic planktonic community at different levels. At the scales of energy input in shallow coastal waters turbulence can increase the flux of nutrients from the sediments (Dade 1993, Grémare *et al.* 2003), can resuspend benthic or settled organisms (Garstecki *et al.* 2002) and organic matter, and can keep planktonic organisms in suspension within the mixed layer (Ross 2006). Resuspension of sediment can, by increasing turbidity, limit the light available for phytoplankton growth (Cloern 1987, Cotner *et al.* 2000). At smaller scales turbulence enhances the uptake of nutrients by large cells (Karp-Boss *et al.* 1996, Peters *et al.* 2006) and the encounter probabilities between particles (Rothschild and Osborn 1988). It can induce changes in plankton community size structure (Cozar and Echevarría 2005), in species composition (Margalef 1978, Estrada *et al.* 1987), in the stoichiometry of particulate organic matter (Maar *et al.* 2002) and in ecosystem metabolism (Alcaraz *et al.* 2002). In a shallow coastal zone, we hypothesize that turbulence events generated by waves or directly by winds will i) have an immediate and transient effect on biomass parameters (such as chlorophyll concentration), because of resuspension of sediment organisms (Carrick *et al.* 1993, Satta *et al.* 1996), and ii) promote a sustained response due to the input of nutrients from the sediment (Grémare *et al.* 2003) and the above mentioned effects of turbulence at the small scale.

The combination of physical forcing and nutrient-enrichment events caused by storms, land runoff or deep water intrusions from open sea, leads to an increase in primary production. This may explain the formation of blooms (Kiørboe 1993), and the subsequent export by sedimentation of a part of the newly produced organic matter, provided that consumers are not tightly coupled to phytoplankton. The response to these unpredictable perturbations can force the system to depart from the seasonal successional pattern. Our aim is to identify responses of the osmotrophic community to episodic events of turbulence and nutrient supply in an oligotrophic open coastal area: Blanes Bay, NW Mediterranean Sea. We have analyzed time series of meteorological, oceanographic and hydrological high frequency data combined with a basic set of chemical and biological variables of the water column collected monthly. These analyses revealed a sequence of responses of the system to these perturbations. In order to extend the results of the time-series analysis, and estimate its validity we performed a more detailed analysis, including a larger set of variables between June 2003 and June 2004, a period noticeable by its dry summer and by strong rainstorms in autumn, which would be expected to singularly affect plankton development.

Material and methods

The study was conducted in Blanes Bay, NW Mediterranean (fig. 1), a well studied open and shallow oligotrophic bay with sandy bottom and a relatively steep slope (2%, Vaqué *et al.* 1997). It is located in front of the town of Blanes, between the submarine Blanes Canyon to the North and the mouth of the river La Tordera to the South. The bay is net heterotrophic (Satta *et al.* 1996b, Duarte *et al.* 2004) and oligotrophic, with production being limited most of the year by phosphorus (Lucea *et al.* 2005, Pinhassi *et al.* 2006). It episodically receives freshwater inputs from the La Tordera River and terrestrial runoff inputs from the town and the surrounding coastal area. Tourism increases the population of Blanes about 5 fold in summer, leading to a nutrient fertilization of the Bay (Duarte *et al.* 1999).

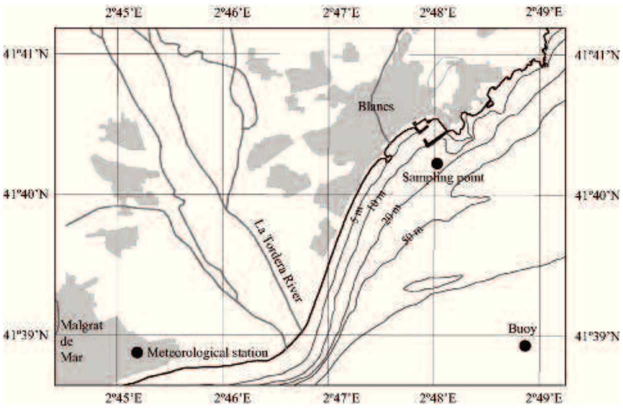

Figure 1: *Map of the studied area in the Bay of Blanes, NW Mediterranean Sea.*

Meteorological and physical data

Meteorological data were acquired from the nearby station of Malgrat de Mar (Catalan Meteorological Service http://www.meteocat.net), situated at 41º38'57"N 2º45'8"E, 520 m from the coast (fig. 1), and at 4 m height above the sea level. The station recorded arithmetically averaged hourly air temperature and relative humidity at 1.5 m height, vector averaged hourly wind speed and direction and global irradiance at 2 m height, and accumulated rainfall at 1 m height. The available time series data span 15 years (from 1990 to 2005).

Wind velocity was rescaled to 10 m height assuming a logarithmic wind profile (Stull 1988):

$$W_{10} = W_n \frac{\log\left({}^{10}\!/_{z_0} \right)}{\log\left({}^{n}\!/_{z_0} \right)} \qquad (1)$$

where W_n is the wind speed at n m height, and z_0 is the aerodynamic roughness length, assumed to be 0.03 m (terrain of class 1 in Trøen and Petersen 1989).

The energy flux from wind forcing was calculated as:

$$E_w = \sigma_a C_{10} W_{10}^3 \qquad (2)$$

where σ_a= 1.2 kg m^{-3} is the density of air, and C_{10} = 1.3 10 $^{-3}$ is the drag coefficient (Gargett 1989). This energy flux was used as a proxy of turbulence directly generated by winds (Mackenzie and Legget 1993), as according to boundary layer model, dissipation rates scale with energy input and distance from the input boundary (i.e., depth) (Turner 1973).

Wave data from 1997 to 2006 were obtained from a scalar buoy (DATAWELL Waverider) placed at 41º38'49"N, 02º48'56"E (fig. 1), over a depth of 74m (Meteorological and Oceanographic Instruments Net of the Catalan Government, XIOM, http://www.boiescat.org/). The buoy registered wave height and period. For our study we used significant wave height (SWH), which is defined as the mean of the height (trough to crest) of the largest one third of waves occurring during the sampling period, in this case one hour (Stewart, 2004).

As an estimate of terrestrial runoff (contribution of rivers and seasonal and ephemeral streams in the area), we used flow data from the river La Tordera, which discharges south of the sampling site (fig. 1). We used data from gauge EA89, in Fogars de la Selva, 10 km upstream from the mouth of the river. Data, provided by the Catalan Water Agency (ACA http:// mediambient.gencat.net/aca/en), were available between October 2001 and December 2004, with daily frequency. Some gaps in the time series were filled with estimates obtained from data of gauge EA81 (in a tributary of the Tordera River in St. Coloma de Farners), after applying a linear regression model predicting EA89 from EA81 data.

Water column parameters

Monthly subsurface water samples were taken from December 1997 to September 2006 in Blanes Bay, with a 2 years gap in 1999 and 2000, within the framework of the Blanes Bay Microbial Observatory (Schauer *et al.* 2003, Unrein *et al.* 2007). Not all parameters used in this study were taken uninterrutedly during the whole time series. Sampling location was at about 1km offshore (41º40'N, 2º48'E) over a depth between 20 and 24 meters (fig. 1).

Water was taken from a depth of 0.5 m, pre-screened with a 200 µm nylon mesh to remove larger mesozooplankton and taken to the laboratory in plastic carboys within 2 hours. Basic parameters included chlorophyll concentration, salinity, dissolved inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and SiO_2), and bacterial concentration. Subsurface water temperature and Secchi disk depth were measured before sampling. Salinity was measured with an YSI 556 MPS Multi Probe system.

Between June 2003 and June 2004 additional parameters were included into the present analysis: particulate organic nutrients, total nitrogen (TN), total phosphorus (TP), autotrophic (ANF) and heterotrophic flagellates (HNF) and microphytoplankton.

Dissolved inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and SiO_2) were analyzed with an Alliance Evolution II autoanalyzer following methods in Hansen and Koroleff (1999) with minor modifications. Samples were kept frozen at -20ºC until analyses.

Chlorophyll *a* concentration (chla) was determined fluorometrically (Yentsch and Menzel 1963). Samples of 20 ml were filtered through Whatman GF/F filters. Chlorophyll was extracted from filters immersed in 6.5 ml of 90% acetone (24 hours at 4ºC in the darkness). The extract was analysed with a Turner Designs fluorometer calibrated with pure chlorophyll *a* (Sigma Co).

For the determination of particulate organic matter, 200 to 500 ml of sea water were pressure-filtered (0.3

atm) through pre-combusted (450ºC, 4h) glass fibre filters (Whatmann GF/F) and immediately frozen in liquid nitrogen, and then stored at -80 ºC. For each sample two subsamples were processed, one for particulate organic nitrogen (PON) and carbon (POC) and the other for particulate organic phosphorus (POP). Before analysis in a Carlo-Erba CHN analyser, the POC/PON filters were thawed in an HCl-saturated atmosphere for 48h to remove carbonates, and dried at 80ºC for 24 h. POP was determined following the oxidation (120ºC, 30 min) of the filter in acidic persulphate and subsequent analysis of dissolved phosphate (Hansen and Koroleff 1999).

For TP determination, water was sampled into acid-cleaned polyethylene bottles and immediately frozen (-20ºC). TP was determined by wet oxidation following the same procedure as for POP. TN was determined after persulphate oxidation following Hansen and Koroleff (1999).

Concentrations of bacteria and cyanobacteria (*Prochlorococcus* and *Synechococcus*) were determined by means of flow cytometry following methods in Olson *et al.* (1993) and Gasol and del Giorgio (2000). Subsamples of 1.8 ml were fixed with 1% paraformaldehyde plus 0.05% glutaraldehyde (final concentrations), left 10 minutes to fix in the dark, deep-frozen in liquid N_2, and then stored at -80ºC. Aliquots were divided into two fractions for counts of bacteria and cyanobacteria in a Becton Dickinson FACScalibur bench cytometer with a laser emitting at 488 nm. To count heterotrophic bacteria, 200 µl were stained with a DMSO-diluted Syto 13 (Molecular Probes) at 2.5 µM final concentration, left for at least 10 minutes in the dark to complete the staining, and run in the flow cytometer at low flow rate (between ca. 15 and 50 µl min^{-1}) for 2 minutes. For cyanobacteria counts, 200 µl subsamples were run at high flow rate (between ca. 60 and 80 µl min^{-1}) without adding any dye for 5 minutes. The abundance was determined using flow speed and the flow was calibrated every ten samples by determining sample volume before and after a 10 minutes run. In all cases 10 µl per sample of a solution of yellow-green 1-µm Polysciences latex beads (10^6 beads ml^{-1}) were added as an internal standard. Bacterial average cell volume was estimated with calibration function provided by Gasol and del Giorgio (2000):

$$B = 7.5 \cdot 10^{-3} + 1.1 \cdot 10^{-1} \left(\frac{F_{bacteria}}{F_{beads}} \right) \qquad (3)$$

where B is the average volume of bacteria in μm^3, F_{bacteria} bacterial mean green fluorescence, and F_{beads} is beads' mean green fluorescence.

Average volume for cyanobacteria was estimated assuming a spherical shape. We used a value of 1 µm for *Synechococcus* sp. (Agawin *et al.* 1998), and a mean diameter of 0.7 µm for *Prochlorococcus* sp. (Vaulot *et al.* 1990).

Heterotrophic and autotrophic nanoflagellates (HNF and ANF) were fixed with 1.5% (final concentration) glutaraldehyde, stained with DAPI (4',6'diamidino-2-phenylindole) at 5 µg/ml and counted using epifluorescence microscopy (Porter and Feig 1980). Briefly, 20 to 30 ml subsamples were stained and concentrated on black polycarbonate filters of 0.8 µm pore size (Millipore). Filters were kept frozen at -20ºC until counting in a Nikon Labophot 2 microscope. Analysis was done within 3 months after the sampling. At least 300 flagellates were counted at 1250X. Organisms were classified in 4 size classes: <4 µm, 4-8 µm, 8-16 µm and >16 µm in diameter. Cellular volume was estimated from the mean size of each class assuming a spherical shape. For the <4µm an average size of 3µm was used. Autotrophic flagellates were distinguished from heterotrophic ones by the presence of chlorophyll *a* detected under blue light.

Microphytoplankton was identified and counted with an inverted microscope (Uthermöhl 1958). A 60 ml sample was fixed with formalin-hexamine (0.4% final concentration) and was kept at 4ºC until analysis. Samples of 12 to 50 ml were transferred to sedimentation chambers, and left undisturbed for 24 hours. The sedimentation chamber was scanned in the inverted microscope at 100X and 400X magnification. Dominant phytoplankton was identified at least to genus level. Width and length of cells were measured for each cell or for each colony or chain. The number of cells per aggregate was also counted. When possible, volume of each cell was calculated applying the formula provided by Hillebrand *et al.* (1999). In radially asymmetrical cells the closest 2-parameter geometrical shape was assumed. This may cause a bias in the estimation of biovolumes, especially when radially asymmetrical cells are dominant.

Calculations and statistical analyses

Osmotrophic plankton biomass-size spectra were calculated based on a Pareto type I distribution (Vidondo *et al.* 1997), from data on concentration and volume of phytoplankton and bacterioplankton. In a Pareto type I distribution, the parameter c is equivalent to the negative slope of the normalized biomass-size spectrum. Brevely, in a normalized biomass-size spectrum particles (i.e. cells, chains, aggregates, etc…) are grouped in logarithmic size classes, and the density of each class, that is the normalized biomass, is plotted against the size. The slope of such spectrum gives relevant information about the size structure of a particular community. For example, communities dominated by small organisms have slopes steeper than those dominated by large organisms. In the present case, in order to apply the Pareto I adjustment proposed by Vidondo et al. (1997) the free living cells and the aggregates or colonies were used as basic items. For autotrophic flagellates <16 µm in diameter epifluorescence microscopy counts were used, whereas

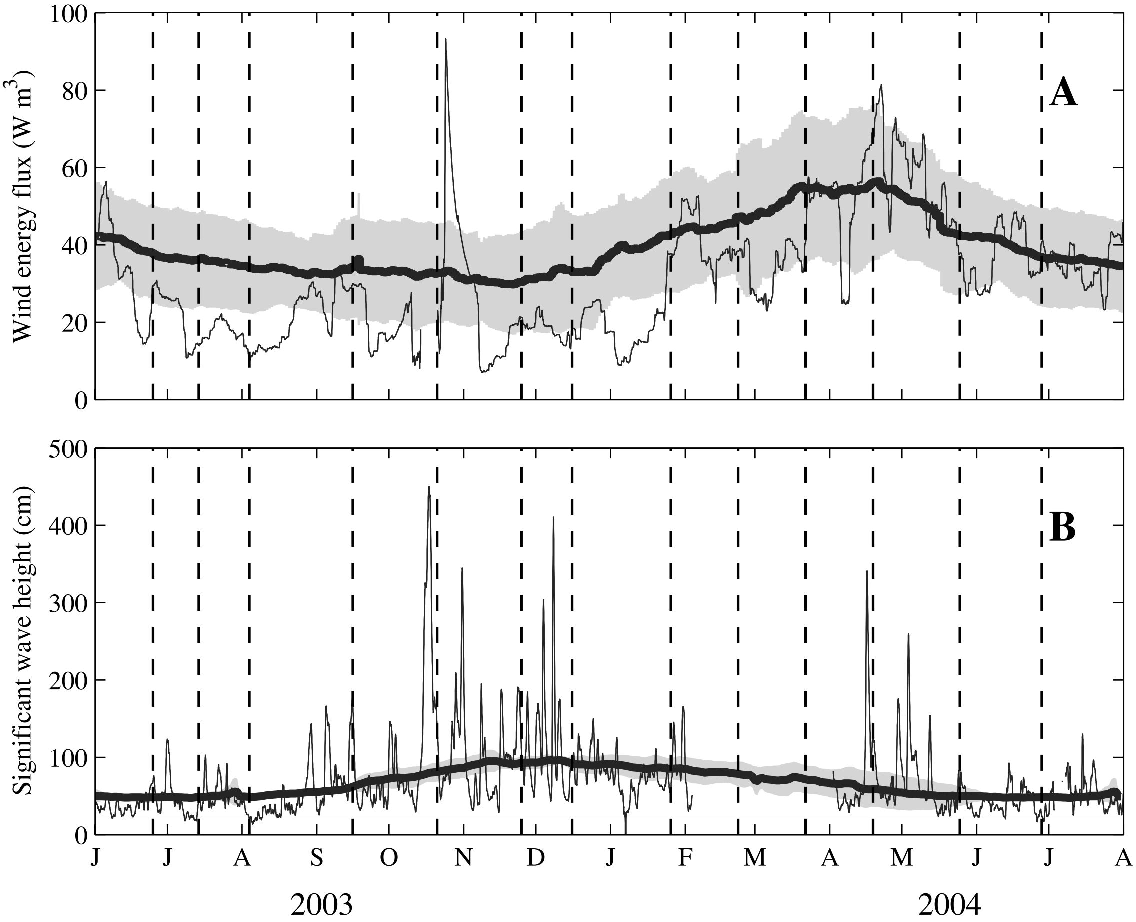

Figure 2: *Seasonal variation of wind energy flux (A) and significant wave height (B) in Blanes Bay. In order to unveil the seasonal patterns the whole time series were smoothed with a 30 day moving average, and then an ensemble average (thick line) and standard deviation (grey area) were calculated for each day of the year. Thin lines show the 7-day moving-averaged values from the period of extended analysis (June 2003 -June 2004).*

Table 1. Correlation matrix between the different water-column parameters sampled. Values shadowed with light grey are significant at p-value<0.05. Values shadowed with dark grey are significant at p-value <0.01.

	Salinity	**Secchi**	**Chla**	**NO_3^-**	**NO_2^-**	**NH_4^+**	**PO_4^{3-}**	**SiO_2**	**POC***	**PON***	**POP***	**TN***	**TP***	**Bacteria**	**HNF***
Temperature	0.07	0.53	-0.51	-0.53	-0.42	-0.07	-0.21	-0.40	-0.14	0.34	-0.41	0.33	-0.59	-0.08	-0.20
Salinity		0.44	-0.36	-0.21	-0.31	-0.11	0.07	-0.24	-0.25	-0.38	-0.82	0.23	-0.54	-0.37	-0.77
Secchi			-0.50	-0.44	-0.45	-0.09	0.10	-0.43	-0.36	0.04	-0.62	0.29	-0.74	-0.33	-0.59
Chla				0.54	0.48	-0.01	0.10	0.47	0.32	-0.04	0.76	-0.45	0.70	0.30	0.35
NO_3^-					0.38	0.10	0.35	0.75	0.20	-0.23	0.56	-0.18	0.81	-0.01	0.32
NO_2^-						0.51	0.28	0.34	-0.02	-0.34	0.46	-0.23	0.53	0.36	0.12
NH_4^+							0.01	0.11	-0.50	0.07	-0.16	0.10	-0.32	0.06	0.05
PO_4^{3-}								0.22	0.18	-0.25	0.19	-0.50	0.17	-0.28	-0.15
SiO_2									-0.07	-0.09	0.19	0.40	0.42	-0.01	0.03
POC*										0.71	0.37	-0.27	0.28	0.22	0.44
PON*											0.32	-0.22	-0.03	0.29	0.56
POP*												-0.40	0.81	0.79	0.67
TN*													-0.26	-0.64	-0.57
TP*														0.43	0.42
Bacteria															0.60

* These parameters include only the 13 months extended sampling between June 2003 and June 2004

for larger flagellates the Utermöhl counts were used.

Carbon content of bacteria was estimated using a conversion factor of 0.350 pgC μm^{-3} (Bjørnsen 1986). *Prochlorococcus sp.* was estimated using a conversion factor 0.133 pgC μm^{-3} (Simon and Azam 1989). For *Synechococcus sp.* we used a mean carbon content of 0.357 pgC μm^{-3} (Arin *et al.* 2002 and references therein). Carbon content of microphytoplankton was estimated applying the formula C=0.109 $V^{0.991}$ (Montagnes *et al.* 1994), where C is the carbon content in pg $cell^{-1}$ and V is the biovolume in $\mu m^3 cell^{-1}$. Similarly, for ANF we used formula C=0.433 $V^{0.863}$ (Verity *et al.* 1992).

Correlations between parameters were performed using Pearson's correlation coefficient (r_p). When the number of samples was low (i.e. n<25) correlations between parameters were estimated using non-parametric Spearman's correlation coefficient (r_s).

