What we know and what we do not know about *Dinophysis*

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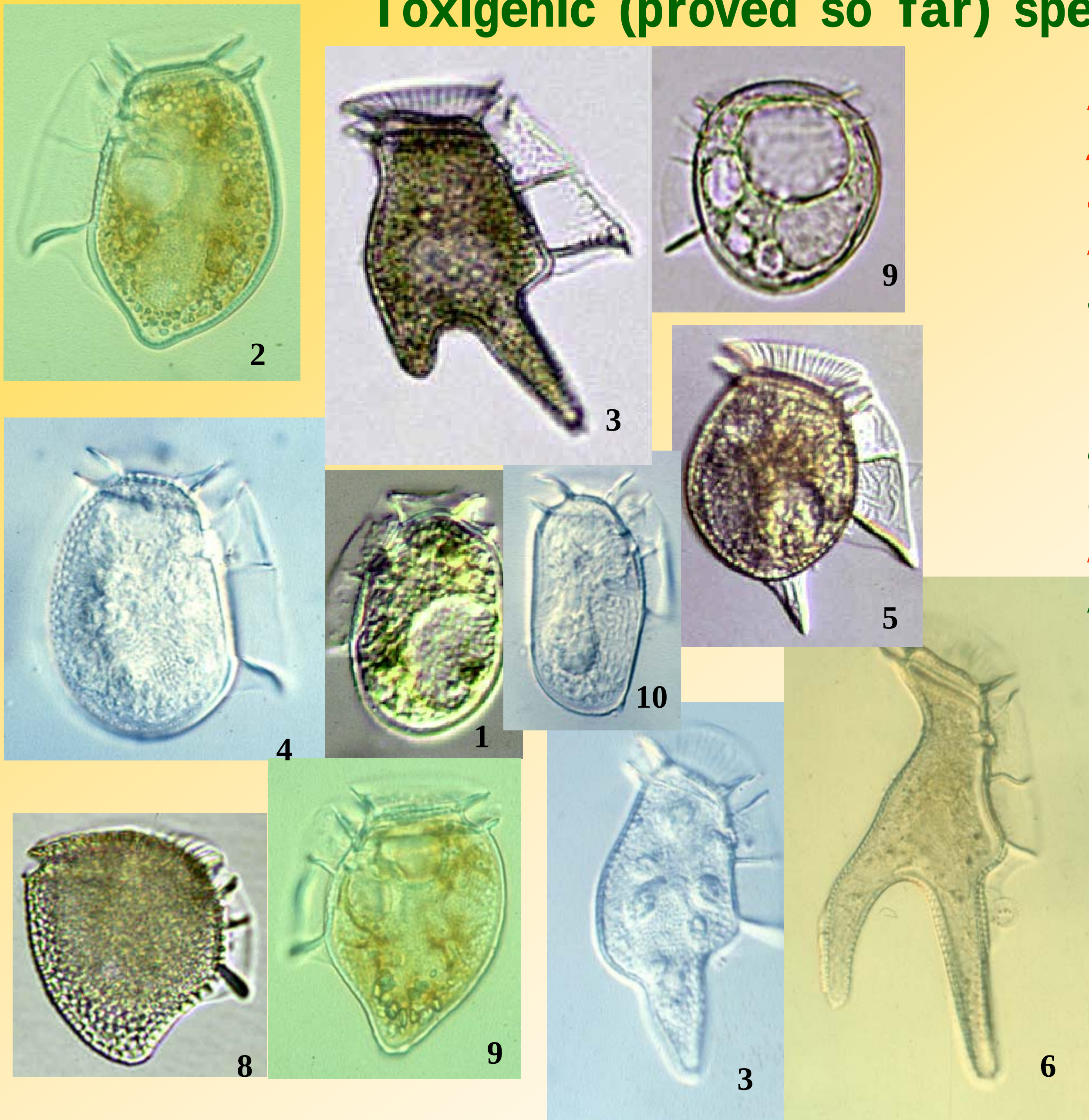
Toxigenic (proved so far) species of Dinophysis

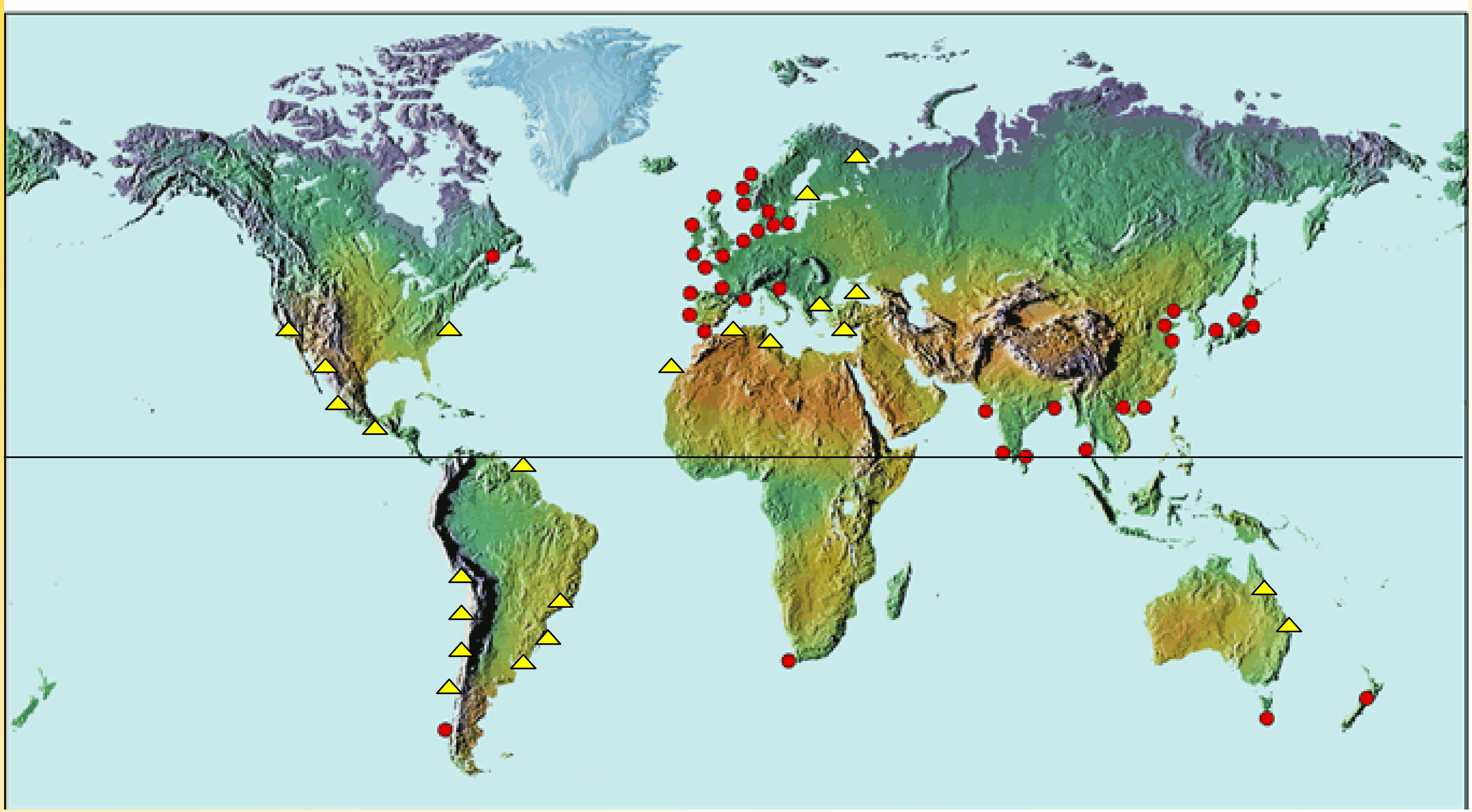


1. *D. acuminata*
2. *D. acuta*
3. *D. caudata*
4. *D. fortii*
5. *D. hastata*
6. *D. miles*
7. *D. norvegica*
8. *D. rapa*
9. *D. rotundata*
10. *D. sacculus*
11. *D. tripos*

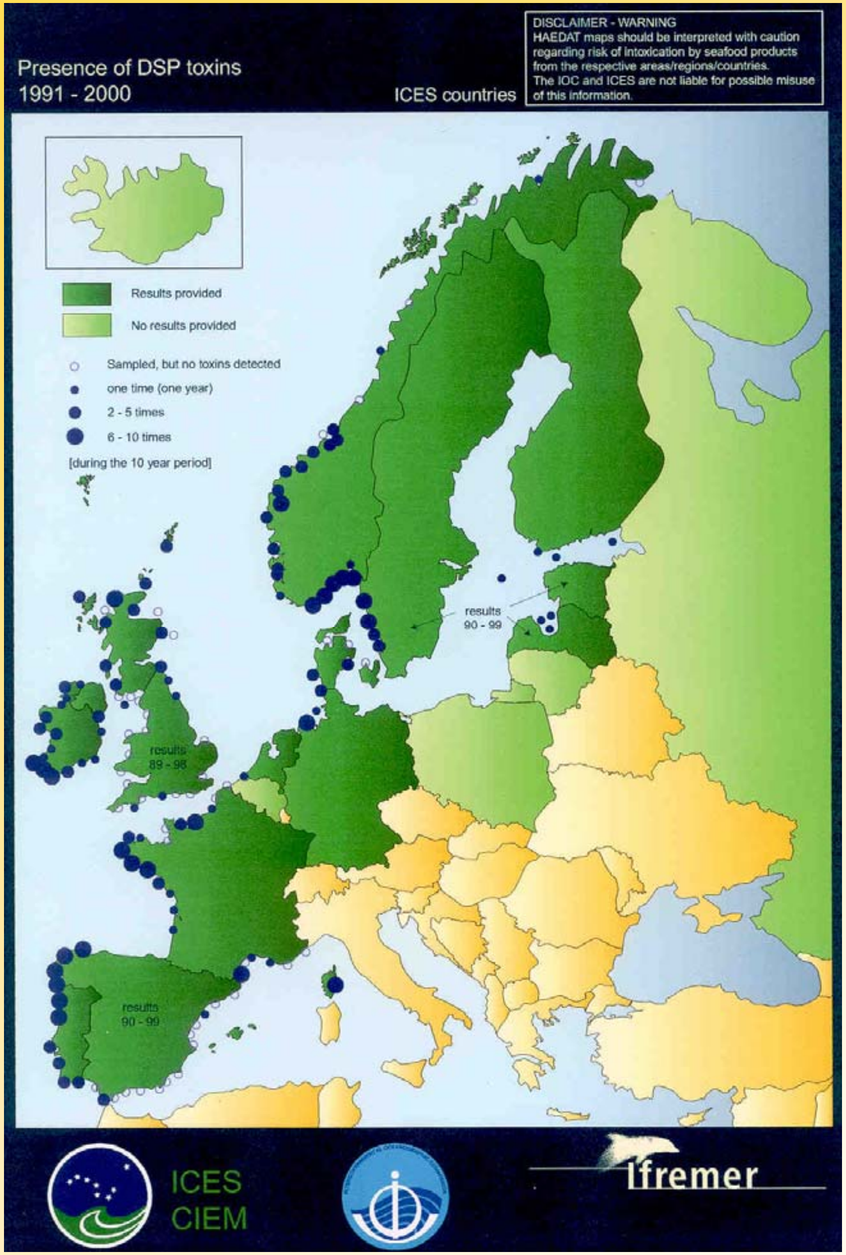
More than 200 species (Sournia 1986) that are usually very scarce (< 100 cel/L)

(Not to scale; photographs by Y. Fukuyo and J. Larsen, IOC.

Temperate-cold seas: *D. acuminata, D. acuta, D. norvegica, D. rotundata*
Temperate-warm seas: *D. acuminata/D. sacculus, D. acuta, D. fortii, D. caudata*
Tropical seas: *D. caudata, D. miles*



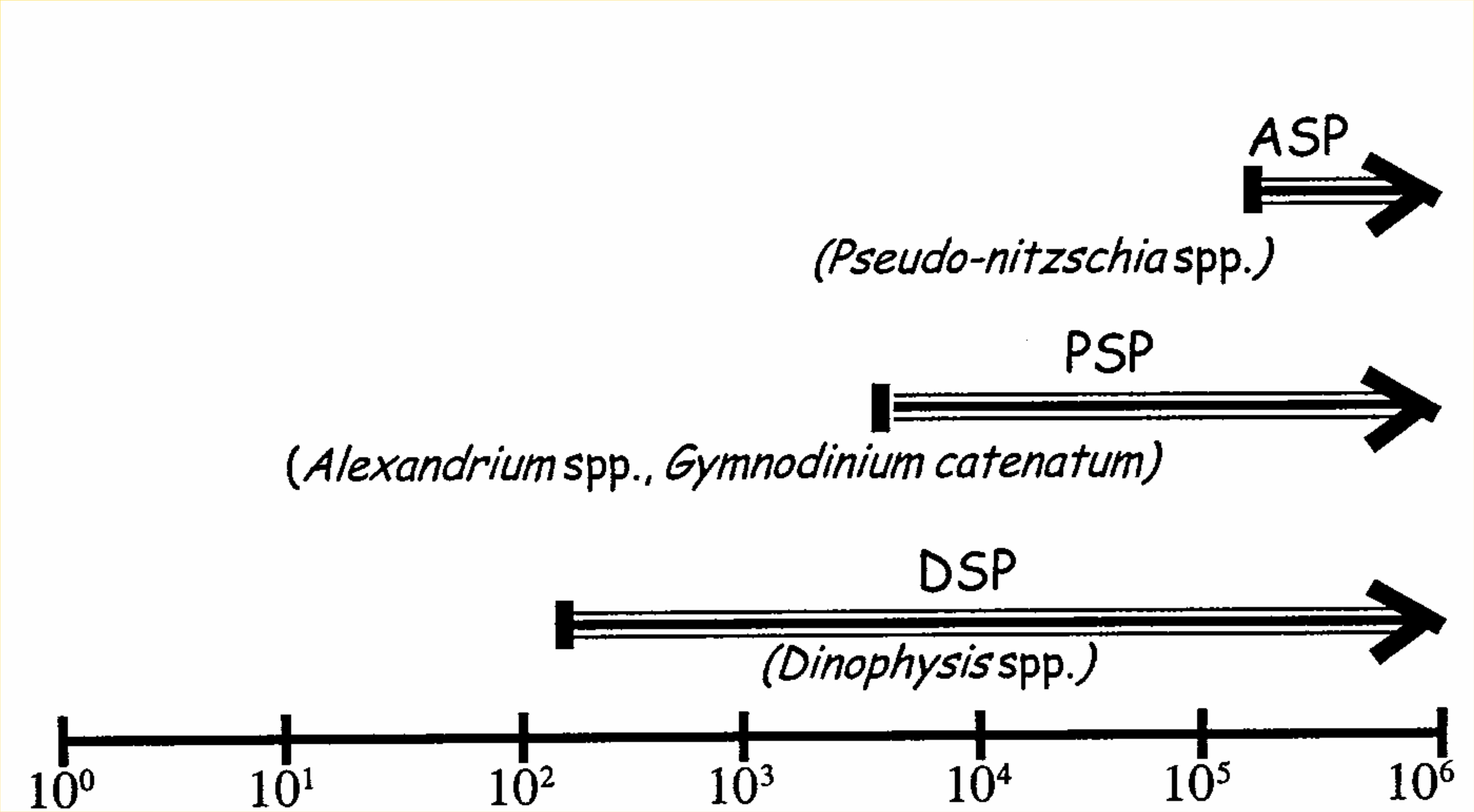
Reported distribution of DSP toxins detected in shellfish



ICES-IOC Decadal Map on DSP Events
1991-2000

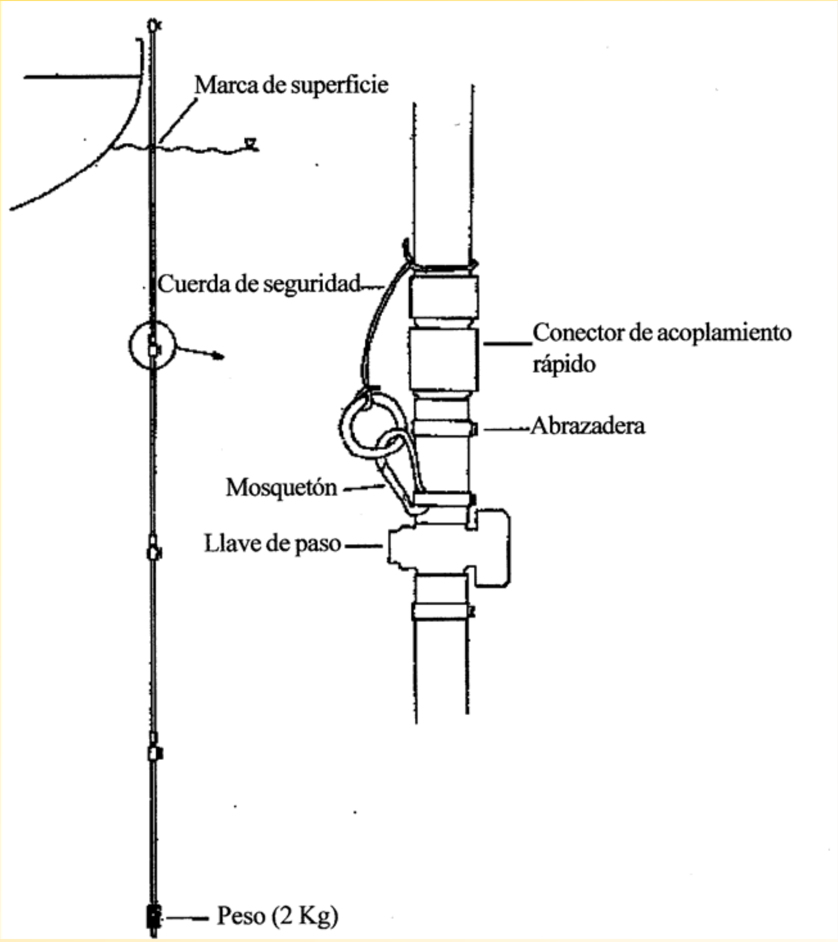
Proliferations of *Dinophysis* spp populations that produce lipophilic toxins (OA, DTXs, PTXs) probably represent, within the different HAB events, the main threat to shellfish exploitations on the Atlantic coasts of Iberia and France, Irish Shelf Seas, Skagerrak-Kattegat, and Norwegian Sea


Ría de Vigo
Galicia, España



Ranges of cellular concentrations (cel · l^{-1}) of HABs in Galician coastal waters within which different toxic outbreaks occur.

