

*Biological rhythmicity in phytoplankton
and interactions with turbulence:
from the cell to the ecosystem scale*

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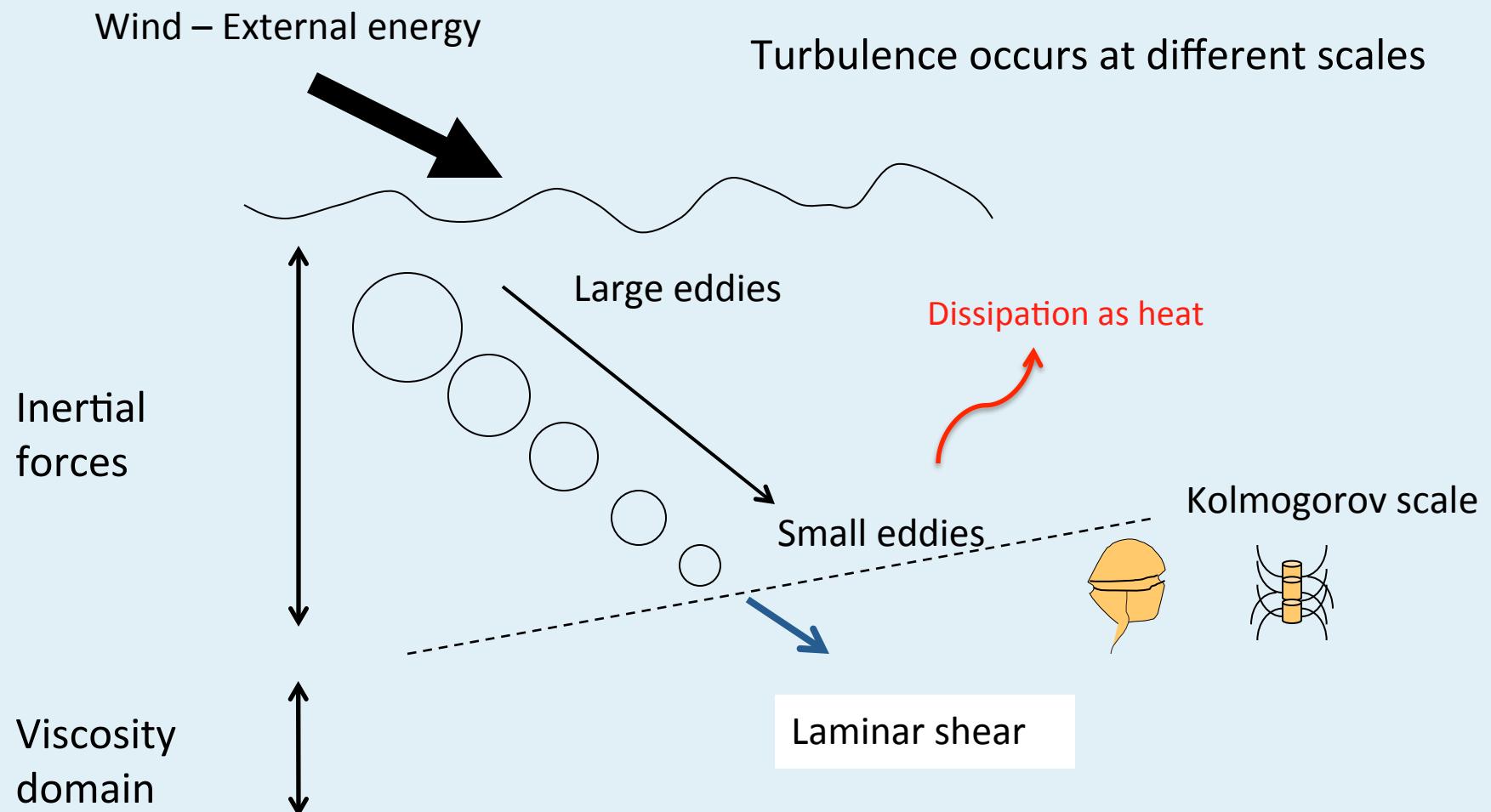


CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

***Biological rhythmicity in phytoplankton
and interactions with turbulence:
from the cell to the ecosystem scale***

- 1.- Scales of turbulence. The Margalef's Mandala**
- 2.- Direct effect of small-scale turbulence on dinoflagellates.
From the lab to the field
- 3.- Studies in Alfacas Bay: plankton system
- 4.- Blooms of the benthic toxic dinoflagellate *Ostreopsis*

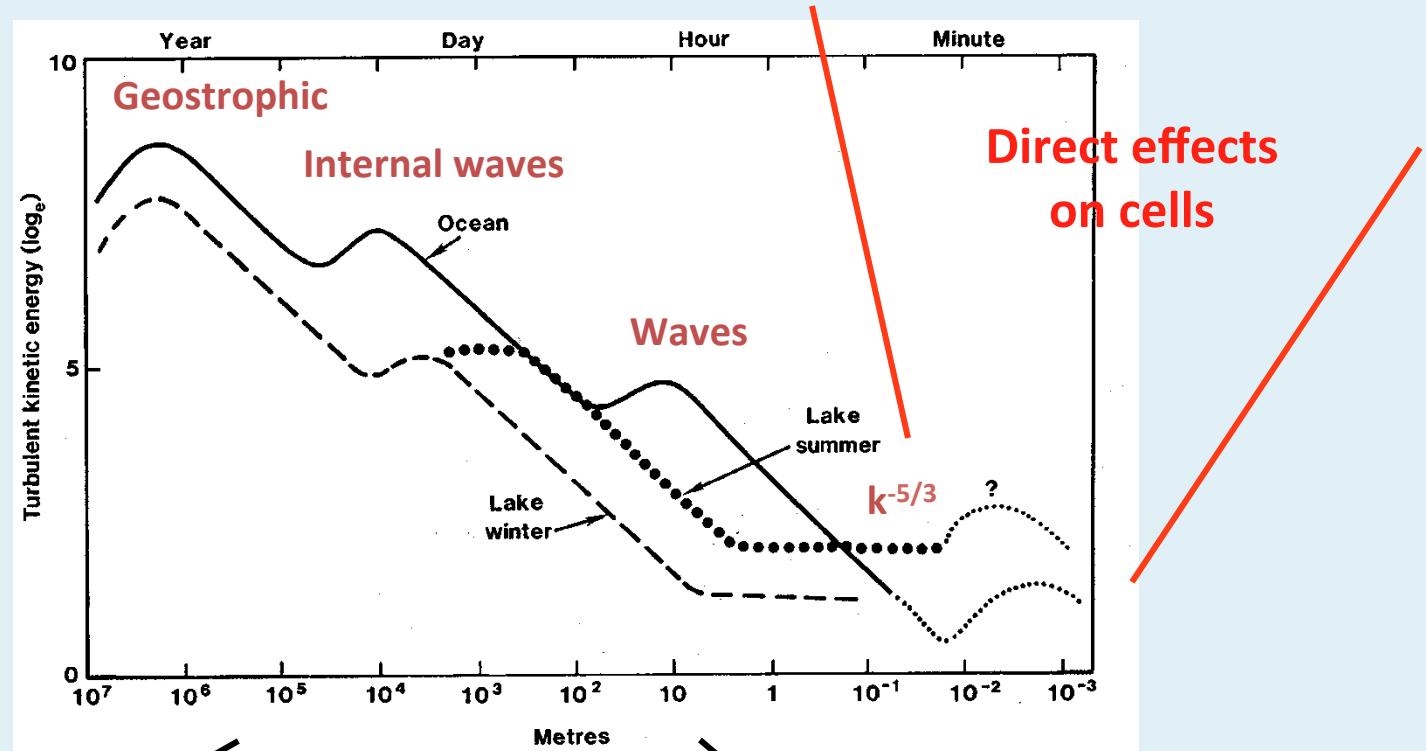
1. Scales of turbulence.



modified from Kiørboe 2006

1. Scales of turbulence.

Scales of turbulent kinetic energy inputs - the energy cascade:



Light exposure

Nutrient supply from deep waters

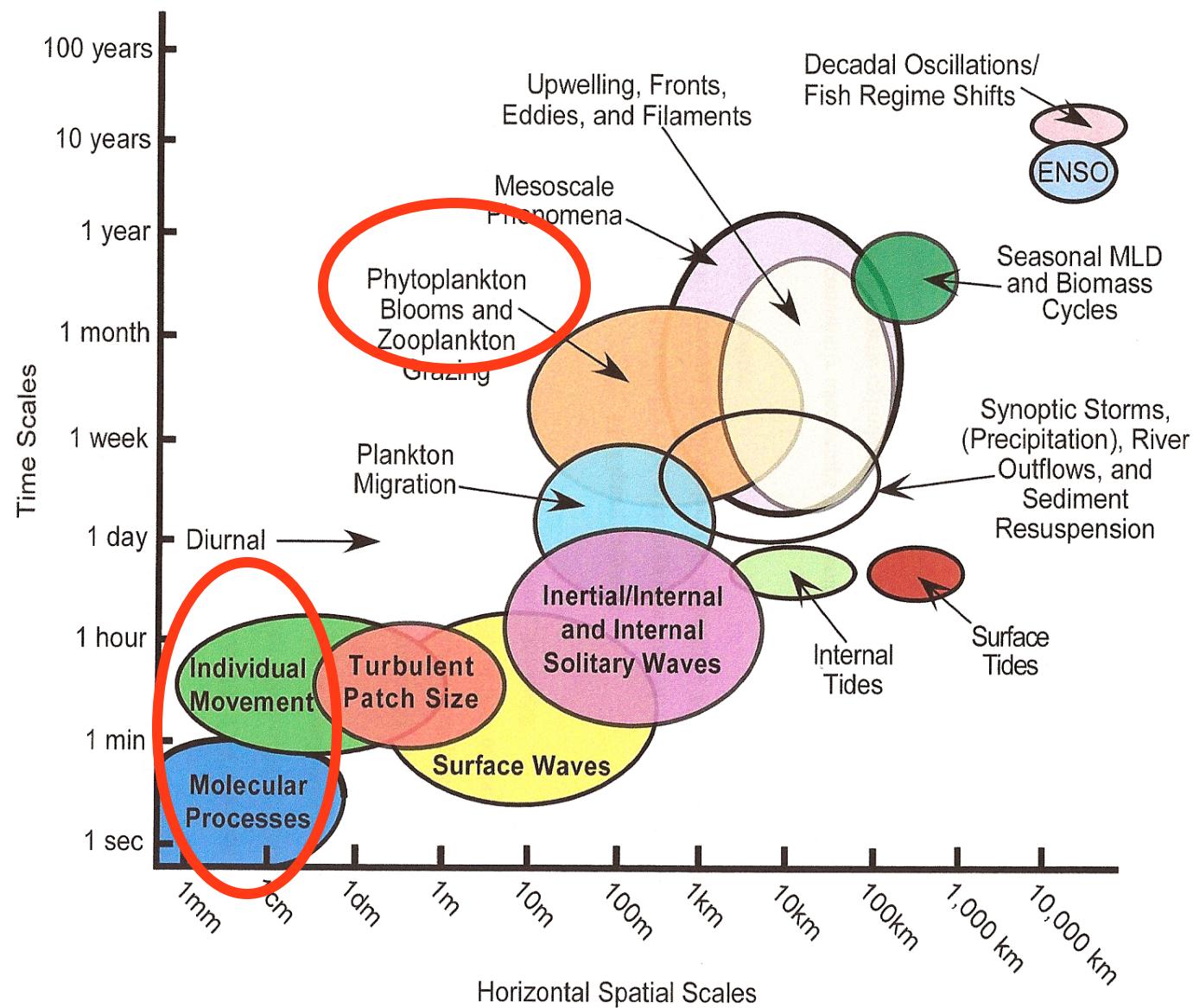
Transport and sedimentation

Environmental microstructure (Intermittency, patchiness)

From Harris (1986)

1. Scales of turbulence.

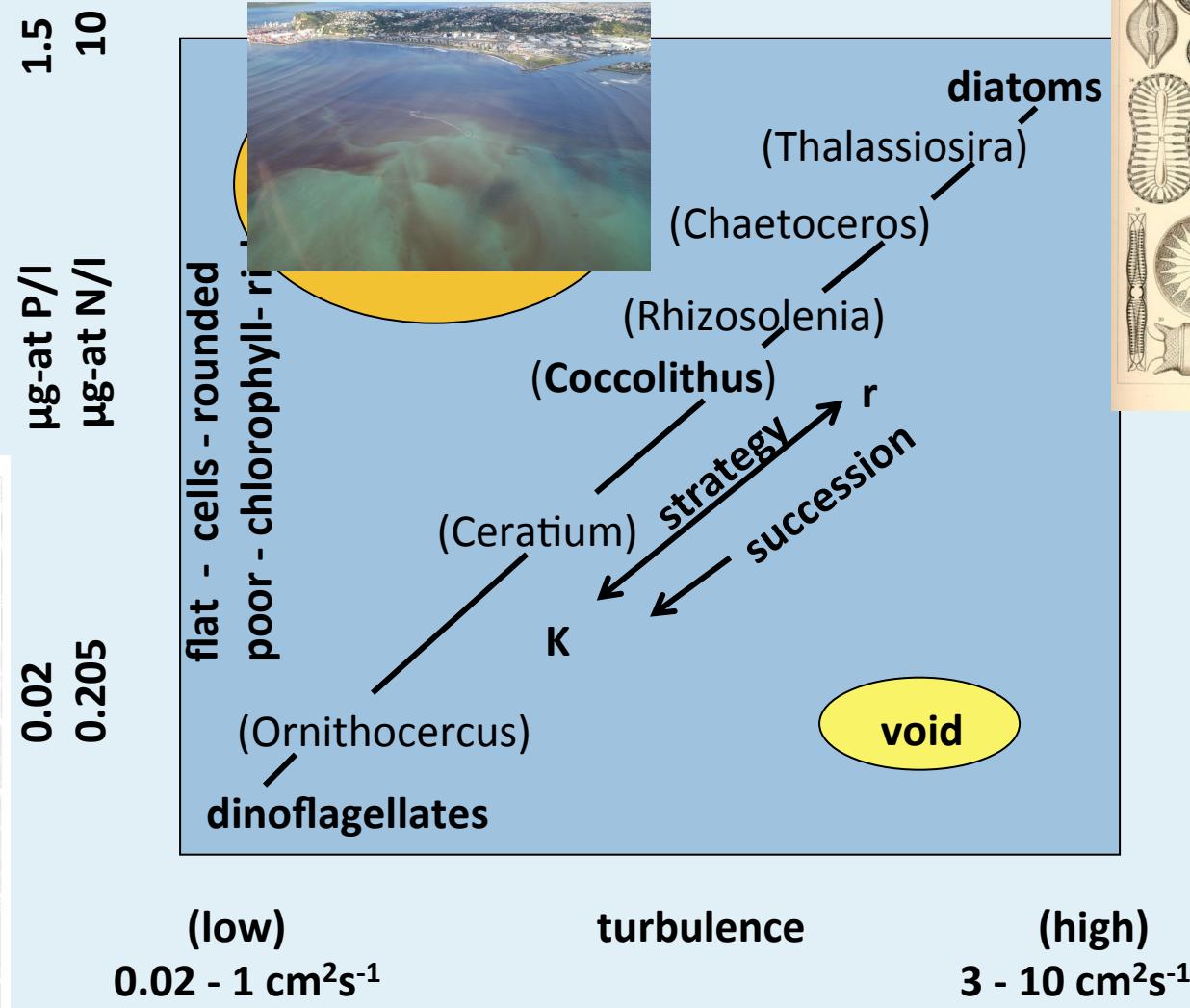
Phytoplankton dynamics results from the interplay among processes that occur at a variety of spatiotemporal scales



Dickey 1991 – modification of Stommel

1. Scales of turbulence. The Margalef's Mandala

the phytoplankton mandala, Margalef 1978
... temptative plot ...