Cross correlations were systematically performed between the 7-day moving averaged meteorological and physical data on one hand, and water sampling data on the other. A cross correlation is a standard method to estimate the degree of covariation of two time series, as a function of the time lag between them (e.g. Jenkins and Watts 1968). By plotting the correlation coefficients versus the time lag (cross correlogram), it is possible to identify the time scales of response of one variable with respect to another. Usually, when performing this type of analyses, the sampling frequency of the two series is the same. In order to retain information contained in the short scales we cross correlated the monthly sampled parameters with the original high frequency physical data series. For example, for a lag of 10 days, we computed the correlation coefficient between our monthly water sampling measurements and the respective values of the high frequency parameters 10 days before each sampling. This decreases robustness of the analysis but allows detection of responses at the weekly scale.

Only water parameters from the Blanes Bay Microbial Observatory were used for these analyses. As both the different meteorological and hydrodynamical time series and the different parameters of the Blanes Bay Microbial Observatory had different time spans and missing points, the number of samples in each cross correlation was different.

Results

Meteorological data

Wind energy flux in the studied area is maximum in spring, between April and May, and minimum in summer and autumn (fig. 2A), a pattern strongly determined by local topography. Most of the year the prevailing winds in Blanes are south-westerlies (locally called "garbins"). These are moderate winds, typical in summer, originated as strong SE sea breezes modified by the Coriolis Effect. Offshore winds are prevailing only in autumn, although they are also very frequent in winter. The E and NE wind ("llevant"), even if not very frequent, can be strong, and because of a potentially long fetch, can be associated with high waves and high turbulence levels. Easterly wind storms usually occur when there is a low pressure system in the South Western Mediterranean and are usually accompanied by persistent rains (Reiter 1975). Whereas in other regions of the Catalan coast autumn is an active season with frequent wind events, in this zone wind energy flux is low, despite very intense events are still relatively more frequent in winter and autumn than in summer (Guadayol and Peters 2006, chapter 1).

Significant wave height (SWH) tends to be slightly higher in autumn than in the rest of the year (fig. 2B). However, high frequency variability is more important than seasonality. High values of SWH were not related with peaks in local wind intensity and were the result of swell.

Precipitation follows the classical pattern of Mediterranean climate (Llasat and Puigserver 1997), i.e. episodic and strong rainstorms in spring and in autumn and dry summers. As a consequence, river discharge also clearly follows this pattern (fig. 3).

Water conditions

Surface water temperature (fig. 4A) was closely linked to air temperature (data not shown), showing a marked seasonality. It showed a maximum around 25ºC between August and September, and a minimum of

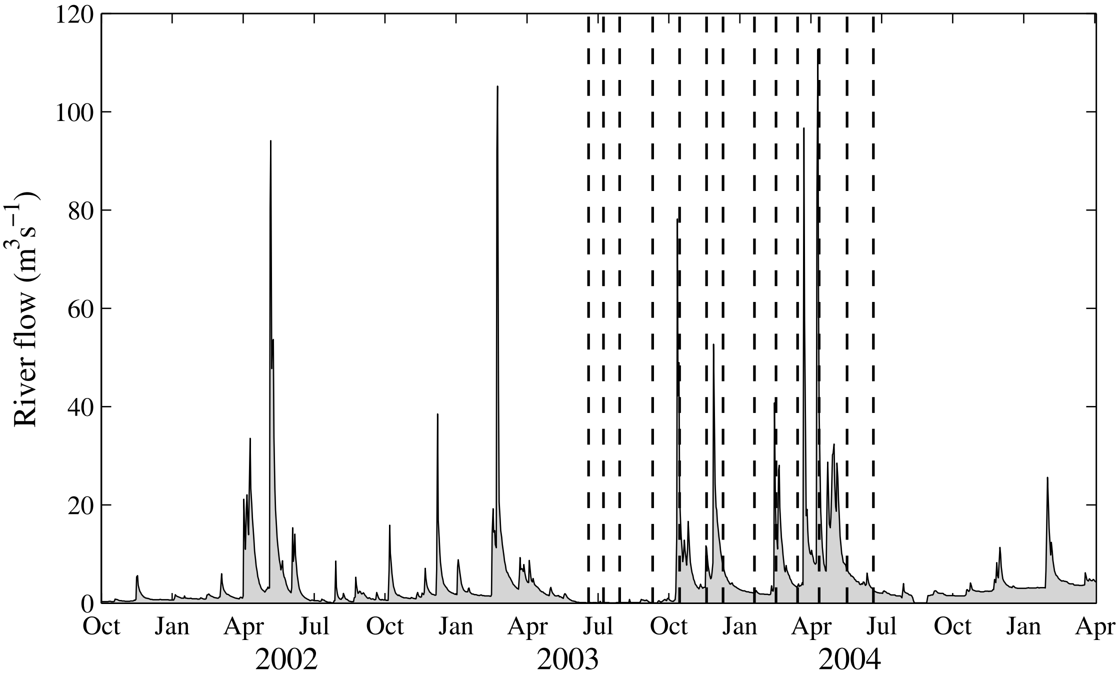

Figure 3: *River discharge at station EA89 in the river Tordera. Vertical bars mark the sampling times during the period of extended analysis.*

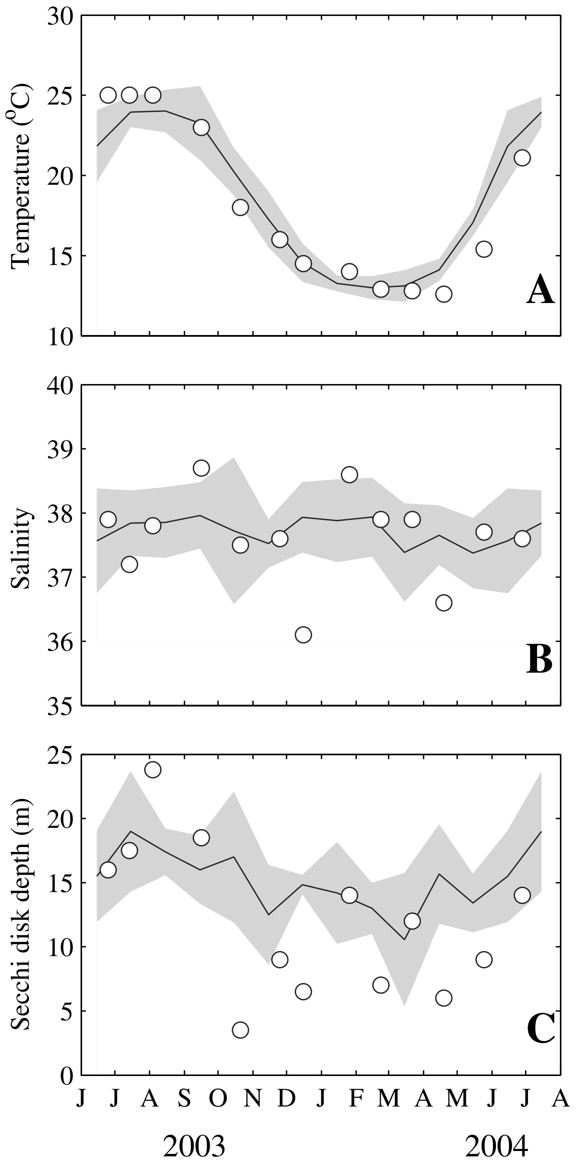

Figure 4: Water properties in Blanes Bay. A) *temperature, B) salinity and C) Secchi disk depths. Lines and grey area show the ensemble average and standard deviation respectively, calculated for each month of the year using the whole time series. Open circles are the values of the extended analysis conducted between June 2003 and June 2004.*

around 12ºC between February and March. Secchi disk depths ranged between the whole water column depth (20-24 m), and 5 meters (fig. 4C). In general they were higher in summer. Chla (fig. 5A) shows a typical seasonal pattern for this area (Estrada *et al.* 1985), with a main peak in late winter, coinciding with the maximum wind energy flux, a minimum in summer and a secondary peak in autumn. Bacterial abundance (fig. 5B) has no clear seasonality.

The correlation matrix between most water parameters is shown in table 1. Several variables correlate significantly with water temperature, indicating their seasonal dynamics. This is the case for Secchi depth, chla, TP, and dissolved inorganic nutrients except ammonium and phosphate.

Cross correlations

Cross correlograms were performed between the available meteorological and physical variables (i.e. river discharge, SWH, wind energy flux, air temperature and rainfall) and the water column parameters. Only river discharge (fig. 6), SWH (fig. 7) and rainfall (not shown) resulted in significant short lagged correlations. Rainfall cross correlograms are not shown because they were very similar to those of river discharge, with slightly longer time responses and lower correlation levels. Air temperature correlated significantly with several of the parameters, but the lag spanned several months, indicating a seasonal relationship. Wind energy flux showed no significant relationship with any of the water column parameters. Note that the number of data points included in each cross correlations is different (figs. 6 and 7). This is because the time span differed for each parameter. For each cross correlation the maximum possible number of values was used.

Salinity and Secchi depths were both negatively correlated with river discharge, as expected (fig. 6). Water temperature was also negatively correlated, but it showed no short-lagged pattern, indicating a seasonal relationship with river discharge. NO_3^- and SiO_2 had positive correlations to river discharge whereas PO_4^{3-} showed no response. Chla concentration and NO_2^- had very similar patterns with a delayed response with respect to the other parameters. Chla showed the highest maximum correlations with river discharge (r_p>0.9, *p-value*<0.0001, *n*=28). Bacterial abundance had the most delayed and weak response to river input.

Cross correlograms with SWH show similar patterns to those with river discharge (fig. 7), although correlations are lower, and time responses are in general longer and weaker. Secchi depth, NO_3^- and SiO_2 showed significant correlations with SWH at lag 0.

Extended analysis of 2003-2004

Air temperature in summer 2003 was higher than usual giving a slight positive anomaly in water temperatures (fig. 4A). Summer was also characterized by low wind energy fluxes (fig. 2A) and an almost null river discharge (fig. 3). Between October and December 2003 there were several events of high waves, and river discharge increased after a peak in October, coinciding with heavy rain events. Runoff showed five major events during this year, two of them in autumn 2003 and the other ones in spring 2004. Salinity ranged between 36.0 and 38.7 (practical salinity scale) and did not show any seasonal pattern (fig. 4B). Salinity minima coincide with the peaks in river discharge. The water column was mixed for most of the year. Only in summer, between June and September, a shallow thermocline at 2-3 meters was formed (Vila-Costa *et al.* in preparation). In spring 2004 there was a negative anomaly in water temperature.

Silicate and nitrate strongly covariated for the year of study (fig. 8). Both presented three major peaks, in October, December and May, after four major runoff events. Silicate concentration decayed to its minimum concentrations in the last two samplings. Nitrite was closely linked to chla concentration. Ammonia was low during the whole year, ranging between 0.06 and 0.48 µmol L^{-1}. However during the first part of the series, when river runoff and wind energy flux were low, NH_4^+ was slightly higher. Phosphate concentration was low (less than 0.03 µmol·L^{-1}) in summer 2003, and then tended to increase to reach values of more than 0.2 µmol·L^{-1} at the end of the series.

Within the year of extended study chla (fig. 5A) ranged between ca. 0.30 and 3.93 µg·L^{-1}. Between June and September 2003 it remained in its summer lowest concentrations. After the wind energy peak that occurred between October and November (fig. 2A), chla raised above its average seasonal levels. The maximum concentration was found in December 2003 coinciding with an event of precipitation and river runoff which took place two weeks before (fig. 3), and with the minimum surface salinity, which also indicates an invasion of freshwater from the river and terrestrial runoff. SWH was also peaking before the December sampling (fig. 2B), coincident with a persistent, although not very intense, NE wind event. This suggests the possibility of a resuspension event.

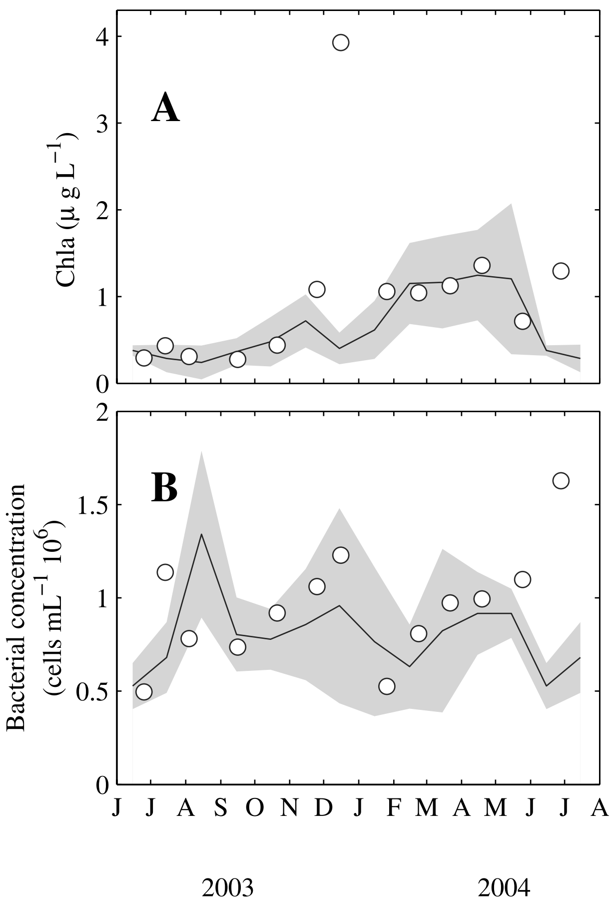

Figure 5: *Chlorophyll a concentration (A) and bacterial concentration (B).Llines and grey areas show the ensemble average and standard deviation respectively, calculated for each month of the year using the whole time series. Open circles are the values of the extended analysis conducted between June 2003 and June 2004. The chla value of December 2003 was excluded from interannual statistics.*

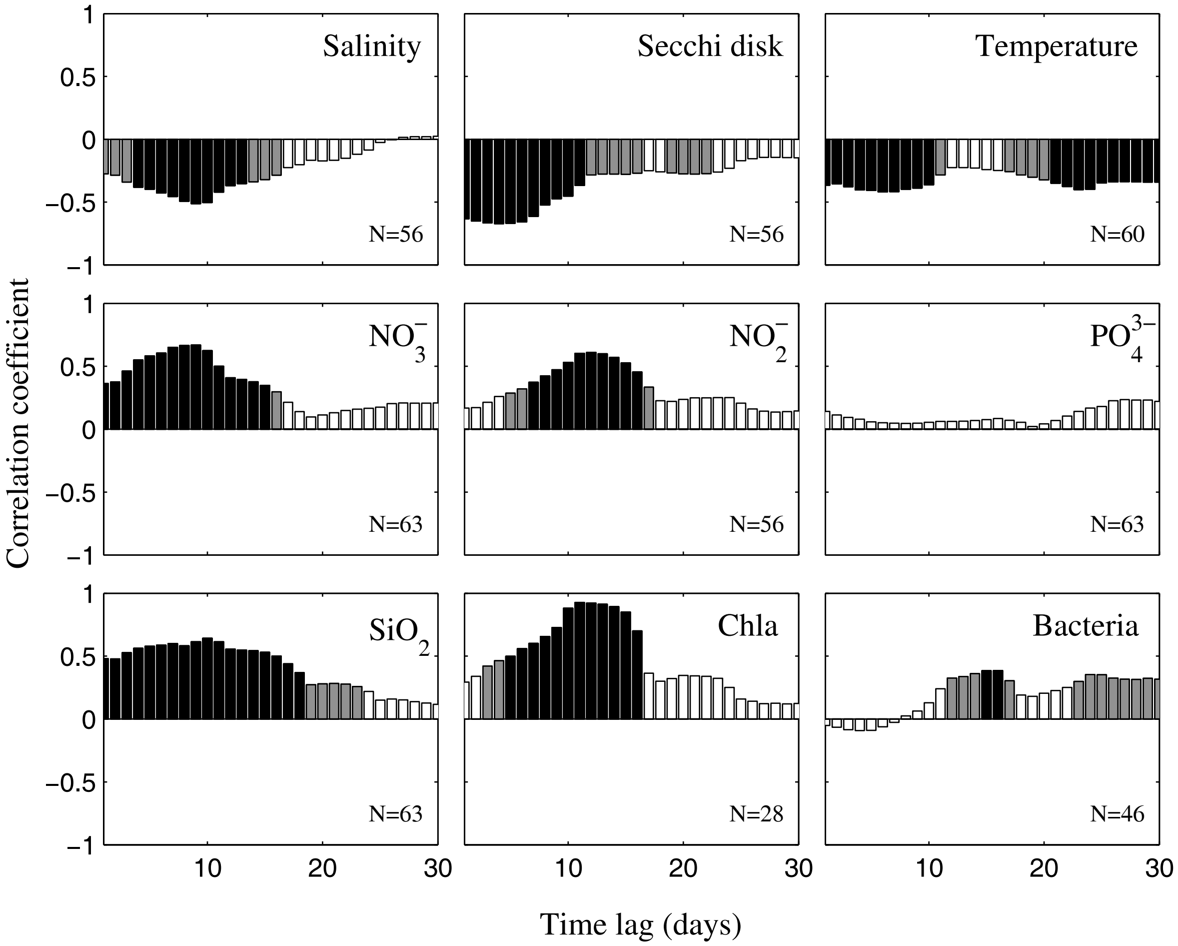

Figure 6: Cross correlograms between river discharge and sampled water column parameters. Black bars represent correlation coefficients at a significant level of p-value <0.01, and grey ones are significant at p-value <0.05.

Bacterial abundance within the year of study ranged between $5.0{\cdot}10^5$ and $1.6{\cdot}10^6$ cells mL^{-1} (fig. 5B). HNF (data not shown) were between $7.3{\cdot}10^2$ and $3.1{\cdot}10^3$ cells mL^{-1}. Both variables are positively correlated (table 1). Between June 2003 and February 2004 HNF were tightly coupled to bacteria (r_s=0.82, *p-value*=0.011, *n*=9).

Phytoplankton composition is shown in fig. 9A. Concentration of *Synechococcus sp.* ranged between $1.4{\cdot}10^3$ and $7.1{\cdot}10^4$ cells mL^{-1}, with a maximum in summer. It was positively correlated with temperature (r_s=0.87, *p-value*<0.001, *n*=13). *Prochlorococcus* sp. appeared after the *Synechococcus* sp. peak, and lasted throughout the autumn and winter, with a maximum in December 2003. ANF peaked also in December 2003, coinciding with the chla peak, and then showed a smaller maximum in March 2004. Concentrations ranged between 1.2 10^3 and 3.2 10^4 cells mL^{-1}. Diatoms reached maximum concentrations at the end of the series, in May and June 2004, due to the great abundance of C*haetoceros* spp. chains. In these months silicate concentration was minimum. Diatom carbon biomass was highly negatively correlated with the ratio Si:N (r_s=-0.94, *p-value*<0.001, *n*=13). The regression model fitted by nonlinear least square method is:

$$C = 1.75{\cdot}10^5 e^{-2.20 Si:N}; R^2 = 0.95 \qquad (4)$$

where *C* is the diatoms concentration in pgC mL^{-1}.

On the other hand the only significant relationship between diatom biomass and nutrient concentration was with PO_4^{3-} (r_s=-0.73, *p-value*=0.007, *n*=13).