Conflicts to sample scarce and patchy populations



1986: ICES Working Group on "*Toxic Phytoplankton and Management of their Effects*"

Recommended method for HAB monitoring (Lindahl 1986)

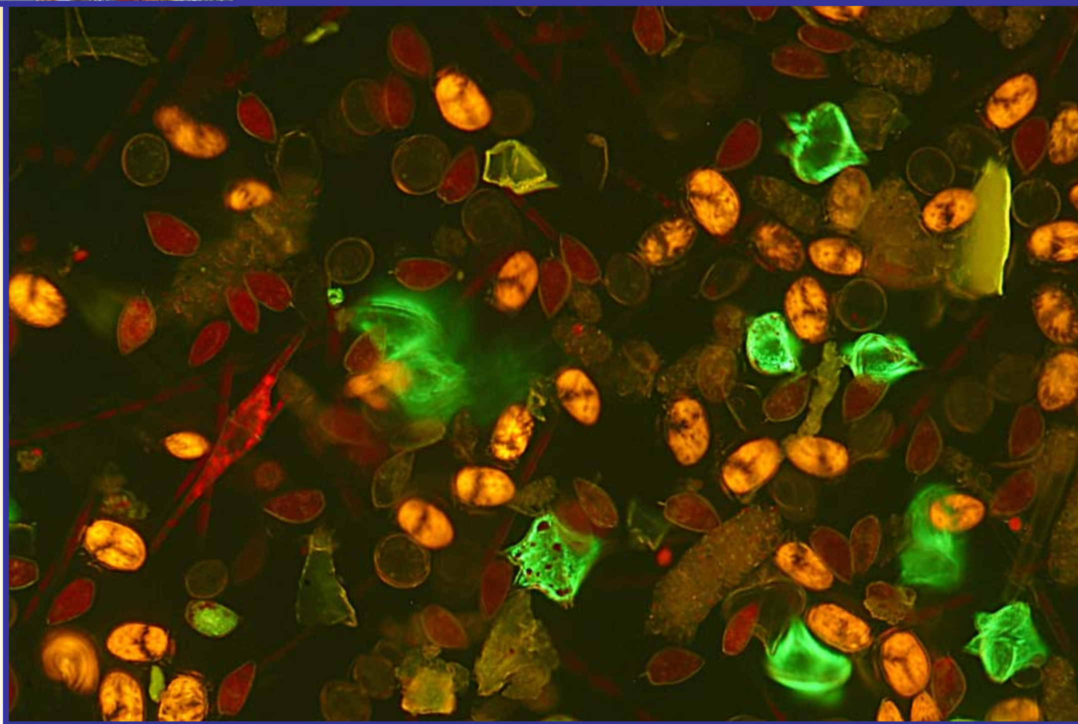
Phytoplankton samples

To obtain a high resolution mapping of *Dinophysis*, even at extremely low concentrations (1-2 cell/ L), we used two sampling procedures with water collected with a peristaltic pump:

- **Whole seawater samples**
- **Size fractionated (20-70 um) quantitative samples (2L reduced to 50 ml)**

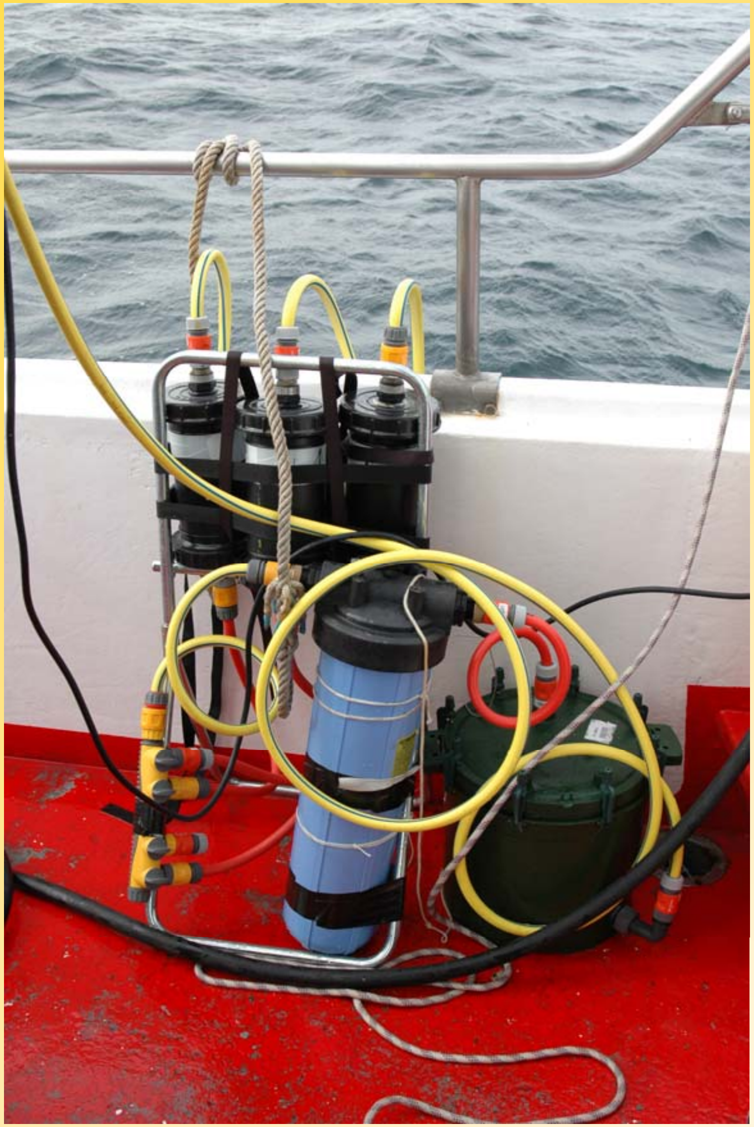
DIC (Nomarski)

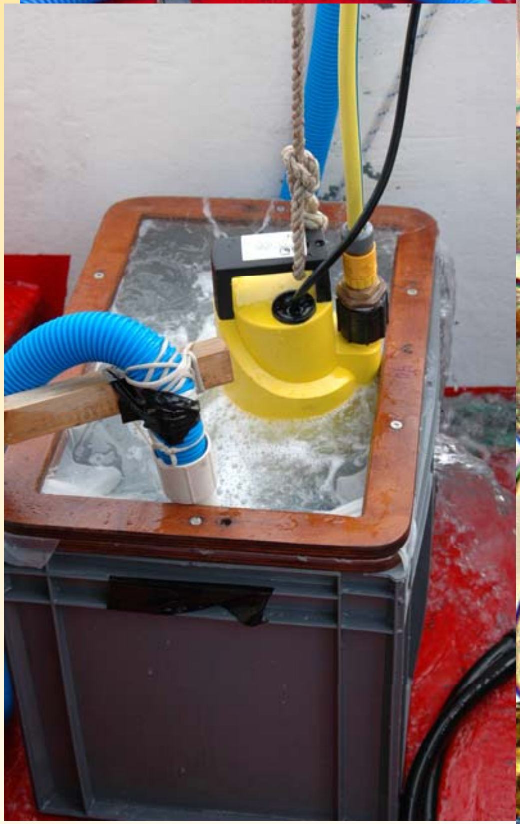
Autofluorescence under blue light epifluorescence



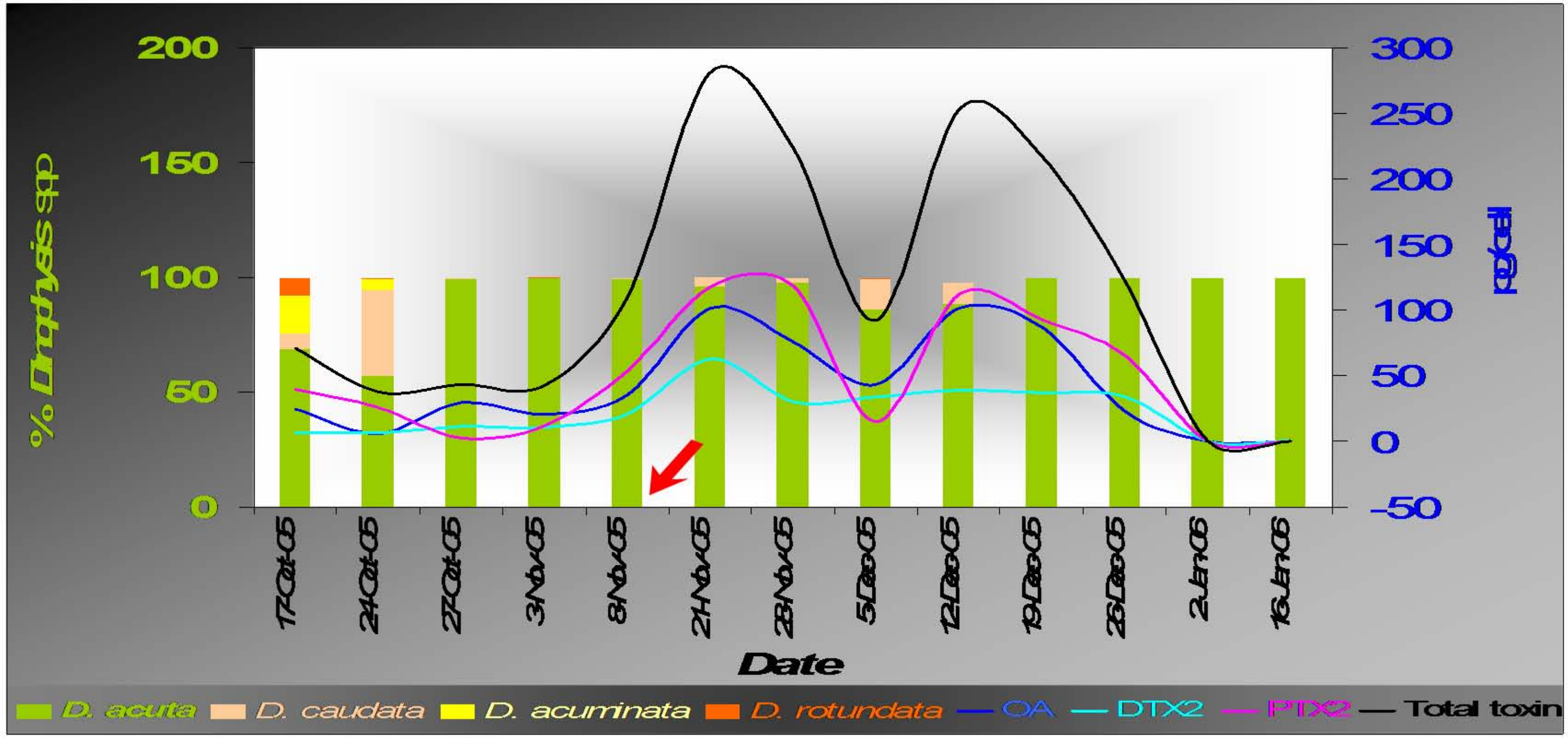
Toxins profile and content

Does *Dinophysis* spp of a given locality have a constant toxin profile??





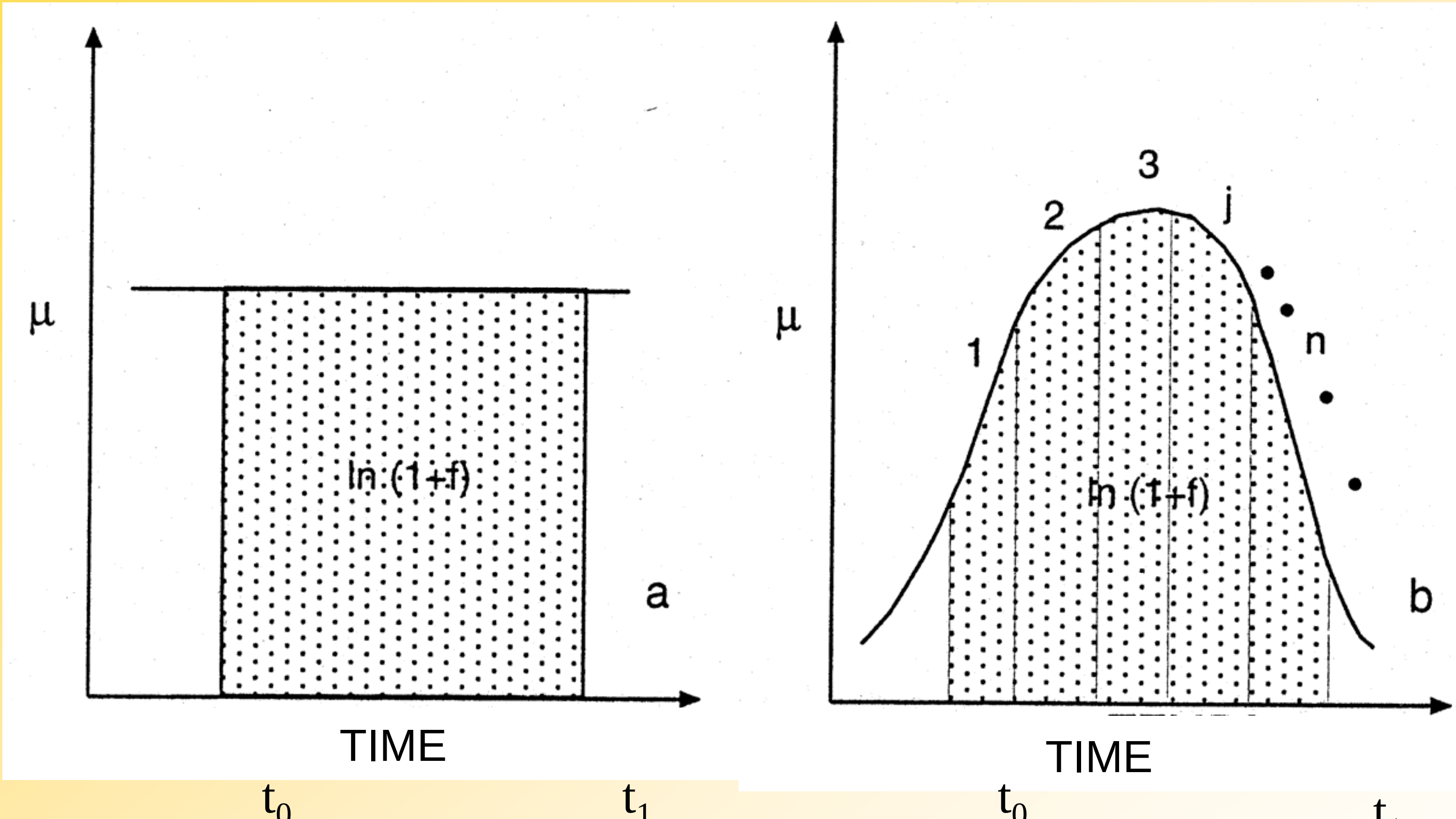
Concentration of D. acuta
Galicia, November 2005



Monitoring of toxin content per cell during a proliferation of *D. acuta*, Ría de Pontevedra, october 2005-January 2006 (Pizarro et al. 2006)

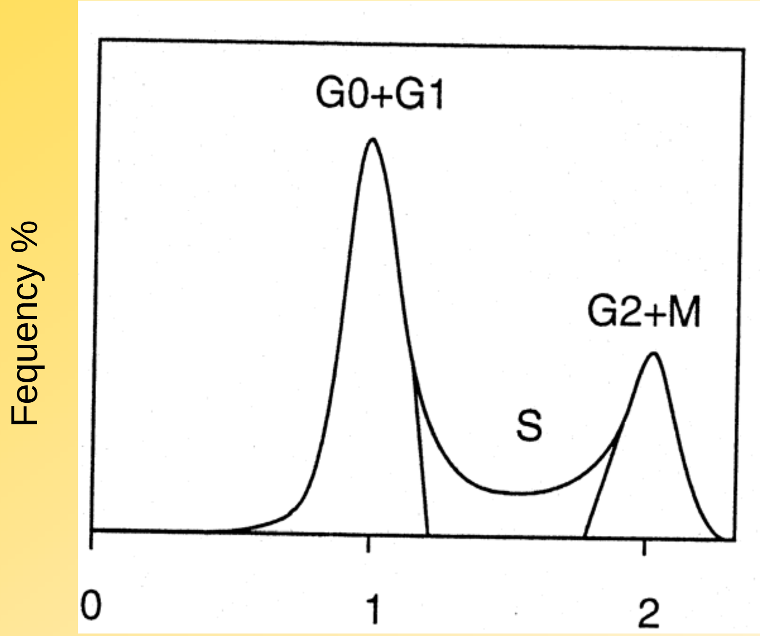
Okadaiates:PTXs ratio is quite variable

Growth Rates



Hypothetic curves of division rate versus time. a) Population with asinchronous division (μ is constant); b) Population dividing in phase. In both cases, the surface under the curves represent the fraction of cells dividing in the time interval (t_1 - t_0).