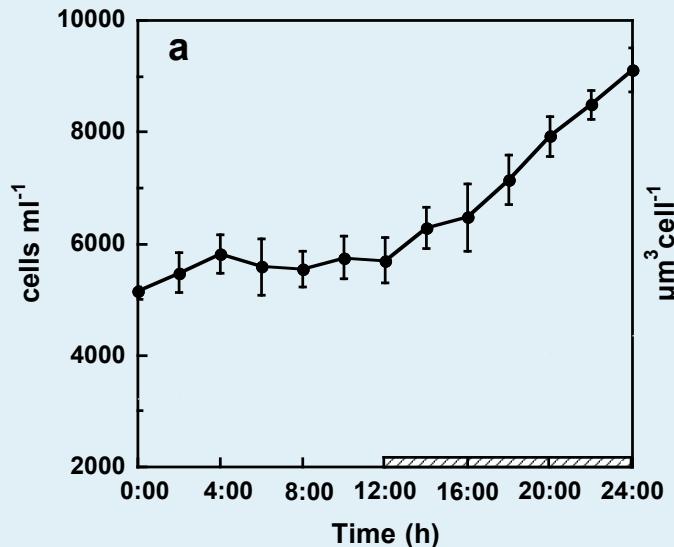
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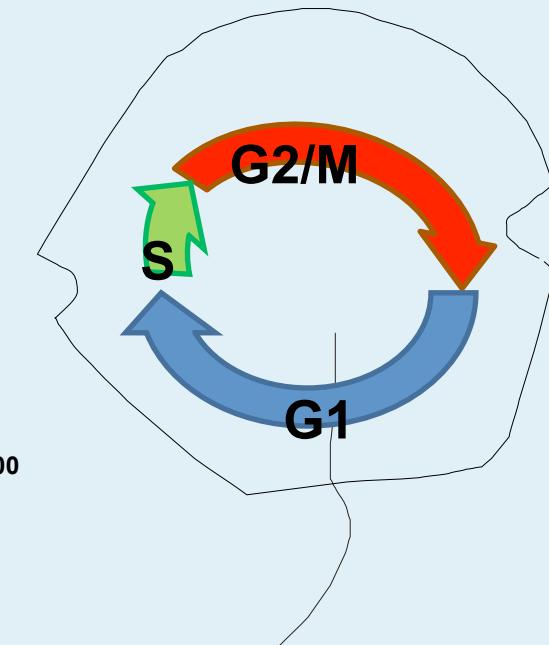
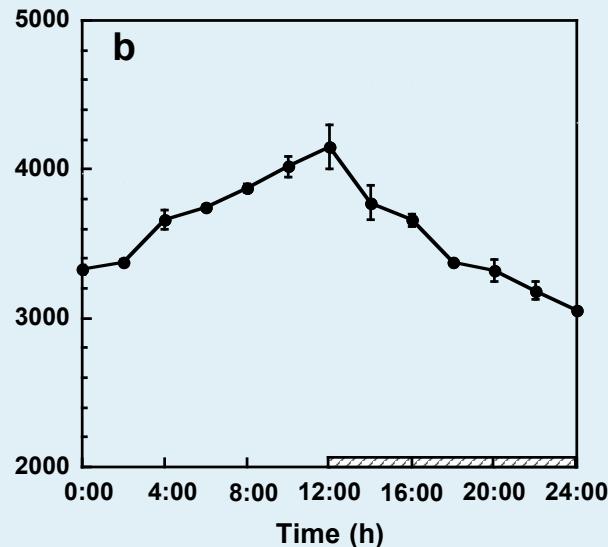
2. Biological rhythms. Dinoflagellates

Berdal et al. 2011

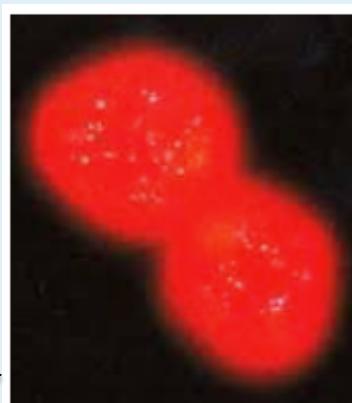
Cell numbers



Cell biovolume



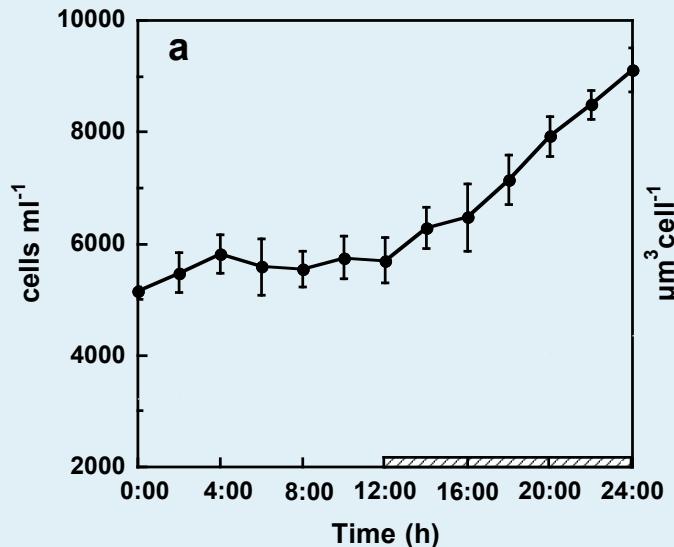
- * Circadian rhythm regulation of several physiological process
- * Cell division: at night
- * Cell growth: during the day
- * via methionine – gonyol – gonyauline - regulation of the bioluminescent circadian rhythm in *Gonyaulax polyedra*



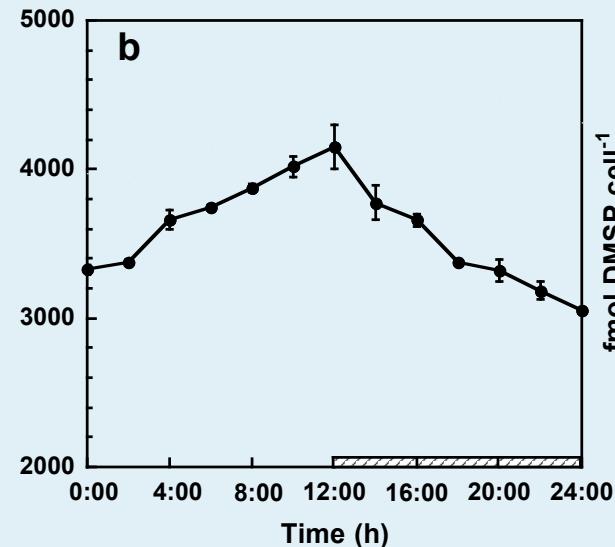
2. Biological rhythms. Dinoflagellates

Berdale et al. 2011

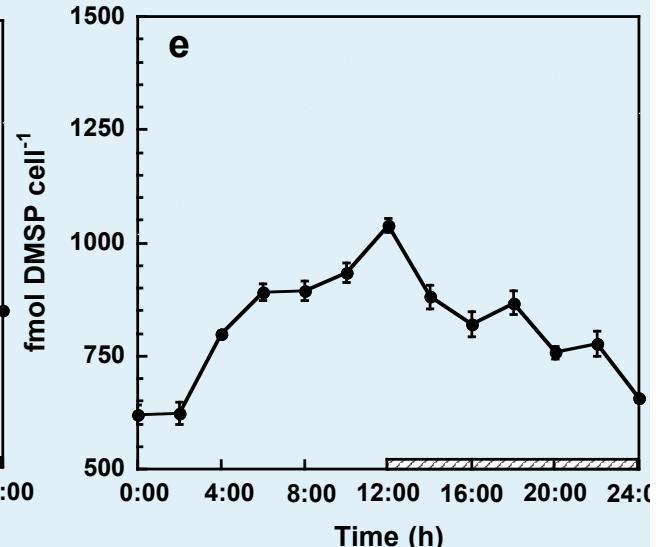
Cell numbers



Cell biovolume

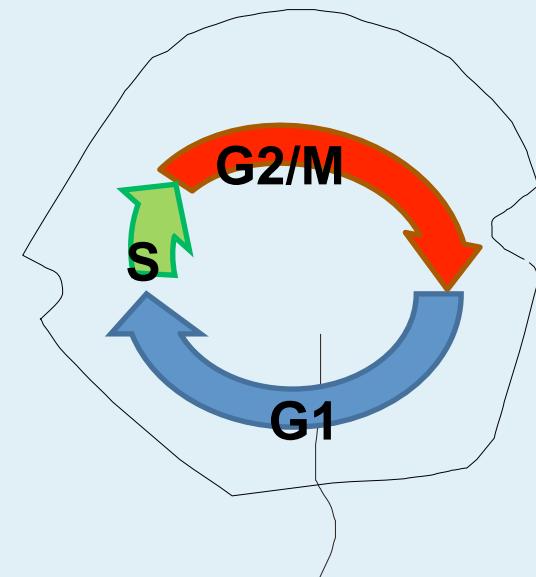


DMSP/cell



- * Circadian rhythm regulation of several physiological process
- * Cell division: at night
- * Cell growth: during the day
- * DMSP was preferentially synthesized during the light period under stillness.

Alexandrium minutum

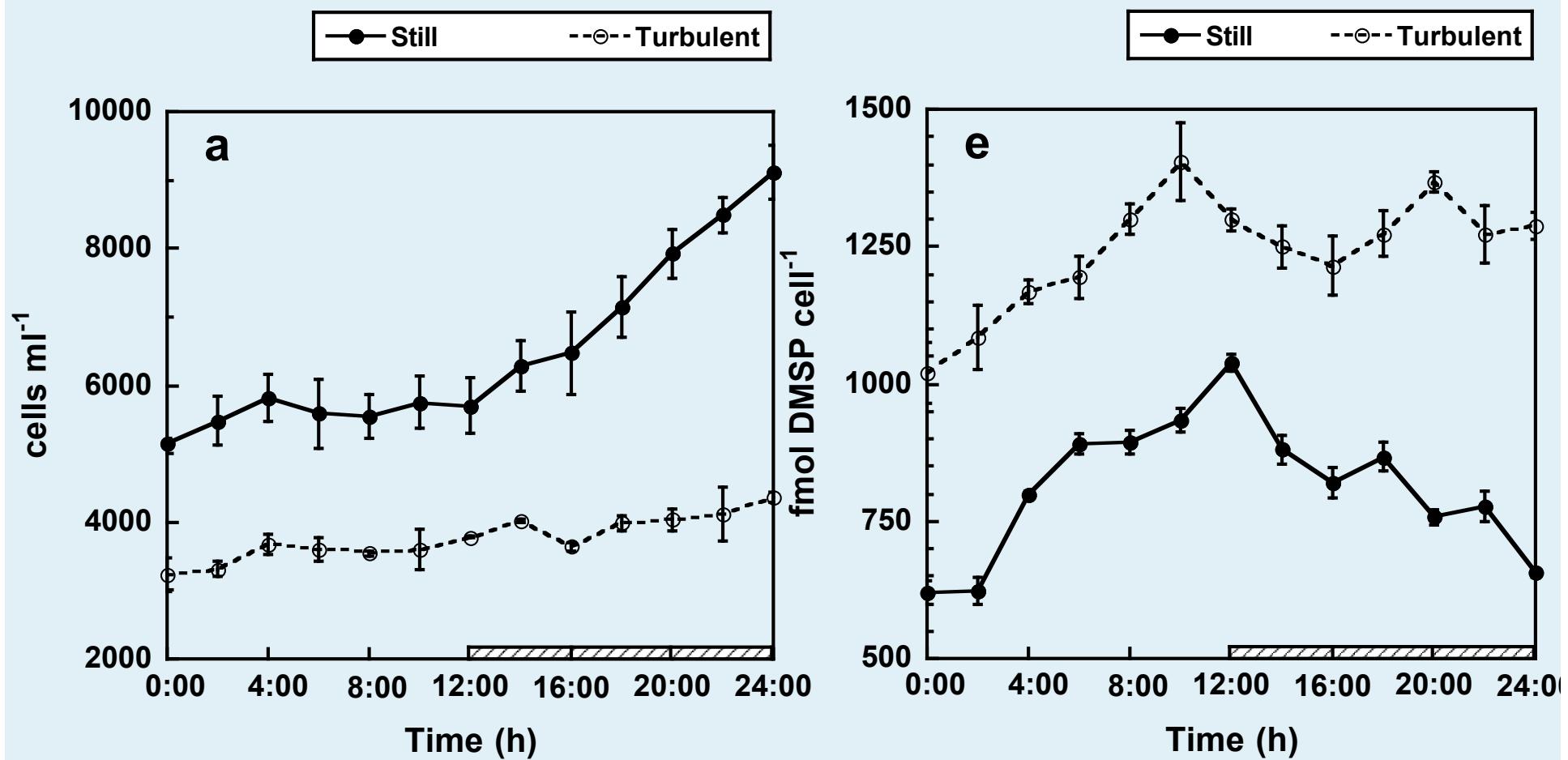


2. Biological rhythms. Dinoflagellates

Berdale et al. 2011



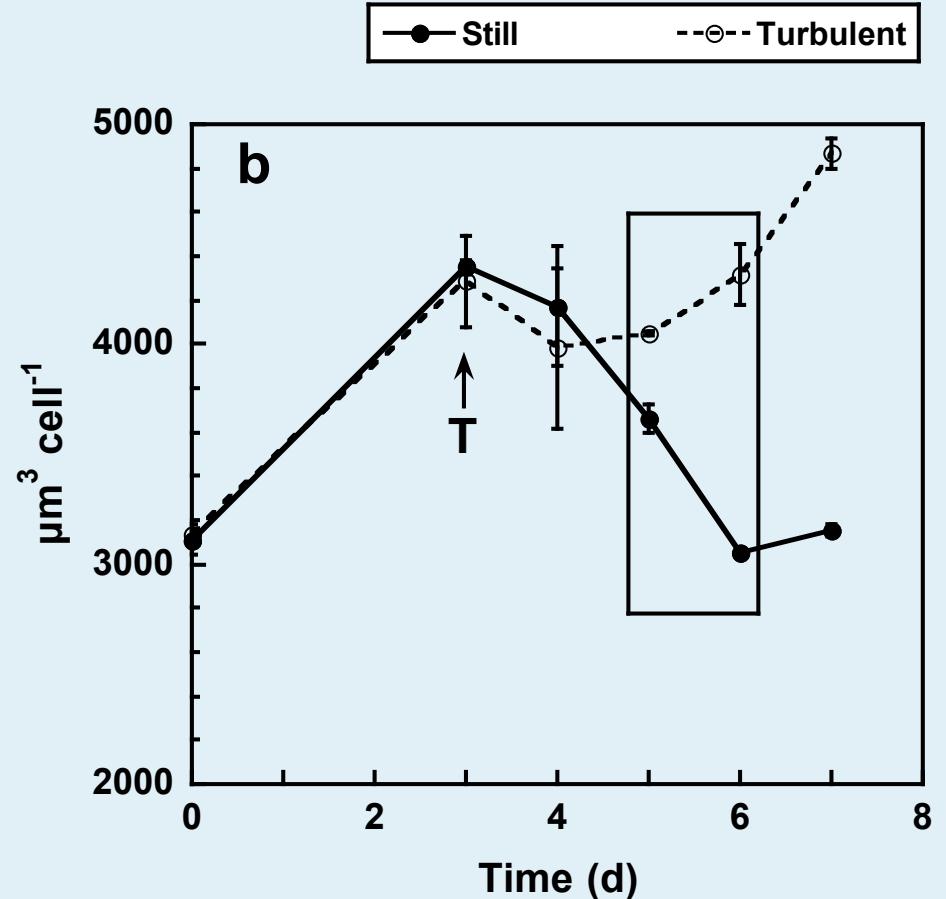
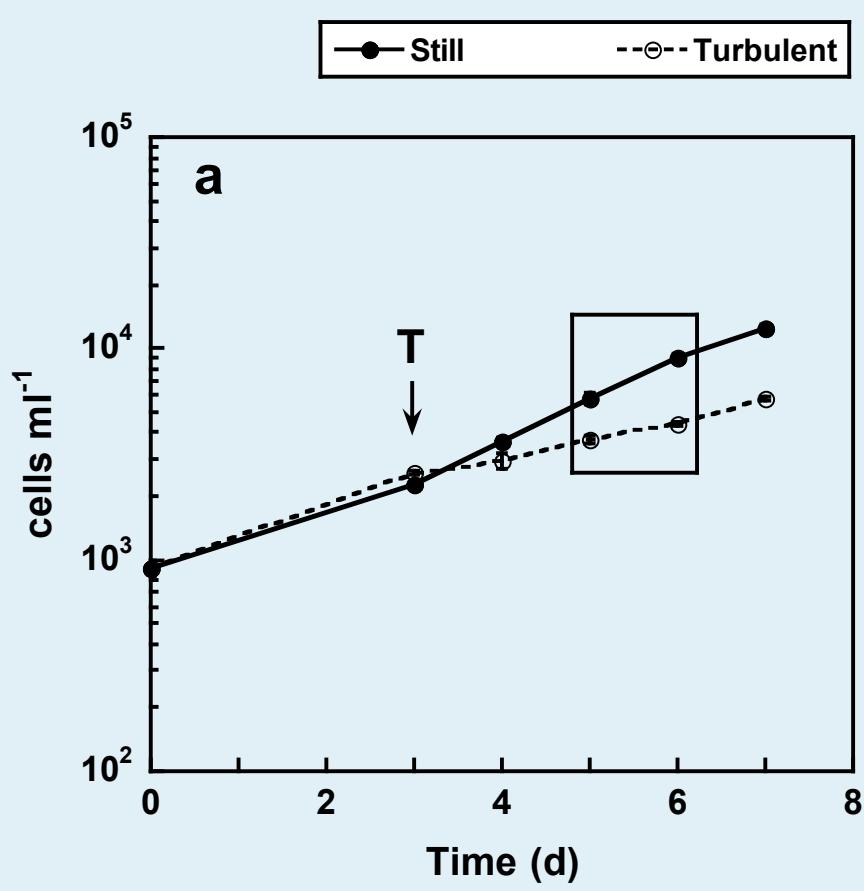
Under turbulence, DMSP accumulated in the cells whose cell division cycle was arrested under stirring



2. Biological rhythms. Dinoflagellates. Interference by small-scale turbulence

Small scale turbulence:

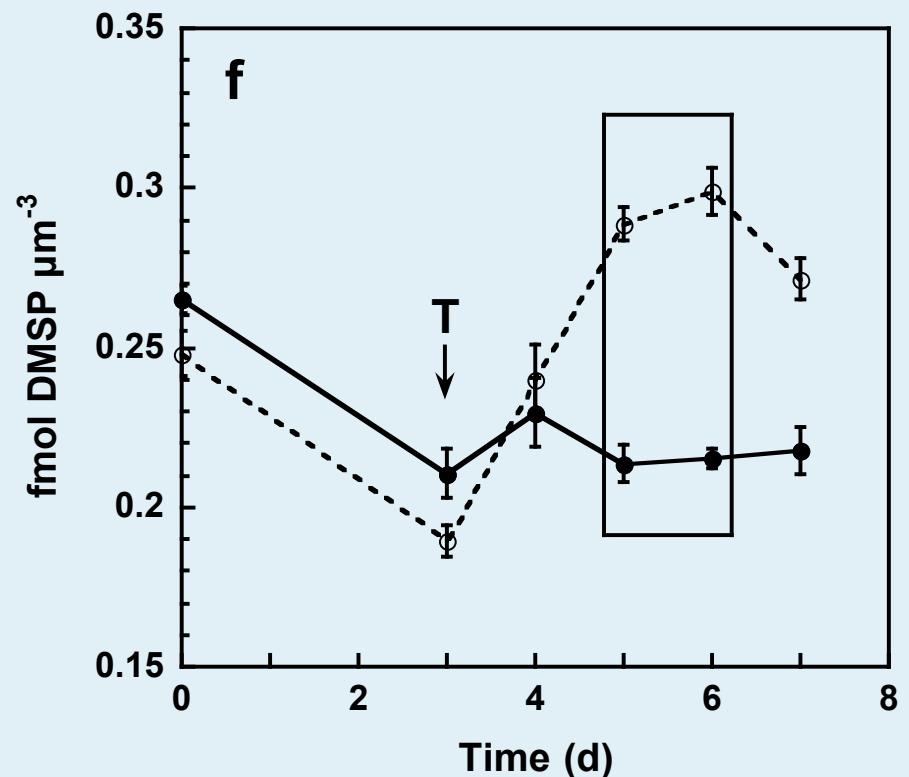
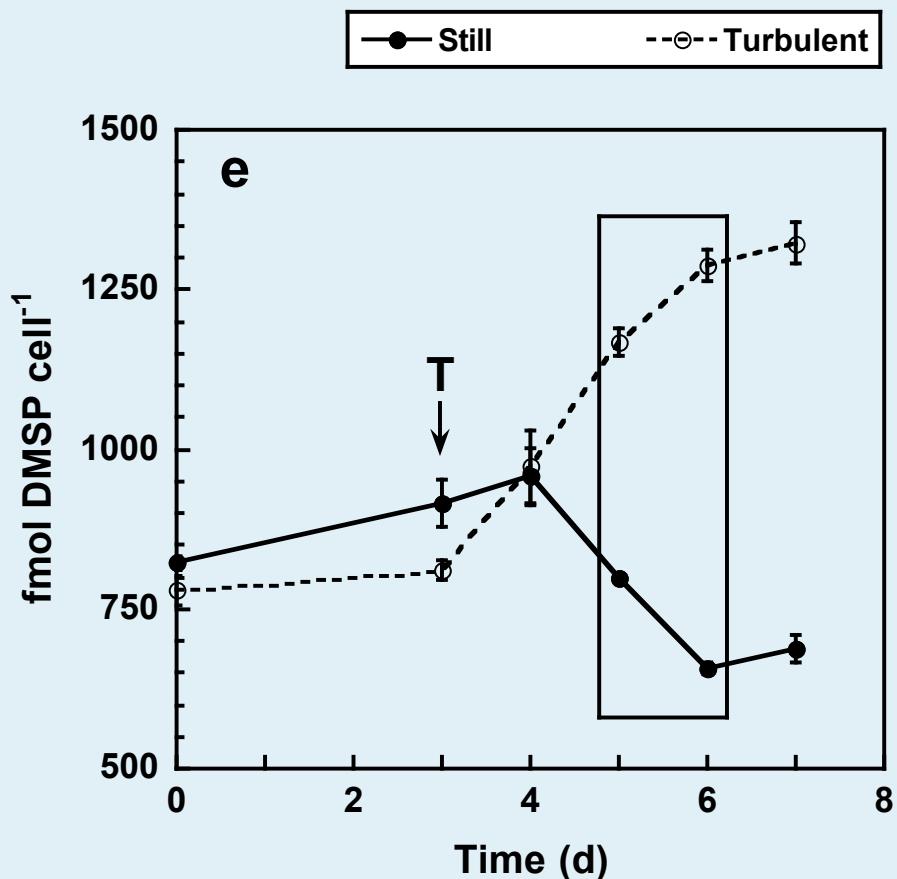
- decreased the net growth rate and cell numbers of *A. minutum*
- increased the cell volume of *A. minutum*.



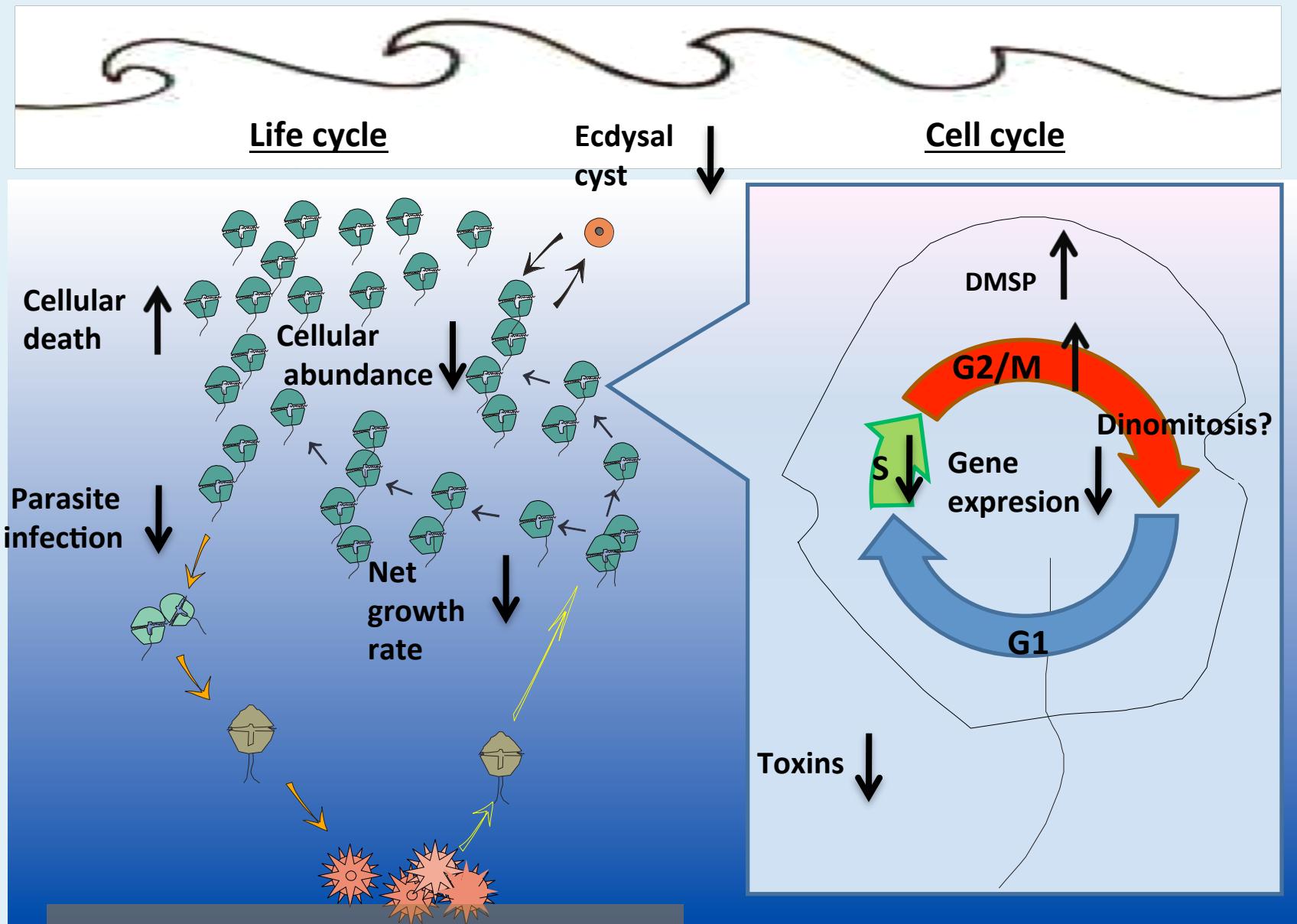
As in former studies (Berdalet 1992, Berdalet et al. 2007, Bolli et al. 2007, Llaveria et al. 2009, 2010)

2. Biological rhythms. Dinoflagellates. Interference by small-scale turbulence

Turbulence increased the cellular DMSP concentration
by ca. 20% (from 0.22 ± 0.01 to 0.27 ± 0.03 fmol μm^{-3})



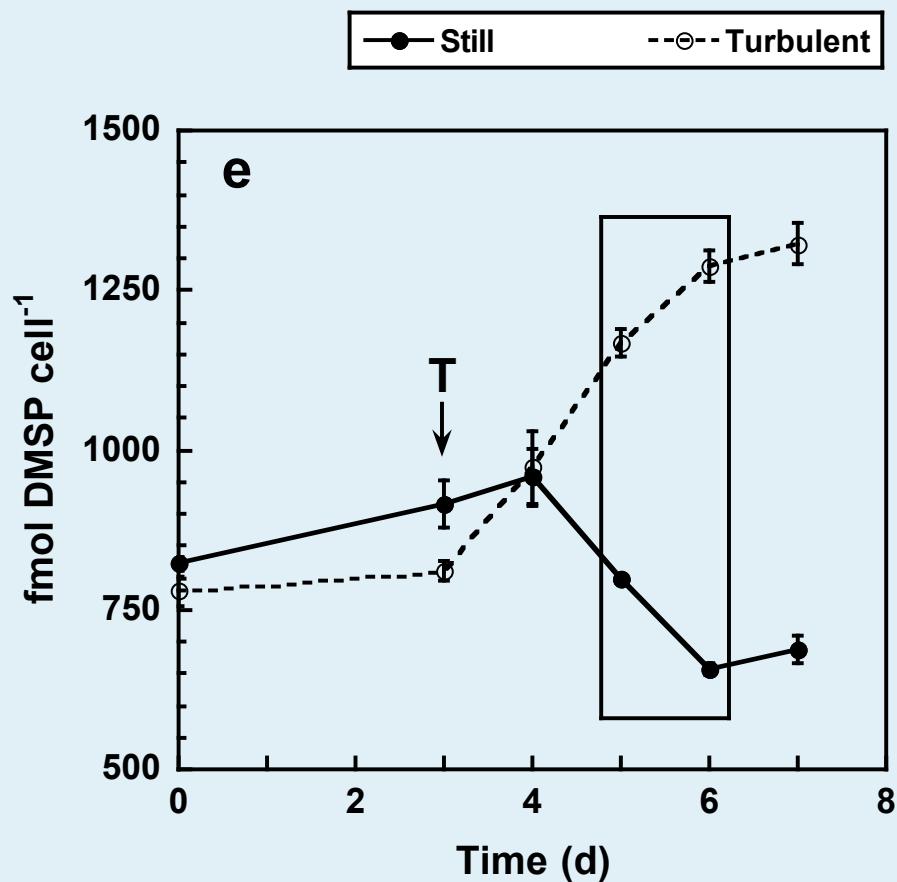
2.- Direct effect of small-scale turbulence: summary of the effects on dinoflagellates



Gisela Llaveria (PhD thesis, 2009)

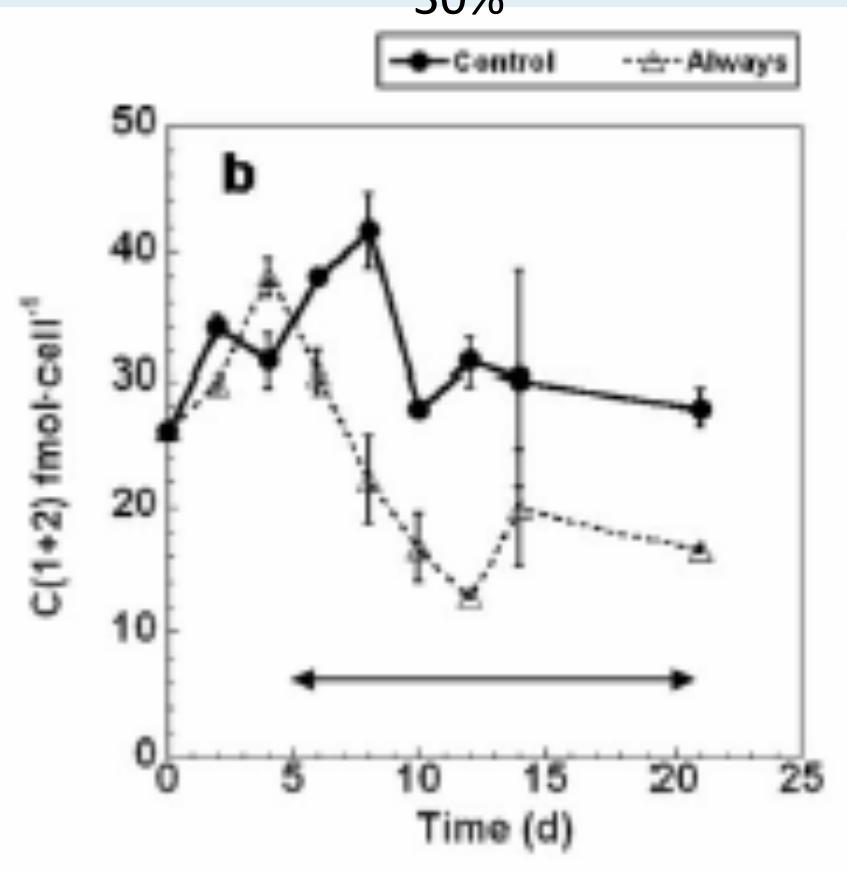
2.- Direct effect of small-scale turbulence: laboratory studies. DMSP

Turbulence increased the cellular DMSP concentration by ca. 20%



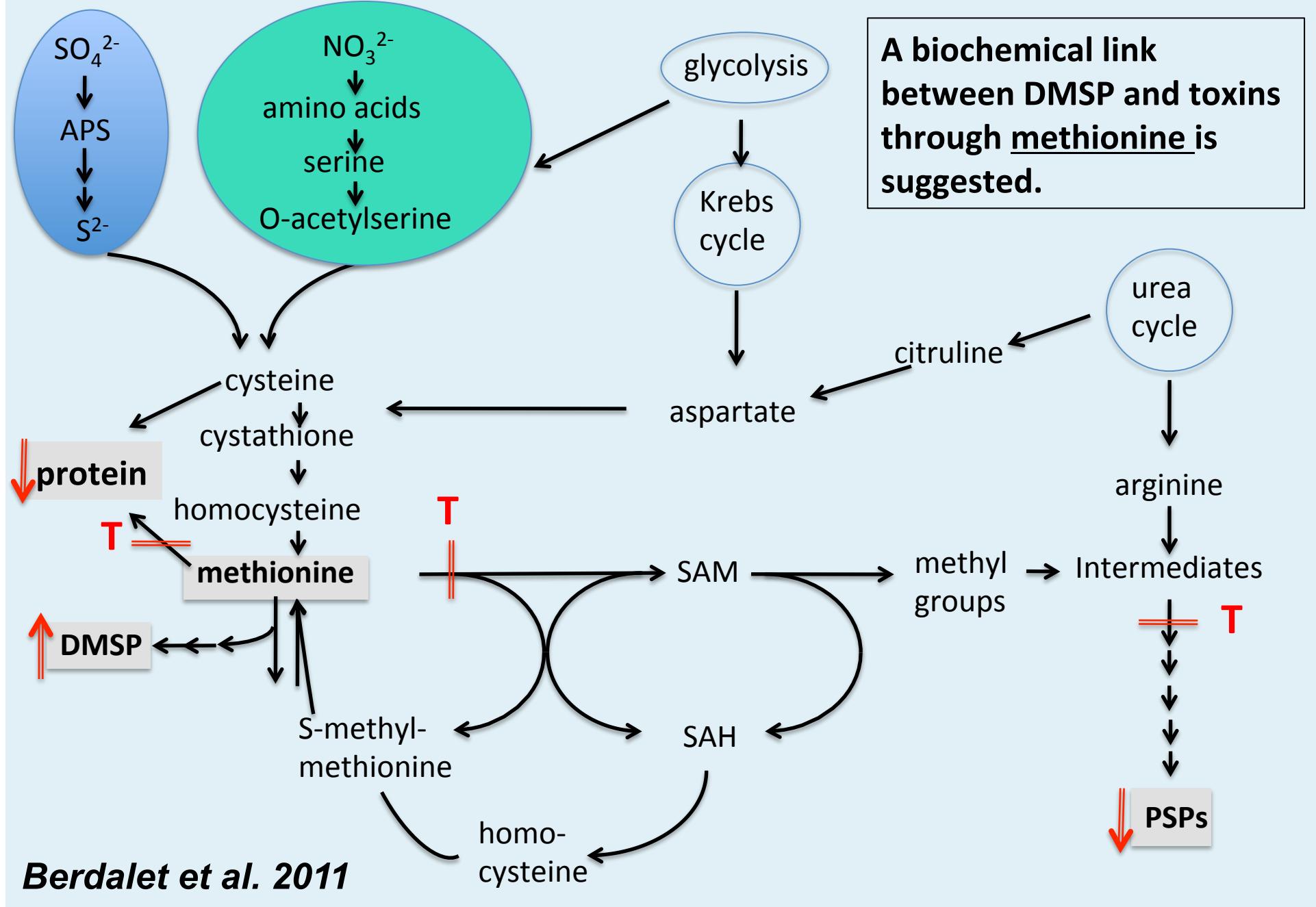
Berdelet et al. (2011).
Harmful Algae 10: 88-95.