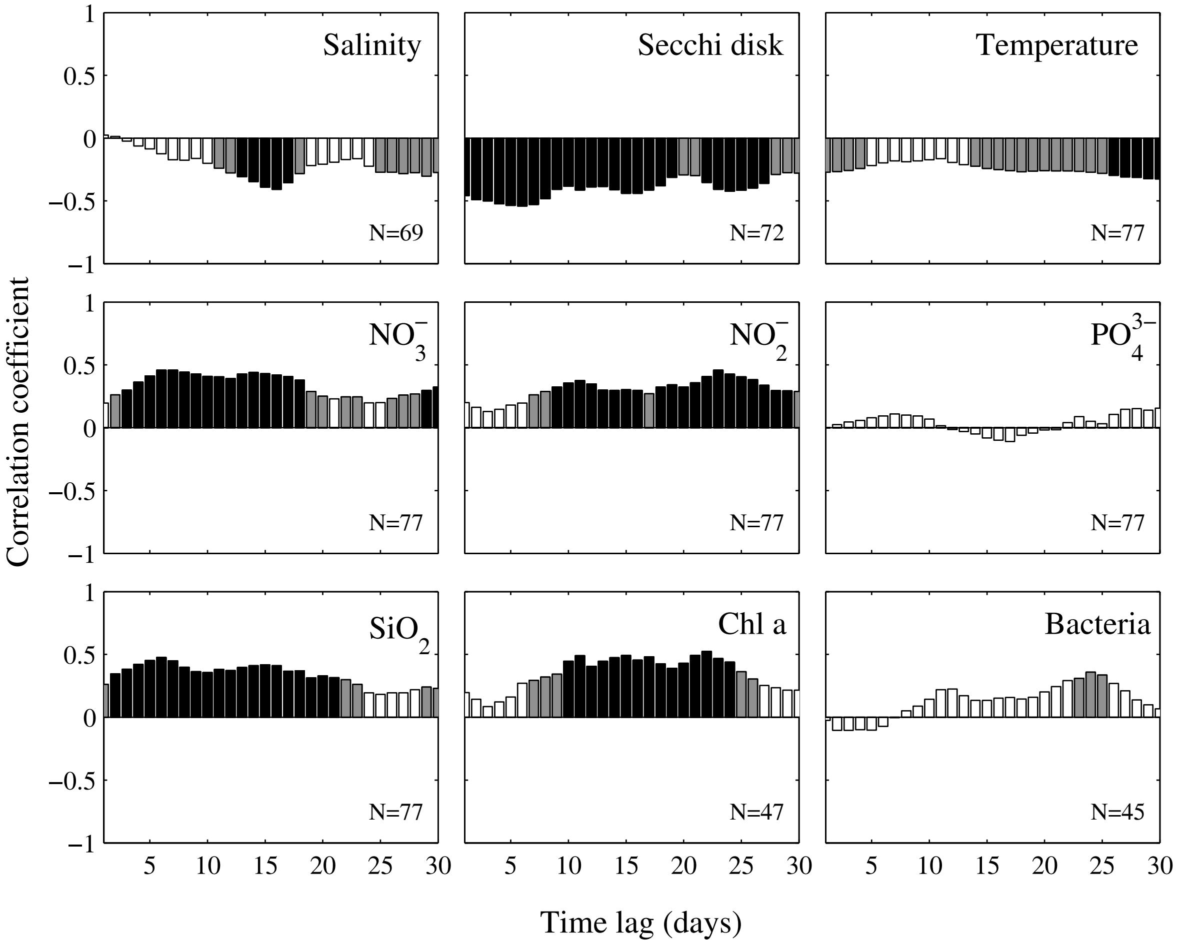

Figure 7: *Cross correlograms between SWH and sampled water column parameters. Black bars represent correlation coefficients at a significant level of p-value <0.01, and grey ones are significant at p-value <0.05.*

Size spectra slopes are shown in fig. 9B. Between June and August 2003 the slope of the size spectra of osmotrophic community was highest. Phytoplankton was dominated by the smallest organisms, i.e. cyanobacteria and ANF, and the contribution of diatoms in terms of carbon was around 10%. In September the slope noticeably decreased as coccolithophores increased in abundance. The rest of the series, from October 2003 to June 2004, diatoms were clearly dominant except in December, when there was a peak in autotrophic nanoflagellates along with an increase in the slope of the size spectrum. In the last three months the slope was low due to an increase in diatom concentration, mainly *Chaetoceros* spp. The living phytoplanktonic C/chl ratio was between 20 and 60, except for December 2003, when it was around 10, and for May and June 2004, coinciding with the bloom of *Chaetoceros* spp. when it was over 100.

Discussion

Cross correlations are a common tool in ecology to investigate lags in responses between different variables (e.g. Olden and Neff 2001 and references therein). The frequency of biogeochemical sampling was not high enough to perform cross correlations between different water column parameters. However, it was possible to establish their sequence of response to hydrodinamic forcing from their cross correlations with high frequency meteorological and hydrological time series. The high frequency time series used in these analyses were air temperature, precipitation, wind energy flux, SWH and river discharge. The water column parameters with a marked seasonal variability, such as chlorophyll *a* or Secchi depth (table 1), were significantly correlated with air temperature. The cross correlograms with air temperature (data not shown) presented low frequency

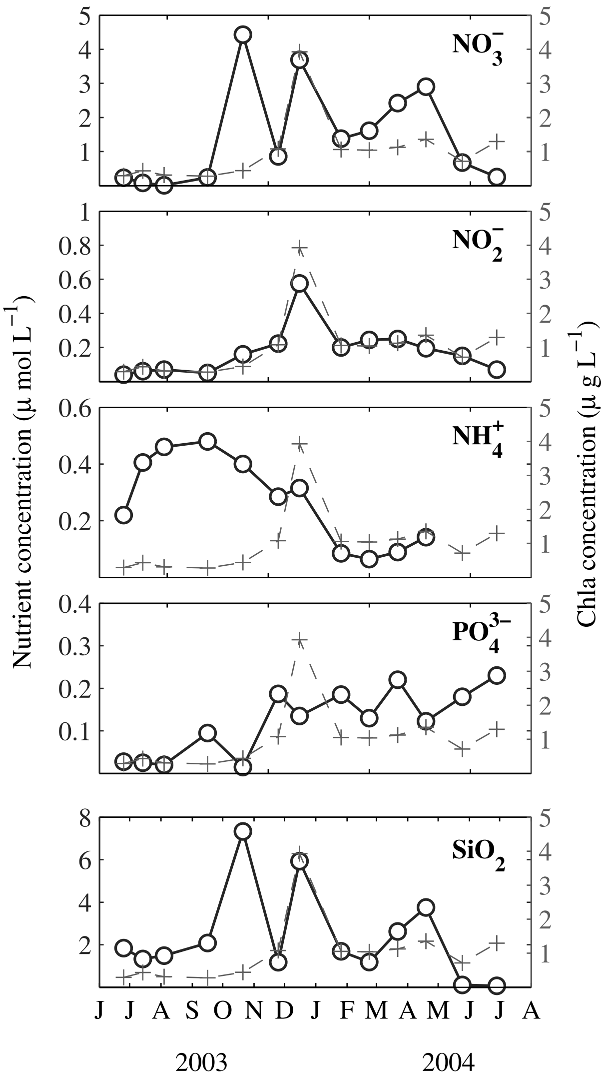

Figure 8: *Nutrient concentrations (open circles) in Blanes Bay during the period of study. Chla concentration (crosses) is represented in each subplot as reference.*

oscillations, with periods of several months, indicating seasonal relationships. The patterns observed with river discharge and SWH (and also precipitation, data not shown), which are parameters characterised by high variability at short time scales (Duarte *et al.* 1999), are completely different. Correlations are relatively short-lagged, of the order of weeks and highly significant (figs. 6 and 7), suggesting rapid responses to episodic events.

Based on the cross correlations with river discharge, we have interpreted the sequence of responses to terrestrial runoff as follows. After a runoff event, surface salinity and Secchi depth, which can be considered as an integrated estimate of water column turbidity, decrease. The concentration of dissolved inorganic nutrients increases because of runoff input. With a few days lag, biomass of autotrophic organisms responds to this increase in inorganic nutrient concentration. This lag is within the same range found by Duarte *et al.* (2000), in a mesocosm experiment conducted in Blanes Bay during July. They found a lag of 4 to 12 days in the response of chla to nutrient additions, depending on the level of nutrient enrichment. The delay in the response to nutrient input could be explained by an initial negative effect on primary production of limited light availability as a consequence of increased turbidity (Cloern 1987, Cotner *et al.* 2000), although in the experimental setup in Duarte *et al.* (2000) there was no increase in turbidity.

Bacteria respond after chla and only weakly. There is no significant correlation at lag 0 between river discharge and bacterial concentration. Vaqué *et al.* (1997) related the bacterial abundances in Blanes Bay with the allochtonous input of dissolved organic matter from runoff flushing after rainstorms. This is consistent with the high negative correlations found between salinity and bacterial and HNF counts (table 1). However, the several days lag in significant cross correlation between river discharge and bacteria suggests that these would be growing on newly produced organic matter, rather than on land-derived detritus input. Given the time resolution of our analyses, however, we can not rule out a quick response of bacteria to terrestrial dissolved organic matter input.

Along with the peak in chla correlation to river discharge, we observed a very similar peak of NO_2^-. Nitrite is excreted from the cells under growth limiting conditions, for example when light is low (Collos 1998), because its further reduction to ammonia needs energy. This covariation could be an evidence for dominant phosphorus limitation in the Bay of Blanes, as in N-limiting conditions nitrite is expected to be significantly taken up by phytoplankton (Serra *et al.* 1978, Collos 1998).

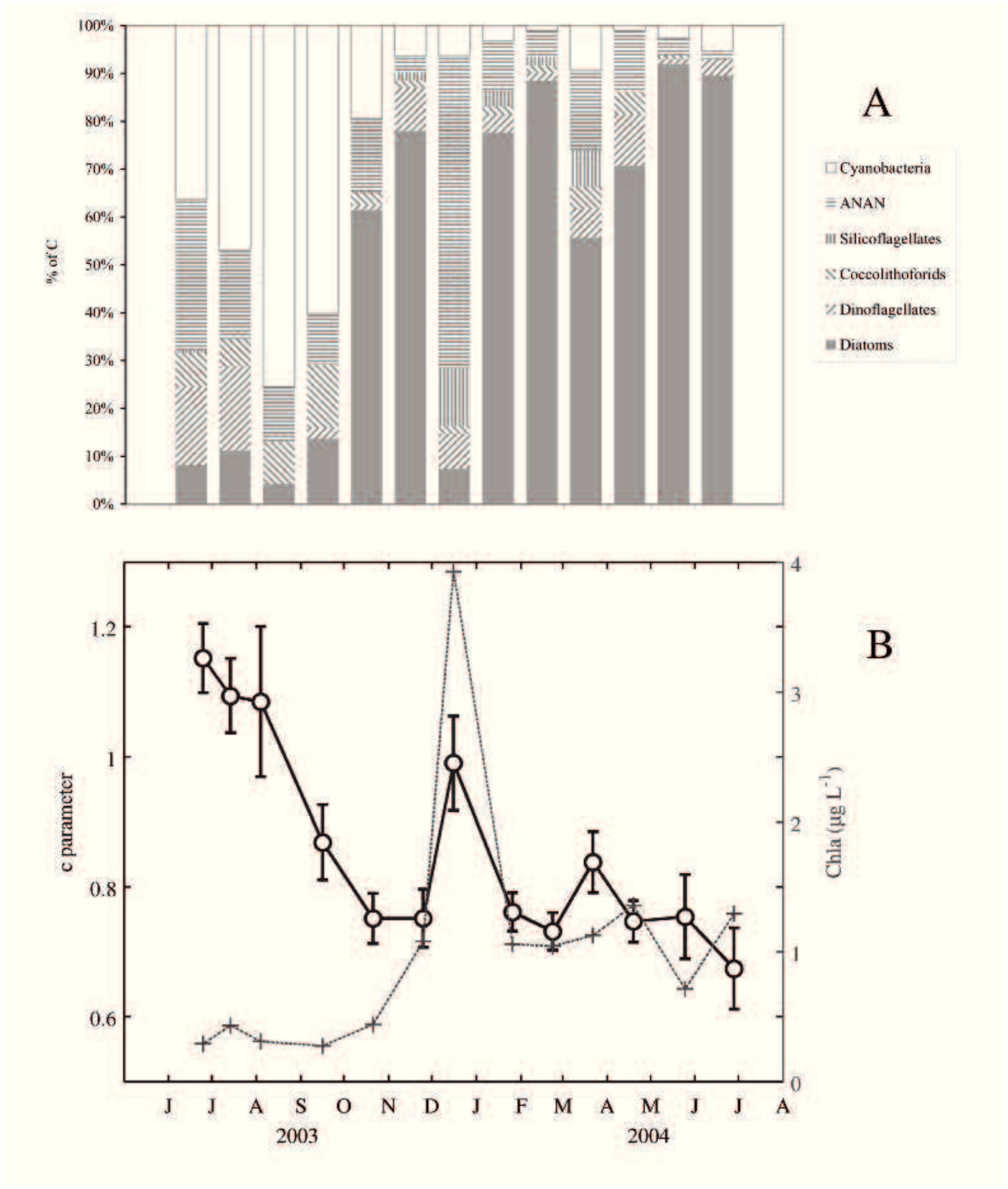

Figure 9*: A: Percentage contribution of each of the main phytoplanktonic groups to total phytoplankton carbon between June 2003 and June 2004; B: c parameter of the adjusted Pareto distribution. Chlorophyll a concentration (crosses) is represented as reference.*

Correlations between SWH and water column parameters show remarkable resemblance with river discharge cross correlations, but with slightly longer delays. In fact, SWH and river discharge are significantly correlated, with a maximum at 3 days lag. Rough seas are often coinciding with torrential rains in strong East storms (Arnau *et al.* 2004). Therefore, both cross correlation analyses could, in fact, be somewhat redundant.

In order to explore this possible redundancy we should examine both correlograms together (figs. 6 and 7). Note that the maximum correlation between salinity and river discharge occurs with aprox. a 10 d lag. Salinity decreases occur because of freshwater inputs, and one would expect a smaller lag with respect to discharge. It has to be taken into account that the discharge gauge is located somewhat upstream (ca. 10 km) but this could probably not account for more than 1 d lag. Our sampling site is located north of the river mouth while the plume extends south. Likely, the salinity signal takes some days to diffuse into our sampling location. The maximum correlation between salinity and SWH occurs several days later than for salinity and river discharge. Since wave height can not affect salinity, its correlation with SWH must be the result of the correlation between SWH and discharge. Storms producing high waves will often be accompanied by rainfall in the La Tordera catchment (894 Km^2) resulting in discharge peaks several days later. This explains the difference in lag between SWH and discharge in the salinity response. However, this difference in lag is not present when considering NO_3^- and SiO_2. maximum correlation peaks. In fact, these occur before the peak in salinity correlation in the river discharge cross correlogram. This indicates that not only river discharge but also sediment resuspension must be affecting the concentrations of NO_3^- and SiO_2.

Rossi and Gili (2005) postulated that terrestrial runoff may be a more important factor than water column mixing in determining seasonal trends in seston composition in coastal and near-bottom water layers. In general, the relative importance of both processes will depend on the hydrography of the location, the proximity of rivers and populated areas, and on the type of sediment and benthic communities. As an example, in another NW Mediterranean Bay, Grémare *et al.* (2003) found an immediate response of bacterial biomass and production, but not of phytoplankton, after a severe storm. They concluded that phytoplankton could not benefit from the increased nutrient concentrations because resuspension caused limitation by light, and bacteria exhausted nutrients long before sedimentation of resuspended material brought back the water column to phytoplankton growing conditions. Again, with our approach, we could not detect any immediate response of bacteria to nutrient enrichments. However, we observed a response of chlorophyll *a* slightly after the response in turbidity, suggesting that there could be some light limitation. This indicates that although chla and Secchi depths are negatively correlated (table 1), particle input from river runoff or resuspension is also strongly determining the turbidity in this location (e.g. Erlandsson and Stigebrandt 2006). However, the increase in turbidity after these events may be not high enough to resemble the situation described by Grémare *et al.* (2003).

Further insight into the possible system responses to turbulence and nutrient enrichment events can be gained from the extended analysis conducted with data between June 2003 and June 2004. The summer of 2003 registered unusual high temperature and low precipitation in Europe (Ciais *et al.* 2005), and the wind energy flux was also particularly low (fig. 2A). As a consequence the discharge in the Tordera river, our estimate for all sources of runoff, was close to 0 (fig. 3) and resuspension of nutrients from sediments was likely to be small. This could explain why during this period the concentrations of nutrients, especially of PO_4^{3-}, were low when compared to the rest of the year, although anthropogenic input in this Bay has been hypothesized to be maximum in summer (Duarte *et al.* 1999). Between June and September 2003, Pinhassi *et al.* (2006) found the highest response of heterotrophic bacteria to nutrient addition for the series, suggesting strong nutrient limitation during this period. In addition only during these months they found a change in the composition of bacterioplankton as a response to nutrient enrichment, implying that the bacterial assemblage was well adjusted to the prevailing low nutrient conditions. In these oligotrophic conditions the microbial food web was dominating, as suggested by the coupling between bacterial and HNF concentration. In fact, the dominance of bacterial biomass may be also related to the antropogenic input of organic matter. The higher ammonium concentration indicates also a preponderance of recycling processes over new production (Sarmiento and Gruber 2006). This could be again explained because ammonium is the dominant form of anthropogenic nitrogen input in summer in Blanes Bay (Duarte *et al.* 2000).

In the NW Mediterranean, high sea surface temperatures in summer are usually leading to heavy rainfalls in autumn (Llasat and Puigserver 1997). Thus, the second part of the series was characterized by several events of rain. From October 2003 to June 2004 there were at least 4 major river discharge events (fig. 3). The values of NO_3^- and SiO_2 measured in the first samplings after the events were maxima (fig. 8) whereas the Secchi depths were minima (fig. 4C), coherently with the results of the cross correlations analyses. Also the concentration of PO_4^- did not show an overall clear response to river discharge events, but steadily increased after November (fig. 8), implying an allochtonous input. Dynamics of chla were less clearly related to events of river discharge and SWH during this period, but they were very significantly correlated to NO_3^- and SiO_2 concentrations (table 1). In the October 2003 sampling, which was performed immediately after a discharge and wave event, no chla increase was observed although nutrients were high. By contrast in other cases, as for example in the December 2003 and May 2004 samplings, performed several days after meteorological episodes, an increase in chla was observed.

As seen in the cross correlation analysis, NO_2^- and chla were strongly linked (table 1). The episodic inputs of nutrients from terrestrial runoff and resuspension (e.g. December 2003) determined phytoplankton succession, pushing the system from an oligotrophic state, dominated by the microbial food web and heterotrophic processes, to a more eutrophic state. The maximum in chla observed in December 2003 could also be due to the resuspension of microphytobentos and detritus rather than to enhanced primary production, as it has already been observed in this location (Satta *et al.* 1996). During the first half of the month a steady wind from NE blew, and the SWH reached values of almost 5 m (fig. 2B).

Between June 2003 and 2004 the system was apparently deficient in phosphorus. Streams episodically provided SiO_2 and NO_3^-, and likely PO_4^{3-} too. Chlorophyll concentration responded to these events after some days. The frequency and intensity of river discharge events determined the concentration of phytoplankton and especially of diatoms. According to Duarte *et al.* (1999), the phosphate input pattern in Blanes Bay is associated with sewage discharge, which is maximum during summer because of the increased tourist population. However, we did not observe a seasonal pattern in PO_4^{3-} concentration during this period, but rather an increasing trend, maybe due to the meteorological anomaly in summer 2003 (Ciais *et al.* 2003). In contrast with NO_3^- and SiO_2, PO_4^{3-} remained relatively invariant even after episodic supplies by runoff or by resuspension. The cross correlation analysis did not show either a response of PO_4^{3-} to either river discharge or SWH (fig. 6 and 7). On the other hand, TP and POP were significantly correlated with Secchi depth, chla and NO_3^- (table 1). This suggests that the dominant form of riverine P input was either particulate (Benitez-Nelson 2000), or PO_4^{3-} that was rapidly depleted by phosphorus-limited osmotrophs through luxurious consumption after an episodic input in P. In the latter case, TP or POP would be better indicators of phosphorus dynamics because any input of PO_4^{3-} would be quickly consumed and therefore difficult to detect. Finally, chlorophyll shows no significant relationship to POC and PON, whereas it is highly correlated to POP. Again, this points to the importance of P in this system. The stoichiometry of particulate organic matter may change throughout the year, but the amount of chlorophyll in the long term is basically limited by the amount of available P.

The strong relationship between diatom concentration and Si:N ratios is puzzling, because there is no such relationship with silicate or with nitrogen alone. This would imply no general limitation of diatoms by these elements. In fact, diatoms were positively correlated with PO_4^{3-}, suggesting again a limitation by P. The correlation between diatoms and Si:N could be partly explained by seasonal changes in the stoichiometry of continental runoff inputs, following human agricultural and touristic practices (Duarte *et al.* 1999, Chattopadhyay *et al.* 2005). Alternatively, the Si:N ratio could be determined by the amount of the diatoms in relation to other groups of phytoplankton. The Si:N ratio for marine diatoms is around 1:1 (Brzezinski 1985). However diatoms have been seen to grow even at limiting Si concentrations, whereas at low phosphate concentrations they are outcompeted by flagellated forms (Egge 1998). In May and June 2004, when PO_4^{3-} was at its highest concentrations, diatoms were dominant in Blanes regardless of Si:N ratios below 0.5. In these P-deficient waters, the proportion of diatoms with respect to other phytoplanktonic groups can dramatically change during the year following changes in PO_4^{3-} concentrations (fig. 8 and 9). While N is taken up by all phytoplankton, Si is significantly used only by diatoms. Therefore, the overall relative consumption of Si to N will change with the relative amount of diatoms present. The strong relationship found between diatoms and the Si:N ratio (eq 4) could be used to predict one variable as a function of the other, as long as P remains the limiting nutrient.