MITOTIC INDEX APPROACH (McDuff & Chisholm, 1988)



Histogram of frequencies of cells on different stages of the cell cycle (G1, S, G2 and M)

$$(T_c + T_r) = (t_0 - t_1)$$

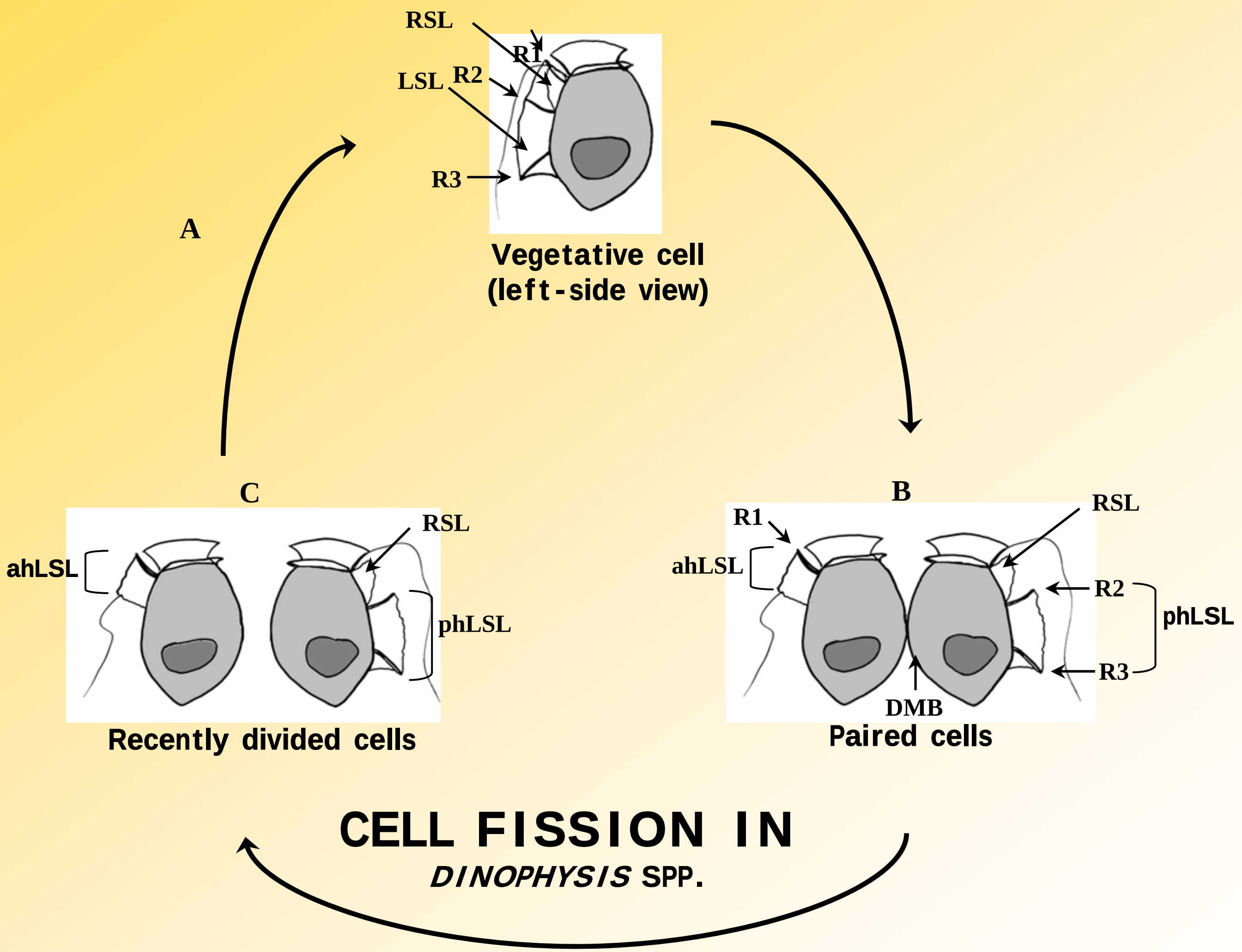
Interval of time necessary for a cohort of cells to pass from one phase to the next;

<u>Or the distance between two maxima</u>

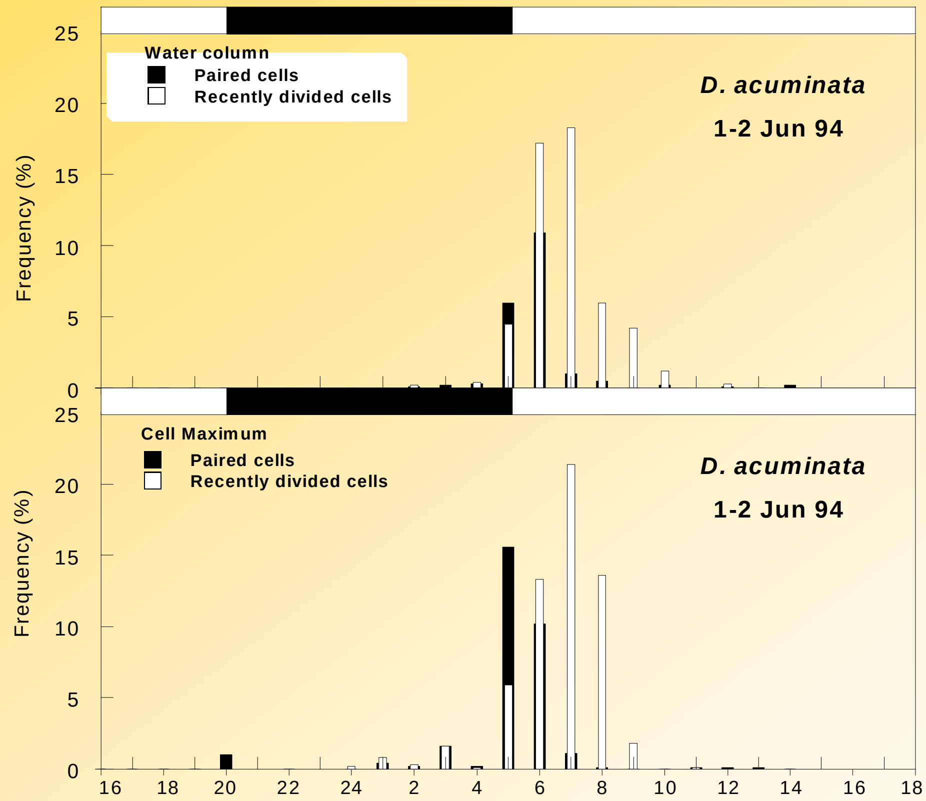
(in our case, the time interval between the time t_0 when the fraction of cells undergoing cytokinesis f_c is maximum, and the time t_1 when the fraction of recently divided cells f_r is maximum)

$$\mu = \frac{1}{n(T_c+T_r)} \sum_{i=1}^{n} (t_s)_i \ln\left[1+ f_c(t_i) + f_r(t_i)\right]$$

(Model of Carpenter & Chang, 1988)



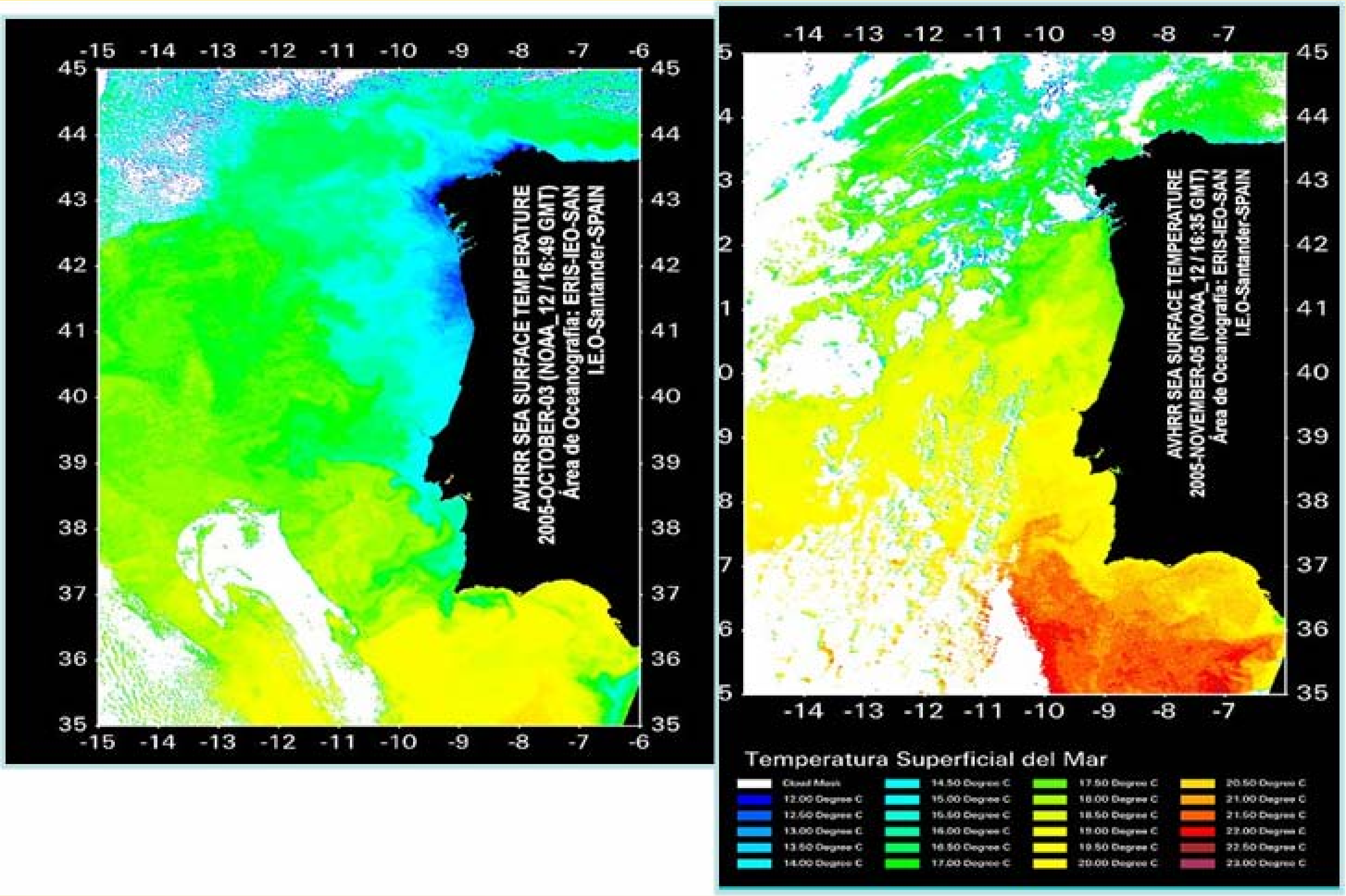
Simplified diagram of morphological changes following cell fission in *Dinophysis* that are used as markers of cytokinesis and recent division



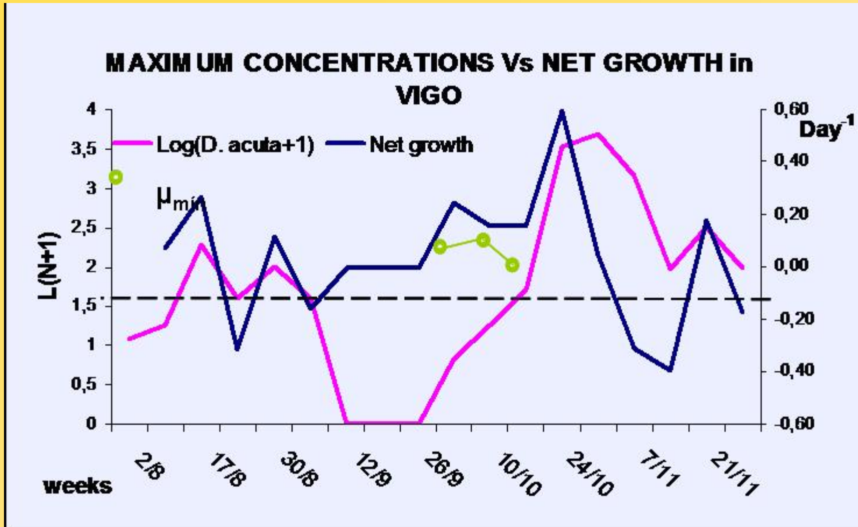
Distribution of frequencies of paired-cells and recently divided cells of *Dinophysis acuminata* in: Top, integrated samples (water column); Bottom, at the cell maximum.

Species	Location	Month	μ_{mi} (d^{-1})	μf_{c+r} (d^{-1})	References
D. acuminata	Long Island, USA	Jul		0.54	Chang *et al.* 1991
	Galician Rias, Spain	Jun	0.25	0.28	Reguera *et al.* 1996, 2003
		Oct	0.13	0.26	
		Jun	0.08	0.09	
		Jun		0.52	González-Gil et al. 2006
	Gullmar fjord, Sweden	Oct		0.75	Gisselson *et al.*, 1999
D. acuta	Galician Rias, Spain	Oct	0.20	0.65	Reguera *et al.* 1996, 2003
			0.17	0.33	
			0.02		
D. caudata			0.19	0.24	
			0.25		
D. fortii	Los Angeles, USA	Jun	0.50		Weiler, 1976
D. norvegica	Baltic Sea	Aug	No growth observed		Carpenter 1995
		Jul-Aug	0.1-0.4		Gisselson et al. 2002
D. tripos	Galician Rias, Spain	Oct	0.19	0.50	Reguera *et al.* 1996, 2003
D. sacculus	Ebro Delta, Spain	May	0.13	0.42	Garcés *et al.* 1997
		Jun	0.14	0.28	
		Jun	0.14	0.38	
		Oct	0.10	0.20	

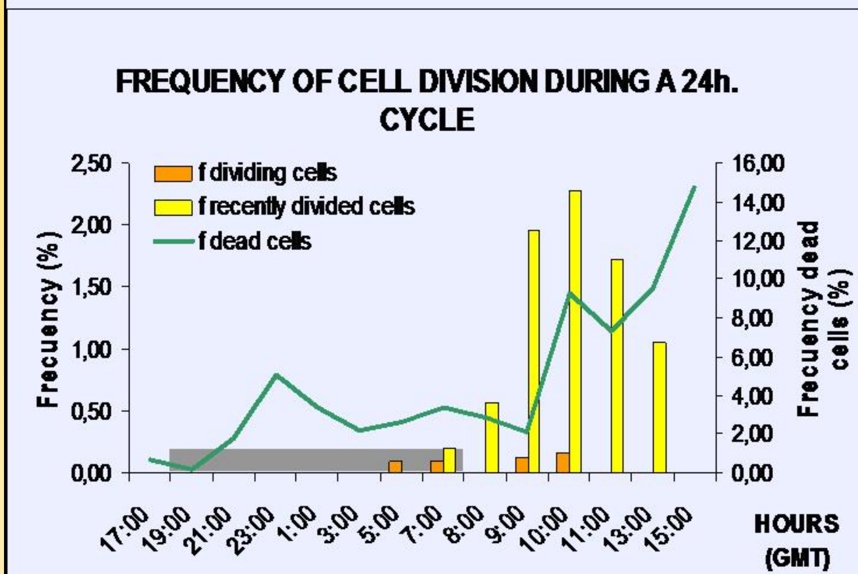
Estimates of *in situ* division rates of several species of *Dinophysis* by the mitotic index approach. A wide range of values observed for the same species in the same growing season.