Turbulence decreased the cellular toxin (PSP) concentration by ca. 30%



Bolli et al. (2007). Biogeosciences 4:
559-567.
Also decrease in ecdysal cyst formation

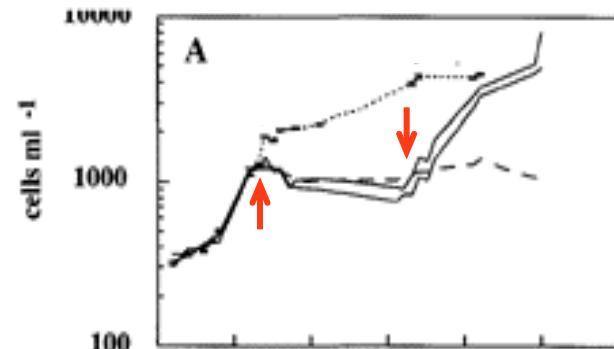
2.- Direct effect of small-scale turbulence: laboratory studies. DMSP and toxins



2.- Direct effect of small-scale turbulence: laboratory studies. Growth rate and population



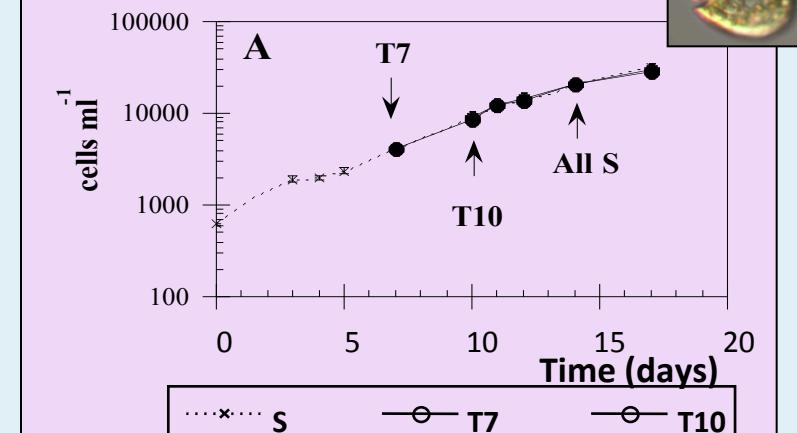
Akashiwo sanguinea



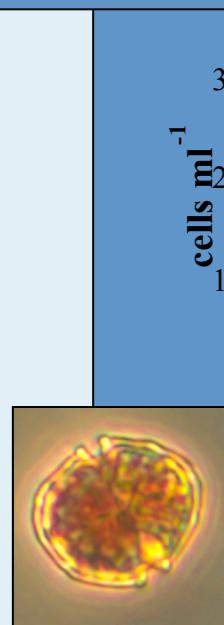
Berdalet (1992)

- Orbital shaker
- Identical design

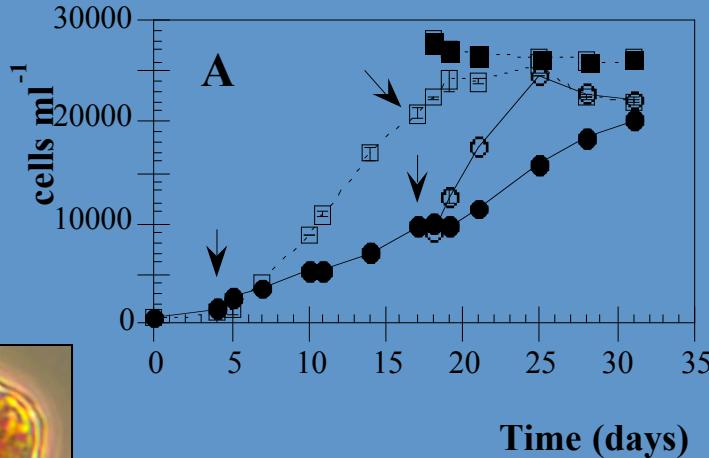
Gymnodinium sp.



Berdalet et al. 2007

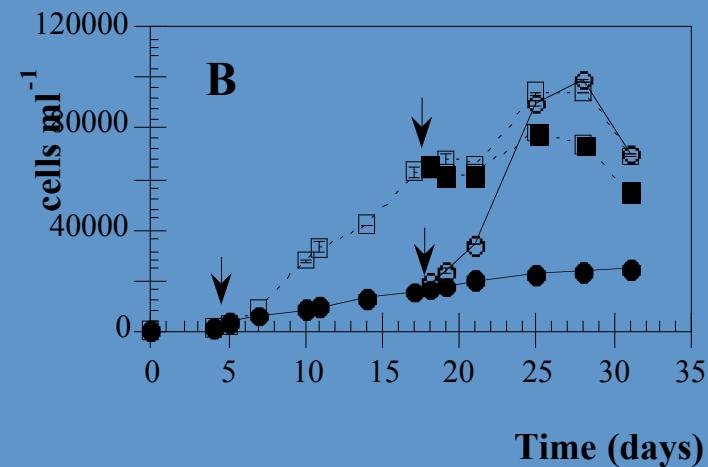


Alexandrium minutum



...□... S ...■... ST ●— T ○— TS

Prorocentrum triestinum



...□... S ...■... ST ●— T ○— TS



2- Direct effect of small-scale turbulence: laboratory studies

Ceratocorys horrida

Orbital shaker

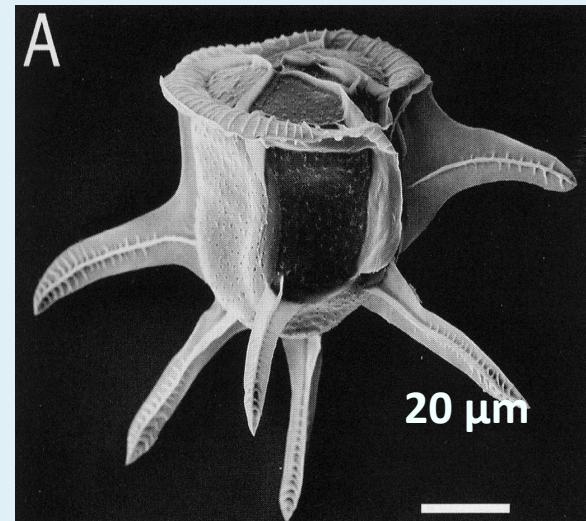
Zirbel et al. 2000. Direct effects on cells: shape

A. Still conditions:
cells with 6 large spines

After turbulence stopped:

- cell morphology recovered
- cell size increased

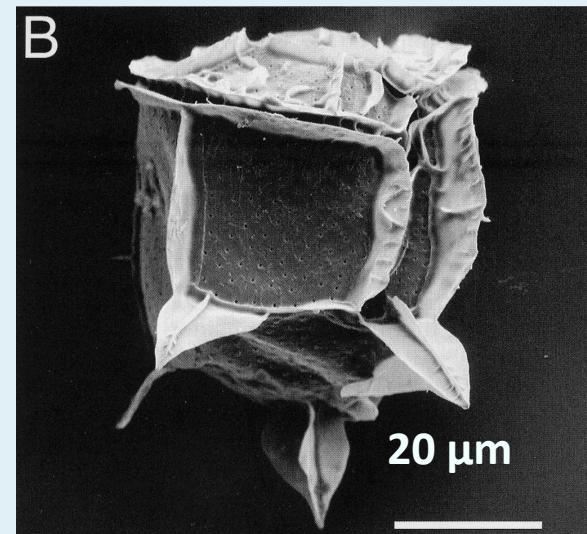
- but: no cell duplication occurred!



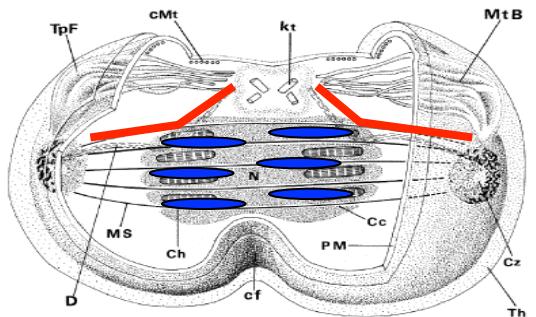
Direct effects on cells: shape

B. Turbulent conditions:
Orbital shaker at 75 rpm
(average $\varepsilon = 0.1 \text{ cm}^2\text{s}^{-3}$)
corresponding to light wind

- decreased net growth rate
- decreased cell size
- short spined cells



2.- Direct effect of small-scale turbulence: Why this sensitivity in dinoflagellates?



Perret et al., 1993

Trying to elucidate a dinomitus
alteration at microtubule level
(Llaveria et al. 2010)

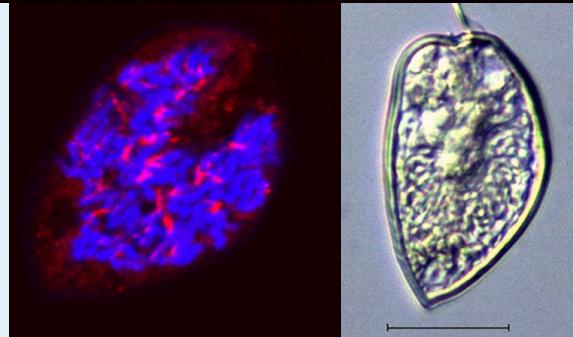
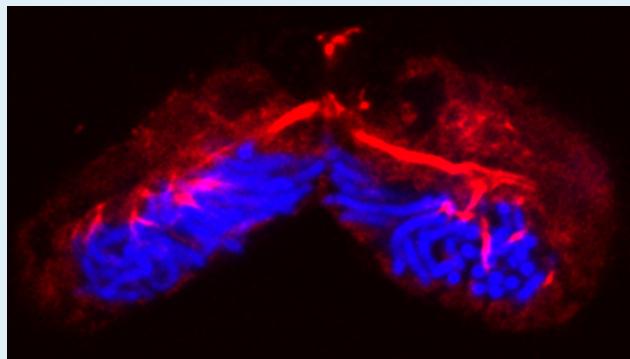
A. minutum, A. sanguinea, P. micans...

Immunocytochemistry

α -tubulin antibody DM1A

Rhodamine Microtubules

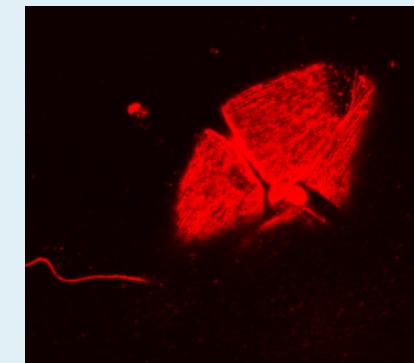
Hoechst 33258 Chromosomes



Prorocentrum micans



Akashiwo sanguinea
Scale bar: 20 um



2.- Hydrodynamic control of dinoflagellate dynamics in the field

U. Polliger & E. Zemel (1981). *In situ* and experimental evidence of the influence of turbulence on cell division processes of *Peridinium cinctum* f. *westii* (Lemm.) Lefèvre.
British Phycological Journal 16(3): 281-287



Table 1. The timing of the mixed period (shaded area) and the fluctuations of the number of *Peridinium* cells ($\times 10^6 \text{m}^{-2}$, monthly averages) in the trophogenic layer (obtained by planimetry).

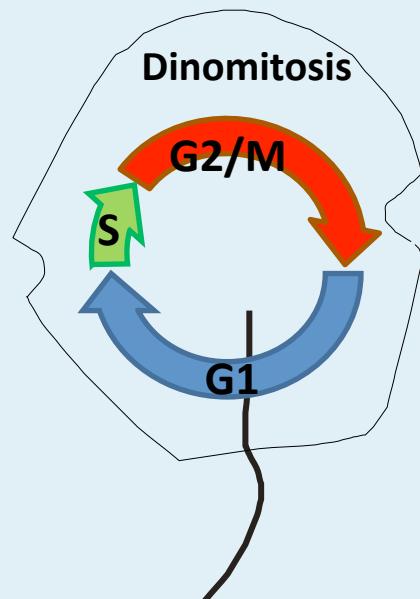
	1969	1970	1971	1972	1973	1974	1975	1976	1977
January	146	1320	460	356	140	175	33	25	101
February	417	2040	877	1210	1473	400	52	258	655
March	2040	4780	2345	3193	3053	1343	788	740	1761
April	4505	4870	3705	3430	4027	2793	2033	2683	2068
May	3700	2885	3850	2990	1270	3148	2912	2158	2542
June	1640	2017	2015	1787	1315	1087	1274	1362	1391

Cell numbers were kept low during wind blowing periods

U. Polliger & E. Zemel (1981).

2.- Hydrodynamic control of dinoflagellate dynamics in the field

Table 2. Wind velocity (m/s) and the division rate of *Peridinium cinctum* f. *westii*, *in situ* (dividing cells/total population, %)



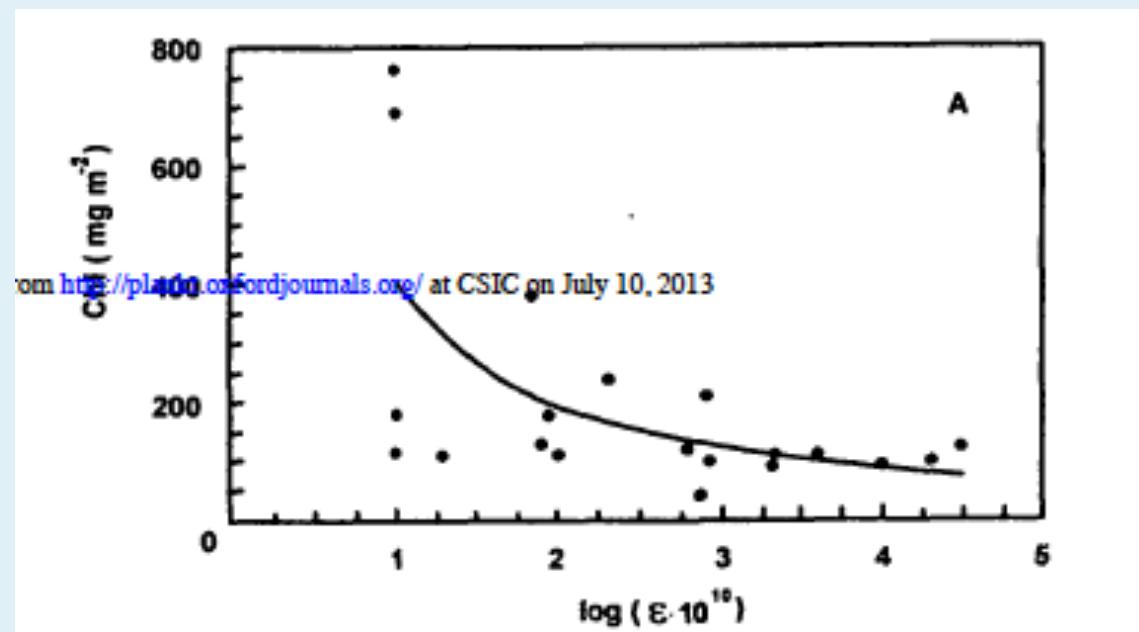
Decreased division rate when wind blew during the period of DNA duplication

Date	Interphase 14h - 17h	18h-22h Synthetic phase	23h-02h Mitosis	03h - 06h Cytokinesis	Division rate (%)
25/03/74	7.2				37
14/04/75	4.8				27.6
07/05/75	4.6				15.4
01/03/72		4.1			3
25/05/72		4.8			2
11/07/72		5.4			1.5
09/05/73		4.6			3.6
23/05/73		4.8			6.1
08/05/74		5.3			5.9
03/06/75		5.3			6.4
02/05/76		5.2			7.2
29/03/73			5.5		13.7
01/05/74			4		10.5
10/02/75			7.8		4.6
28/03/73				9.1	21.2
01/05/74				6.8	10.5
27/02/76				4	37.3
28/02/73					28.7
23/01/74					19
04/03/74					30.6
13/03/74					37
08/01/75					26.4
13/03/75					24.4
25/03/75					25.5

U. Polligher & E. Zemel (1981).

2.- Hydrodynamic control of dinoflagellate dynamics in the field

T. Berman & Shteinman (1998). Phytoplankton development and turbulent mixing in lake Kinneret (1992-1996)
Journal of Plankton Research 20: 709-726

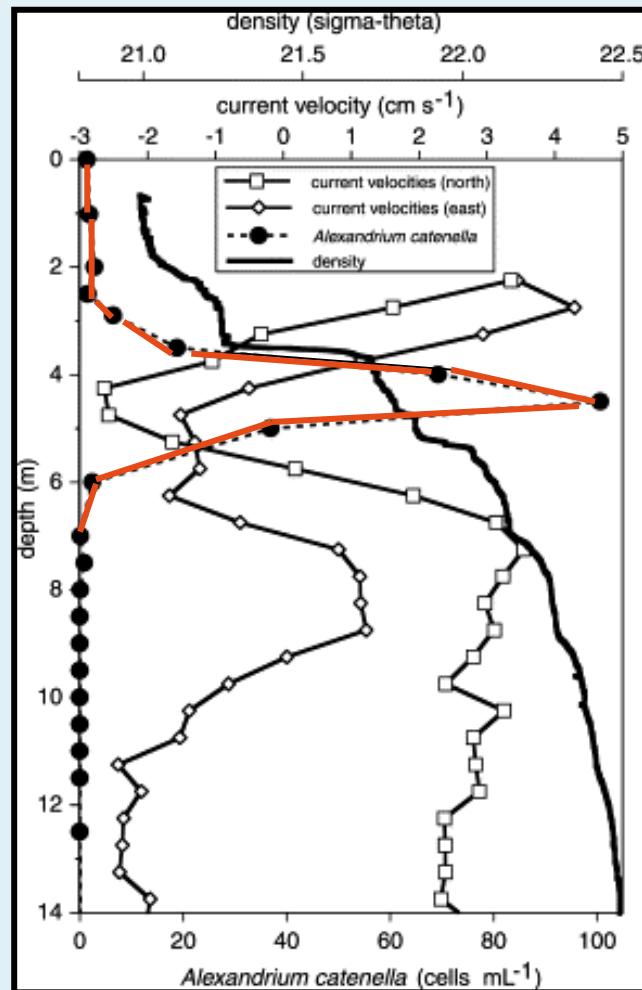


*Turbulent kinetic energy dissipation rate determined changes in phytoplankton succession and population composition
Absence of Peridinium bloom when TKE excessively high.
Record of dinoflagellate abundances when TKE were very low.*

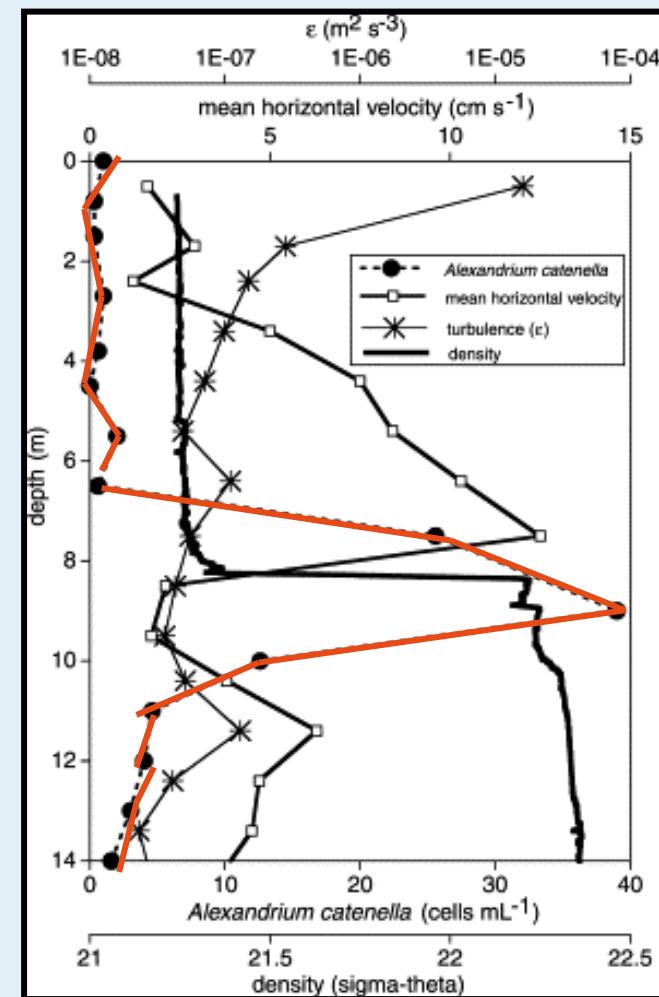
2.- Hydrodynamic control of dinoflagellate dynamics in the field

Sullivan, J.M., E. Swift, P.L. Donaghay, J.E.B. Rines. 2003. Small-scale turbulence affects the division rate and morphology of two red-tide dinoflagellates. *Harmful Algae* 2:183-199.