In summary, the cross correlation analyses presented in this study were successful in detecting responses of several parameters to forcings characterized by their episodic nature, that is, terrestrial runoff, precipitation and waves, showing the potential for building predictive tools. The robustness of such analyses will significantly increase with higher frequency biogeochemical data from automated stations. Short-scale variability in abiotic forcings must be taken into account when interpreting trends in biological activities in coastal ecosystems even for longer seasonal scales (Rossi and Gili 2007, Seymour *et al.* 2005). The results of this and other studies (Nogueira *et al.* 2000, Grémare *et al.* 2003, Rossi and Gili 2005), show that the episodic nature of terrestrial runoff and of turbulent forcing is a key factor for understanding the dynamics of coastal planktonic communities.

Acknowledgments

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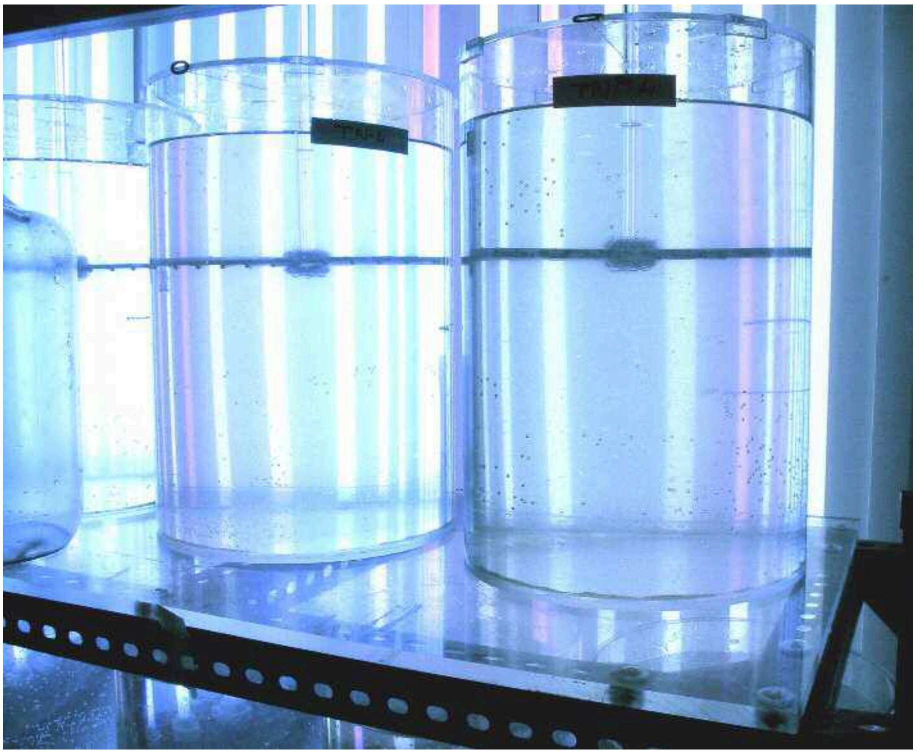
Chapter 4: Episodic meteorological and nutrient load events as drivers of coastal ecosystem dynamics: an experimental simulation.

Episodis meteorològics i d'entrada de nutrients com a conductors de la dinàmica de l'ecosistema costaner: una simulació experimental.



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Manuscript

Photo: experimental containers used in this study

Abstract

A one year long series of monthly experiments with laboratory enclosures was conducted with water from Blanes Bay (NW Mediterranean) to analyse the change in the short time response of the osmotrophic planktonic community to simulated turbulence and nutrient input events. Both experimental factors triggered a relative increase of biomass in the enclosures, in terms of chlorophyll *a*, bacteria and particulate organic matter. Ratios of particulate organic nitrogen to particulate organic phosphorus were higher in the water than in the sediment, although turbulence partially smoothed out this difference. This dynamics is likely caused by different net sedimentation rates between particulate organic matter fractions. The short time responses to experimental forcing were depending on initial physico-chemical environmental conditions, and were not found to be related to seasonal changes in phytoplankton composition and size structure. The response to turbulence, in terms of chlorophyll *a*, was maximum in spring, and weakly related to day length. The response to nutrient enrichment was found to be seasonal, being correlated to the photoperiod, and also strongly correlated to nitrate and silicate initial concentrations and negatively correlated to Secchi depth, which are proxies of recent inputs of nutrients by episodes of resuspension and river discharge.

KEYWORDS: turbulence, nutrients, osmotrophic plankton, microcosms experiments, Blanes Bay.

Resum

Per tal d'analitzar els canvis en la resposta a curt termini de la comunitat planctònica osmotròfica a episodis simulats de turbulència i entrada de nutrients. es va dur a terme una sèrie de 13 experiments mensuals amb aigua de la Badia de Blanes (Mediterrani nord-occidental) reclosa en recipients de laboratori. Els dos factors experimental van estimular un increment de biomassa, en termes de clorofil·la *a*, bacteris i matèria orgànica particulada. El quocient entre el nitrogen i el fòsfor orgànics particulats era més alt en l'aigua que en el sediment, encara que la turbulència suavitzava aquesta diferència. Aquesta dinàmica probablement es deu a diferències en les taxes de sedimentació netes entre les diverses fraccions de matèria orgànica particulada. Les respostes a curt termini dels factors experimentals depenen de les condicions ambientals, i van resultar no estar relacionades amb canvis estacionals en la composició i l'estructura de mides de la comunitat fitoplanctònica. La resposta a la turbulència, en termes de clorofil·la *a*, fou màxima a la primavera, i estava feblement relacionada amb el fotoperíode. En canvi, la resposta a l'enriquiment en nutrients va resultar ser estacional, estant positivament correlacionada amb el fotoperíode, i també amb les concentracions inicials de nitrat i silicat, i negativament amb la profunditat del disc de Sechi, que són indicadors de les les entrades recents de nutrients per episodis de ressuspensió i descàrrega de rius.

PARAULES CLAU: turbulència, nutrients, plàncton osmotròfic, experiments de microcosmos, badia de Blanes.

Introduction

The dynamics of plankton in coastal areas is related to the variability in external energy input (Margalef 1978). In the upper layer of the ocean the input of mechanical energy is due to many different processes, such as winds, tides, surface wave breaking or Langmuir circulations (Gargett 1989). This energy fuels turbulence that shows time and space length scales from those of energy input to those of dissipation as heat. As a consequence, effects of turbulence on planktonic organisms operate at different levels (Thomas *et al.* 1997, Peters and Marrasé 2000).

At the largest scales of energy input, of the order of tens of meters to hundreds of kilometres, turbulence is a key factor to explain the distribution of phytoplankton with respect to their resources, namely light and nutrients (e.g. Gran 1931, Sverdrup 1957, Huisman *et al.* 2002, Diehl *et al.* 2002). Mixing enhances the diffusion of nutrients from bottom nutrient-rich layers, and may alter the light regime that phytoplanktonic cells experience. Large eddies can also keep planktonic organisms in suspension within the mixed layer (Ross, 2006), favouring those cells with high settling velocities, like diatoms and in general large non-motile cells, in front of those which control their position into the water column by swimming, as it is the case of dinoflagellates.

These large scale motions break into progressively smaller eddies without loss of kinetic energy. As eddies get smaller viscosity starts to be important, until they reach the Kolmogorov length scale, where the viscous forces equal the inertial forces. Below this scale energy starts to be dissipated as heat, and eddies are no longer formed. There remains a laminar shear field, changing in direction and intensity. Kolmogorov scales typically range between some cm and some mm. At these small scales and below, the direct effects of turbulence on planktonic organisms have been seen to derive from 4 main processes: (1) an increase in uptake rate of nutrients (Lazier and Mann 1989, Jumars *et al.* 1993, Karp-Boss *et al.* 1996), (2) an increase in the encounter rates between particles potentially leading to an enhancement of grazing rates (Rothschild and Osborn 1988), of mating (Visser and Jackson 2004), and of particle aggregation (Jackson and Burd 1998, Beauvais *et al.* 2006); (3) an increase of sedimentation rates due to increased aggregation (Kiørboe 1997) or to enhanced settling velocities (Ruiz *et al.* 2004); and (4) an alteration of a variety of physiological processes (Berdalet and Estrada 2005).

All these effects, both at large and small scales, which operate mainly on individuals, can lead to changes at the ecosystem level. These include changes in the species composition of the community (Margalef 1978, Estrada *et al.* 1987), in its size structure (Arin *et al.* 2002, Malits *et al.* 2004, Cozar and Echevarría 2005, Peters *et al.* 2006), and elemental stoichiometry (Maar *et al.* 2002), and even in the ecosystem metabolism (Peters *et al.* 2002, Alcaraz *et al.* 2002).

In addition, the input of external energy is often associated to nutrient loads. As stated above, mixing of the euphotic layer can bring in nutrients from the deep layers. In shallow coastal waters it can increase the flux of nutrients from the sediments (e.g. Dade 1993, Mann and Lazier, 1996). Often, episodic land input of nutrients is also associated to turbulence. For example in the NW Mediterranean the occurrence of East storm winds, which because of its long fetch generate the highest waves in the zone, are often associated to torrential rains (Arnau *et al.* 2004, Chapter 3) that derive in strong episodes of terrestrial runoff.

The main mechanical energy input in the NW Mediterranean, where the tides are relatively small, comes from the wind, which generates turbulence directly by its stress on the water surface, or indirectly, generating wind waves. The distribution of wind events is not homogeneous either in space or in time (Guadayol and Peters 2006). Particularly in the coastal area, the influence of land and its orography can considerably alter the spatial distribution of winds and of turbulence. As a result turbulence and the nutrient loads associated with it are highly episodic. Because the effect of turbulence is species-specific (Peters and Marrasé 2000, Sullivan and Swift 2003) and size-dependent (Karp-Boss *et al.* 1996), the response of the pelagic ecosystem to these episodic perturbations depends on the initial composition and structure of the planktonic community (Estrada *et al.* 1987). We hypothesize that, as a consequence, the response to such disturbances will change throughout the seasonal cycle following changes in the planktonic community. To test this hypothesis we assessed the short time reactivity to turbulence and to nutrient additions of the different planktonic assemblages during an annual cycle. We monitored the reaction in terms of biomass of the osmotrophic planktonic community to simulated events of turbulence and nutrient load in a series of monthly laboratory experiments performed between June 2003 and July 2004 in a coastal area (Blanes Bay, NW Mediterranean).

In order to experimentally approach the effects of turbulence events we used an oscillating grid system. This kind of systems has been traditionally used as a mean to generate isotropic small-scale turbulence in studies of plankton dynamics (Peters and Redondo 1997, Sanford 1997, Chapter 2), but also in studies of resuspension of sediments (Hopfinger and Toly 1976). By using oscillating grids, we could generate small-scale turbulence, and also maintain cells in suspension, simulating a turbulent event both at the small and at the large scales.

Material and methods

Experimental design

From June 2003 to July 2004 a monthly experiment was performed (table 1). Subsurface water sampled in Blanes Bay (41º40'N, 2º48'E, 800 meters offshore, 20-24 meters depth), was screened through a 200 µm nylon mesh to remove mesozooplankton and brought to the laboratory in plastic carboys within 2 hours. In the laboratory, water was distributed in 15 L cylindrical transparent methacrylate containers with an inner diameter of 24.2 cm. The basic experimental design consisted of 6 containers, 3 of them subjected to natural levels of turbulence, and the other 3 kept under still conditions. Of each set of 3 containers, two received an addition of nutrients and the other one was left as control.

Experiments lasted for 3 days and were conducted in a temperature-controlled chamber set at the *in situ* water temperature. We used a combination of white and gro-lux fluorescent lights giving a measured light intensity inside the experimental containers of 225 µmol photons $m^{-2}s^{-1}$, which is above the range of saturating irradiance for this system (Satta *et al.* 1996, Lucea *et al.* 2005). The light/dark cycle was adjusted to the *in situ* conditions. Details of the sampling procedure and the initial conditions are given elsewhere (Chapter 3).

Nutrient enrichment

Additions of inorganic nutrients were made to follow molar Redfield Ratios (Si:N:P=15:16:1). NO_3^- was added at 4 µmol L^{-1}, PO_4^{3-} at 0.25 µmol L^{-1}, and SiO_2 at 4 µmol L^{-1}. The addition of SiO_2 declined during the annual series as there was precipitation in the stock solution, until it reached 0.3µmol L^{-1} in experiment 9. From there on a new stock solution was used. Metals were prepared as in the f/2 medium (Guillard 1975) and added maintaining their proportion to nitrate. These concentrations are well below the maximum concentrations of nutrients found in this area (Blanes Bay Microbial Observatory time series, Chapter 3), which are 7.1 µmol L^{-1} for NO_3^-, 1.0 for PO_4^{3-}, and 7.6 for SiO_2. After addition, all containers were gently stirred with a sterile plastic pipette to favour their homogenization. Samples for determination of inorganic nutrient concentration were taken from each container after about 2 hours from the addition. In experiment 6 (November 2003) nutrients were added by mistake to all containers.

Turbulence generation

Turbulence was generated by means of grids oscillating vertically as described in Peters *et al.* (2002). Stroke length and frequency of oscillation were set at 13 cm and 2.5 revolutions per minute respectively. The turbulent kinetic energy dissipation rate obtained with these settings, estimated applying equations in Peters and Gross (1994), was 10^{-2} cm^2s^{-3}. As water volume was reduced owing to sampling during the experiment, settings were adjusted daily to maintain the initial dissipation rate. Measurements done *a posteriori* with an

***Table 1**: Experimental dates and initial water conditions.*

Experiment number	Date	Temperature (ºC)	Salinity	Secchi depth (m)	Stratification	Photoperiod (h)
1	25/06/2003	25.0	37.9	16.0	yes	14.95
2	14/07/2003	25.0	37.2	17.5	yes	14.69
3	04/08/2003	25.0	37.8	23.8	yes	14.06
4	16/09/2003	23.0	38.7	18.5	no	12.05
5	21/10/2003	18.0	37.5	3.5	no	10.34
6	25/11/2003	16.0	37.6	9.0	no	9.23
7	16/12/2003	14.5	36.1	6.5	no	9.03
8	26/01/2004	14.0	38.6	14.0	no	9.60
9	23/02/2004	12.9	37.9	7.0	no	10.70
10	22/03/2004	12.8	37.9	12.0	no	12.1
11	19/04/2004	12.6	36.6	6.0	no	14.54
12	24/05/2004	15.4	37.7	9.0	yes	14.71
13	01/07/2004	21.1	37.6	14.0	yes	14.92

acoustic Doppler velocimeter with these conditions gave an integrated dissipation rate within the region delimited by the movement of the grid of $10^{-3}cm^2s^{-3}$ (Chapter 2). Thus, depending on the method used, our estimates of the generated turbulence were in the range 10^{-3}-$10^{-2}cm^2s^{-3}$.

Sampling procedure

Between 1.5 and 2 L of water was taken daily from each container at 10 a.m. local time, for determination of inorganic nutrients, particulate organic carbon (POC), nitrogen (PON), and phosphorus (POP), total nitrogen (TN), and phosphorus (TP), chlorophyll *a* (chla), bacteria, cyanobacteria, and microphytoplankton. Samples for autotrophic and heterotrophic nanoflagellates (ANF and HNF) were taken from the initial water and the last day. After sampling the last day, all containers were gently stirred with a sterile pipette until the sedimented material was completely resuspended. Again sample was taken from the water column for chla, particulate organic matter (POM), TN and TP, and microphytoplankton. From the sampling after resuspension it was possible to estimate the concentration of these parameters in the sediment the last day. Sampling was done by siphoning water through an autoclaved, milliQ rinsed, glass tube.

Dissolved inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and SiO_2) were analyzed with an Alliance Evolution II autoanalyzer following methods in Hansen and Koroleff (1999) with minor modifications. Samples were kept frozen at -20ºC until analyses.

Chlorophyll *a* concentration was determined fluorometrically (Yentsch and Menzel 1963). Samples of 20 ml were filtered through Whatman GF/F filters. Chlorophyll was extracted from filters immersed in 6.5 ml of 90% acetone (24 hours at 4ºC in the darkness). The extract was analysed with a Turner Designs fluorometer calibrated with pure chlorophyll *a* (Sigma Co).

For the determination of particulate organic matter, 200 to 500 ml of water were pressure-filtered (0.3 atm) through pre-combusted (450ºC, 4h) glass fibre filters (Whatmann GF/F) and immediately frozen in liquid nitrogen, and then stored at -80 ºC. For each POM sample, two subsamples were processed, one for PON and POC and the other for POP. Before analysis in a

Carlo-Erba CHN analyser, the POC/PON filters were thawed in an HCl^{-}saturated atmosphere for 48h to remove carbonates, and dried at 80ºC for 24 h. POP was determined following the oxidation (120ºC, 30 min) of the filter in acidic persulphate and subsequent analysis of dissolved phosphate (Hansen and Koroleff 1999).

TP was determined by wet oxidation using the same procedure as for POP. TN was determined after persulphate oxidation following Hansen and Koroleff (1999).

Concentrations of bacteria and cyanobacteria (*Prochlorococcus* and *Synechococcus*) were determined by means of flow cytometry following methods in Olson *et al.* (1993) and Gasol and del Giorgio (2000). Further details are given in chapter 3. Bacterial average cell volume (B [µm^3]) was estimated using the calibration function provided by Gasol and del Giorgio (2000):

$$B = 7.5{\cdot}10^{-3} + 1.1{\cdot}10^{-1}\left(\frac{F_{bacteria}}{F_{beads}}\right) \qquad (3)$$

where F_{bacteria} bacterial mean green fluorescence, and F_{beads} is the mean green fluorescence of the yellow-green 1-µm Polysciences latex beads used in this study.

Average volume for cyanobacteria was estimated assuming a spherical shape. We used a value of 1 µm for *Synechococcus sp.* (Agawin *et al.* 1998), and a mean diameter of 0.7 µm for *Prochlorococcus sp.* (Vaulot *et al.* 1990).

Heterotrophic and autotrophic nanoflagellates (HNF and ANF) were fixed with 1.5% (final concentration) glutaraldehyde, stained with DAPI (4',6'diamidino-2-phenylindole) and counted using epifluorescence microscopy (Porter and Feig 1980). Organisms were classified in 4 size classes: <4 µm, 4-8 µm, 8-16µm and >16µm. Cellular volume was estimated from the mean size of each class assuming a spherical shape.

Microphytoplankton was identified and counted with an inverted microscope (Utermöhl 1958) following the same procedure described in chapter 3. Dominant phytoplankton was identified at least to genus level. Width and length of cells were measured for each cell or for each colony or chain, and the number of cells per aggregate was also counted. When possible, volume of each cell was calculated applying the formula provided by Hillebrand *et al.* (1999). In radially asymmetrical cells the closest 2-parameter geometrical shape was assumed. This may cause a bias in the estimation of biovolumes, especially when radially asymmetrical cells are dominant.

Calculations and statistical analyses

The responses of the phytoplankton to the experimental forcings were quantified from chla values of the water column as follows. We assumed that within the short temporal frame of the experiments, the increase in chla growth in response to experimental forcings was going to approach an exponential function. Estimations of the net growth rate of chla in the water column for each container were thus calculated adjusting an exponential model to the experimental values of chlorophyll *a* concentration from daily samplings. The adjustment was made by non linear least square estimation. The difference between the apparent net growth rate of turbulent and still treatments was considered as a proxy of the net response of the phytoplankton biomass to turbulence. Similarly, the difference in growth rates between enriched and control treatments was considered a proxy of the response to nutrient additions.