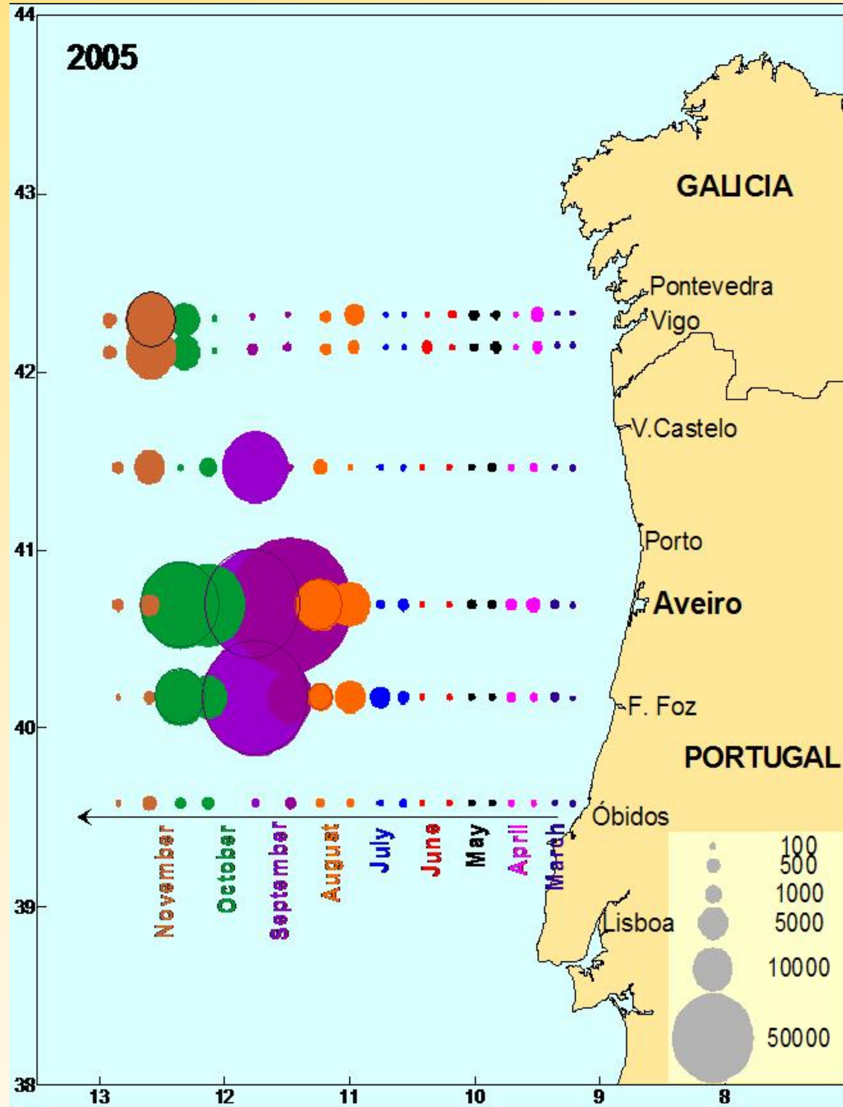
Contrasting images during upwelling conditions (left) and downwellin (right) in Western Iberia, October 2005



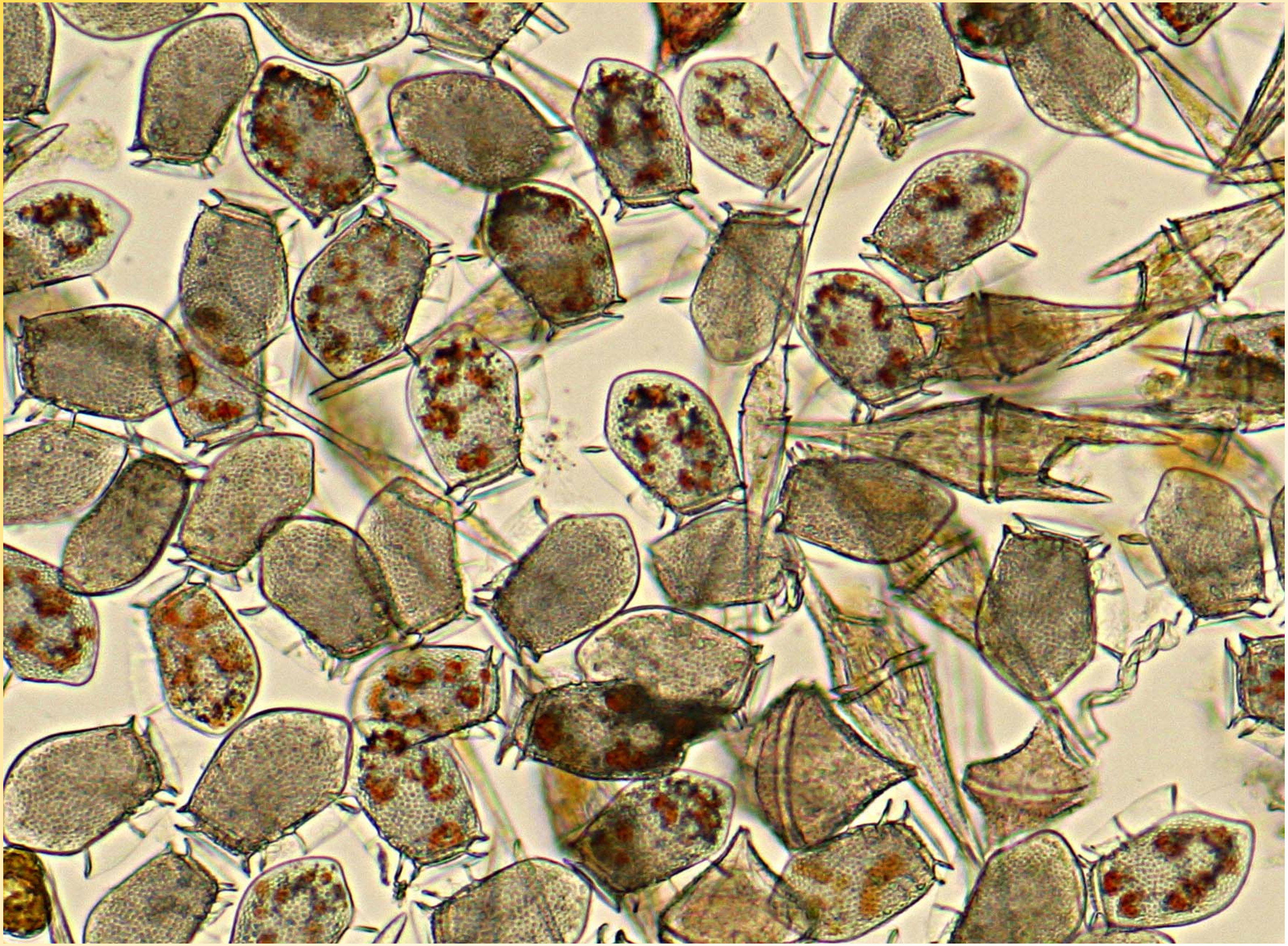
MAXIMUM CONCENTRATIONS Vs NET GROWTH in VIGO
Log(D. acuta+1)
Net growth
μmin
L(N+1)
Day-1
weeks
2/8
17/8
30/8
12/9
26/9
10/10
24/10
7/11
21/11



FREQUENCY OF CELL DIVISION DURING A 24h. CYCLE
f dividing cells
f recently divided cells
f dead cells
Frecuency (%)
Frecuency dead cells (%)
HOURS (GMT)

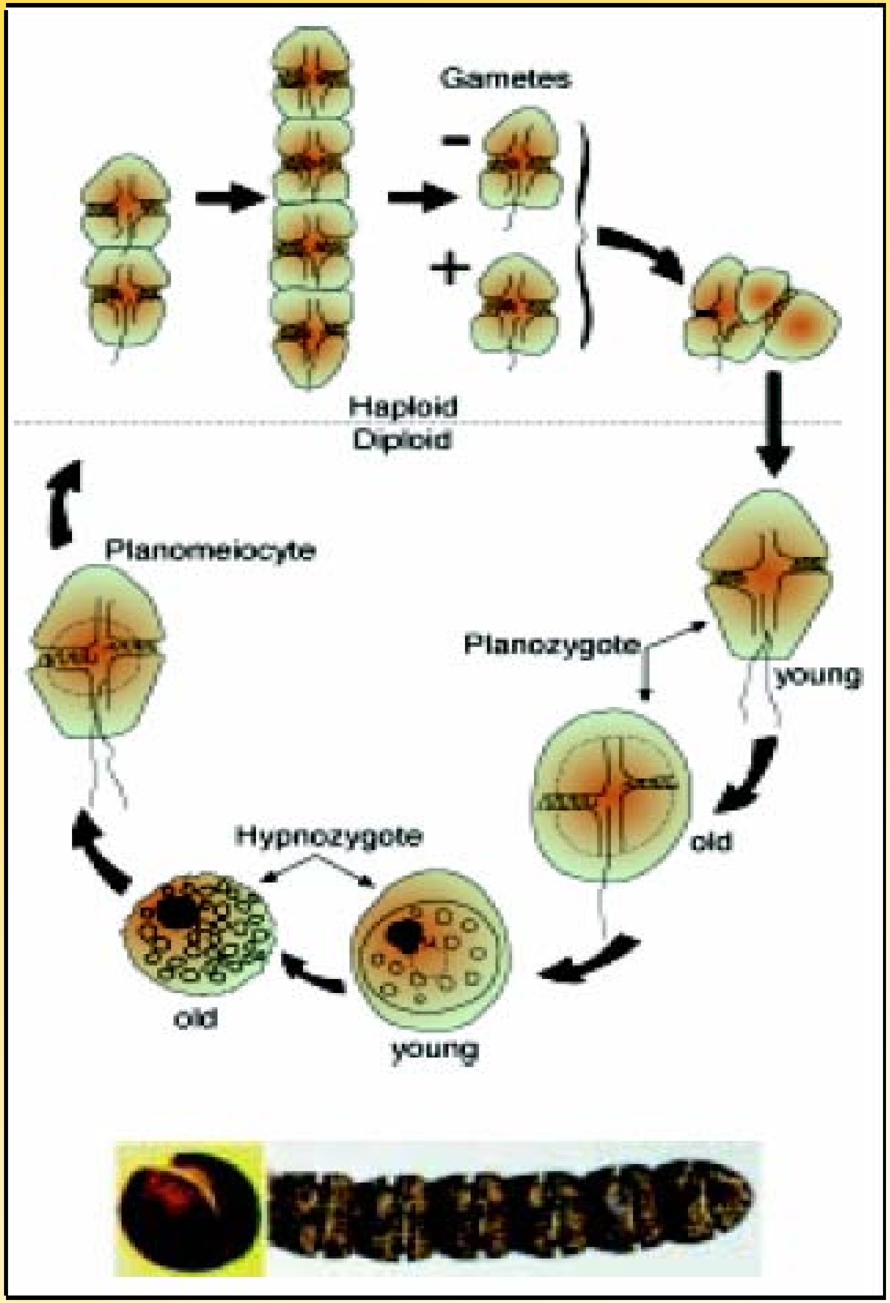


2005
GALICIA
Pontevedra
Vigo
V.Castelo
Porto
Aveiro
F. Foz
PORTUGAL
Óbidos
Lisboa
November
October
September
August
July
June
May
April
March
100
500
1000
5000
10000
50000



Concentrated population of dinoflagellates from the Galician Rías (November 2005). Notice the differences in cell content in *D. acuta* cells

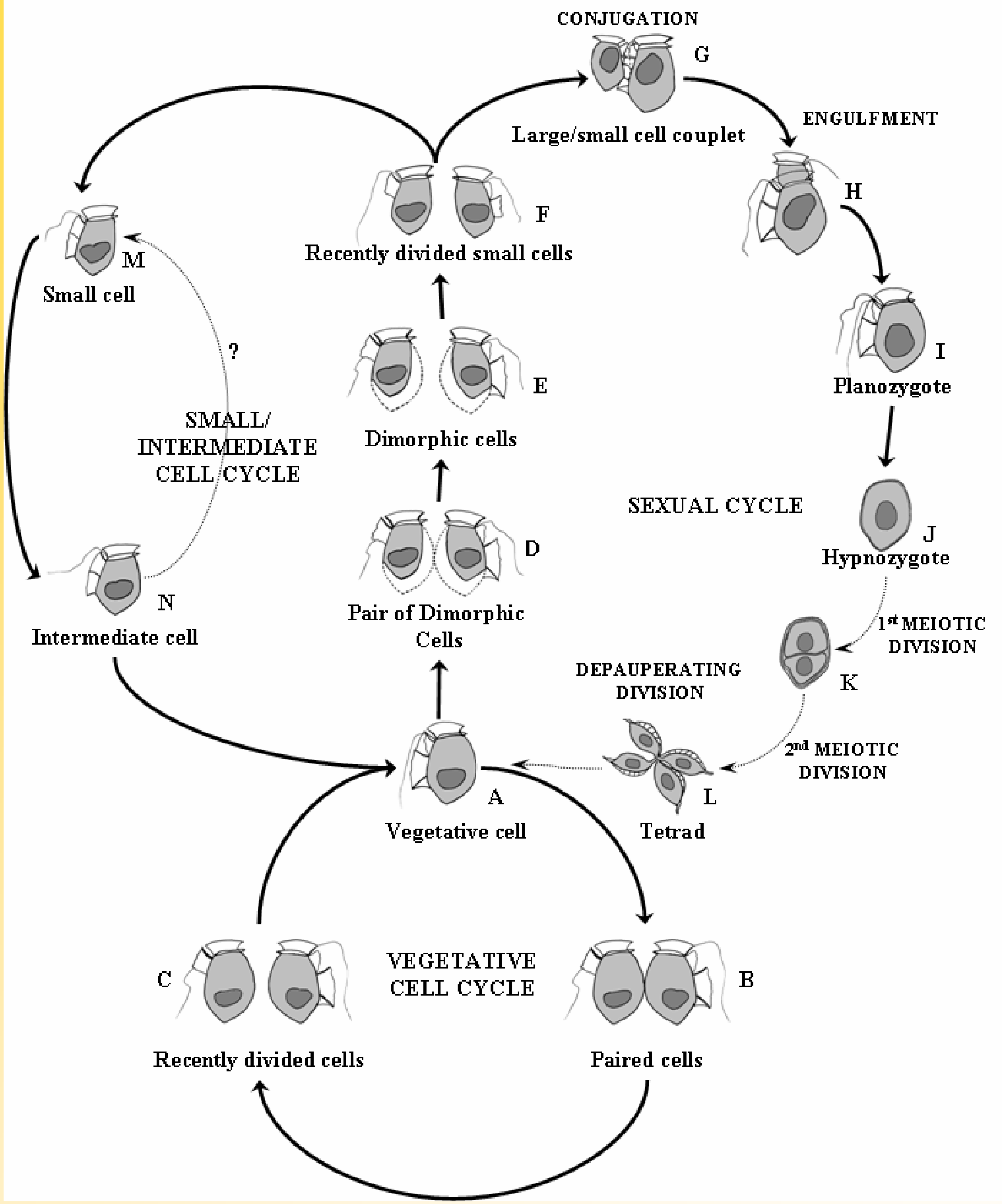
Life History



Life cycle of *Gymnodinium catenatum* (Blackburn et al. 1989)

To study the population dynamics of a target species we must be able to recognize ALL its life cycle stages.

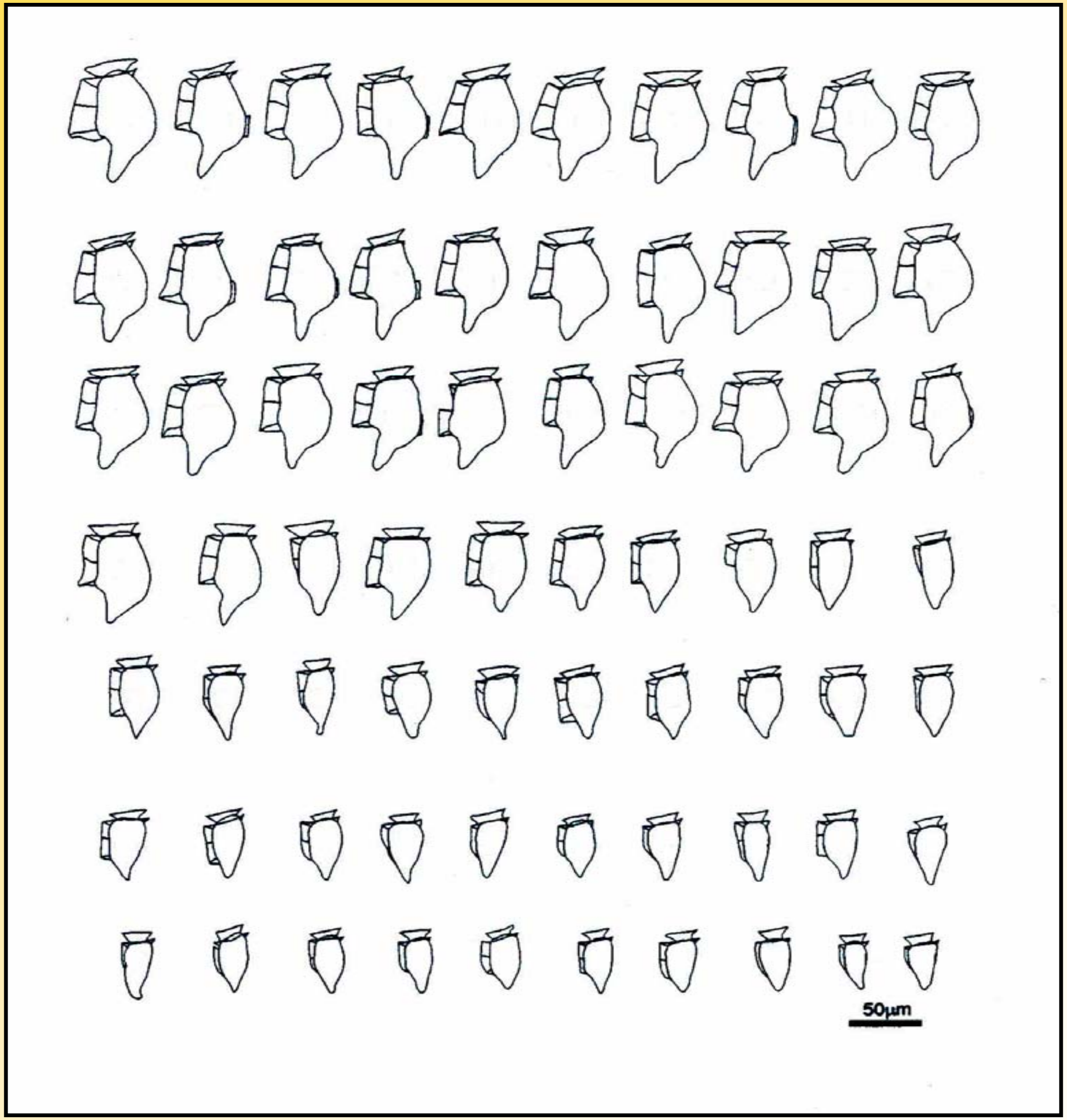
To follow changes in vegetative stage numebrs IS NOT ENOUGH



Life cycle of *Dinophysis* spp

Reguera & González-Gil 2001

Intraspecific Morphological Variability



Morphological variability of *Dinophysis caudata*, in one sample from Delta del Ebro, shows a c*ontinuum* of shapes between *D. caudata* y *D. diegensis*

(Reguera, González-Gil & Delgado 2005)