East Sound, WA, 1997



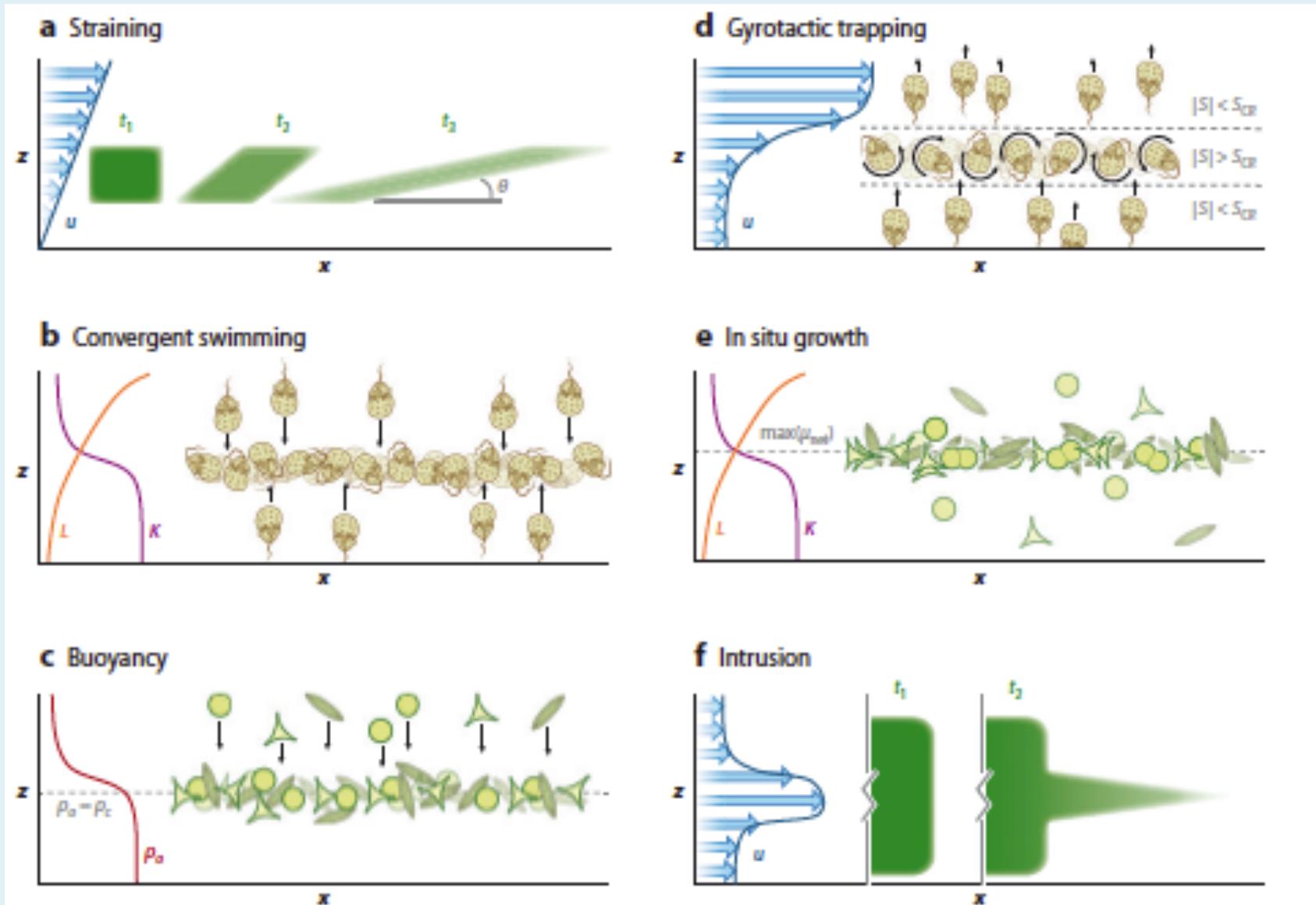
East Sound, WA, 1998



- *Alexandrium catenella* layer in the region of minimum current shear
- Highest ϵ (ADV measured) above and below the *A. catenella* layer

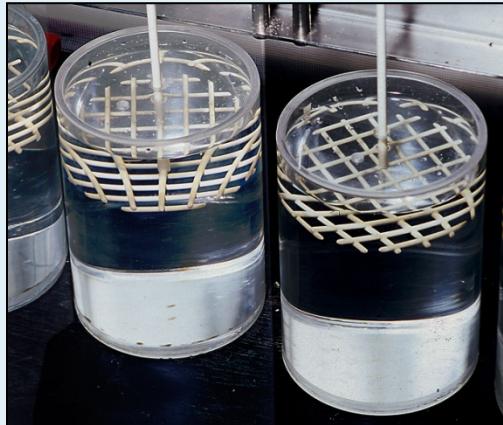
2.- Hydrodynamic control of dinoflagellate dynamics in the field

MECHANISMS OF THIN LAYER FORMATION (Durham et al. 2011)



2.- Direct effect of small-scale turbulence: encounter based processes

Encounter based processes: aggregation and predator-prey interaction



ϵ levels: 0.05-1 cm²s⁻³

Havskum et al. 2005

Prey: *Ceratium tripos* - horns

Predator:
Fragilidium subglobosum
spherical



Turbulence

Sedimentation
Patchiness

Increased feeding rate

Increased encounter rate

Increased feeding rate

Rothschild & Osborn 1988
Marrasé et al. 1990

2.- Direct effect of small-scale turbulence: encounter based processes

Encounter based processes: parasite - host interactions (Llaveria et al. 2010)

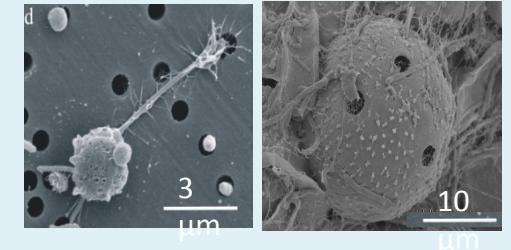
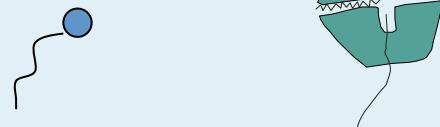
Lower infectivity under turbulence:

1) Direct effect at cellular level: entrance to the cell

STILL



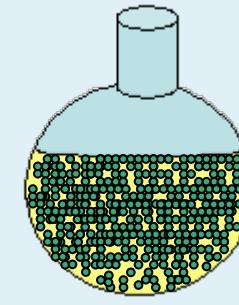
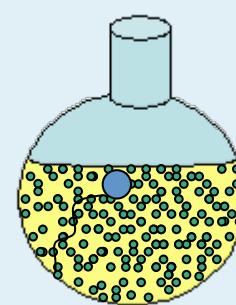
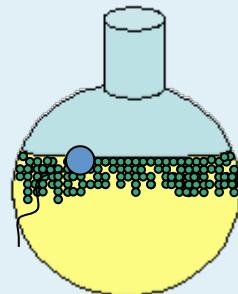
TURBULENCE



Parviluciferans sinerae

Alexandrium minutum,
Scrippsiella trochoidea

2) Indirect effect at population level: patchiness distribution



3) Superimposed: physiological state of the community

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3.- Studies in Alfacs Bay: plankton system



Ebre Delta



Mussel rafts



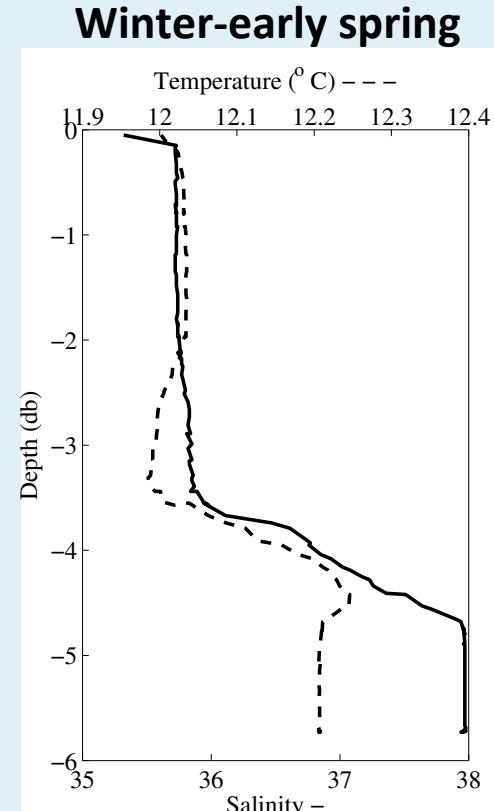
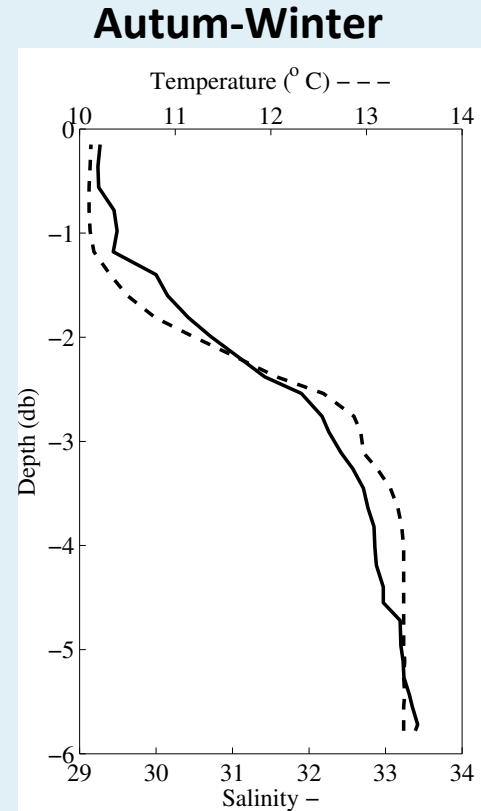
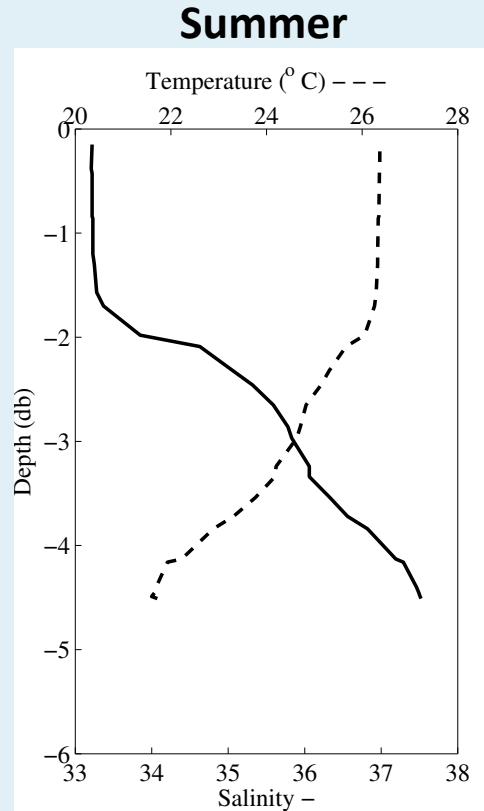
12 km x 6 km x 6m

3.- Alfacs bay: seasonal scale

ESTUARINE CIRCULATION

- Temperature and salinity-driven **stratification**
- **Freshwater inputs** from irrigation channels create **buoyancy**
- **Small tides** (0.2 m), not relevant for mixing
- “Typical” **positive stratified estuary**:

Flow seaward at surface & landward in the deeper layer

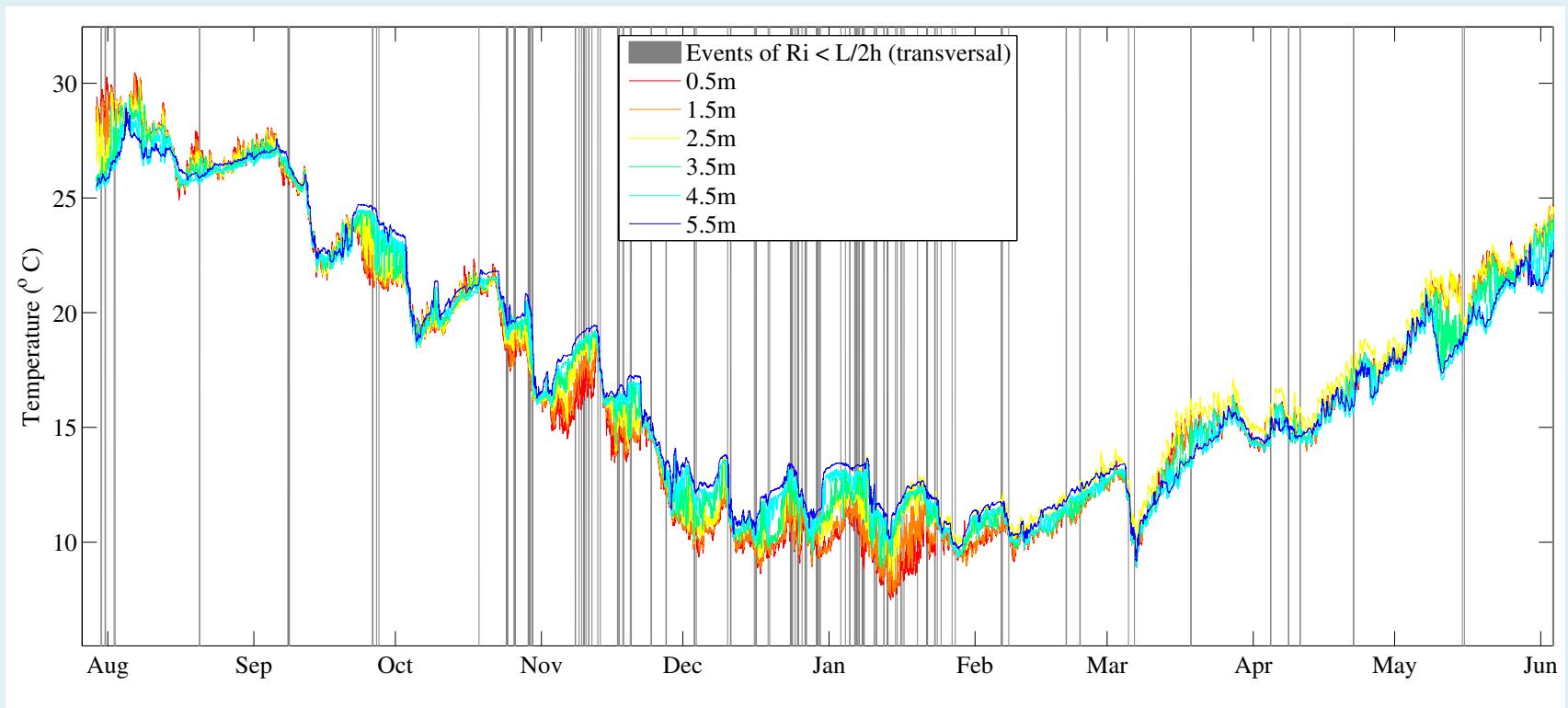


3.- Alfacas bay: seasonal scale

ESTUARINE CIRCULATION

The stratification is broken sometimes over the year by wind forcing

1-year series of Temperature data every 0.5 m (max depth 6m)
Grey bars: events of strong winds that induce high shear regime