Sedimentation rates were derived from total phosphorus (TP) measurements. It was assumed that unlike nitrogen, which is abundant in atmosphere and may be exchanged through air-water interface, phosphorus, had no extraneous supply or sink in the containers (Maar *et al.* 2002). Therefore the amount of TP remained constant within each container, independently of any physical, chemical or biological processes, except for the sampling. The decay in the concentration of TP in the water column is due to sedimentation dynamics only. The calculation of net sedimentation rates was done with an exponential model of water column TP concentration against time from day 0 to day 3. The adjustment was done by non-linear least square estimation. Sedimentation rates could only be reliable calculated for nutrient enriched containers, because differences in TP from day to day in the non-enriched containers were too close to the sensitivity of the method of analysis.

Analyses of covariance (ANCOVAs) and variance (ANOVAs) were performed to assess the effect of turbulence (TUR) nutrient enrichment (NUT), and initial conditions (experiment, EXP) taken as factors, to several of the sampled parameters. The sampling time (TIME), that is the time of sampling from the microcosms counting from the beginning of the experiment, was used as the continuous variable in ANCOVAs. Data were standardized dividing by the initial values of each experiment, and then time 0 data were excluded from the analyses of covariance. Level of significance was *p-value*<0.05 unless otherwise indicated. For simple comparisons two-tailed student's T tests were performed. Degrees of covariation were estimated through non-parametric Spearman's correlation coefficient (r_s).

Osmotrophic plankton biomass-size spectra were calculated adjusting a Pareto type I distribution (Vidondo *et al.* 1997) to data on concentration and volume of phytoplankton and bacterioplankton. In a Pareto type I distribution, the parameter *c* is equivalent to the negative slope of the normalized biomass-size spectrum. Thus large values of *c* indicate dominance of small organism, whereas low values appear when large organisms are abundant. Items used were the free living cells and aggregates or colonies, based on epifluorescence microscopy counts for autotrophic flagellates <16 µm in diameter and Utermöhl counts for larger flagellates.

Results

Initial conditions

A detailed description of the annual cycle in the Bay of Blanes is given in chapter 3. Briefly, during summer 2003, the river discharge from the nearby river La Tordera and thereby the input of nutrients from terrestrial runoff, were low (chapter 3, fig. 3) because of anomalous high air temperatures, unusual atmospheric stability and reduced precipitations. In addition, the low wind energy flux and wave height indicated low resuspension from the sediments (chapter 3, fig. 2). As a consequence during the first part of the experimental series, between June and September 2003, dissolved inorganic nutrient concentrations were very low (chapter 3, fig. 8). With autumn came the typical Mediterranean episodes of intense rain and subsequent increases in river flow, which drove the system to less oligotrophic conditions. Initial water conditions for the experiments are given in table 1. The water column was mixed between October 2003 and April 2004. In summer a shallow thermocline appeared at 2-3 meters. Salinity variations were due to river discharge events (chapter 3) and maybe to intrusion of salty water from the submarine Blanes Canyon (Masó and Tintoré 1991).

Chlorophyll *a* concentration tended to increase throughout the year of study (chapter 3, fig. 5A). Maximum concentration (3.93 µg L^{-1}) was found in December 2003 during Exp 7, following an episode of river discharge occurred two weeks before, and also a prolonged period of high significant wave heights that reached values of almost 5 m. This indicates that some resuspension had taken place. During the sampling for Exp 6 in November 2003 a large amount of the brown alga *Codium* sp. was observed on the beach and macrophyte detritus were seen under the microscope, which points to a resuspension event occurred before this experiment, although meteorological records do not show large storms in the previous weeks.

Dynamics of both NO_3^- and SiO_2 were strongly linked to terrestrial runoff (chapter 3, fig. 7). They showed three major peaks: in Exp 5 (October 2003), Exp 7 (December 2003) and Exp 11 (April 2004). These peaks followed periods of high waves and/or river discharges. In the last two experiments, coinciding with a persistent diatom *Chaetoceros* spp. bloom, silicate concentrations were at their minimum (0.07 µmol L^{-1}). By contrast, PO_4^{3-} was apparently not related either to terrestrial runoff or to sediment resuspension driven by waves or wind, but increased throughout the period of study, from values close to the analytical detection limit in summer 2003 (between 0.02 and 0.03 µmol L^{-1}) to

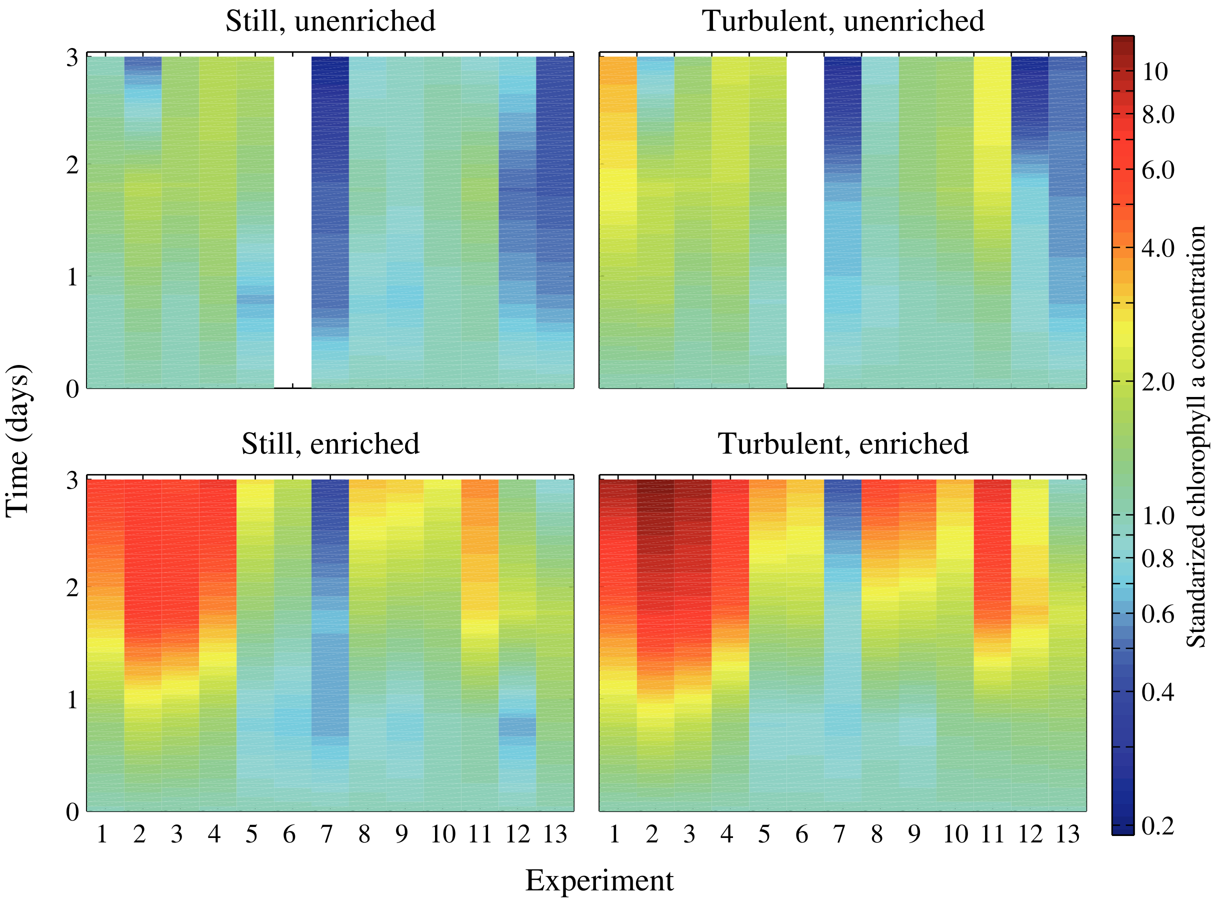

Figure 1: *Temporal evolution of chlorophyll a concentration for each experiment. Different subplots show the 4 different experimental conditions. Data was standardized by dividing by the initial values. Note that colour-scale is in logarithmic mode. Values between samplings were obtained by linearly interpolating in a grid of one hour resolution.*

maxima in June 2004 (0.23 µmol L^{-1}). As a consequence of these different dynamics of dissolved inorganic N and P, the ratios N:P (mol:mol) showed a wide range, from less than 2 in June 2004 to more than 300 in October 2003.

In summer 2003 phytoplankton was dominated by small microorganisms, mainly cyanobacteria and autotrophic nanoflagellates, whereas the contribution of diatoms in terms of carbon was only around 10% (chapter 3, fig. 9A). For the remainder of the series, from October 2003 to June 2004, diatoms were clearly dominant except in December, when there was a peak in autotrophic nanoflagellates. In the last three months there was a persistent dominance of *Chaetoceros* spp., which reached abundances of more than 1000 cells mL^{-1}. The living C/chl ratio was between 20 and 60, except for December 2003, when it was around 10, and for May and June 2004, coinciding with the dominance of *Chaetoceros* spp. when it was over 100.

Bacterial dynamics showed no seasonal pattern (chapter 3, fig. 5B). As a consequence the percentage of heterotrophic carbon followed inverse dynamics to that of chlorophyll *a*, being maximum in summer 2003, and then decreasing along the series.

Water column

Both experimental factors, nutrient addition and turbulence, had a significant effect on all the biomass parameters monitored during experiments (ANCOVA,

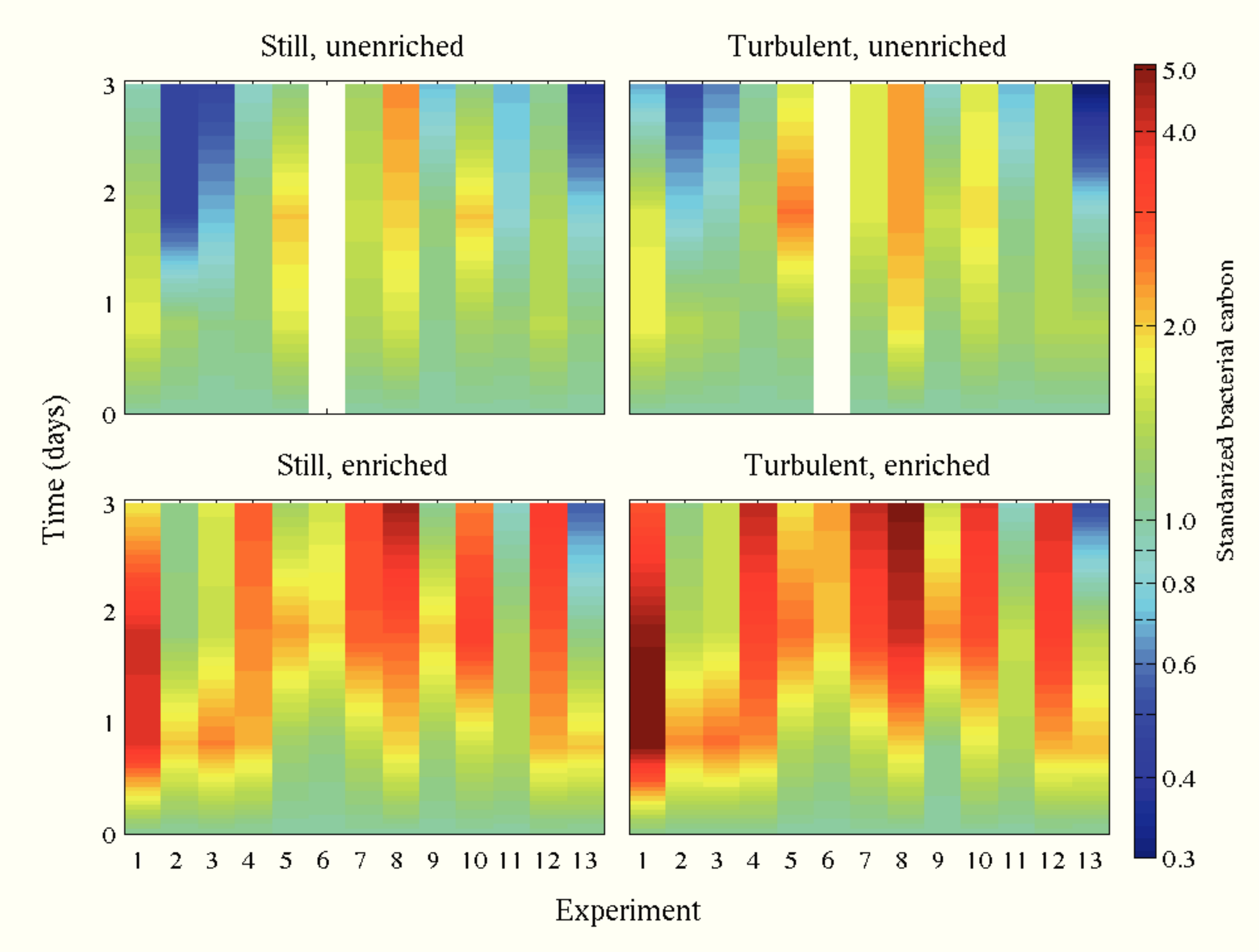

Figure 2: *Temporal evolution of bacterial abundance for each experiment. Different subplots show the 4 different experimental conditions. Data was standardized by dividing by the initial values. Note that colour-scale is in logarithmic mode. Values between samplings were obtained by linearly interpolating in a grid of one hour resolution.*

table 2). Except for POP, the factor that explained more variability in the dependent parameters was experiment (EXP), which is expected to include the variability due to initial conditions. As expected, in all cases except for Exp 7 (December 2003), chla increased after addition of nutrients (fig. 1). This increase adjusted well to an exponential curve, and no decay was observed before the experiment was terminated. In the non-enriched treatments, chla either increased or remained stationary, except again in Exp 7 and in Exps 12 and 13. Observed accumulation of chla in the water column implies that sedimentation rates were lower than growth rates.

Bacterial concentration also responded positively to nutrient addition and turbulence (ANCOVA table 2, fig. 2), although the response was weaker than with chla. In most experiments, the concentration increased at the beginning and then went down before the end of the experiment.

Diatom concentration in the water column decreased because of sedimentation in all treatments from day 0 to day 3, except in the first 4 experiments, in which it increased in the enriched treatments (data not shown). These were also the experiments in which initial diatom concentration was lowest, and Si addition was closer to the intended values. Consistently, the slope of the size spectrum (c) increased from the initial sample to day 3 in all experiments except in the first ones when initial diatom concentration was minimum. An increase in the slopes indicates that smallest organisms become relatively more important by the end of the experiments.

Table 2: Summary of ANCOVAs. All dependent variables were standardized dividing by the initial values, and linearised by taking natural logarithm. Continuous predictor is TIME, the experimental sampling time. TIME=0 were excluded from the analysis. EXP represents the 13 experiments. NUT (nutrient enrichment) and TUR (turbulence) are treated as on-off variables. Degrees of freedom (df) and p-values (p) are shown.*(p-value<0.001).

		Parameter									
		Chla **N=366** **Adj. R^2=0.70** **df=52**		**Bact** **N=310** **Adj. R^2=0.59** **df=52**		**POC** **N=233** **Adj. R^2=0.50** **df=52**		**PON** **N=242** **Adj. R^2=0.61** **df=52**		**POP** **N=370** **Adj. R^2=0.81** **df=52**	
Treatment	df	SS	*p*	SS	*p*	SS	*p*	SS	*p*	SS	*p*
Intercept	1	1.023	0.045	17.770	*	3.500	*	0.744	0.017	13.348	*
TIME	1	27.821	*	0.692	0.014	13.953	*	12.342	*	0.522	0.023
EXP	12	101.349	*	26.647	*	20.514	*	18.348	*	31.758	*
NUT	1	40.219	*	15.942	*	6.519	*	14.204	*	81.555	*
TUR	1	4.550	*	0.893	0.006	0.894	0.032	1.156	0.003	0.755	0.006
EXP X NUT	12	12.823	*	4.695	*	3.303	0.151	2.472	0.090	20.191	*
EXP X TUR	12	1.777	0.855	0.165	0.999	1.161	0.909	0.686	0.941	0.890	0.706
NUT X TUR	1	0.000	0.970	0.014	0.727	0.008	0.843	0.001	0.921	0.006	0.801
EXP X NUT X TUR	12	1.056	0.980	0.251	0.999	0.479	0.998	0.654	0.951	0.315	0.994
Error	313	79.416		29.312		34.383		24.096		31.492	

The slopes of the size spectra showed no significant change due to turbulence or nutrient addition from day 0 to day 3 (ANOVA). The relative change between initial time and day 3 was inversely related to the initial slope ($[c-c_0]/c_0=1.52-1.41c_0$; $r^2=0.78$; $n=54$). That is, when c was low at the beginning of the experiment it tended to increase in all treatments, whereas when it was high it tended to decrease.

Measured increase in PO_4^{3-} concentration after 2 hours of addition was lower than calculated from nutrient addition (0.17 ±0.05 µmol L^{-1} ave±sd), indicating a high uptake rate of P. The ratio $NO_3^-:PO_4^{3-}$ increased in the enriched treatments of all experiments from June 2003 to March 2004, except for experiment 2 in July 2003. On the other hand, it decreased in the last 3 experiments, where *Chaetoceros* spp. was dominant and the initial ratio $NO_3^-:PO_4^{3-}$ was already low. The ratio $SiO_2:NO_3^-$ did not change from time 0 to time 3 in most of the experiments. Again, only in Exp. 2 and in the last 4 experiments this ratio increased, suggesting a quicker uptake of nitrogen than of silicate. Thus, system was limited by P most part of the year, except for the last months, between March and June 2004, in which diatom concentration was highest, and punctually in exp. 2. In these experiments the system was rather limited by nitrogen.

The fraction of P which was in the particulate form (i.e. POP/TP) increased in the nutrient enriched treatments. Changes in this ratio throughout the year were most conspicuous when comparing the first sampling of each experiment, after ca. 16 hours of the addition of nutrients (fig. 3). At this time, POP/TP ratios were within a relatively narrow range in the control containers (fig. 3A) whereas in the fertilized treatments (fig. 3B) they were positively correlated to photoperiod (r_s=0.75, *p-value*=0.005 for still treatments, r_s=0.73, *p-value*=0.006 for turbulent treatments). In subsequent days this pattern was smoothed and eventually disappeared.

Variability in the responses

Responses of phytoplankton biomass to the experimental factors, assessed as the difference in

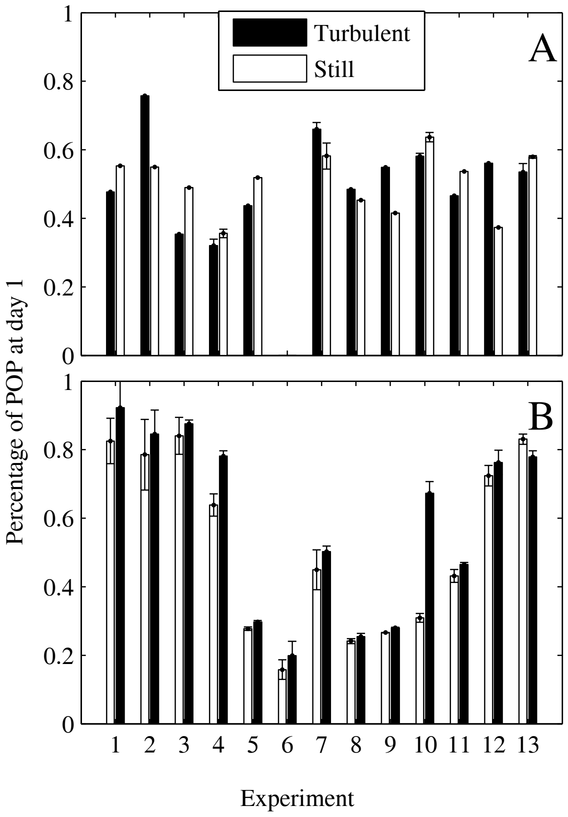

***Figure 3:** Fractions of TP in the particulate organic form (POP) for each experiment, in the control treatments (A) and in the nutrient enriched treatments (B). Error bars represent standard deviations.*

chlorophyll *a* net growth rate in the water between treatment and control, are shown in fig. 4. Both factors triggered positive responses, although the response to nutrient addition was higher than to turbulence.

Except for a weak significant correlation with the photoperiod, no significant relationship could be found between the response of phytoplankton biomass to turbulence and any of the initial conditions (table 3). However, there were significant differences between the seasons whereas no differences were found due to enrichment in the response to turbulence (ANOVA, Adj. R^2= 0.28, *p-value*=0.028). The *post-hoc* Tukey analysis showed the response in spring to be higher than in summer (*p-value*=0.027) and in autumn (*p-value*=0.075).