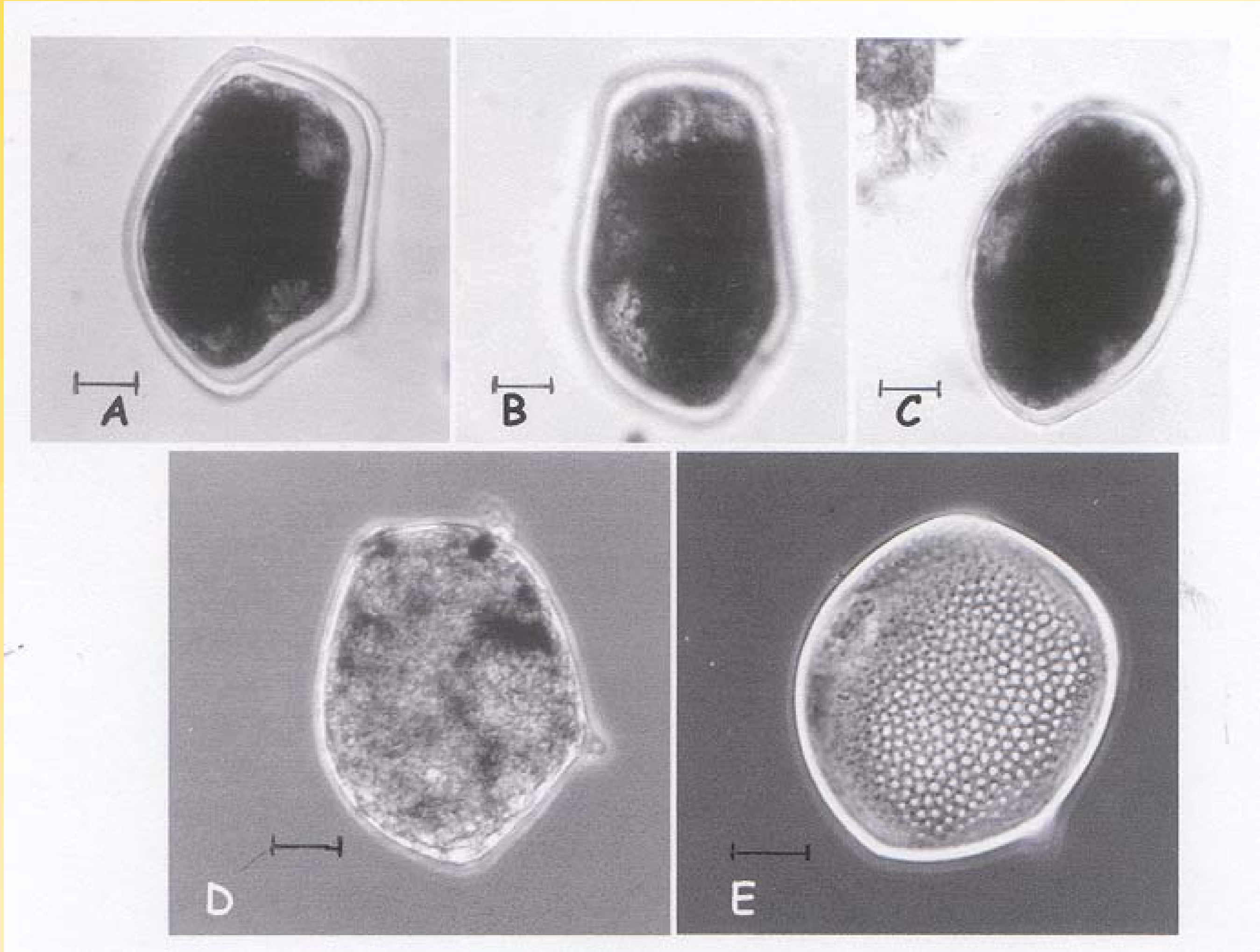
Ocasionally, high percentages of small cells (10-50%) have been observed in field populations of *Dinophysis acuminata* and *D. acuta*

(McKenzie, 1991; Reguera et al. 1995; Hajdu et al. 2006; Moita et al. 2006)

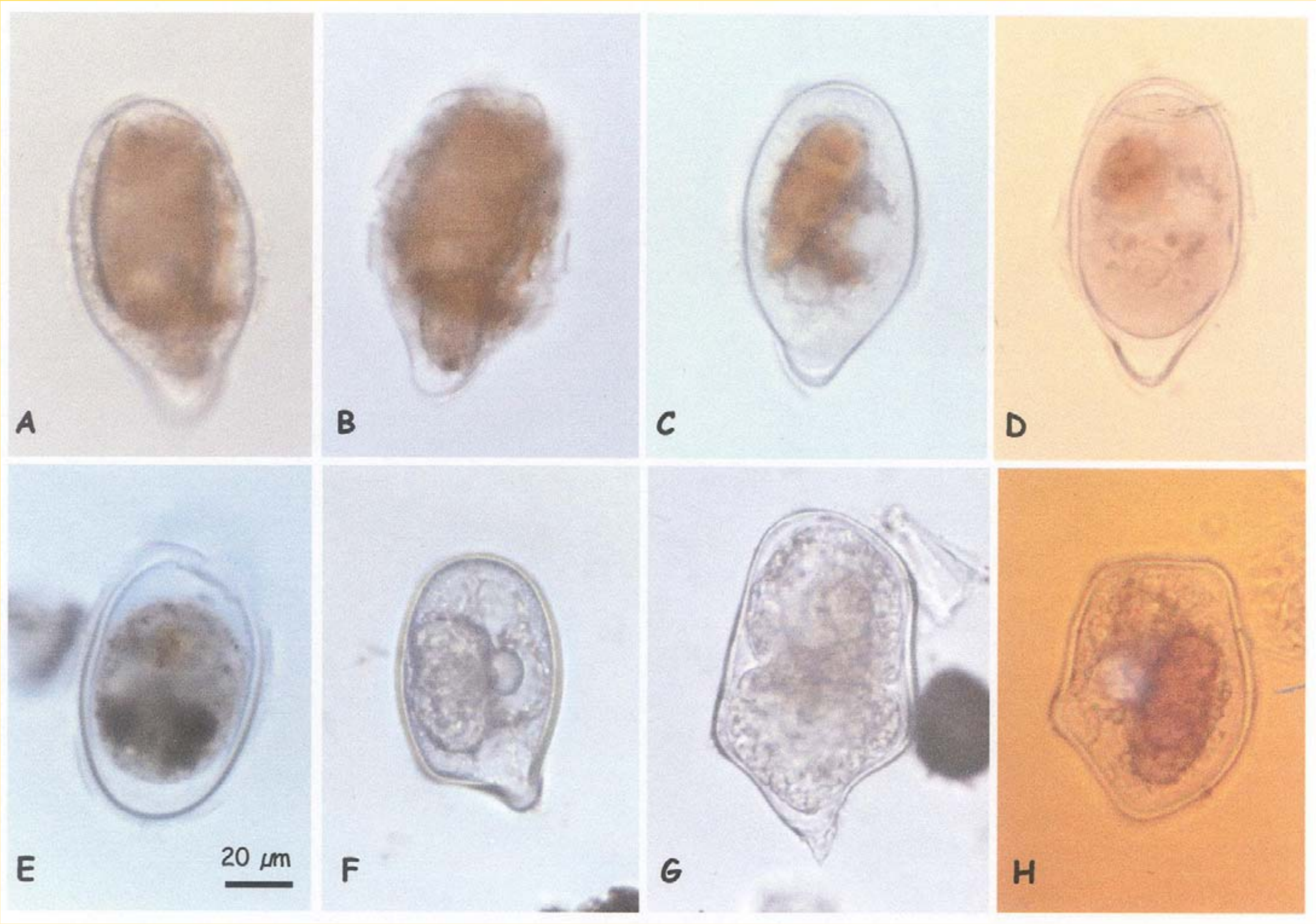
BUT observations of a few hypotetical cysts is an unusual event (Moita & Sampayo 19933; Reguera el al 1990,1995)

Do Dinophysis spp. rely on cysts to initiate new populations??

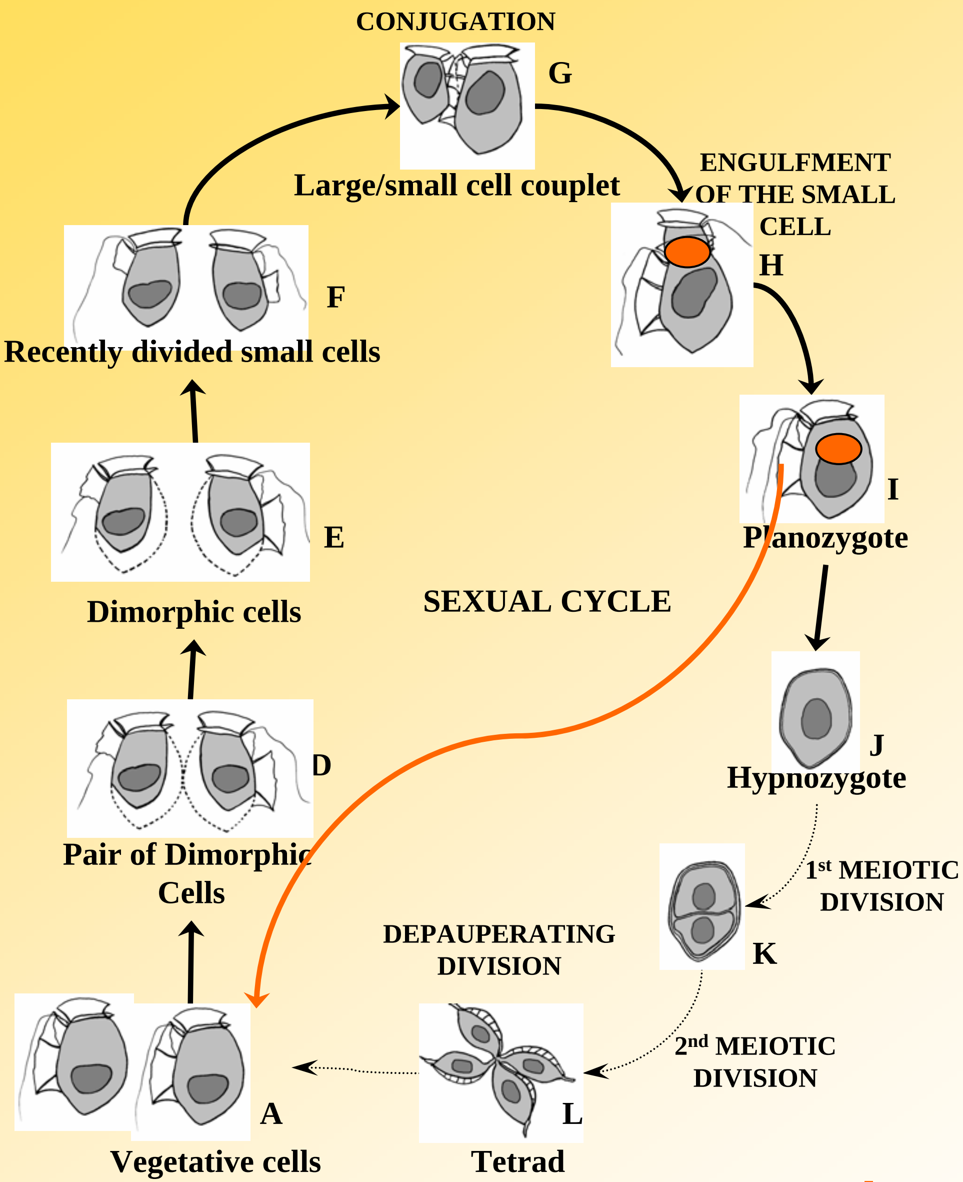
Quistes (?) de *Dinophysis acuta*



A-D. Distintos estadios de hipotéticos quistes de *D. acuta.* E. Detalle de la reticulación, similar a a la de las células vegetativas.
(Reguera, Bravo & Fraga 1995)

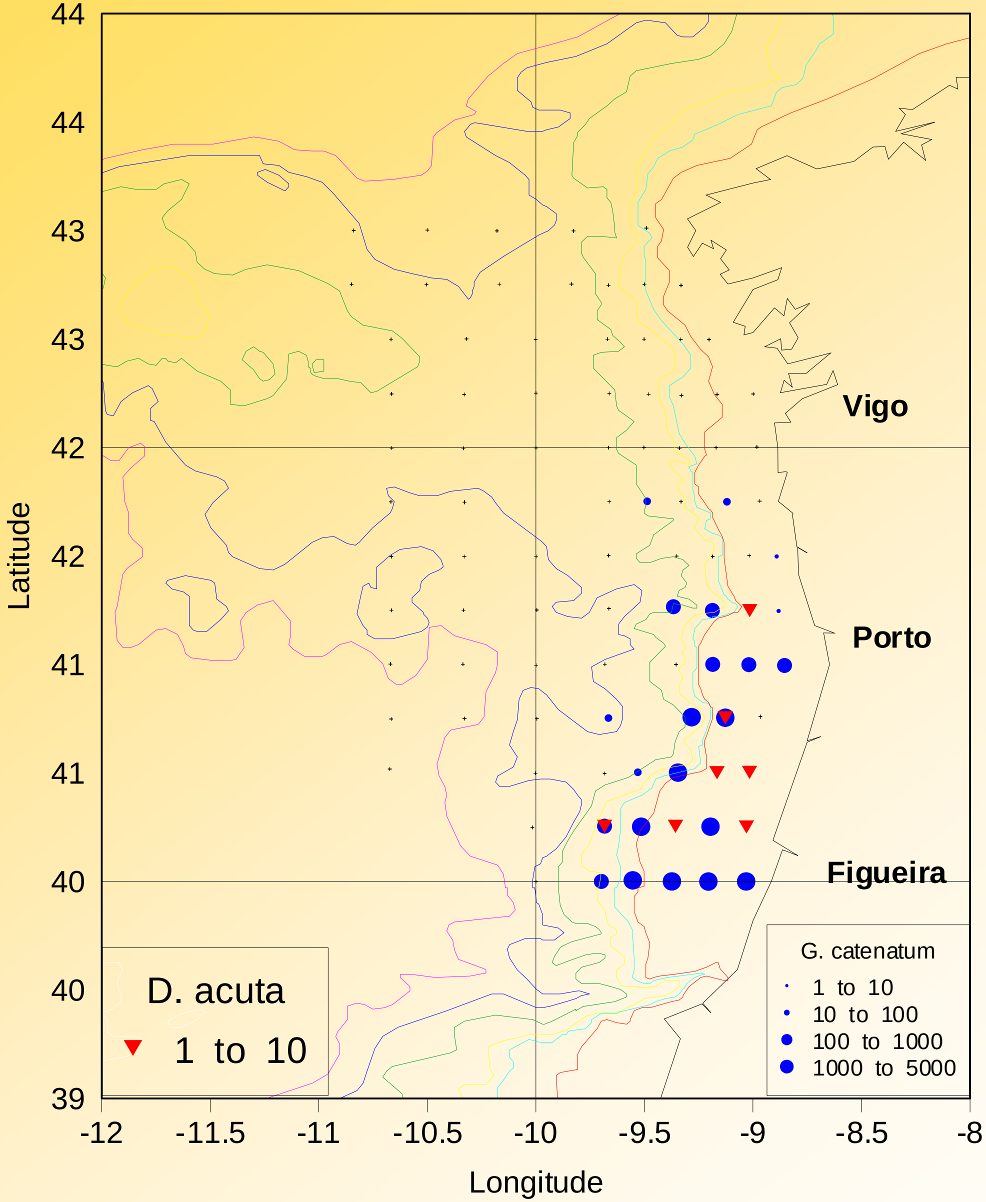


Hipotéticos quistes de *Dinophysis* spp. A-F: Evolución temporal de un quiste de *D. caudata*. B: *D. tripos*. C: *D. acuta* (Reguera, 2003)

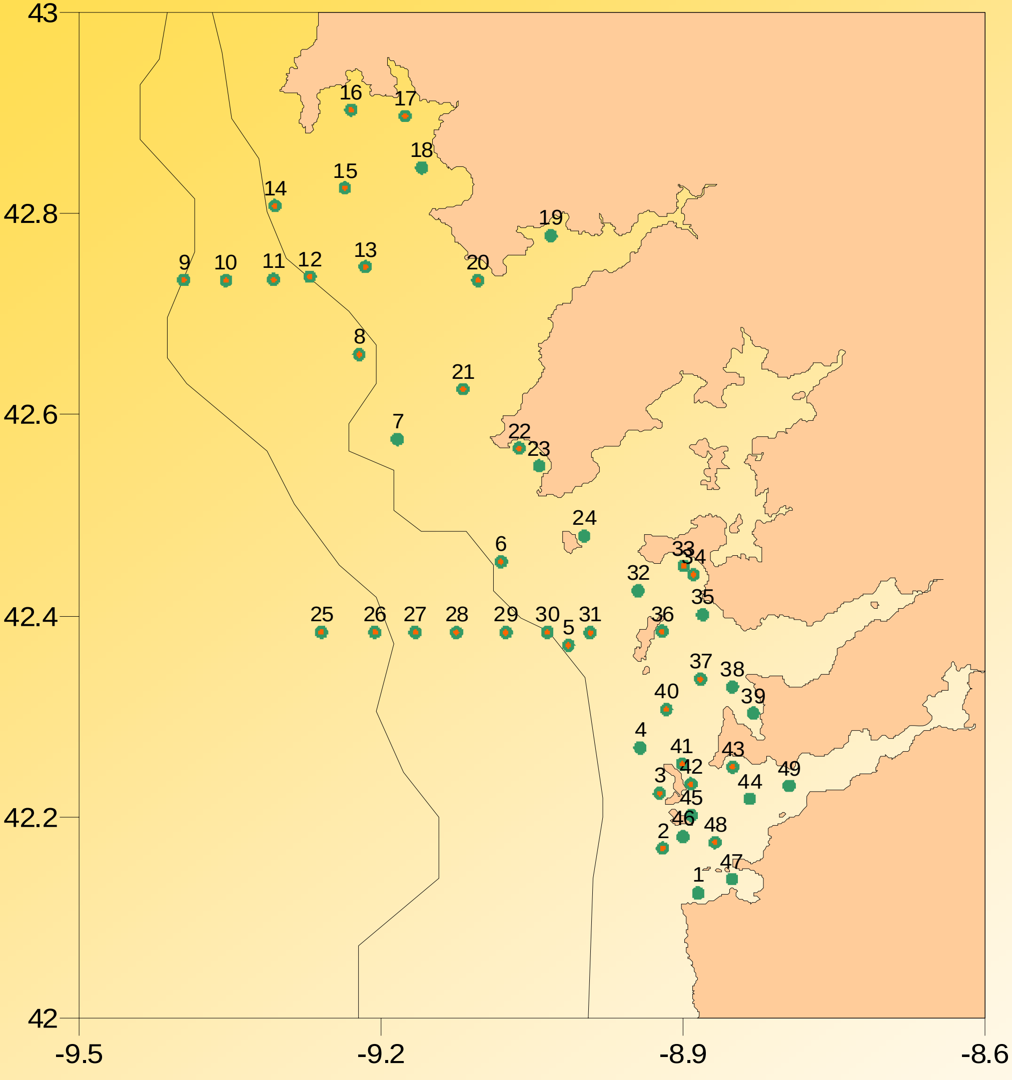


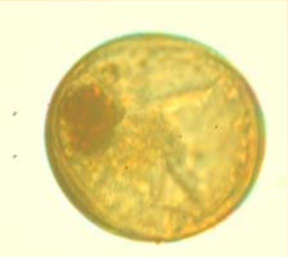
New observations on the life cycle of *Dinophysis* spp.