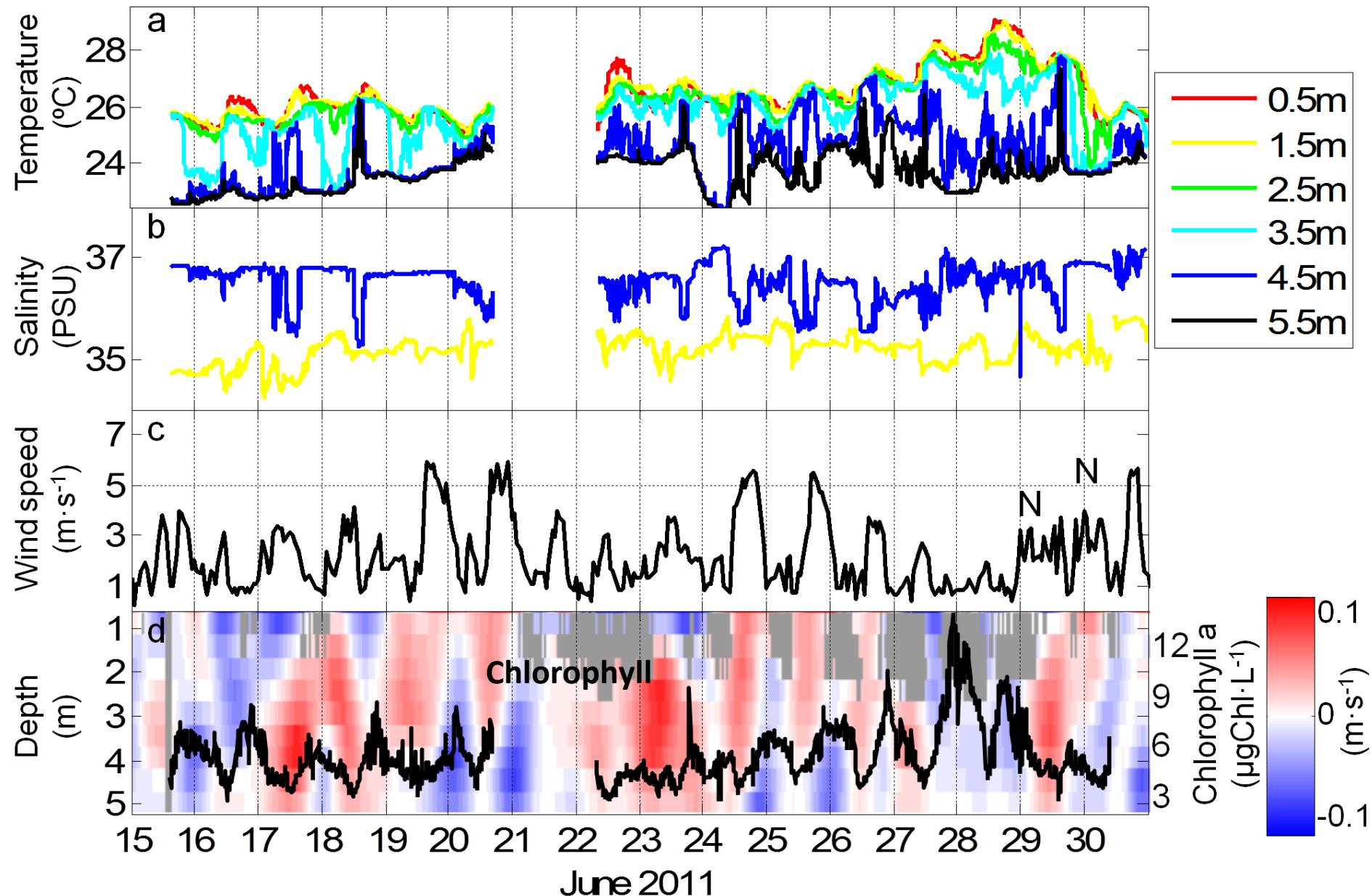


Llebot et al. 2010

3.- Alfacs bay: short time scale

ESTUARINE CIRCULATION

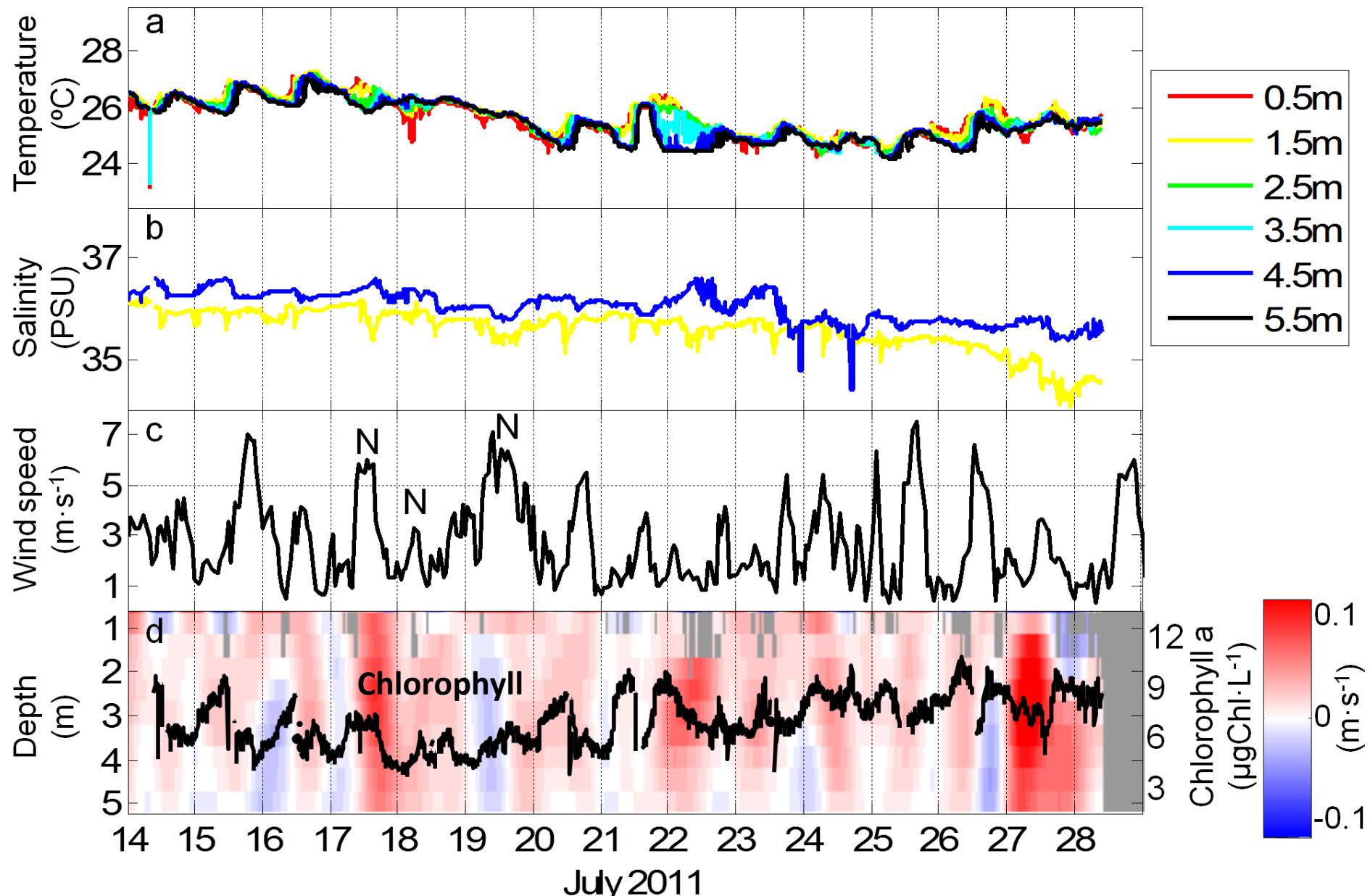
Berdalet, unpublished



3.- Alfacs bay: short time scale

ESTUARINE CIRCULATION

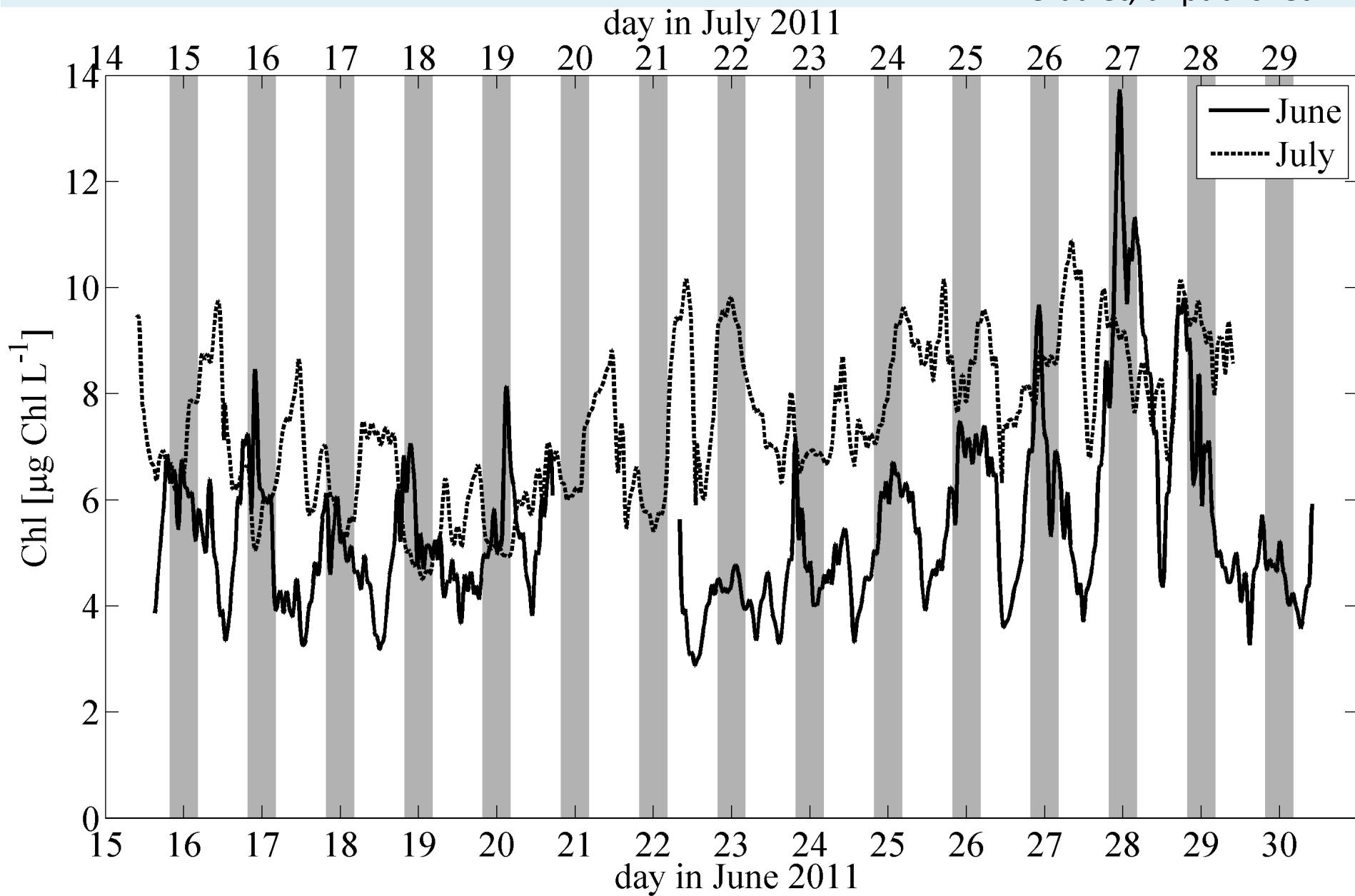
Berdalet, unpublished



3.- Alfacas bay: short time scale

ESTUARINE CIRCULATION

Berdalet, unpublished



3.- Phytoplankton variability: short time scale

Mathematical equation for modelling population dynamics

$$\frac{\partial n}{\partial t} = \mu n - mn - \nabla(n\bar{v}) - \nabla(n\bar{u})$$

$\partial n / \partial t$: time rate of change of n (cells·l⁻¹)

μn : growth by cell division (μ), combination of:

- * intrinsic genetic factors modulated by
- * environmental factors: light history, turbulence, temperature, salinity, ...

mn : direct loss (m) of organisms through mortality (grazing, virus, parasites, mechanical damage, ...)

$\nabla(n\bar{v})$: 3D, transport of cells by water flow

$\nabla(n\bar{u})$: motion of organisms relative to water (sinking, swimming, rising by buoyancy, slippage)

3.- Phytoplankton variability: short time scale

Mathematical equation for modelling population dynamics

Cell division



$$\frac{\partial n}{\partial t} = \mu n - mn - \nabla(n\bar{v}) - \nabla(n\bar{u})$$

Mortality:
- grazing
- parasites
- virus



Fig. 1.8. Tintínido (*Favella* sp.) con células de *Dinophysis acuminata* (fondo) y *D. acuta* (parte superior) en el interior de su lorica.

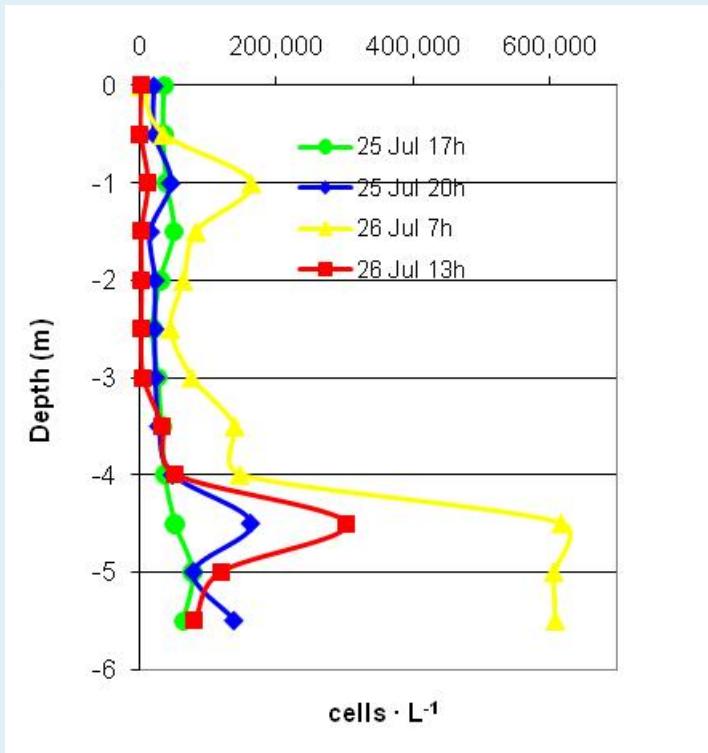
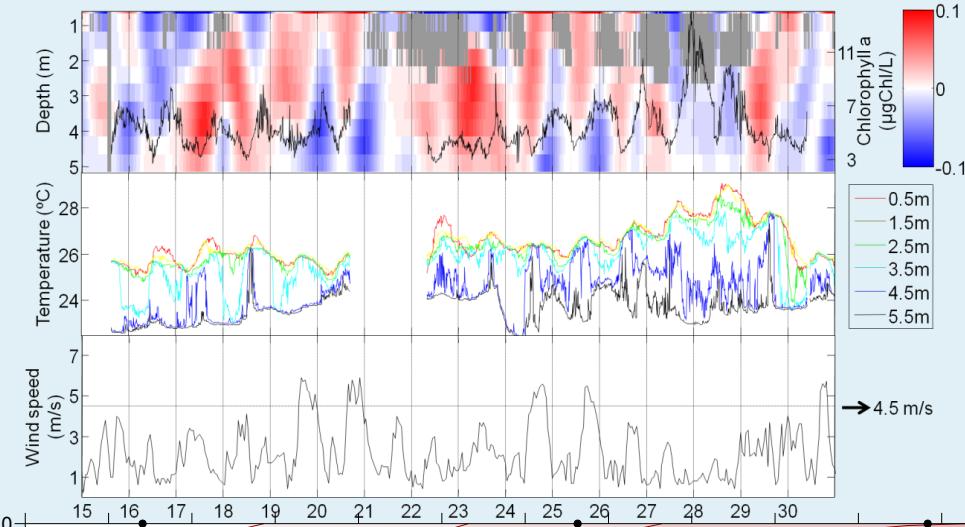
Favella sp. a tintinnid
predator of *Dinophysis*
(Reguera, 2002)

3.- Phytoplankton variability: short time scale

Mathematical equation for modelling population dynamics

$$\frac{\partial n}{\partial t} = \mu n - mn - \nabla(n\bar{v}) - \nabla(n\bar{u}) \longrightarrow$$

↓ Transport (in / out)

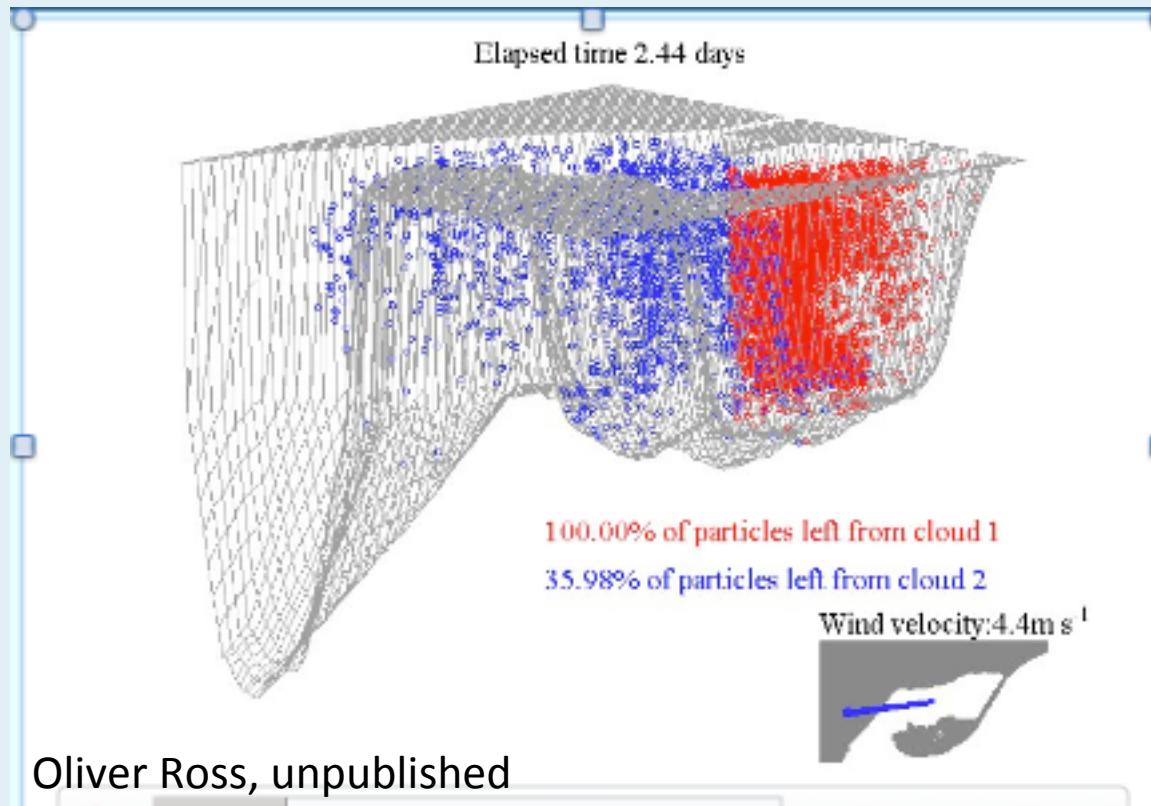


Vertical migration

Berdalet, unpublished

Role of circulation patterns

Lagrangian model – Example of particle tracks for July 2007



We observe the inflow at the bottom of Mediterranean water and the outflow occurring at the surface