The response to nutrient addition was positively correlated to photoperiod, temperature and Secchi depth (table 3). It was negatively correlated to the concentration of dissolved inorganic N (particularly to NO_3^-) and to SiO_2. An ANOVA (Adj. R^2= 0.48, *p-value*=0.006) revealed that differences in the response between seasons were also significant whereas no differences were detected between S and T treatments. The *post-hoc* Tukey analysis showed that response in autumn was lower than in spring and summer. Levene's test indicated that the data had no homogeneous variance, but the non-parametric Kruskal-Wallis test ($H_{(3,24)}$=12.98, *p-value*=0.005) confirmed the results of the ANOVA.

Sedimentation

All biomass parameters (chla, and POM) except POP were significantly higher after resuspension in turbulent and/or enriched treatments than in controls (ANOVA, table 4). Figure 5 shows chla in T vs. S treatments of all experiments separated by sampling day. Spearman's correlation between chla in turbulent treatments and in

***Table 3**: Spearman's correlation coefficients of initial values with the mean response to turbulence and nutrient-enrichment. Correlations significant at p<0.100 are highlighted in light grey, and those significant at p<0.050 in dark grey.*

	Response to turbulence		**Response to enrichment**	
	r	*p*	*r*	*p*
Photoperiod	0.48	0.097	0.58	0.052
Temperature	-0.12	0.692	0.55	0.064
Secchi depth	-0.07	0.816	0.72	0.008
Salinity	-0.05	0.865	0.11	0.744
Size spectra slope	0.04	0.906	-0.28	0.379
Chla	-0.09	0.779	-0.27	0.404
Bacteria	-0.07	0.830	0.06	0.850
NO_3^-+NO_2^-+NH_4^+	-0.22	0.470	-0.84	0.001
PO_4^{3-}	0.07	0.821	0.06	0.852
SiO_2	-0.34	0.263	-0.76	0.007

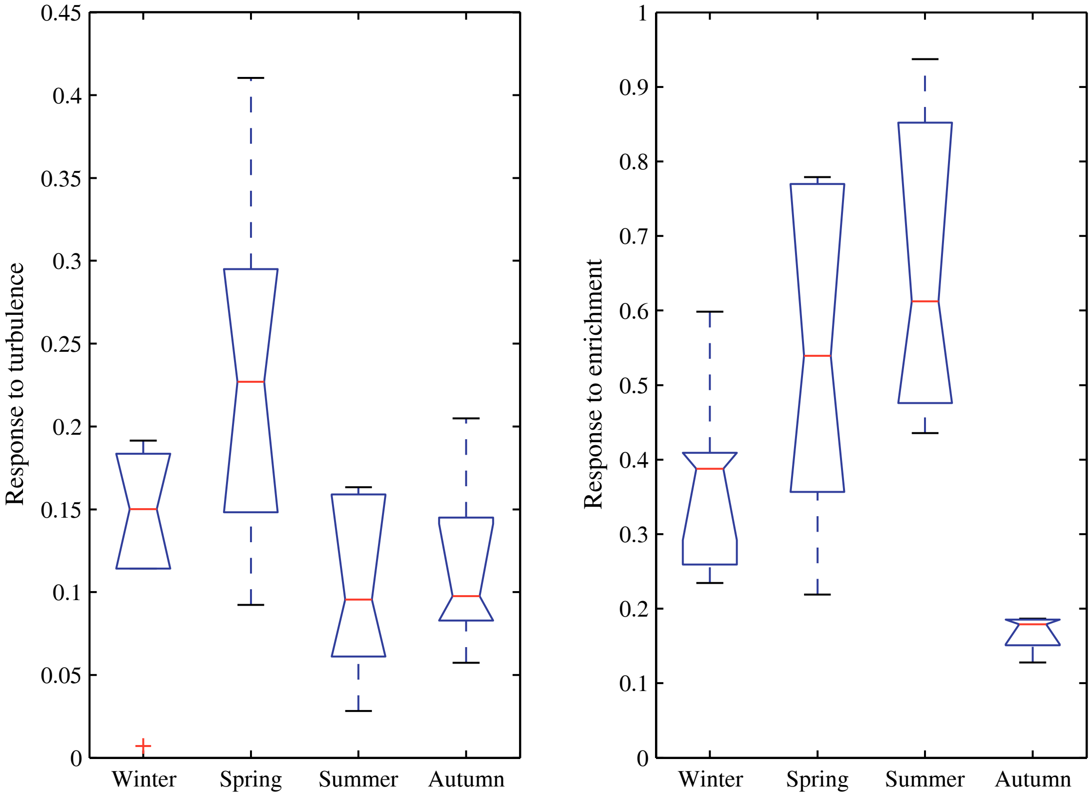

Figure 4: *Boxplot of responses to turbulence (A) and to nutrient enrichment (B) grouped by seasons. Responses were calculated as differences in exponential growth rates calculated from chlorophyll a concentration in the water column (see text). Boxes have lines at the lower quartile, median and upper quartile values. Whiskers show the minimum and maximum values of the group. Crosses represent outliers.*

still ones for all days was significant (r_s >0.92; *p-value* <0.001). The slope of the best fit regression linear model (y=a·x) between S and T treatments increased during the experiment, from 1 at the initial time, to ca. 1.6 at day 3, and then decreased back to ca. 1.1 after resuspension (fig. 6). The same pattern was repeated for POC and PON, and less clearly for TN (fig. 6). On the other hand the slopes for POP and TP were close to 1 at all sampling times, and even lower after resuspension. As a consequence the ratios PON:POP and POC:POP should be higher in the sediment than in the water. Actually, an ANOVA with the ratio PON:POP of day 3 revealed differences between sediment and water and between enriched and control treatments. The *post-hoc* Tukey test confirmed that ratios were higher in the sediment. In fact, at day 3 most of the treatments in all experiments showed PON:POP values lower than the Redfield ratio in the water. Interaction between turbulence level and compartment (water column or sediment) was significant at a *p-value*=0.07 for non-enrichment treatments. A *post-hoc* Tukey test showed that the difference in PON:POP ratios between water and sediment was larger under still conditions. Turbulence relatively increased the PON:POP ratio in the water and reduced it in the sediment.

Sedimentation rates calculated from TP ranged between ca. 0.09 and 0.25 d^{-1}. Only rates for enriched containers were calculated. In unenriched containers the differences between initial and final TP concentrations were very small, and exponential decay in them was often not clearly observed. In turbulent treatments sedimentation rates were significantly lower (paired t_{12}=3.22, *p-value*=0.007), although differences in rates

Table 4: *Summary of ANOVAs. EXP represents the 13 experiments. NUT (nutrient enrichment) and TUR (turbulence) are treated as on-off variables. Degrees of freedom (df) and p-values (p) are shown.*(p-value<0.001).*

		Parameter							
		Chla **N=96** **Adj. R^2=0.98** **df=51**		**POC** **N=65** **Adj. R^2=0.76** **df=51**		**PON** **N=65** **Adj. R^2=0.86** **df=51**		**POP** **N=98** **Adj. R^2=0.98** **df=51**	
Treatment	df	SS	*p*	SS	*p*	SS	*p*	SS	*p*
Intercept	1	148.287	*	64.863	*	64.484	*	56.447	*
EXP	12	56.546	*	10.073	*	13.099	*	20.295	*
NUT	1	25.122	*	5.438	*	7.306	*	26.666	*
TUR	1	0.780	*	0.023	0.588	0.150	0.115	0.005	0.538
EXP X NUT	12	5.524	*	1.876	0.104	1.173	0.140	13.229	*
EXP X TUR	12	0.828	*	0.392	0.922	0.295	0.901	2.601	*
NUT X TUR	1	0.000	0.878	0.056	0.405	0.063	0.295	0.210	*
EXP X NUT X TUR	12	0.290	0.189	0.097	1.000	0.260	0.932	1.349	*
Error		0.7446		0.984		0.683		0.662	

between still and turbulent treatments were relatively small (0.03 d^{-1} on average).

Discussion

Common trends

Our experimental setup simulated the effects at different scales of a standard intensity turbulent event in a coastal zone. Therefore we used turbulence levels and nutrient enrichments within the range of conditions that could be reasonably found in the Blanes Bay coastal ecosystem. Depending on the method used, our estimates of the generated turbulence were in the range 10^{-3}-10^{-2} cm^2s^{-3}. The wind necessary to generate these average levels of dissipation at 1m depth (to avoid the wave-affected-surface layer, Stips *et al.* 2005) applying eq. 5 in Mackenzie and Leggett (1993) is between 2 and 5 m s^{-1}. The frequency of wind events with a mean intensity of 5 m s^{-1} in the zone, calculated applying equations in Guadayol and Peters (2006, Chapter 1) is shown in figure 7. On average there are about 1.5 such events per season at the location. Unlike other zones of the Catalan coast, spring is the season with highest frequency of low intensity wind events, as the ones simulated in this study are, although high intensity events are still more frequent in autumn (Guadayol and Peters 2006, Chapter 1).

Similar studies with water from the NW Mediterranean (e.g. Arin *et al.* 2002, Maar *et al.* 2002) did not consider seasonal variability and in general used higher turbulence intensities and longer experiments. Despite the realistic moderate forcings of the present study, the effect of both turbulence and nutrient enrichment on chlorophyll and particulate organic matter production was found to be highly significant.

Turbulence significantly increased chla within the experimental system at all times of the year, when considering only the water column (ANCOVA, table 2), and also after including resuspended sediment (ANOVA, table 4). The mechanisms for this enhanced production in the water column may include some or all of the following: increased nutrient flux towards large osmotrophs, differential grazing and differential sedimentation. At the relatively low turbulent levels used in this study, sedimentation seemed to play a major role, as suggested by the decrease in diatom concentration during the experiment. The decrease after resuspension in the turbulent *vs.* still slopes shown in figure 6 for several parameters can only be explained by different

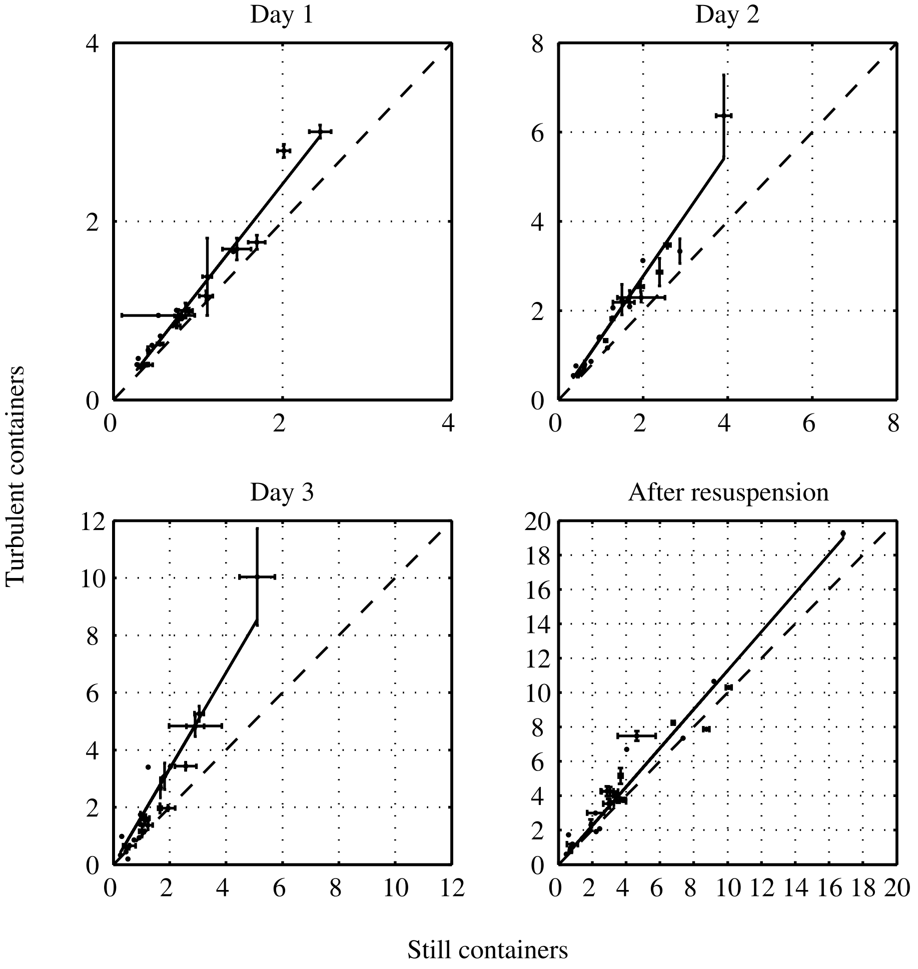

Figure 5: *Chlorophyll a concentration (µg L^{-1}) in the water column in turbulent vs still treatments for each sampling day, and after resuspension. Continuous line show the linear best regression fit model, of the form y=ax. Dotted line is the 1:1, plotted as reference.*

sedimentation-resuspension dynamics between still and turbulent conditions. The robustness of this relationship suggests that these dynamics are, within the experimental containers, fairly constant throughout the year, regardless of different size structures and physiological states of the planktonic community.

The deviation from the 1:1 slope after resuspension (fig. 5 and 6) implies a higher biomass production in the turbulent containers, as already seen from analyses of variance. Once large cells are either kept in suspension and/or resuspended to a higher degree owing to turbulence, the increase in nutrient flux towards cells can keep populations growing and/or in a better physiological state. We did not measure grazing losses, but predators of large osmotrophs, if present, were mostly screened out at the beginning of the experiments. Also, the duration of the incubations was too short to allow any significant copepod development from earlier stages. Thus, we discard grazing as playing a major role in the dynamics of microphytoplankton in this experimental setup.

Bacteria showed a significant response to turbulence and enrichment, although it was low compared to that of phytoplankton. Their growth did rarely exhibit an exponential pattern. Most often they increased in number at the beginning of the experiment, and then stabilized or decreased. This pattern is commonly observed in this kind of experiments (e.g. Arin *et al.* 2002, Malits *et al.* 2004). In longer experiments a second peak of bacterial

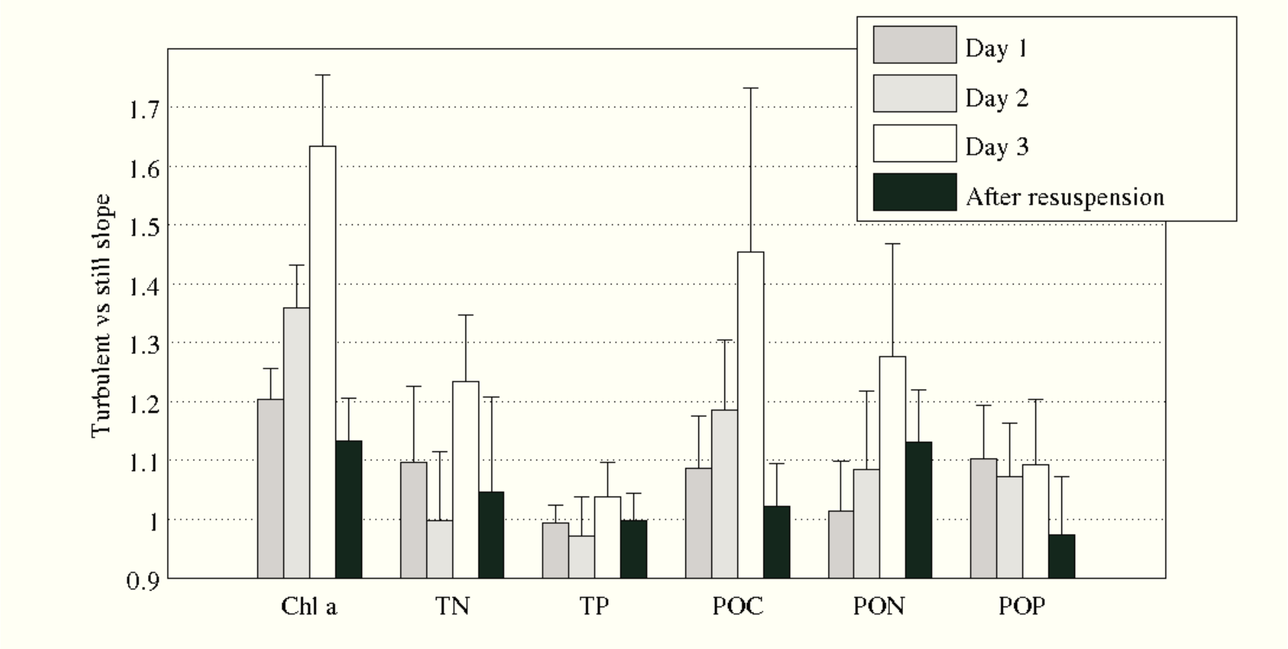

Figure 6: *Slopes of the best fit regression models of the form y=ax, where y is the value of the parameter in the turbulent treatment, and x is the value in the still one. Error bars are the confidence intervals at 95%.*

concentration following the chlorophyll *a* maximum is usually observed. This peak in the post-bloom phase is triggered by the release of dissolved organic material from phytoplankton and their predators (Malits *et al.* 2004, Pinhassi *et al.* 2004). Observed effects on bacterial concentration during this phase can be a consequence of higher phytoplankton growth, rather than of direct influence of turbulence or of nutrient addition. In the short time experiments of the present study, the significant response to the experimental factors is more likely to be reflecting immediate effects in bacterial populations. Because sedimentation-resuspension dynamics are probably much less important than for phytoplankton because of small sizes (Peters *et al.* 1998), observed changes are likely to reflect small-scale turbulence effects

The natural levels of turbulence used in this study should in theory not directly affect bacterial uptake rates (Peters *et al.* 1998, Malits *et al.* 2004). This is because the increase in uptake rates due to small-scale turbulence depends on the size of the organism (Karp-Boss *et al.* 1996). The positive response to turbulence encountered in this study is most likely due to indirect effects through changes of trophic web interactions as suggested by Peters and co-authors (1998, 2002). According to these studies, under turbulent conditions flagellates increase their grazing pressure over cyanobacteria and small autotrophic flagellates, which are more nutritious, diminishing in this way their pressure on bacteria.

Particulate organic matter was richer in P in the water than in the sediment. This suggests either that PON and POC are netly sedimenting at a higher rate than POP, or that P has a shorter residence time in the sediment. In this respect, it is known that organic P is remineralized more rapidly than C both in the dissolved (Clark *et al.* 1998) and in the particulate fractions (Faul *et al.* 2005).

The ratios between biomass parameters in still and in turbulent treatments throughout the experiment (fig. 6) indicate also a different behaviour under turbulent conditions of P with respect to N and C in the particulate fraction. We did not directly measure sedimentation of particulate organic matter but some insight can be gained from budget analysis. Net sedimentation rates of TP were higher in still than in turbulent containers, but the differences were very small (0.03 d^{-1} on average), compared to the difference for chlorophyll *a* (0.15 d^{-1} on average). Moreover, neither TP nor POP showed any difference at any sampling time between still and turbulent treatments, which also indicates a low effect of turbulence in P net sedimentation rates. In contrast, the ratios of POC and PON between turbulent and still treatments were increasing throughout the experiments, and then went back to values close to 1 after resuspension, indicating differences in net sedimentation rates due to turbulence.

As a consequence, POC and PON were higher in the water under turbulent conditions. The final result is that turbulence relatively increased the ratio PON:POP in the water column, decreasing differences with the sediment. In the same line, Maar *et al.* (2002) found higher PON:POP, POC:POP and living C:POP ratios under turbulent conditions after 4 days of experiment. They interpreted this finding as an effect of small-scale turbulence on turnover rates of P, which they found to be higher under turbulent conditions as predicted from theory. In a mesocosm study, Eppley *et al.* (1978) found N:P utilization ratios to be lower in stirred than in unstirred enclosures, implying also a higher turnover rate for P under turbulent conditions. These authors concluded that differences in utilization ratios were likely due to different dynamics of the water column. However, this study may be not comparable to ours, since turbulence was introduced daily for a short time with the objective of avoid sedimentation or promote resuspension of large cells.