Escalera & Reguera *(submitt.)*

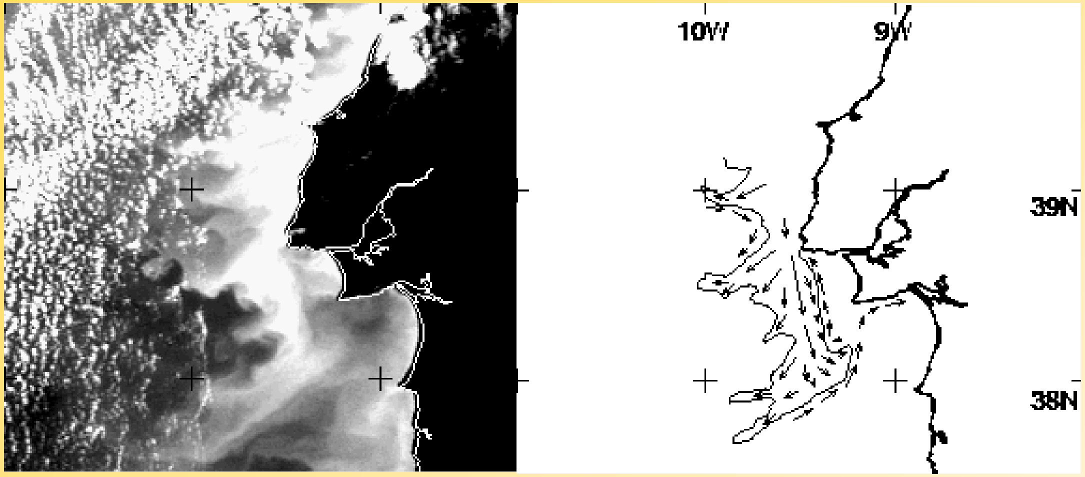


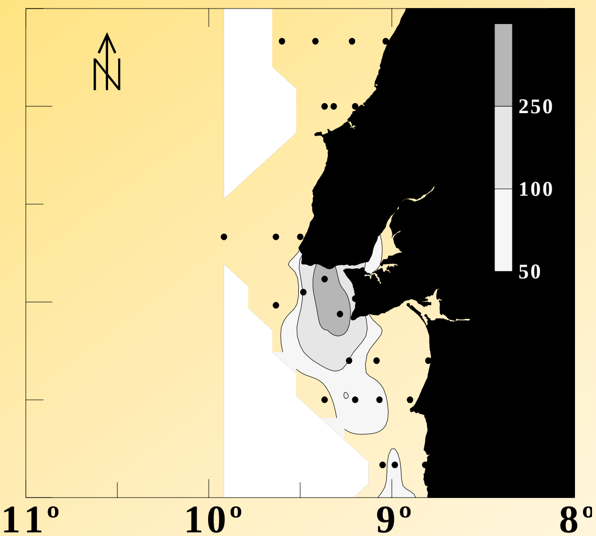
Distribution of *Dinophysis acuta* (red) and *Gymnodinium catenatum* (blue) during the "MORENA 93" cruise (Reguera, Figueiras & Cabanas, 2003)





Winter (1-2 February 2006) distribution of *D. acuta* off the Galician Rías





Map of the central coast of Portugal showing, that *G. catenatum* maxima may be associated with upwelling plumes. (A) Satellite infrared image from July 1, 1994. (B) Sketch of subjectively determined flow field from previous images from June 26 and 28. Continuous line indicates the position of the upwelling front. (C) Distribution of maximum values per station of *G. catenatum* (cells·L^{-1}) in 3 July 1994. (From Moita & Amorim 2002, LIFEHAB Report)

Behaviour

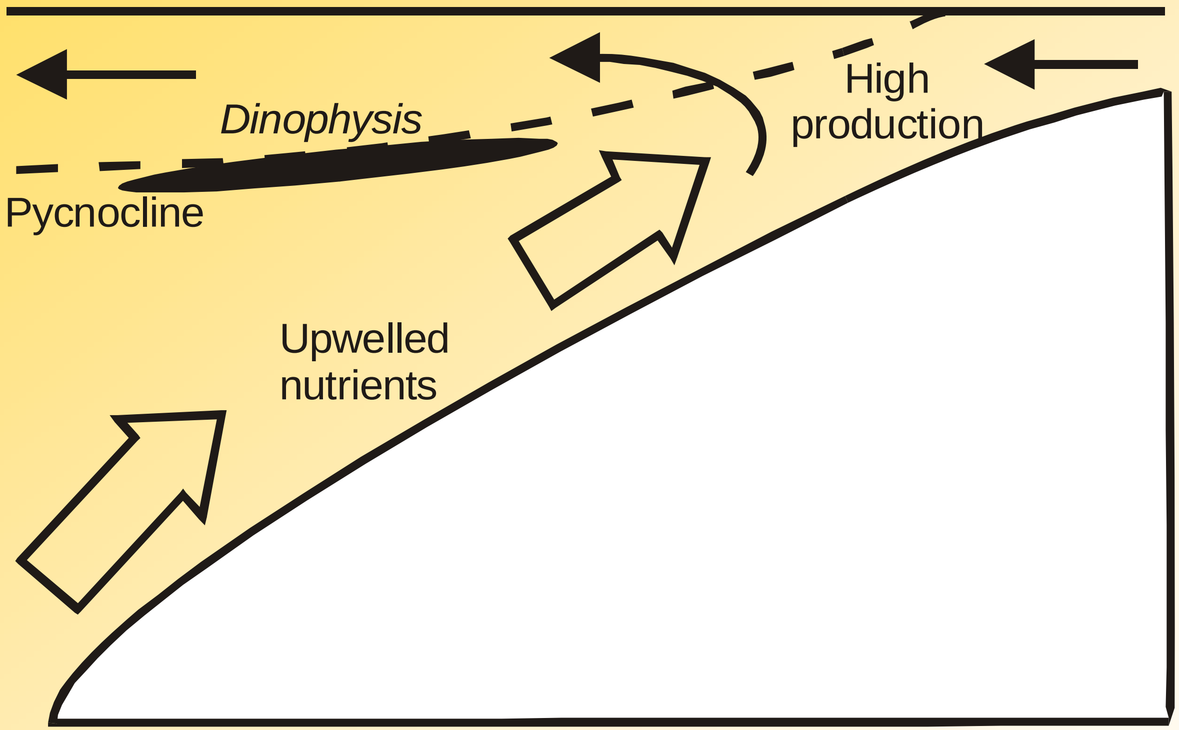
1. *Dinophysis* spp. like to aggregate in depths of marked density-gradients ?

Not always

2. Does Dinophysis spp perform daily vertical migration?

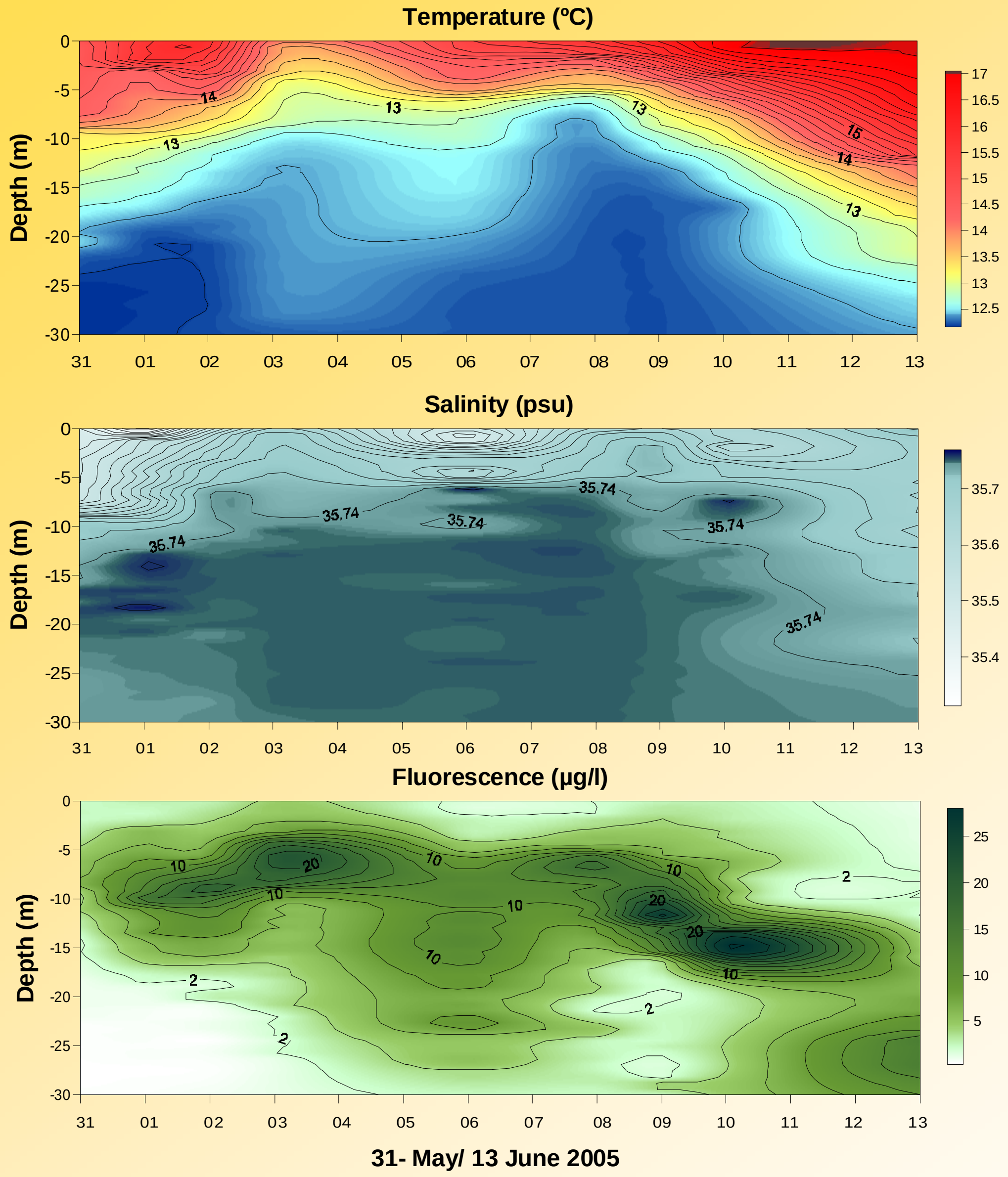
Sometimes

We need to describe behaviour during different phases of the population growth/environmental conditions



Proposed schematic of processes occurring during upwelling with resulting thin layers of *Dinophysis* within the Galician Rias

Results

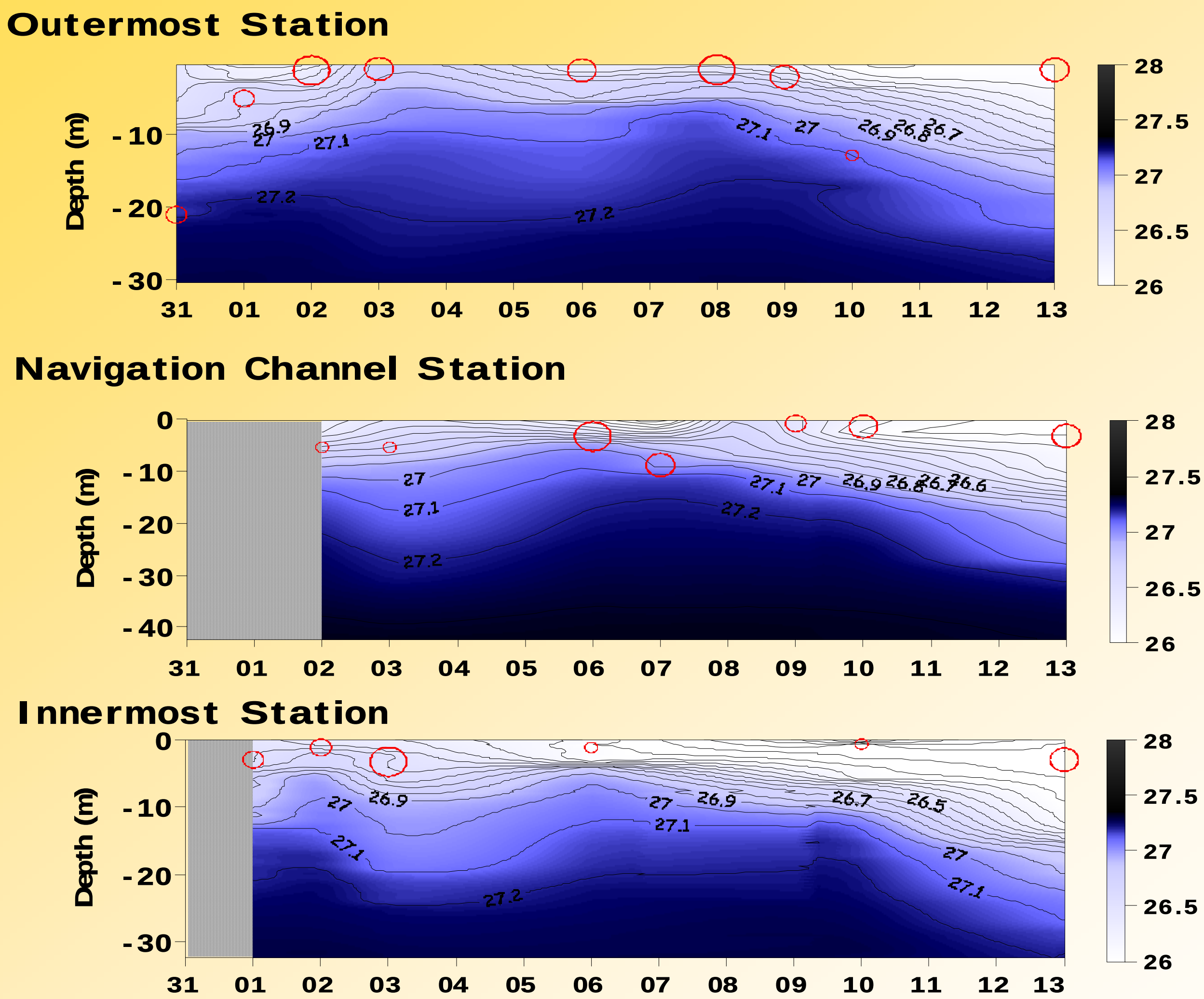


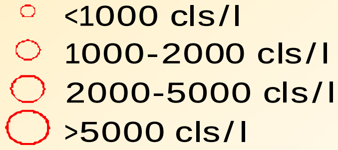
During upwelling events, the TL shoals and intensifies

Chlorophyll maxima, just above the 13º isoline, followed the excursions of the pycnocline

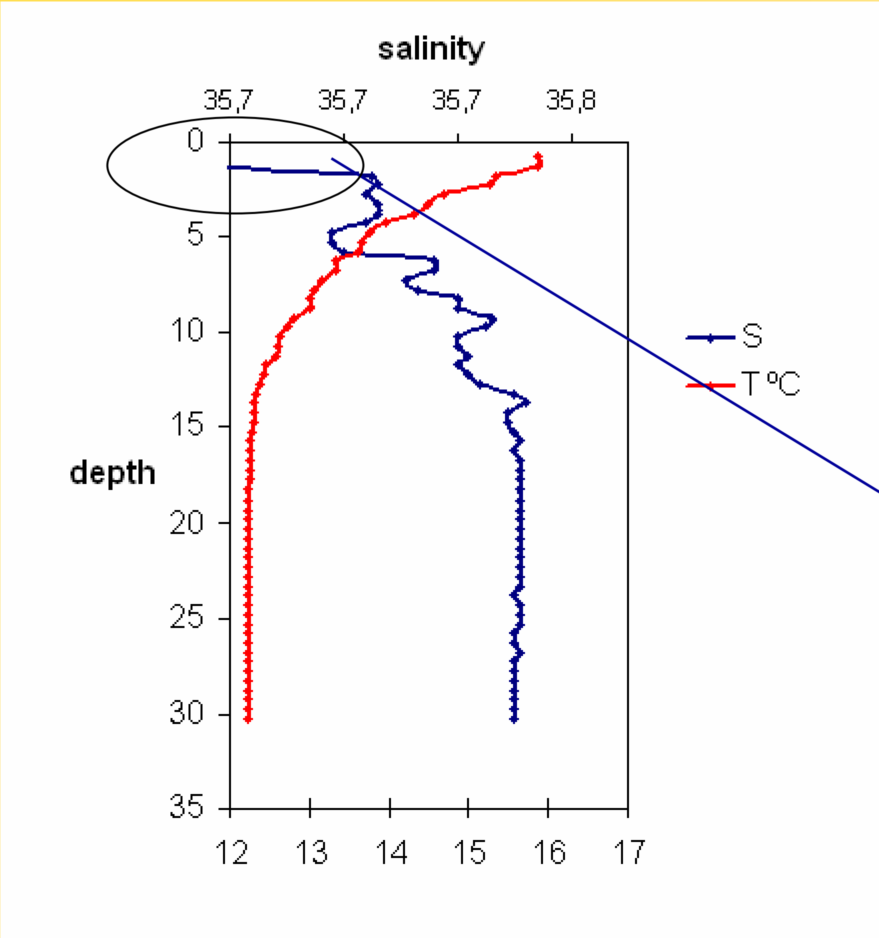
During relaxation and downwelling, the TL descends or even disperses