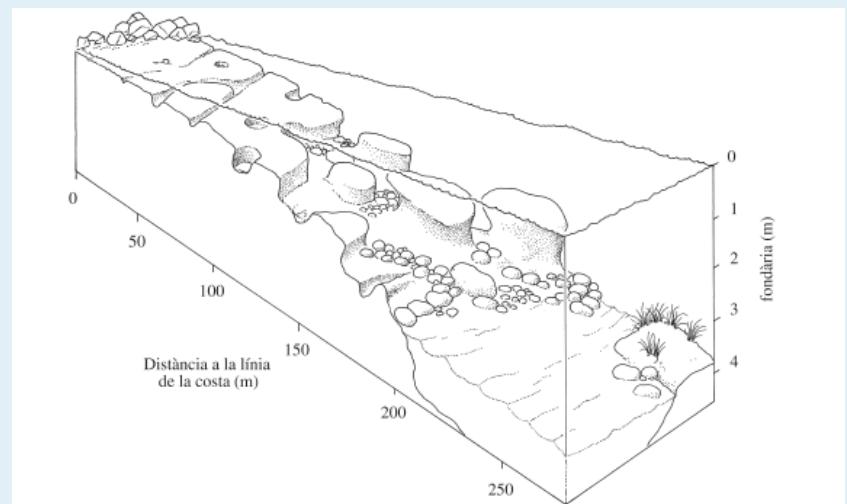
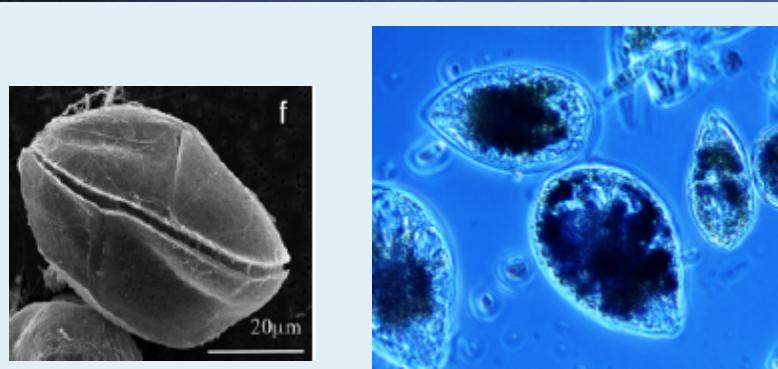
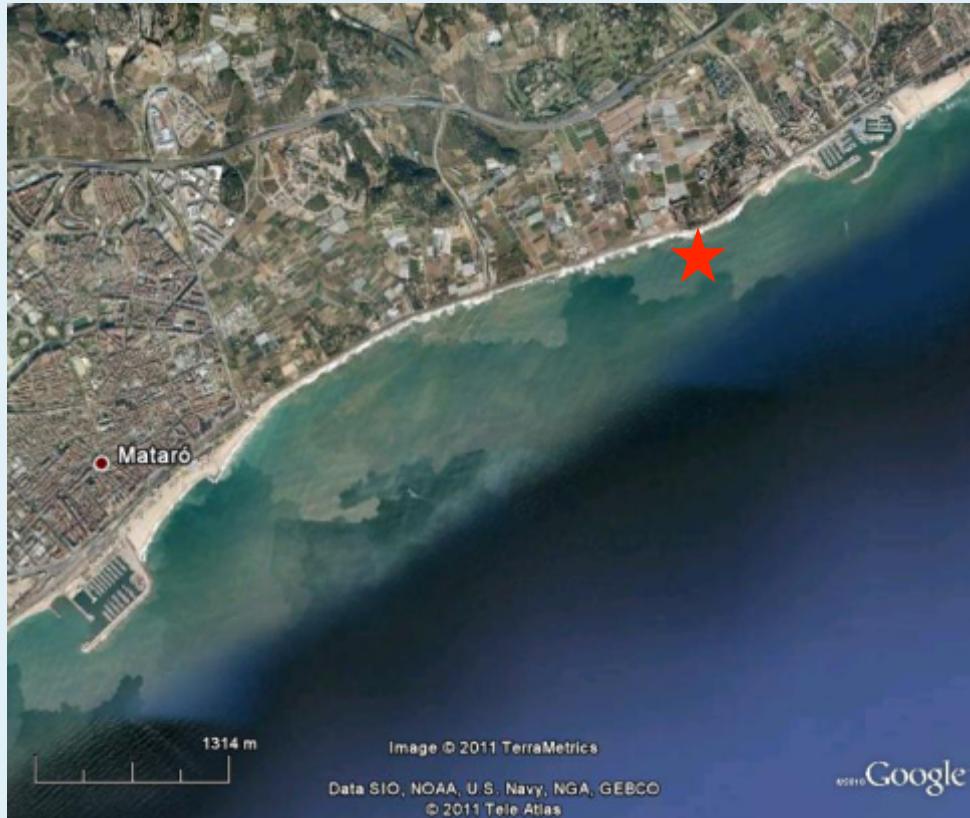
The model shows that the retention is higher during high wind induced mixing events, which break down the estuarine circulation.

Biological rhythmicity in phytoplankton and interactions with turbulence: from the cell to the ecosystem scale

- 1.- Scales of turbulence. The Margalef's Mandala
- 2.- Direct effect of small-scale turbulence on dinoflagellates.
From the lab to the field
- 3.- Studies in Alfacas Bay: plankton system
- 4.- Blooms of the benthic toxic dinoflagellate *Ostreopsis*



4.- Blooms of the benthic toxic dinoflagellate *Ostreopsis*



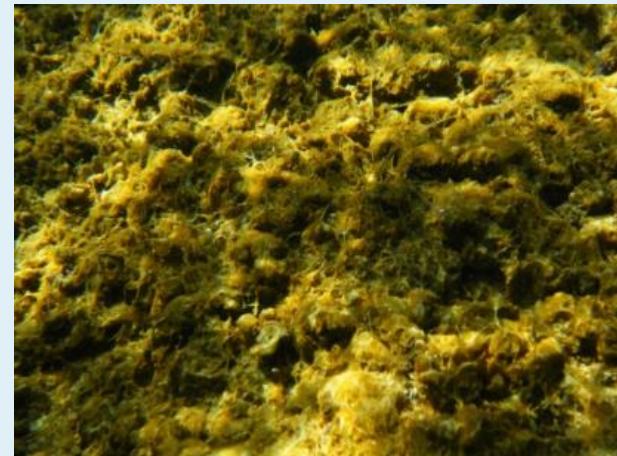
4.- Blooms of the benthic toxic dinoflagellate *Ostreopsis*

Llavaneres: Natural microalgae community (light microscopy)

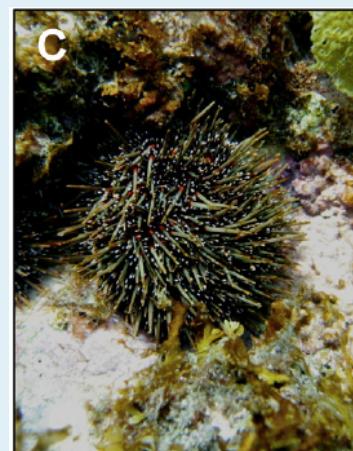
BEFORE the BLOOM



BLOOM



Photos: Vila and Berdalet (ICM-CSIC)



Shears & Ross, 2009

4.- Problem overview

- ✓ ***Ostreopsis* benthic HABs:**
 - It produces palytoxin (PLTX) and analogues (ostreocin, ovatoxin, ...) (e.g. Ciminiello et al. 2008; Taniyama et al. 2003)
 - In tropical latitudes: associated to serious seafood intoxications clupeotoxisms (Randall 2005)
 - Detection of huge concentrations and blooms in temperate latitudes (Rhodes 2011): risk of PLTX intoxication (detected in certain macrofauna, not well characterized yet; Amzil et al. 2012; Biré et al. 2013), lag of legislation on BHAB related toxins (EFSA, 2009 warning)
- ✓ ***Ostreopsis* blooms in the Mediterranean Sea:**
 - associated to respiratory and cutaneous irritations (Brescianini et al. 2006; Barroso et al. 2008, etc.)
 - macrofauna mortalities (Sansoni et al. 2003; Shears & Ross 2009- New Zealand)



Self-produced mucilage, dense, sticky, attachment to surfaces (macroalgae, sea urchins, rocks, sand, ...).

Photos: Vila and Berdalet (ICM-CSIC)

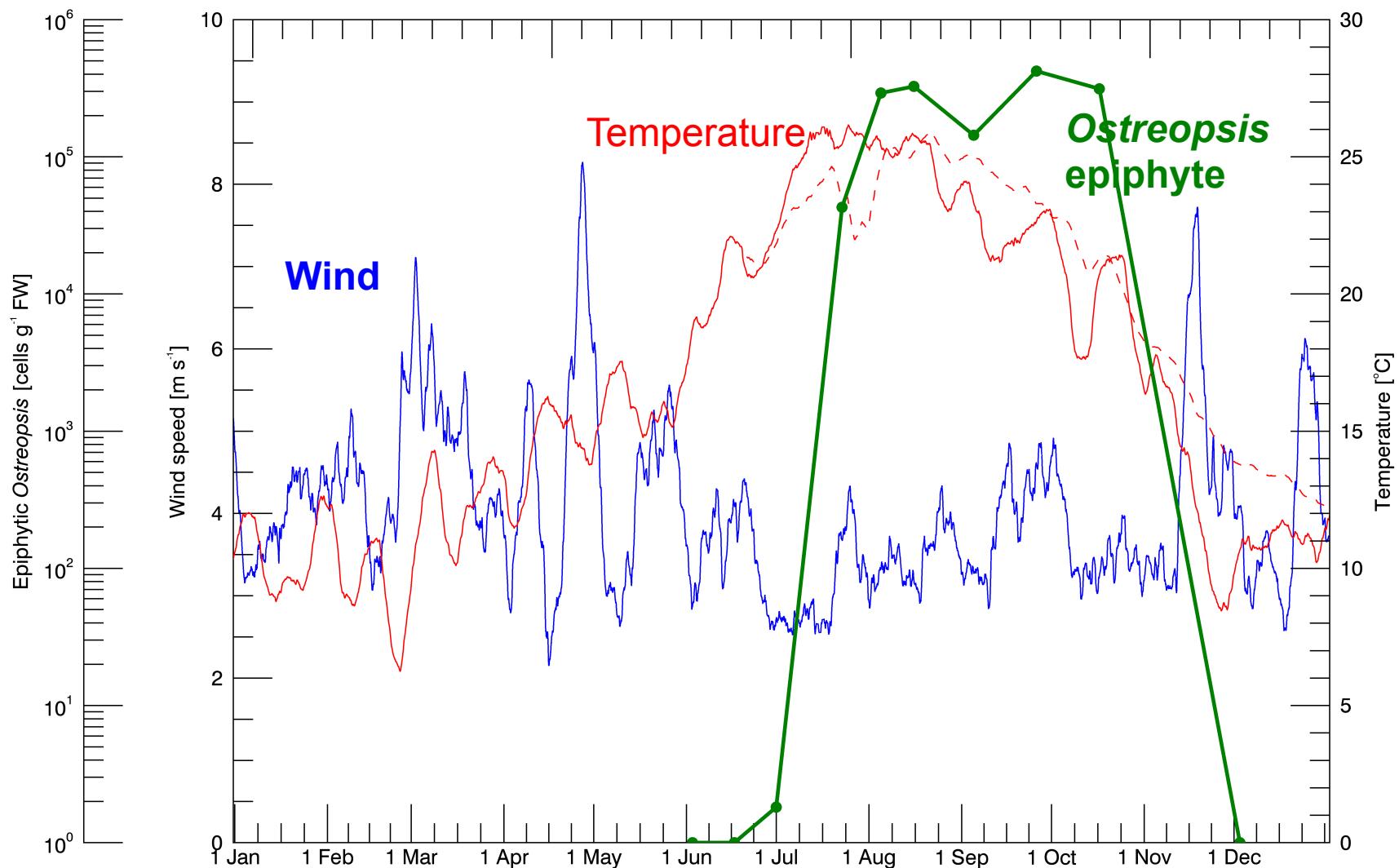
4.- The problem: in the Mediterranean, respiratory irritation symptoms in people exposed to aerosols coinciding with high cell numbers

Year	Location	Human cases	Ecosystem impacts	Ostreopsis Cells/L (max)	Reference
1998	Spain (Llavaneres)	?	Sea urchins, mussels	$20 \cdot 10^4 / L$	Vila et al. 2008
1998, 2000, 2001	Italy (Tirren.)	~100	Yes		Sansoni et al. 2003
2001, 2003, 2004	Italy (S Adria.)	28	-		Gallitelli et al. 2005
2004	Spain (Llavaneres)	74 (~200)	No	$2,3 \cdot 10^4 / L$	Vila et al. 2008; Álvarez et al. 2005
2005, 2006	Italy (Genoa)	228, 19	-		Brescianini et al. 2006; Durando et al. 2007
2006	Spain (Llav.)	37	No	? – $0,2 \cdot 10^4 / L$ (2 days after)	Álvarez 2006
2006-2009	France	47	-	$> 3 \cdot 10^4 / L$	Tichadou et al. 2010
2006	Spain (Almería)	57	-	$0,12 \cdot 10^4 / L$	Barroso et al. 2008
2009	Algeria	150-200	Yes	$8 \cdot 10^4 / L$	Illoul et al. 2012
2013	Spain (Llav.)	13	No	$5 \cdot 10^4 / L$	Abós-Herràndiz et al. 2014
2014	Spain (Llav.)	7	No	$200 \cdot 10^4 / L$	Berdalet, Vila, Abós-Herràndiz

The bloom in Pins Mar, 2013

Annual bloom, peak in summer.

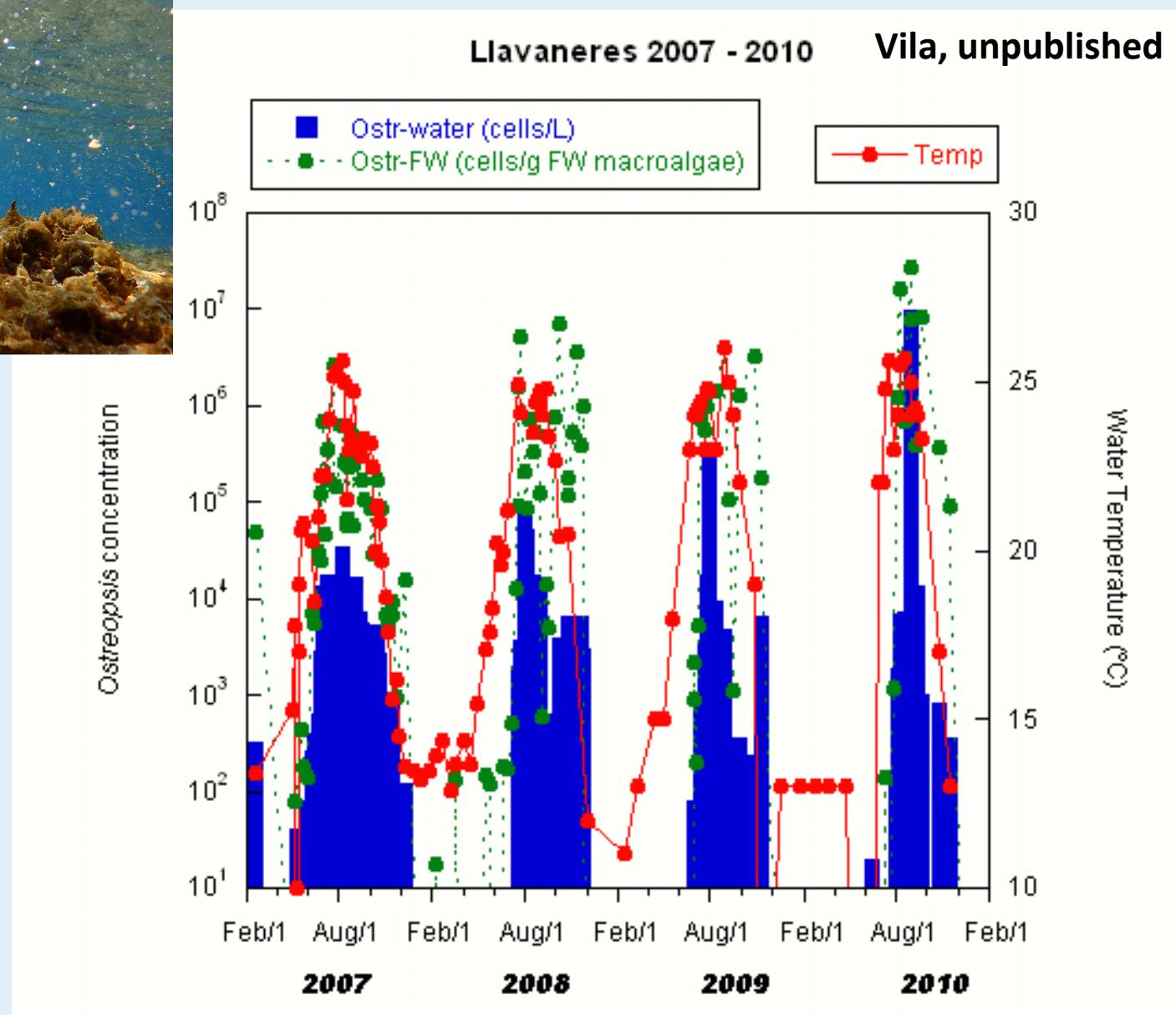
Data of the three environmental parameters: low-pass filtered using a cut off period of 7 days (running mean).