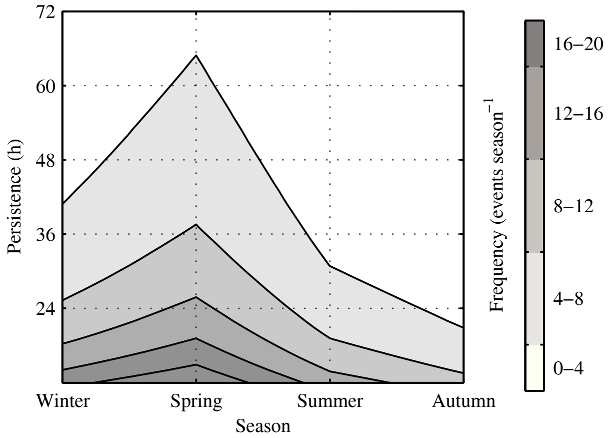

Figure *7: Frequency of wind events with a mean intensity of 5 m s^{-1} in the zone, as a function of its minimum persistence (h) and grouped by season. Calculated using equation 8 in chapter 1 with parameters from the nearby meteorological station of Malgrat de Mar (41º38'57"N, 2º45'8"E) between years 1990 and 1997.*

Sedimentation-resuspension dynamics can also add to the interpretation. Small particles, such as bacteria and cyanobacteria, settle at a slower rate than larger phytoplankton. In fact, no differences in sedimented bacteria can be observed between still and turbulent treatments, even at the higher levels of turbulence used in other studies (Peters *et al.* 1998). Thus, if there is a differential sedimentation of nano- and microphytoplankton due to turbulence but not of bacteria, the percentage of bacteria in the water is expected to be higher in still than in turbulent conditions. Bacteria have N:P ratios of around 10:1 (Fagerbakke *et al.* 1996 and references therein), lower than phytoplankton. Consequently, an increase in the proportion of bacteria in the water can partly explain the decrease of PON:POP in the water column, particularly in still conditions. However, the percentage of heterotrophic bacteria in these experiments was found not to be significantly affected by turbulence (ANCOVA). Arin *et al.* (2002) by contrast found lower percentages of heterotrophic C under turbulent conditions.

In the field, some increases in biomass associated to turbulence episodes are not related to enhanced growth rates, but to resuspension from the sediment of benthic autotrophic flagellates (Garstecki *et al.* 2002). This could explain the dynamics found in Exp. 7, which were very different from the other experiments. A decline in biomass parameters was observed, which was caused by sedimentation. Initial chlorophyll *a* was unusually high for this system, and the phytoplankton community was dominated by autotrophic nanoflagellates. It coincided with the absolute maximum in NO_2^-, and also with relative maxima in NO_3^-, NH_4^+ and SiO_2 concentrations. This different behaviour can be explained by previous meteorological conditions. During the first half of the month a steady wind from NE blew, and the significant wave height reached values of almost 5m (chapter 3). This likely led to an intense resuspension event, detected by a minimum in Secchi disk record, which explains the maxima in chla and nutrients observed and the tendency to sink during the experiment.

Temporal dynamics of the responses

We did not seek to detect changes along the year in the phytoplankton composition due to experimental forcings. Our aim was to evaluate the role of initial phytoplankton compositions on the short time responses, assessed from bulk biomass parameters like chlorophyll *a* concentration. In long time enclosure experiments it is usual to find that during the first stages the response to experimental factors in terms of chlorophyll *a* is mainly due to diatoms (Estrada *et al.* 1987, Estrada *et al.* 1988, Estrada *et al.* 1996). Other phytoplankton groups, like haptophytes, dinoflagellates and nanoflagellates become dominant in later stages of the succession. Diatoms are particularly favoured by turbulence, because of their size (Peters *et al.* 2006) and life strategy (Margalef 1978, Smetacek 1985). Thus, the absolute and relative amount of diatoms should be reasonably good predictors of the capability of the system to quickly respond to turbulence and nutrient enrichments. However, in the present study the responses in terms of chlorophyll *a* growth rate were not found to be significantly related neither to the proportion and concentration of diatoms, nor to the size structure of the community (table 3). The response to experimental forcings, particularly to nutrient fertilization, was related to initial physicochemical conditions. This may be in part caused by the low Si concentration of enrichments, which could limit diatom growth in some of the experiments, or stimulate sedimentation (Bienfang *et al.* 1982). However, the ratio $SiO_2:NO_3^-$ in the water column did either not change throughout the experiment or increase, suggesting no general limitation by silica. Moreover, the differences in response due to initial community composition are usually detected after several days (Estrada *et al.* 1987) or under more intense forcings (Estrada *et al.* 1996). It was the purpose of this study to adjust the intensity and duration of experimental factors to realistic levels, which may hinder the detection of changes due to community composition. Episodic events of turbulence and/or nutrient fertilization are indeed affecting the trophic status of the ecosystem, and can therefore influence the successional pattern at a seasonal time scale (chapter 3). But the initial response to these events seems to be independent of the stage of the succession.

The maximum responses of the chla to turbulence throughout the year were found in spring (fig. 6A), coinciding with the period of maximum frequency of wind events (fig. 7). Nevertheless, this does not imply a better adaptation of the community to turbulence. For example in Exp. 1 (spring 2003), the response was the maximum of the whole series, but wind speeds and waves were unusually low (chapter 3). Moreover, although frequency of wind events is lowest in autumn in this particular area of the Catalan coast (Guadayol and Peters 2006, chapter 1), the wave height in 2003 during this season was maximum, reaching over 4 m. The size structure of the community and its composition did not explain the changes in the responses either. These were not found to be directly related to any seasonal parameter, except for a weak relationship with the photoperiod (table 3). Thus the response of the planktonic community to turbulence has been seen to be independent of other external factors.

By contrast, the response to nutrient enrichment was more dependent on initial conditions. It showed a strong seasonal component, being positively correlated to photoperiod, as well as to water temperature (table 3). A similar pattern is observed in the ratio POP/TP after 1 day of nutrient enrichment (fig. 3B), which was also correlated to photoperiod. Coherently, in the analysis of covariance (table 2) the interaction EXP X NUT was found to be always significant, indicating a different response to nutrient enrichment throughout the year. These seasonal patterns are not related to the structure of the community, which did not follow itself a seasonal curve, because in summer 2004 diatoms (*Pseudonitszschia* spp.) were dominant and the slopes of the size spectra were low (chapter 3). Correlations with seasonal parameters, like temperature or photoperiod, suggest that the changes throughout the year in the responses, especially to enrichments, could be due to the differences in conditions of growth not related to the experimental factors. Particularly, we would expect that any environmental change with a positive effect in phytoplankton growth, as nutrient enrichment or turbulence, will be magnified by the number of hours to grow, i.e. by the photoperiod. Simply, given that there are some differences in specific growth rates due to experimental factors, the divergences in terms of absolute biomass between the treatments will be larger as phytoplankton has more time to grow.

The influence of other parameters, not directly related to seasonality, into the pattern of variability of the responses to nutrients can not be ruled out. Actually the correlations of the responses to enrichment with Secchi depth and with concentrations of N and Si were the strongest found. The fact that the response was inversely related to the initial water concentrations of NO_3^- and SiO_2, and not of PO_4^{3-} suggests that there was a general limitation either by N and/or by Si. However, this is a system generally found to be limited by P (Lucea *et al.* 2005, Pinhassi *et al.* 2006, Chapter 3). Moreover the ratio of consumed N:P in the enriched treatments was above the Redfield ratio only in 4 of the 13 experiments (data not shown), and the ratio NO_3^-:PO_4^{3-} similarly increased in the enriched treatments in most of the experiments. The concentration of PO_4^{3-} in the water, due to its high turnover rates (Benitez-Nelson 2000 and references therein), may be a poor estimator of the P dynamics in the water column (Pomeroy 1960, Hecky and Kilham 1988), and therefore of any systemic limitation by this element. The high turnover rates, together with the luxury consumption, make the PO_4^{3-} signal after an enrichment episode highly ephemeral. NO_3^- and SiO_2 have been shown to be better indicators of recent nutrient inputs in this system (chapter 3), likely because they are not so rapidly taken up by osmotrophs. In fact both nutrients, as well as the Secchi depth, are closely linked with the terrestrial runoff and the wave, and have therefore episodic dynamics. The correlation they show with the response to nutrient enrichment could be reflecting the importance of nutrient input history. Response to nutrient enrichment is lower if the system has recently come through a nutrient fertilization event, whereas is larger when the community is nutrient starved, whatever the limiting element. For example, in October 2003, after a strong river discharge event, bacterioplankton was non-limited by phosphorus (Pinhassi *et al.* 2006), despite an N:P ratio much higher than Redfield ratio.

Conclusions

Even under the moderate, “realistic” experimental conditions used in this study, both nutrient addition and turbulence triggered a highly significant increase in chlorophyll *a* concentration. In the case of turbulence these increments could be due to direct effects of small-scale turbulence or to altered sedimentation-resuspension dynamics under turbulent flow, as expected in natural conditions as well. POP had different sedimenting dynamics with respect to POC and PON. In general, POC and PON were settling at a higher rate. Turbulence slightly modified this pattern by relatively increasing the ratio N:P of the particulate organic matter in the water.

There were considerable changes in the phytoplankton response to experimental factors throughout the year, but at the short time scales considered no relationship was apparent between these responses and the initial phytoplankton composition and size structure. The changes in the response to nutrient fertilization were related to seasonal forcings, namely photoperiod and temperature, and also to the recent availability of nutrients, whereas the response to turbulence was found to be only weakly seasonal. The input of nutrients from runoff in coastal zones has been increasing during the last century due to anthropogenic environmental perturbations (Duarte *et al.* 1999, Ver *et al.* 2002). The frequency and intensity of extreme hydrological phenomena is predicted to increase during the next decades (IPCC 2007). The ability of the planktonic community to respond to these forcings is of importance to understand the flux of carbon in coastal systems. The results of this study suggest that the capacity of phytoplankton to quickly react to episodic nutrient events depends on the trophic state of the system. We found the maximum response when the frequency of nutrient inputs was lower. By contrast, the phytoplankton response to turbulence could not be related to the frequency of turbulent episodes.

Although laboratory experiments are only approximations that can not fully reproduce the complexity of natural turbulent flows, ecologically relevant information can be obtained from these studies. The response of the community to laboratory turbulence is quantitatively variable in time, but does show very robust qualitative regularities.

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Chapter 5: Summary and general discussion.

Resum i discussió general.

Picture: Starry Night

Vincent Van Gogh, 1889

This thesis has examined the effects of turbulence on plankton dynamics, focusing on the episodic pattern of turbulent forcing in the coastal zone and the effects of this pattern on the osmotrophic planktonic community. In chapter 1 the distribution of wind events has been studied as a proxy of the variability of turbulent energy input in a coastal zone. In chapter 2 the actual turbulence levels achieved in the laboratory systems used for experimental research have been measured. In chapter 3 the variability in the response of plankton to meteorological forcings has been studied *in situ* with a time series approach. Finally, in chapter 4 the information obtained in the preceding chapters has been combined to experimentally evaluate the changes in the sensitivity to turbulence and nutrient enrichment of the natural community in a one year long series of microcosm experiments.

The research in this thesis has highlighted the large spatial and temporal variability of turbulent forcing derived from wind in the Catalan coastal zone (chapter 1). An empirical model has been developed which allows predicting the distribution of wind events with an ecologically relevant intensity. That is, it was not sought to characterise the frequency of catastrophic extreme events, as is often the case in the engineering literature. Instead the aim was to characterize the frequency and the persistence of intermediate intensity events, which are strong enough to have a noticeable effect on planktonic organisms, and at the same time are frequent and long enough to be of relevance at the ecosystem level.

The use of wind speed as the only estimator of turbulence intensity must be understood as a first approximation. The empirical models of Mackenzie and Leggett (1993) result from a compilation of published turbulence profiles from a wide range of locations and situations. Thus, they have the advantage of being successful in explaining the variability of turbulence with a minimum input information for a high diversity of situations and regardless of methodological biases. However, we must take into account that the sampling frequency of wind speed input in these models is of one day. The models are likely giving the correct dissipation rates, within an order of magnitude, when daily integrated wind speeds are used, but may give biased estimates when used directly with high frequency resolution wind speed data. Other limitations of these models are related with biases in the turbulence profiles dataset. It is probably not correct to apply them in the first centimetres of column, because measurements in this layer are not possible with free-falling profilers. Also, measurements done in strong turbulent conditions and with high waves are very scarce. Thus, when applying the model caution must be put for the highest values, because we are extrapolating the original model.

Further efforts must be put in understanding the mechanisms of generation and development of turbulent flows, and the importance of waves (as has become evident from chapter 3) and of wind direction in this process. The mooring of acoustic Doppler sensors next to meteorological stations (VARITEC project efforts) may aid in developing better models. In any case, the determination of wind variability in chapter 1 is extremely useful to understand the variability of energy input in the surface layer, although how this energy translates into turbulent mixing is still a matter for discussion.

As a second step of this thesis, the turbulent kinetic energy dissipation rates generated with the oscillating-grids experimental system used in chapter 4 have been measured. A qualitative and quantitative understanding of the turbulence achieved in laboratory systems is crucial to design and interpret realistic experiments. With the aim to extend the usefulness of measurements to the wider scientific community, these analyses have been extended to a range of different systems frequently used in experimental studies of plankton and turbulence. These include other oscillating-grid systems with tanks of different volumes and grids of diverse geometry, and orbital shakers with an array of common laboratory containers. In the case of oscillating grid systems, measurements have shown that turbulence is isotropic, at least at the smallest scales. Moreover, turbulence is distributed homogeneously within cylindrical containers as long as the stroke length is comparable to the water column height. Away from the volume covered by the grid oscillation, dissipations decay following fairly well the empirical model of Hopfinger and Toly (1976).

Turbulence in the oscillating grid systems has been shown to be trustfully estimated using theoretical considerations about the energy entered into the system by the oscillating grid. We have also provided a list of drag coefficients for different grids, including those used in chapter 4, empirically derived from turbulence measurements.

Future enhancements of this part of the thesis should include the explicit calibration of grid drag coefficients. As theoretical models have been shown to adequately estimate the turbulence variability, and because we have now a large dataset of velocity measurements, such calibration would allow to precisely determine the several parameters implied in the different methods used for the estimation of turbulence. Moreover, such an exercise would allow an increased precision of turbulence estimates in the containers based on theoretical calculations without the need for further measurements.

Once the variability of turbulent input in the Catalan coast was known, and natural dissipation rates could be reproduce with confidence in the laboratory, the aim was to identify the variability in the responses of planktonic community to realistic episodes of turbulence. Because effects of turbulent flow on plankton are size-dependent and species-specific, we hypothesized that the response was going to change throughout the year following successional patterns. To investigate this we combined a time series analysis of several field parameters with a series of 13 monthly microcosm experiments simulating such events. Blanes Bay (NW Mediterranean) was chosen as the study site, because there was an ongoing joint effort to keep up a biogeochemical time series, and because it was very close to one of the meteorological stations used in the wind events analysis, namely Malgrat de Mar.

In chapter 3 the biological time series with a monthly frequency (Blanes Bay Microbial Observatory) was analyzed in combination with several physical and meteorological high frequency time series, to assess the responsiveness *in situ* of the osmotrophic planktonic community to episodic forcings. River discharge and wave height were found to be the two high-frequency hydrodynamical variables with an episodic pattern that have a clearer effect on plankton dynamics. The influence was likely due mainly to an increase in nutrient concentrations because of sediment resuspension (in the case of waves) and/or of terrestrial runoff. Increased nutrients lead to increased chlorophyll *a* concentrations after several days. Since the forcing variable are interconnected through meteorological variability, namely precipitation and wind speed, they strongly co-vary. Thus, it is difficult to assign a quantitatively precise and discernible cause-effect relationship between them and the response of plankton.

Potentially good predictive tools of the coastal ecosystem may be statistically derived from high frequency data of river discharge and significant wave height. Much of the variability in the ecosystem is explained at frequencies higher than seasonal. These conclusions have implications for the management of coastal systems. However, in order to generalize the results of this study, and to further develop and refine the empirical relationships to predict the ecosystem state, it is necessary to use high frequency ecological/ biogeochemical data. This effort can be achieved by high frequency sampling of the desired variables, but logistical, economic and manpower constraints restrict this approach to a few days of high frequency sampling at a time, mainly during research cruises. In my view, there is no doubt that the future lies in remote acquisition of high frequency data from moored stations and online transmission to a laboratory control. Obviously, not all desired variables can be remotely acquired but one can get a long way with just fluorescence data as a proxy for chlorophyll biomass. Even taxonomic information identifying the larger microplankton is almost operational. One such system (SOL, Littoral Observing System) will soon become operational at our laboratory as part of efforts to establish a fixed station acquiring physical as well as biological data in quasi-continuous mode. As mentioned above this system will also allow the development of better predictive models of turbulence in the water based on meteorological and oceanographic information.

In the experimental study of chapter 4, a consistent and significant effect of turbulence has been observed even in the non-enriched treatments in spite of the low dissipations used in the turbulent containers. The coincidence of mixing with nutrient enrichment always led to an increased phytoplankton response. The changes in the response to the same experimental forcings had a seasonal component, probably linked to the photoperiod, but were not directly related to the community composition or size structure. The historic record of nutrient input by terrestrial runoff and sediment resuspension was strongly determining the response of the community to new nutrient enrichments.

The wind velocity which would generate the values of dissipation used in these experiments is between 2 and 5 m s^{-1} applying Mackenzie and Leggett's empirical model (1993). The empirical equation developed in chapter 1 can be used to obtain the number of events expected to occur with an average wind intensity of 3.5 m s^{-1}. The estimated frequency is of ca. 22 events per season for the area of study. This is once every 4 days and approximates the frequency of meteorological fronts crossing the area.

As happened with field data (chapter 3), it was not possible to clearly separate the effects of resuspension from those of small-scale turbulence in the microcosms. Although in our case this is magnified by the low turbulence levels applied, this is likely to happen in all turbulence generation systems. The aim was to enclose a water portion, and avoid having vertical structuring mechanisms, like thermocline, or light or nutrient profiles. The use of oscillating-grid systems to study the effects of turbulence is particularly suited for this purpose since one can achieve an almost homogeneous turbulence distribution within the container. However, because turbulence induces resuspension of cells from the bottom, there are more cells in the water column in stirred containers. If, as it is expected, cells grow faster in the water than in the sediment, the final biomass achieved in the turbulent microcosms will be higher. Knowing the particle sedimentation rates would be useful to separate the influence of both processes. An estimation of the net sedimentation rates can be obtained from the analysis of phosphorus dynamics (Maar *et al.* 2002), but as seen in chapter 4, this may not be representative of the particulate organic matter dynamics, because of non uniform behaviour of the different elements.

Possible experimental workarounds were discarded for different reasons. In order to avoid sedimentation, Havskum *et al.* (2005) periodically mixed the water in the different containers. A recirculating flow from the bottom to the top of the container, or bubbling air would also avoid the settling out of material. The drawback of all these "solutions" is the introduction of mostly uncontrolled energy and hence turbulence in the containers confounding the experimental treatment if turbulence level is the key variable of interest.

A different approach consists of trying to scale a deep water column and separate the upper layer where turbulence mixes the water from the lower still water layer. This will prevent the resuspension of material in the turbulent treatments. This can be achieved with containers that are long (tall) and thin (Estrada *et al.* 1987). But then one has a range of turbulence conditions and other vertically structuring cues that influence the positioning of biological populations, making it hard or even impossible to assess the effect of a certain level of turbulence. In addition even if one can scale the dimensions of a natural water column to a few meters in height, neither the size of the planktonic organisms nor their abundance can be scaled posing additional puzzling interpretation problems.

Turbulence variability and plankton structure

According to the intermediate disturbance hypothesis (IDH, Connell 1978) the maintenance of high diversity in an ecosystem can be explained by the occurrence of disturbances of intermediate frequency and/or intensity. In systems with no disturbances, or with low intensity disturbances, the diversity is expected to decrease because of competitive exclusion (Hardin 1960). When disturbances are too intense or frequent, on the other hand, only species with high growth rate may prosper.

At the scales of plankton, aquatic systems had been traditionally seen as relatively unstructured environments (e.g. Hutchinson 1961). Under such conditions one would expect the principle of competitive exclusion to operate, and the diversity in plankton to be small. However, planktonic diversity is very large. This apparent contradiction was highlighted by Hutchinson, who named it "the paradox of plankton". Of course, the paradox may be solved considering the aquatic environment heterogeneous, even at the tiny scales of plankton. At least from a hydrodynamic point of view, planktonic organisms live in a continuously changing environment.