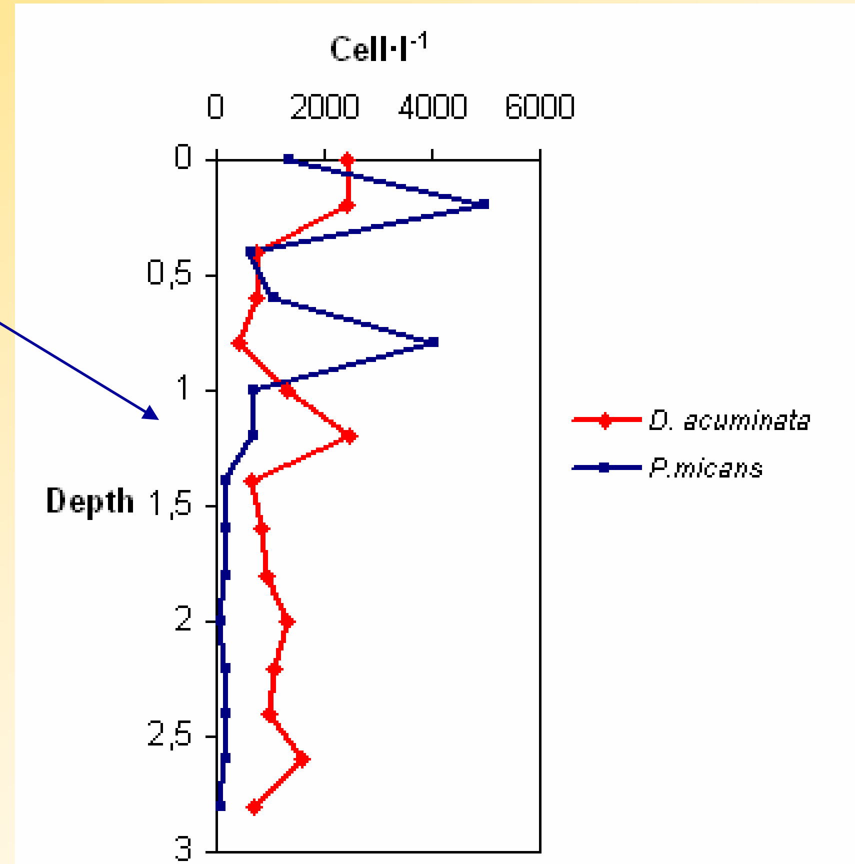
Temporal variation of oceanographic parameters in Boca Norte sampling points from 31 May to 13 June 2005 (L. Velo *et al*. in prep).



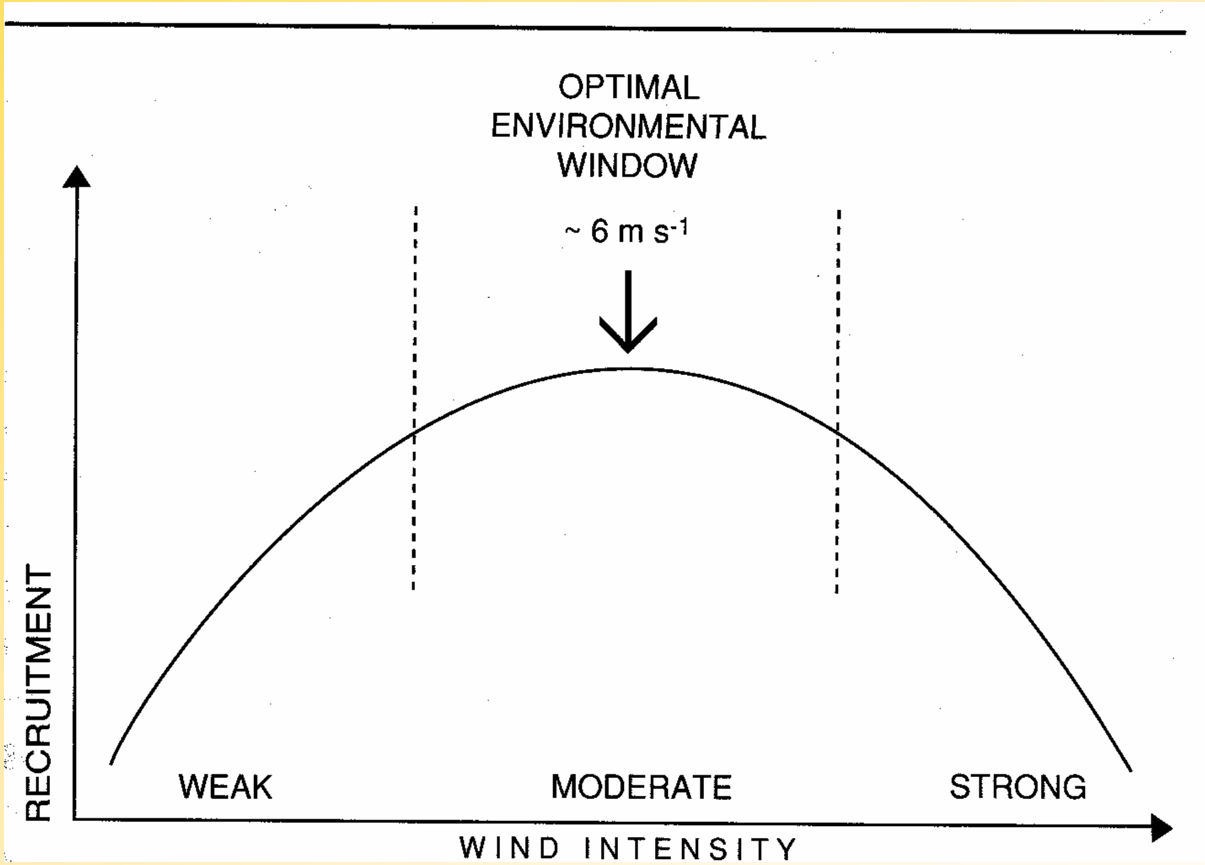


Sigma t contours and *Dinophysis acuminata* distribution



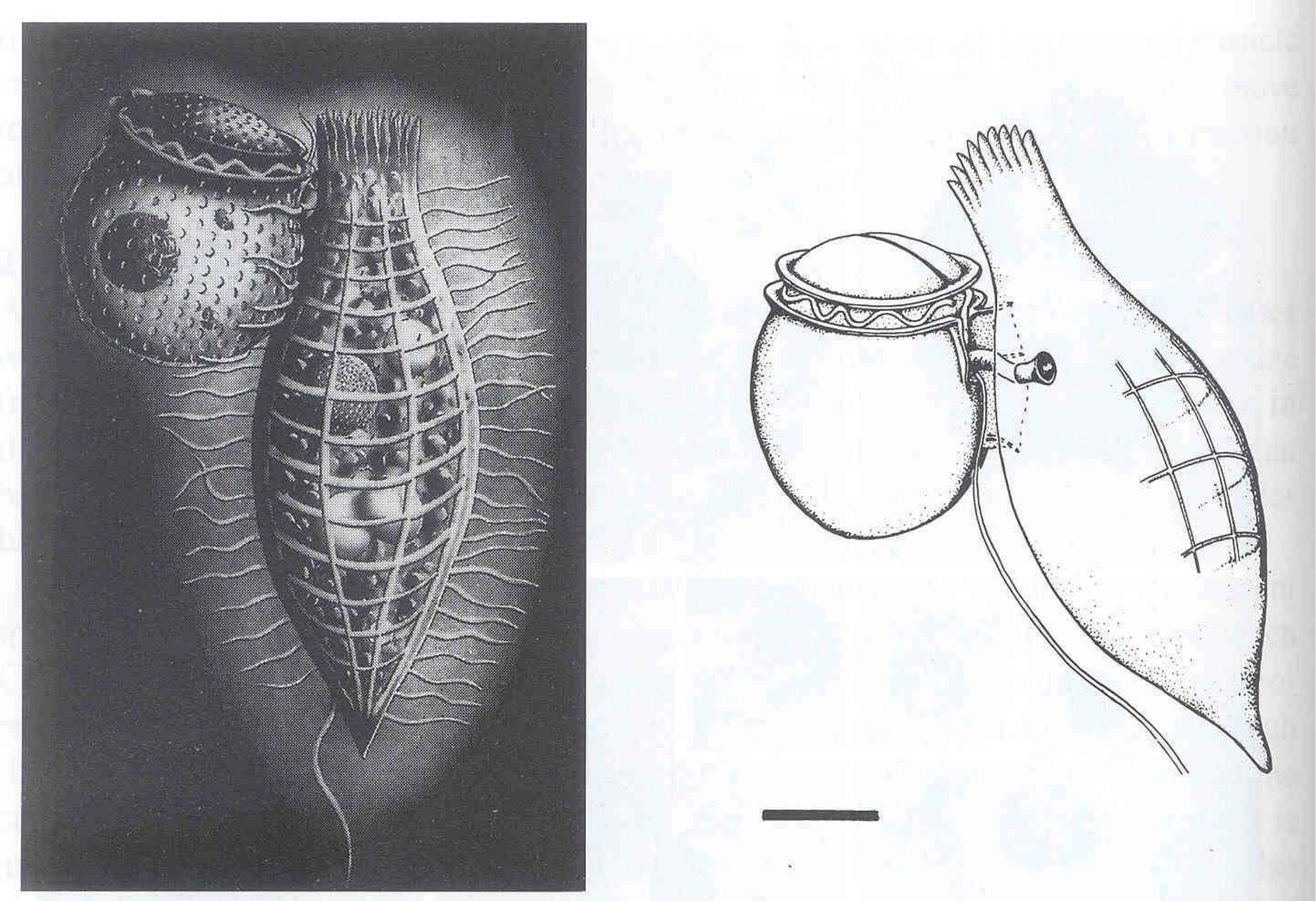


Fine (FSS) vertical distribution of *D. acuminata* and *P. micans* cells in the top 3 m in the northern mouth of Ria de Pontevedra, 9 June 2005.

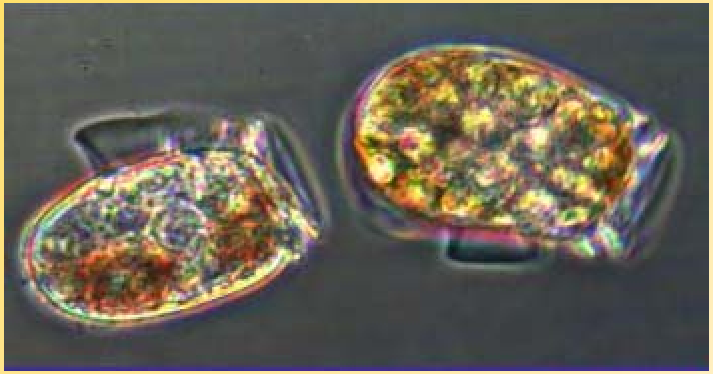


The environmental window hypothesis (Cury & Roy 1986) is being used as an approach to describe OEW for *Dinophysis*

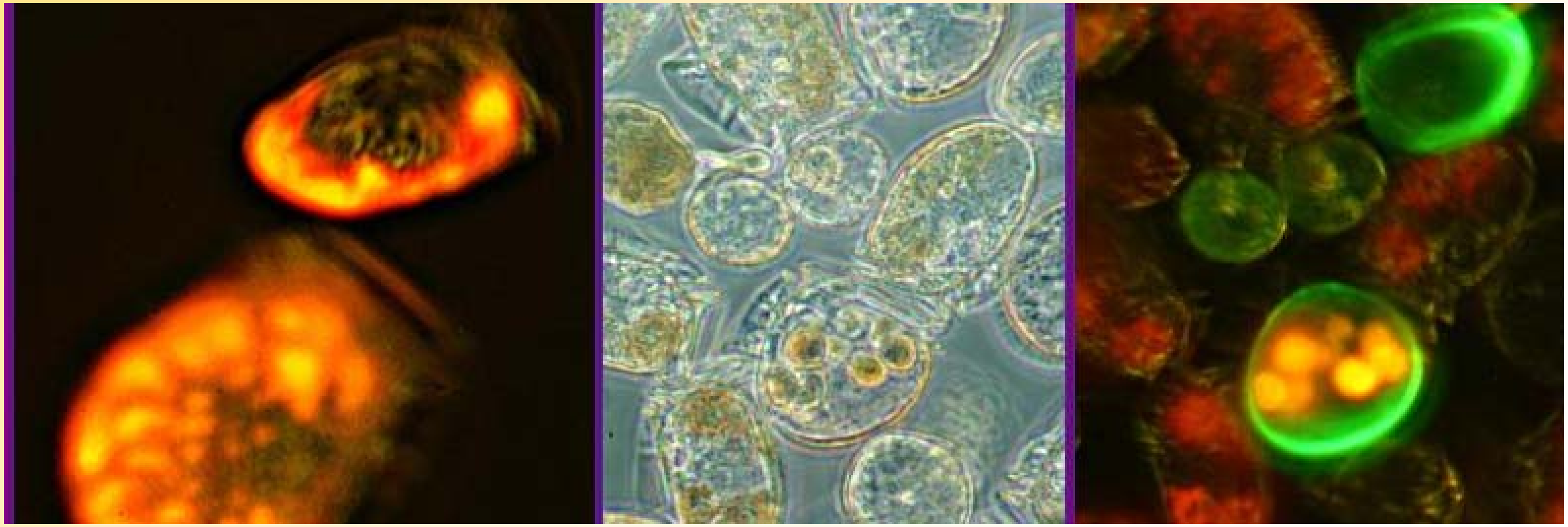
Nutrition



The hunter hunt: *Dinophysis rotundata* (heterotrophic) piercing the lorica of the prostomatid ciliate *Tiarina fusus* with a feeding peduncle and sucking its content (*myzocytosis*) (Hansen, 1991). T. fusus feeds on nanoflagellates, cryptophytes and dinos (including HAB species *L. polyedrum, H. akashiwo*).



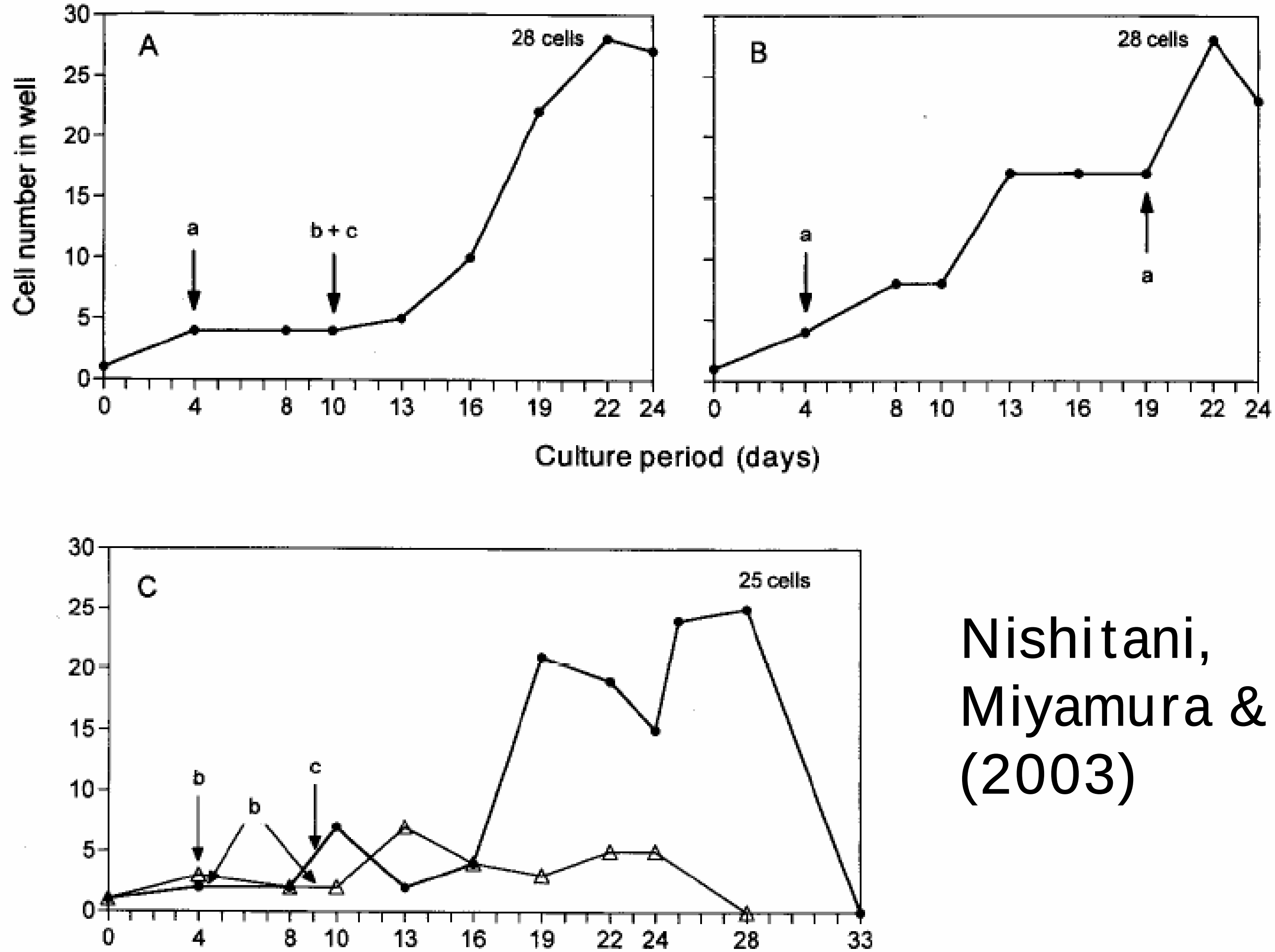
What does Dinophysis eat ?