4.- The problem: Recurrence of the proliferation in Llavaneres Beach (40 km, N BCN)

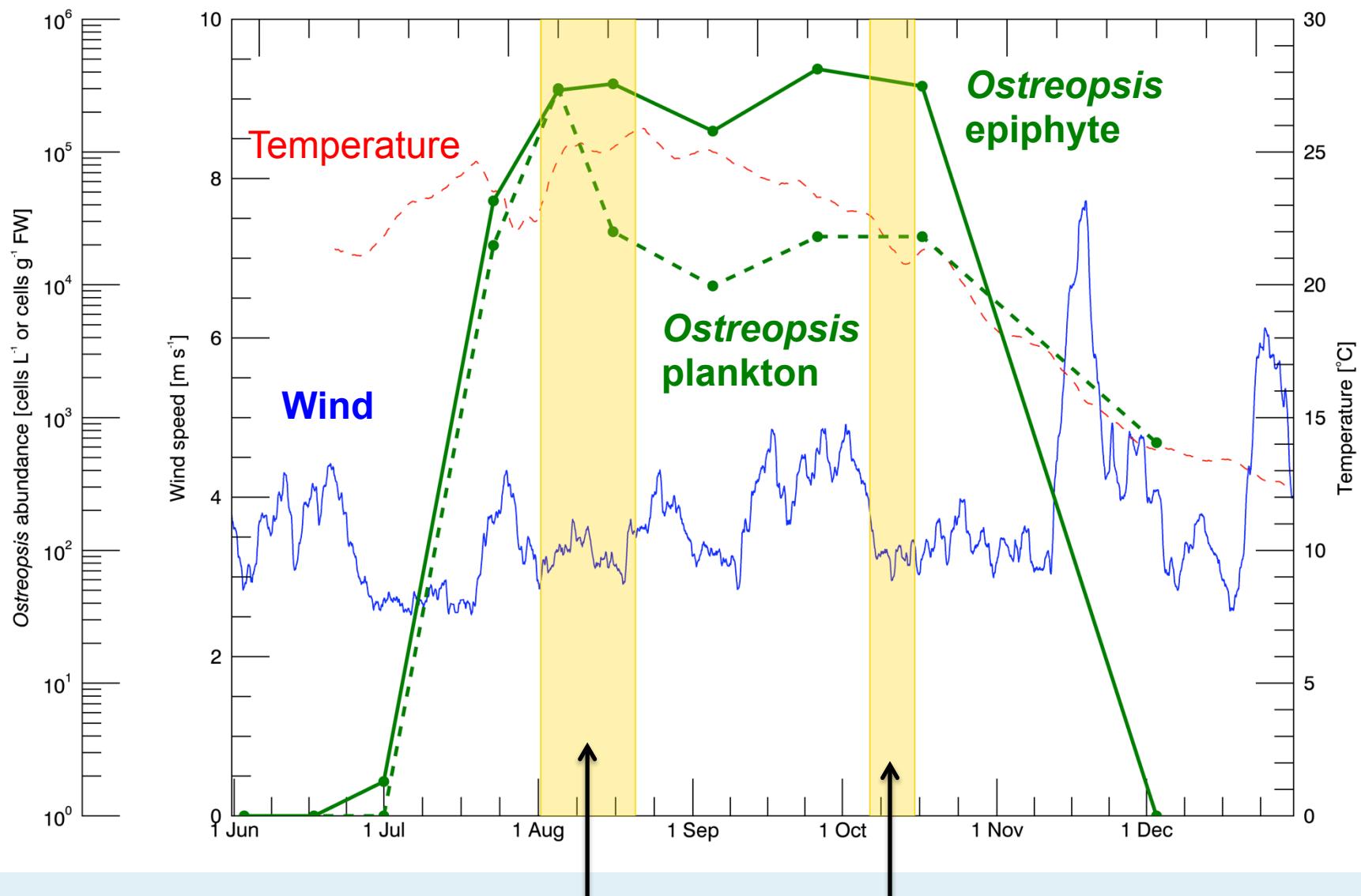


Photos: Vila and Berdalet (ICM-CSIC)



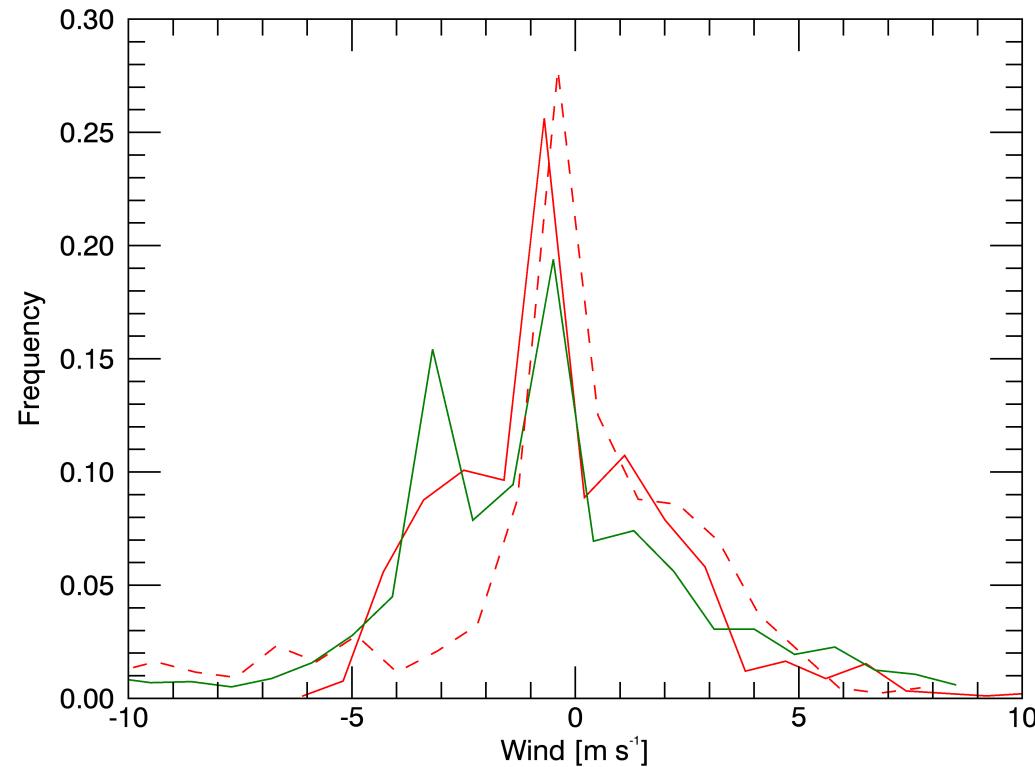
Epidemiology study – Pins Mar June a Descember 2013

Vila, Abós-Herràndiz, Isern-Fontanet, Àlvarez, Berdalet. 2016, *Scientia Marina*



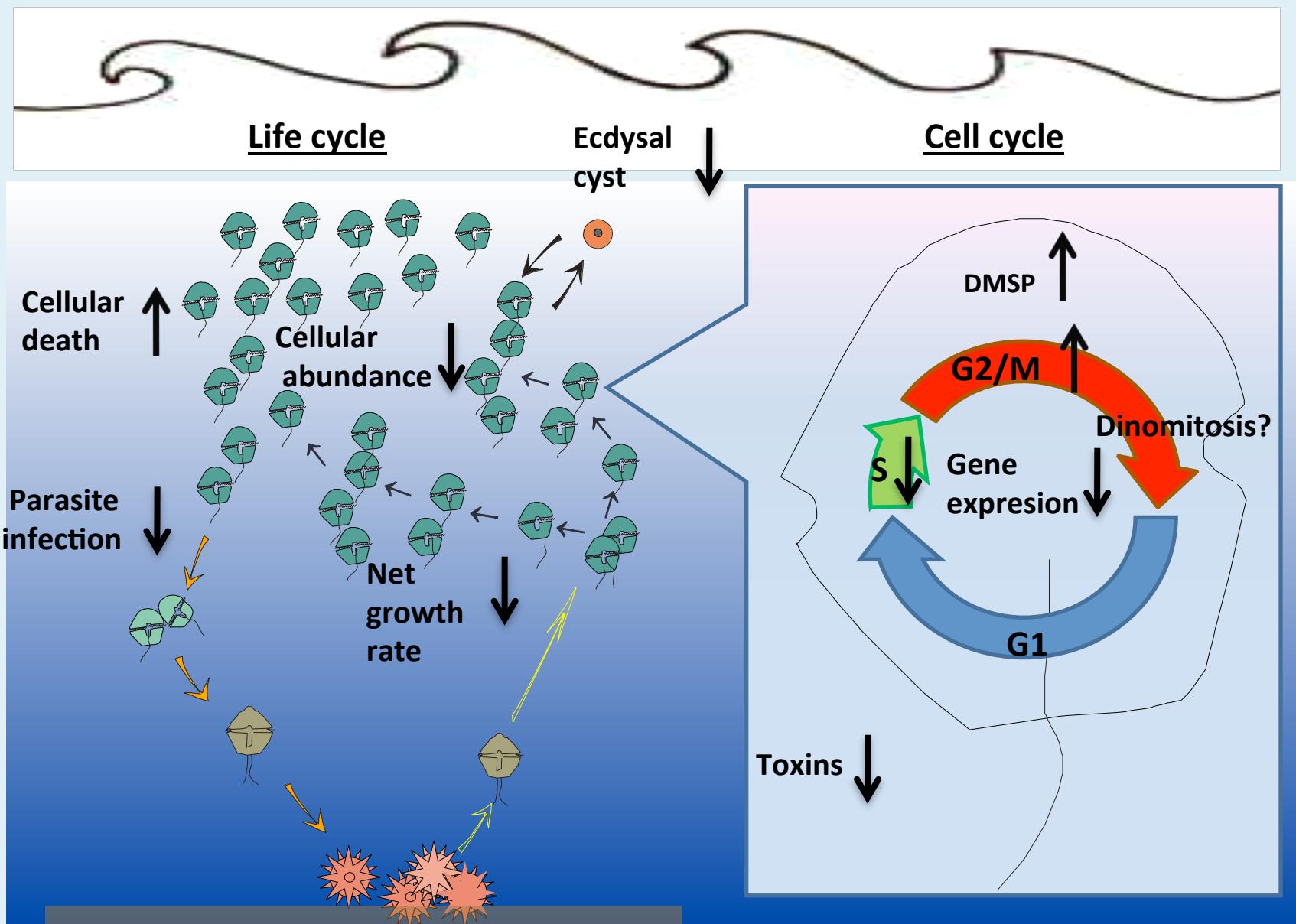
Period of respiratory affection and general malaise

Wind is necessary for aerosolization and dispersion, but not clear trend in the affection period



- Histograms of the wind component perpendicular to the coast corresponding to:
 - * the first affection period (**solid red line**),
 - * the intermediate period (**green line**)
 - * the second affection period (**dashed red line**).
- Negative values indicate landward winds and positive values correspond to seaward ones.

Ongoing and future steps include the study of the physiological rhythmicity and the modulation by small scale turbulence and in situ, as we conducted on plankton species



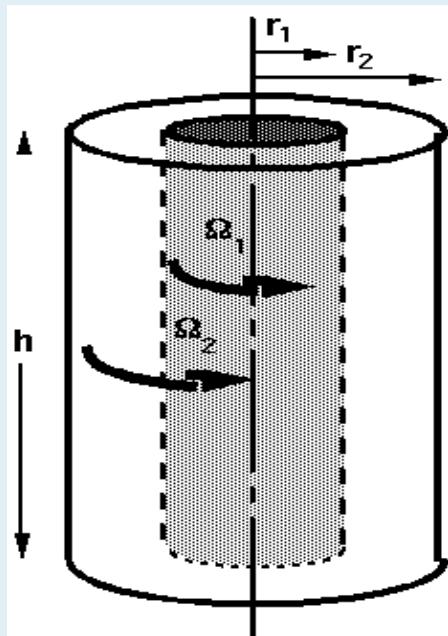
Gisela Llaveria (PhD thesis, 2009)

Moltes gràcies!!!
Thanks for your attention!
¡Muchas gracias!

Direct effect of small-scale turbulence: laboratory studies

Experimental setups to generate turbulence

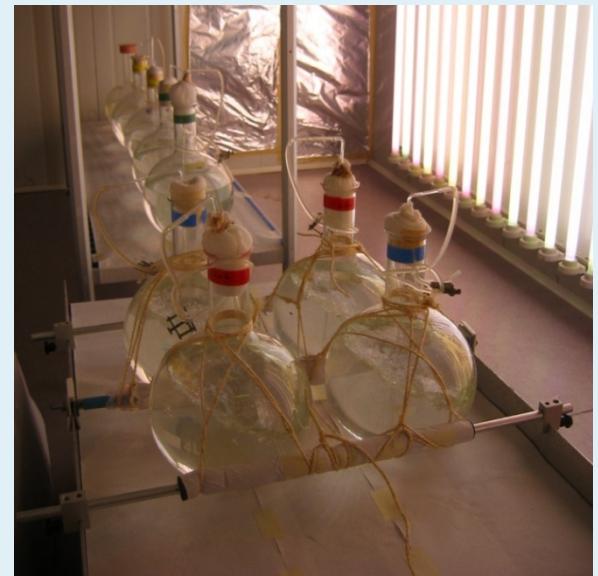
Couette cylinders



Vertically oscillating grids



Orbital shaker



- +: very low energy dissipation rates (ϵ);
- +: laminar shear $du/dz = C(\epsilon/v)^{1/2}$
- +: physics well characterized
- : swimming cells can escape and avoid turbulence

- +: low (ϵ)
- : swimming cells can escape and avoid turbulence

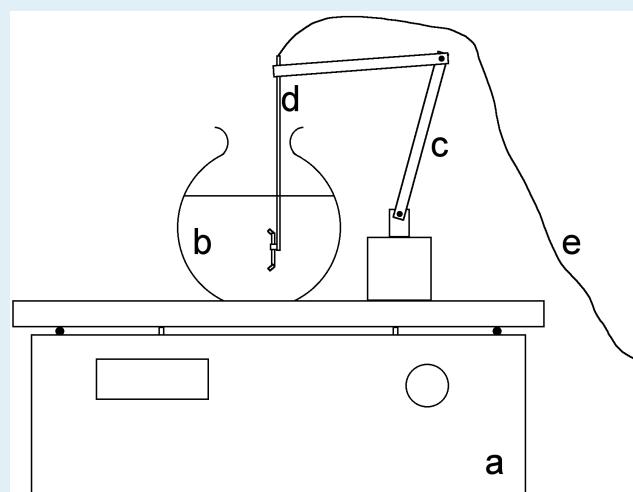
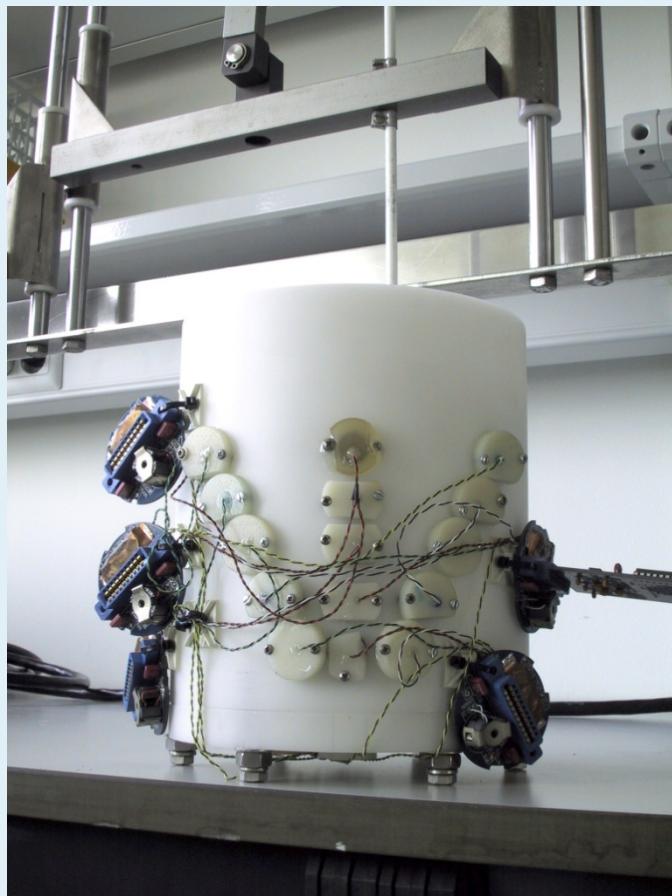
- +: swimming cells cannot escape
- : high (ϵ)

Direct effect of small-scale turbulence: laboratory studies

Efforts to estimate experimentally generated turbulence

Ò. Guadayol, F. Peters, J.E. Stiansen, C. Marrasé, A. Lohrmann. 2009.

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. LOM 7: 287-303-



Miniaturized ADV