Competitive exclusion, and therefore the achievement of equilibrium, can occur only if the time between disturbances, as experienced by organisms, is much different from the time it takes for competitive replacement to happen (Hutchinson 1961). On the other hand, if the frequency of disturbances is comparable to biological characteristic rates, no equilibrium may be achieved, and high diversity is maintained. Unlike terrestrial ecosystems, where biomass is dominated by organisms with generation times of at least one year, and often much longer, marine ecosystems are dominated by very small organisms with generation times much shorter than seasonal variability patterns. To understand the diversity of plankton we must look for potentially disturbing forcings with frequencies of the same order of magnitude than plankton growth rates. As seen in chapter 1, one such variable forcing could be turbulence.

If we accept that turbulence may be a factor in the maintenance of high diversity of plankton, as some theoretical models indicate (Kemp and Mitsch 1979), we may ask: ¿Which turbulent episodes occur at frequencies and persistences comparable to those of planktonic organism time scales? This could set a limit to the level of intensity expected to produce an effect on organisms.

Effects of turbulence are strongly size-dependent (fig. 1B). Also, growth rates (and other demographic parameters) are related to organism size in a non-linear way, being higher the smaller the organism. On the other hand, the frequency and persistence of turbulent events decreases as their intensity increases (fig. 1A, equation 8 in chapter 1). Thus, for small organisms with short generation times, such as bacteria, the probability of experiencing a noticeable turbulent event will be very small, since the event must be very intense in order to produce a significant effect, and therefore very rare. Consequently, turbulence at these scales of variability

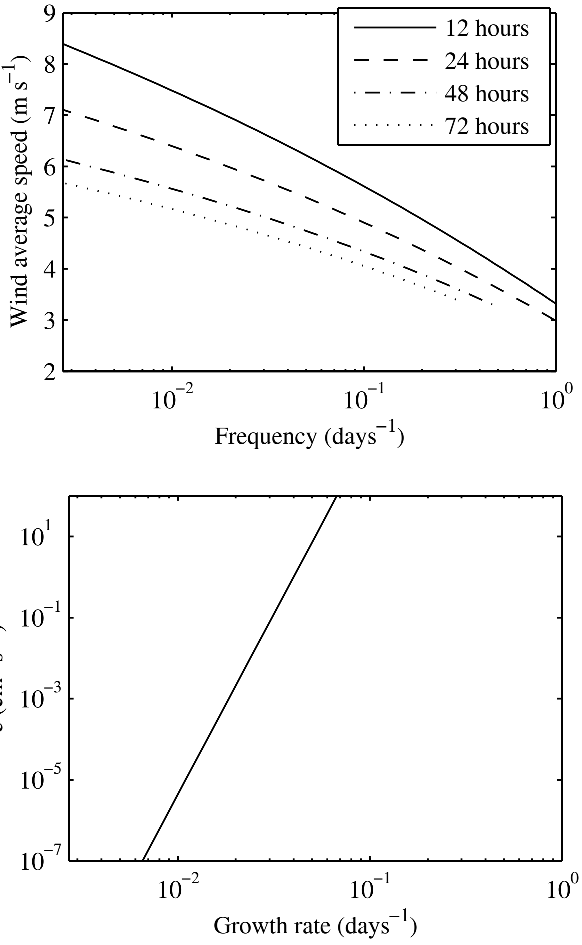

Figure 1: *A) Intensity of wind events against its frequency for 4 different persistences in Malgrat de Mar (NW Mediterranean). Calculations used equation 8 in chapter 1. B) Threshold of dissipation rate triggering an effect on a planktonic organism against the growth rate of this organism. For practical reasons, the threshold has been taken as the dissipation rate that results in the Kolmogorov length scales equal to the size of the organism. Growth rate (μ) was calculated from organism cell volume, assuming a spherical shape, and applying the formula for phytoplankton μ=1.24V-0.15 (Kagami and Urabe 2001).*

will probably have little effect on the diversity of picoplankton. On the other hand, fast growing organisms are quicker to respond to very short (in the order of hours) high intensity events.

As we consider larger organisms, the threshold of turbulent intensity for a significant effect to occur decreases. At the same time, biological processes are slower. There will be a critical size for which there will be noticeable effects of turbulent events with frequencies and persistences comparable to specific biological rates. At this size, turbulent events can be considered as intermediate disturbances, and may explain, at least in part, high diversity maintenance. Also, organisms are expected to be ecologically adapted to variations on time scales comparable with their generation times (Marra 1978). For those organisms over the critical size, turbulence will be an evolutionary force. But, what is this critical scale?

To our knowledge there are few studies systematically addressing the effects of different levels of turbulence on planktonic organisms, aiming to determine the threshold for the effect, this critical scale. This is in part due to the difficulty in assessing dissipation rates in experimental systems. Measurements done in chapter 2 will allow undertaking such studies with a higher degree of certainty. However, assessing the response curve is inherently difficult because turbulence is operating at very different scales and has many possible effects, physiological and ecological, sometimes counteractive. Meanwhile we can extract some conclusions from already published data (see Peters and Marrasé 2000 for a review), as well as from data gathered in chapters 3 and 4 and theoretical analyses. For example, we observed a response in chlorophyll *a* to turbulence and/or nutrient fertilization events both in the field (chapter 3) and in the laboratory (chapter 4), whereas the response of bacteria was weaker and delayed. This sequence of responses has been observed before (Arin *et al.* 2002, Malits *et al.* 2004, Pinhassi *et al.* 2004). It is an indication that bacteria, because of their small size, are not directly affected by turbulence, whereas phytoplankton is. Some influence on bacteria may occur through food web interactions (Peters *et al.* 1998, 2002), or after changes in the bacterial population size-structure have taken place (Malits *et al.* 2004). However, in our systems, delayed response of bacteria is more probably related to increased dissolved organic matter concentrations rather than to a response to the episodic environmental forcings.

In the experimental series presented in chapter 4, low levels of turbulence, representative of frequent events in the Catalan coast, were used. Even so, turbulence consistently triggered a significant response at the system level, with or without nutrient enrichment. The observed increases in chlorophyll *a* were not related to diatoms, which in most of the experiments were settling, but to smaller phytoplankton. Applying the empirical model developed in chapter 1, we obtain frequencies as high as 0.25 d^{-1} in Malgrat de Mar, comparable to phytoplankton growth rates. Therefore, coherently with the intermediate disturbance hypothesis, these events must be affecting the diversity of microphytoplankton and even smaller organisms. In accordance, experimental studies have shown that intermediate disturbances with frequencies between 0.14 and 0.29 d^{-1} are optimal to maintain high phytoplanktonic diversities (Sommer 1995, Flöder and Sommer 1999, Flöder *et al.* 2002).

Another approach to the issue of critical scale is to look at the organisms which are known to be especially sensitive to turbulence, or that have developed some turbulence related life strategy. This includes for example microphytoplankton, basically diatoms and dinoflagellates (Margalef 1978, Thomas *et al.* 1997). Both have maximal growth rates around 1 d^{-1} (Banse 1982, Peters and Marrasé 2000), although *in situ* rates are generally much lower. In general diatoms have relatively higher growth rates than dinoflagellates. Whereas the latter are often negatively affected by turbulence at least in experimental conditions, diatoms seem to be particularly well adapted to turbulence. Besides their documented occurrence in turbulent conditions (Margalef 1978), they are adapted to fluctuating light conditions, as encountered under strong mixing, and have a wide window of saturating irradiance (Litchman 2000). They are non-motile, unlike dinoflagellates, and relatively large, and thus may benefit from the increase in nutrient uptake generated by turbulence. Much of their cytoplasm is occupied by a single vacuole, which has been related to buoyancy regulation (Smetacek 1985), to adaptation to fluctuating conditions (Stolte and Riegman 1995), and to a mechanism to increase cell size without increasing nutrient requirements (Thingstad *et al.* 2005). Thanks to this vacuole moreover, their nutrient requirements are lower (Thingstad *et al.* 2005) and their specific growth rates are higher (Egge 1998) than would be expected from their external volume. In this way, they have at the same time low-intensity thresholds of effect of turbulence (because they are large), and fast growth rates, comparable with frequencies of such low-intensity levels of turbulence. It is not surprising that they are so well-adapted to turbulent conditions.

Turbulence is intermittent at all scales, even at the smallest. We have based our study on the synoptic scales of variability (see fig. 7 of introduction), on intermediate episodes with persistences of the order of days, and frequencies of several events per season, comparable to planktonic specific growth rates. But there is always a strong intermittency or patchiness at the smallest scales, even when the flux of turbulent energy at the largest scales is constant. The direct effects of this variability must be on processes with comparably short time scales, namely nutrient uptake, particle encounters, excretion, etc... This does not mean that short-scale variability is not influencing longer time-scale processes, like growth or predation. Changes in these processes integrate the effects occurred at shorter time scales. However, our level of observation did not permit to study the effects of such short-time phenomena. The huge genetic diversity of the smallest components of the planktonic community, including bacteria, cyanobacteria, and small autotrophic flagellates, is now beginning to be unveiled (Pedrós-Alió 2007). As we have discussed, this diversity of pico- and nano-plankton can hardly be explained by the distribution of episodic events of turbulence and nutrient load at the synoptic scales of this study. The intermittency at the smallest scales may however be a factor to take into account, because it affects the distribution of nutrients and creates a heterogeneous environment for these organisms (Seuront *et al.* 2002).

Turbulence variability and ecosystem dynamics

In coastal zones, dinoflagellate blooms have been seen to generally occur after a period of calm weather (Wyatt and Horwood 1973, Margalef 1978, Berman and Shteiman 1998) and/or tend to occur in waters of restricted exchange such as harbours or embayments. Moreover, dinoflagellates are a taxonomic group especially sensitive to turbulence (e.g. Berdalet and Estrada 1993, Peters and Marrasé 2000), and therefore their blooms are expected to be mitigated by the occurrence of storms. The frequency and intensity of turbulence events in a given region may be determining the development of harmful algal blooms (HABs). In this sense, chapter 1 offers a useful tool for the study of the geographic distribution of areas more likely to form HABs. The geographical variability in the pattern of winds in the Catalan coast has been shown to be even more important than the seasonal dynamics, because of the influence of local relief. This may be a special feature of the Catalan coast, because of its irregular orography, but we would expect some degree of geographical variability to occur in any coast. It would be interesting to compare the observed distribution of HAB occurrence along a given coast with the geographic changes in frequency, intensity and persistence of wind episodes. Of course there are other factors affecting this distribution, for example the degree of confinement of the water, or the ecological history, but such an analysis could help explain the occurrence and/or maintenance of HAB, and could also give a clue on the *in situ* sensitivity thresholds of the different dinoflagellates species to turbulence.

In addition, dinoflagellates could also benefit in some way from turbulence episodes, especially if these are followed by periods of relatively calm conditions. This benefit could come from the resuspension of nutrients from the sediments, as well as of dormant cysts of dinoflagellates. Thus, another question to be investigated could be if turbulent episodes may stimulate the occurrence of dinoflagellate blooms.

In the Catalan coast nutrient input episodes are often associated to turbulent events, although there is not always a direct relationship between the two forcings (chapter 3). When nutrient fertilization coincides with turbulent events, the response of phytoplankton is faster and larger (chapter 4). This effect is explained by small-scale effects on phytoplankton cells, and also by turbulence in shallow waters maintaining settling phytoplankton (like diatoms) in the water column. The faster dynamics of phytoplankton and the consequent increase in abundance may favour the uncoupling with zooplankton and the development of a bloom. When turbulence stops, large non-motile phytoplankton is expected to increase its settling rates, farther escaping from predation. Thus, the episodic pattern of external energy forcing, in conjunction with nutrient inputs from terrestrial runoff or resuspension, may favour the export of C to the sediment in coastal zones.

Patterns of terrestrial runoff are changing at a global scale. The natural courses have been modified by massive pavement and urbanization, and by changes in the use and demand of water and soils. The construction of dams and artificial channels leads to an increase in the predictability of the flow and a decrease in its sediment charge (Cloern 2001). The concentration of nutrients in the runoff waters has been increasing in the last decades, leading to a slow process of eutrophication of coastal waters (Cloern 2001). The input of nutrients is thus higher and more regular. Because it becomes less episodic it can be uncoupled from turbulent events. Simultaneously, it seems that the frequency and intensity of extreme events (for example storms) is going to increase in the context of the global climate change (IPCC 2007). Thus, turbulence, may become the modulating factor of the phytoplanktonic dynamics in coastal areas (Peters 2003), determining the usage of excess nutrients and the export of carbon.

Another consequence of global change in terrestrial runoff is the decline in the Si:N ratios (Cloern 2001). This decline could lead to competitive advantage of non-siliceous taxa over diatoms (Svensen *et al.* 2001), and to a decrease in the frequency and intensity of diatom blooms. Because diatoms have relatively high settling velocities, this has implications in the episodic pattern of fixation and exportation of C in costal zones. However, in chapter 3 we observed a strong negative relationship of the amount of diatoms with the Si:N ratio (fig. 2), along with a positive relationship with P. Sustained bloom concentrations of diatoms between May and July 2004 were coincident with the lowest Si and the highest P concentrations of all the year of study. This suggests that at least in this particular location, P is still the dominanly limiting nutrient for diatom growth.

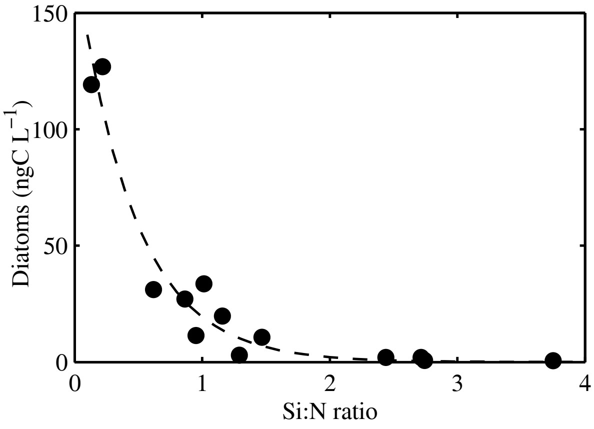

Figure 2: *Diatom concentration against the dissolved Si:N ratio in Blanes Bay between June 2003 and July 2004. Plotted line is the model fitted by the nonlinear least squares method.*

Final remarks

The study of turbulence and plankton has been essentially based on the assessment of effects of constant levels of turbulence on planktonic organisms and communities, particularly through experimental studies. Such work is extremely important to understand the mechanics of such effects, and to test theoretical models (e.g. Rothschild and Osborn 1988, Karp-Boss *et al.* 1996). The present thesis gives the relevance of these studies a dimensional leap in a natural variability context. Thanks to this, the body of evidence and conclusions regarding the influence of turbulence on plankton is now more robust. The dynamic nature and spatial distribution of turbulence in the coastal ocean and especially, the contribution of the different sources of energy is still far from being completely understood by physicists. In the meantime, this thesis has started to address how turbulence and the temporal variability in different meteorological and hydrological forcing factors affect the biogeochemical dynamics of a coastal system. I hope that this new approximation will be a stepping stone in the long way to understand how the spatial and temporal variability in environmental forcings, and particularly in turbulence, influences the dynamics of planktonic ecosystems.

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Appendix: Matlab functions

```
%% thresholds.m
%% This function calculates the frequency of wind events as a function of their persistence and mean intensity
%% syntax: [results,beta,J,R2]=threshold(w);
%% w=wind speed vector with frequency in hours
%% results=[persistencies,means,thresholds,frequency,estimated_frequency,residuals];
%% beta=[b'1,b'2,b'3,b'4, confidence intervals at 95%]
%% J=jacobian matrix;
%% calls functions cut.m, persistence.m and stretched_exponential.m
%% to calculate predictions with confidence intervals, type:
%% [yp, ci] = nlpredci(stretched_exponential,[persistence_in_hours wind_speed],beta(:,1),results(:,4),J)

function [results,beta,J,R2]=thresholds(w)

%% SETS THE MINIMUM AND MAXIMUM THRESHOLDS TO BE EXPLORED
minim=floor(10*nanmean(w))/10;
maxim=15;
thresholds=(minim:0.1:maxim-0.1);

%% IDENTIFY AND REGISTER THE EVENTS ABOVE EACH THRESHOLD %%%
for j=thresholds
  % identify the values above the threshold %%
  events=zeros(length(w),1);
  events(w>=j)=1;
  events(find(isnan(w==1)))=-1;

  % cut out events starting or ending with NaN values %
   events=cut(events);

  %%% registers the persistence of each event and calculate its mean
  %%% intensity
   [p,m]=persistence(events,w);

  %% percent of the series that is valid. It depends on the threshold.
  valids=length(find(events>-1))/length(events);
```

```
    % number of seasons of the series without taking into account NaN values
    series_length=length(events)*4/365/24*valids;

    % fills the matrix of frequencies and of means
     for i=1:max(p)
            frequencies(i,thresholds==j)=length(find(p>=i))/series_length;

       % seeks all events of equal persistence and calculates the mean intensity
       means(i,thresholds==j)=nanmean(m(p>=i));
     end
end

means(means==0)=NaN;
frequencies(frequencies==0)=NaN;%% Deletes events with frequency 0
thresholds=(minim:0.1:length(frequencies(1,:))/10+minim-0.1);
    %% Readjusts the length of the "theshold" vector taking into account
    %% only the events that do not start or end in NaN

X=[];
y=[];
t=ones(length(frequencies(:,1)),1);

for i=1:length(thresholds)-1
    X=[X;[1:length(frequencies(:,1))]',means(:,i),t*thresholds(i)]; % X=[persistencies,means,thresholds]
    y=[y;frequencies(:,i)];    % =[frequencies]
end

%% DELETES ROWS WITHOUT VALUE
y(isnan(X(:,3)))=[];
X(isnan(X(:,3)),:)=[];

%%% ADJUSTS THE MODEL
beta=[1000 -0.2 0 0];
[beta,residuals,J]= nlinfit([X(:,2),X(:,3)],y,stretched_exponential@,beta); % multiple non-linear regression model
ci=nlparci(beta,residuals,J);
yhat=beta(1)*exp(-beta(2)*X(:,3)).*exp(-X(:,2).^(beta(3)+beta(4)*X(:,3))); %estimated frequency

%%% R2 %%%
```

```
RSS=nansum(nansum((y-yhat).^2)); % residual variation
TSS=nansum(nansum((y-nanmean(nanmean(y))).^2));% total variation
R2=1-RSS/TSS;

%% OUTPUT %%
results=[X(:,1:3),y,yhat,residuals];
beta=[beta',ci];
```

%% cut.m

```
%% marks as non-valid the events that start or end with a NaN
%% syntax: [events]=cut(events);
%% called by thresholds.m
```

function [events]=cut(events)

```
   for i=1:length(events)-1
      if events(i)==-1
         if events(i+1)==1
            events(i+1)=-1;
         end
      end
   end

   for i=0:length(events)-2
      if events(length(events)-i)==-1
         if events(length(events)-i-1)==1
            events(length(events)-i-1)=-1;
         end
      end
   end
```

%% persistence.m

```
%% Registers the persistences of each of the events and calculates the mean intensity of each event
%% va anotant les persistencies de tots els events que es troba
%% syntax: [p,m]=persistency(events,w)
```

```
%% called by thresholds.m

function [p,m]=persistence(events,w)

p=[];
m=[];
c=0;

    for i=1:length(events)-1
       if events(i)==1
         c=c+1;
         if events(i+1)<1
            p=[p;c];
            m=[m;mean(w(i-c+1:i))];
            c=0;
         end
      end
    end
```

```
%% stretched.m
%% STRETCHED EXPONENTIAL model
%% syntax: [beta,residuals,J]=nlinfit([X(:,2),X(:,3)],y,stretched_exponential@,beta);
%% called by thresholds.m

function yhat = stretched_exponential(beta,X)

x1=X(:,1); %% persistences
x2=X(:,2); %% means

yhat=beta(1)*exp(-beta(2)*x2).*exp(-(x1.^(beta(3)+beta(4)*x2)));
```

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Com que sóc un covard, no vull carregar jo sol amb la culpa d'aquesta perla que teniu a les mans. És més, no seria just, perquè hi ha moltes persones implicades en major o menor grau. Com a homenatge al seu mèrit, i perquè no puguin fugir, arribat el cas, del dit acusador de la societat que vol saber en què ens gastem els seus quartos, m'agradaria fixar sobre el paper els noms d'aquestes persones. Ve't-ho aquí.

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