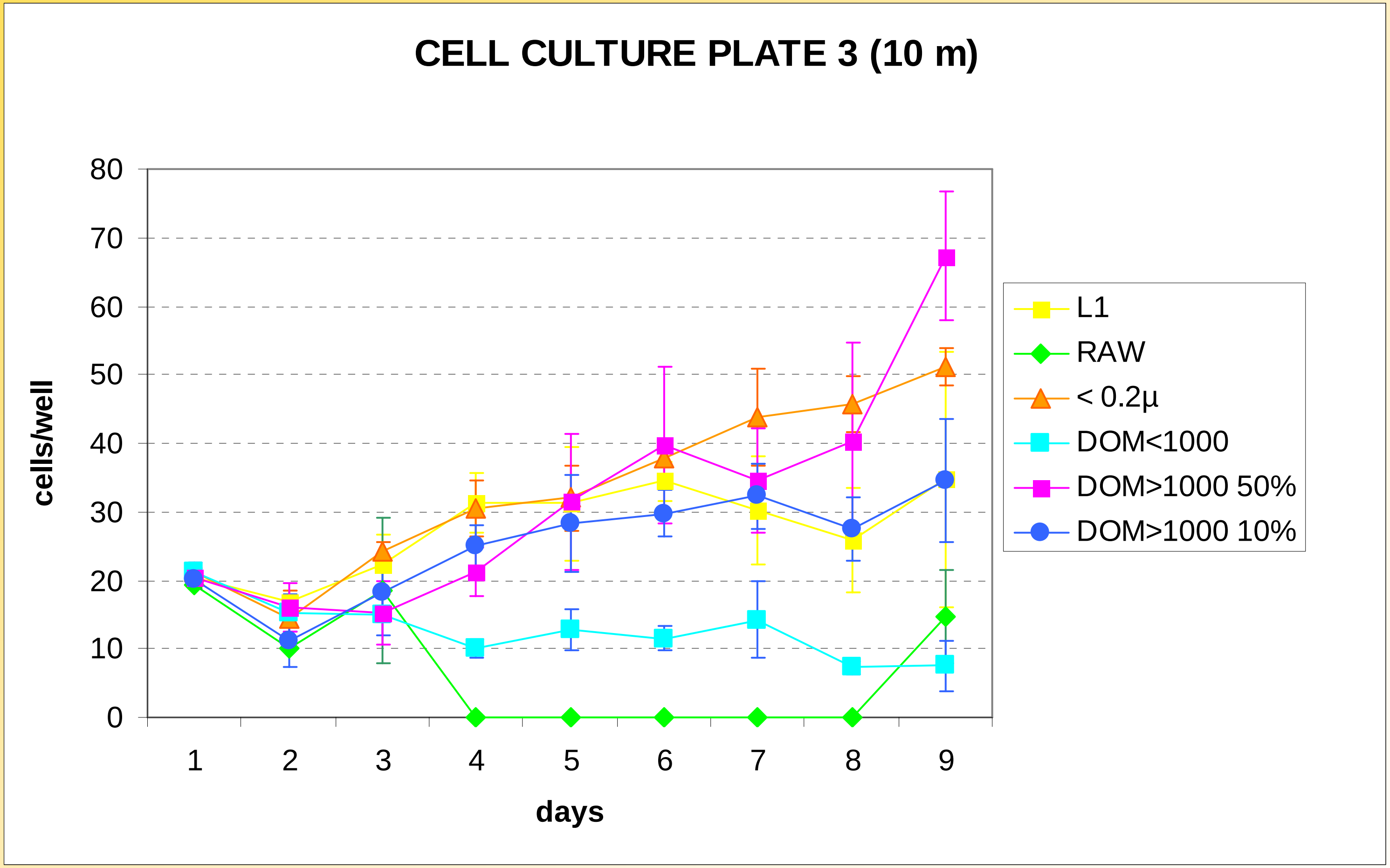
Different treatments (DOM, small alive/dead phytoplankton, bacteria…) have been used by different authors to maintain *Dinophysis* spp for observations on their life cycle or to try to cultivate them.

- **2 to 5 generations (doublings) obtained in the best cases**
- **Duplications did not continue after replication**
- **Often, formation of small cells observed.**

D. caudata fed with crushed and frozen picoplankton



Nishitani, Miyamura & Imai (2003)

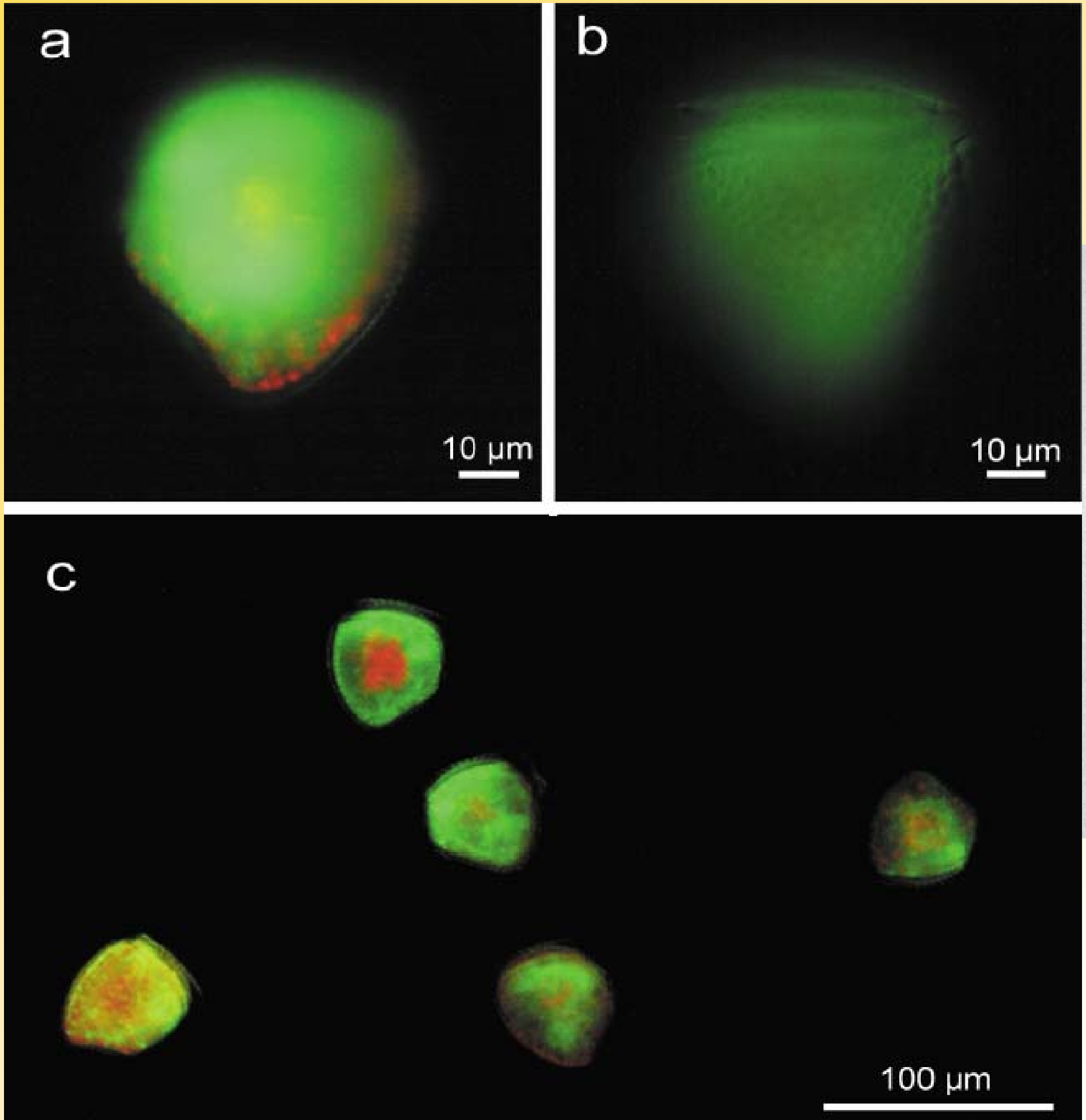


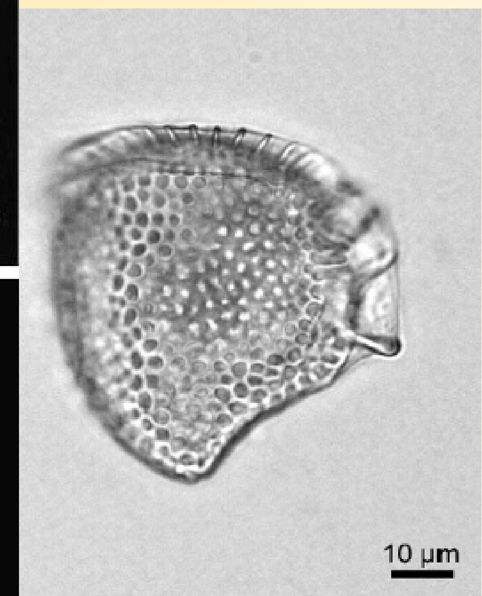
Incubations of picked *D caudata* cells under different treatments including concentrated DOM (ultrafiltration) (Reguera et al., 20005)

Sven Janson

Molecular evidence that plastids in the toxin-producing dinoflagellate genus *Dinophysis* originate from the free-living cryptophyte *Teleaulax amphioxeia*

Environmental Microbiology (2004) **6**(10), 1102–1106





Kleptoplastids of haptophyte origin in *Dinophysis mitra* (Koike et al., *Protist* 2005)

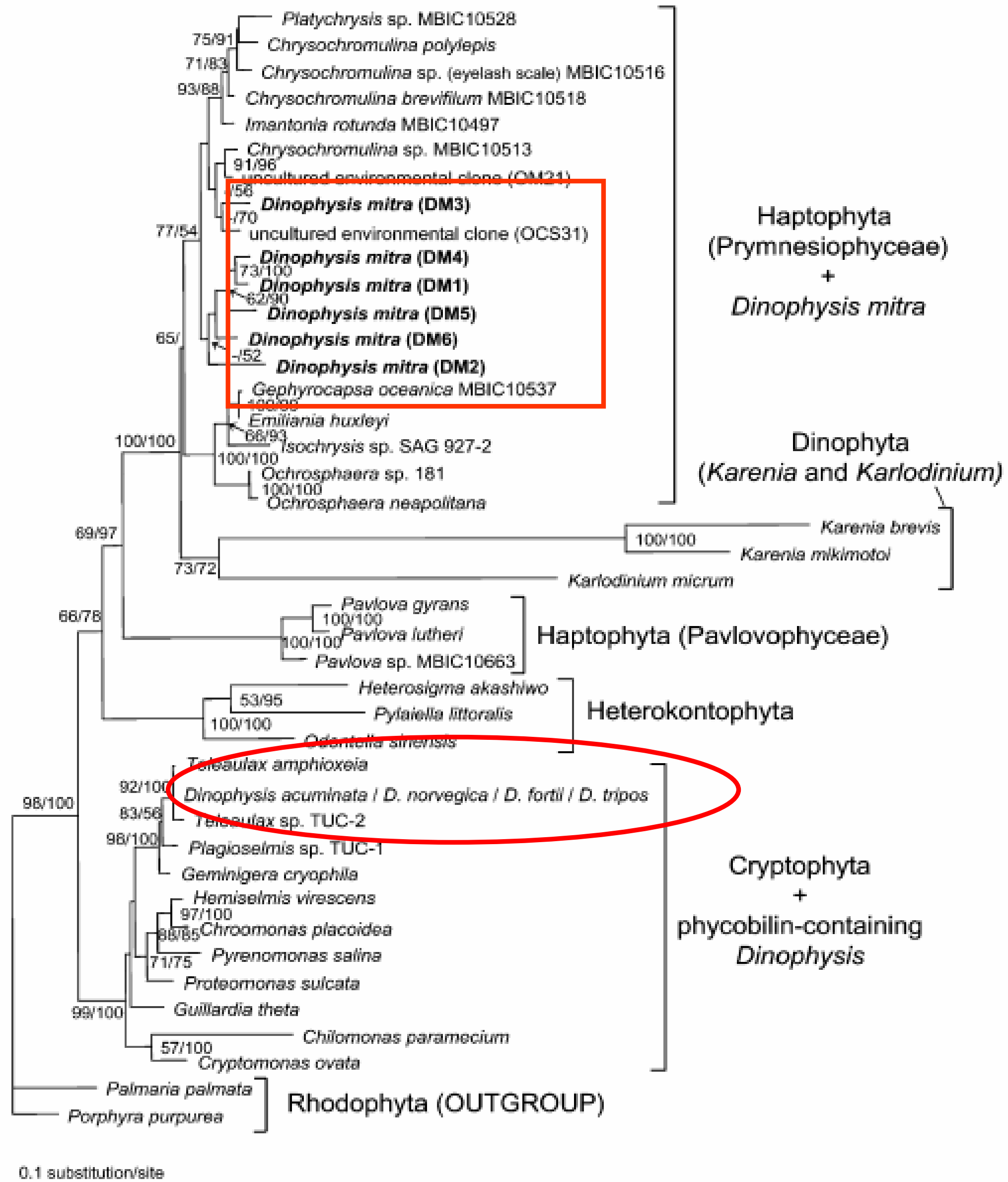
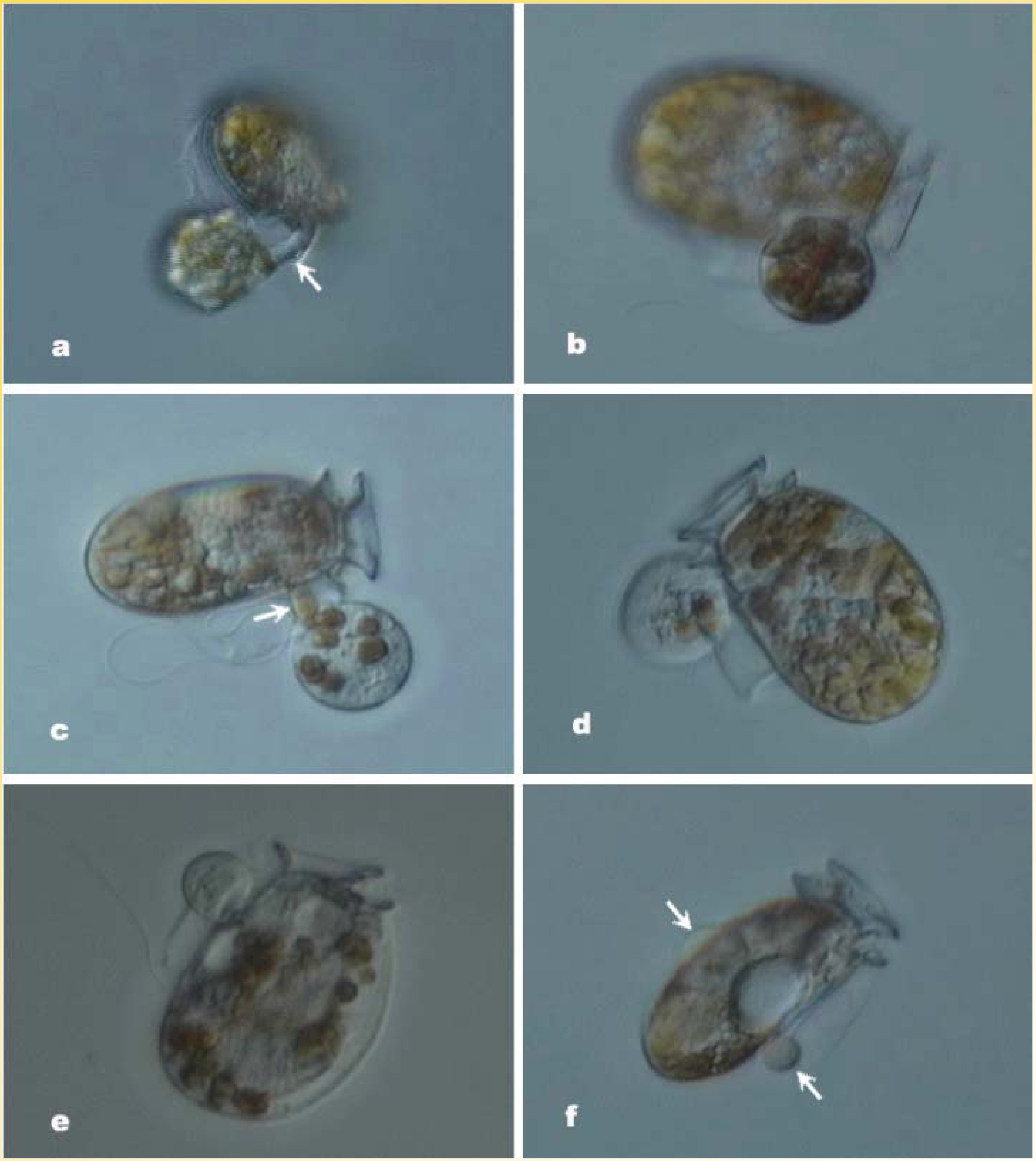


Figure 6. Maximum likelihood phylogeny (PhyML) of plastid SSU rDNA. Bootstrap probabilities of PhyML and posterior probabilities of MrBayes are shown for nodes with support over 50% (dashes represent support lower than 50%). Major photosynthetic groups are labeled on the right.

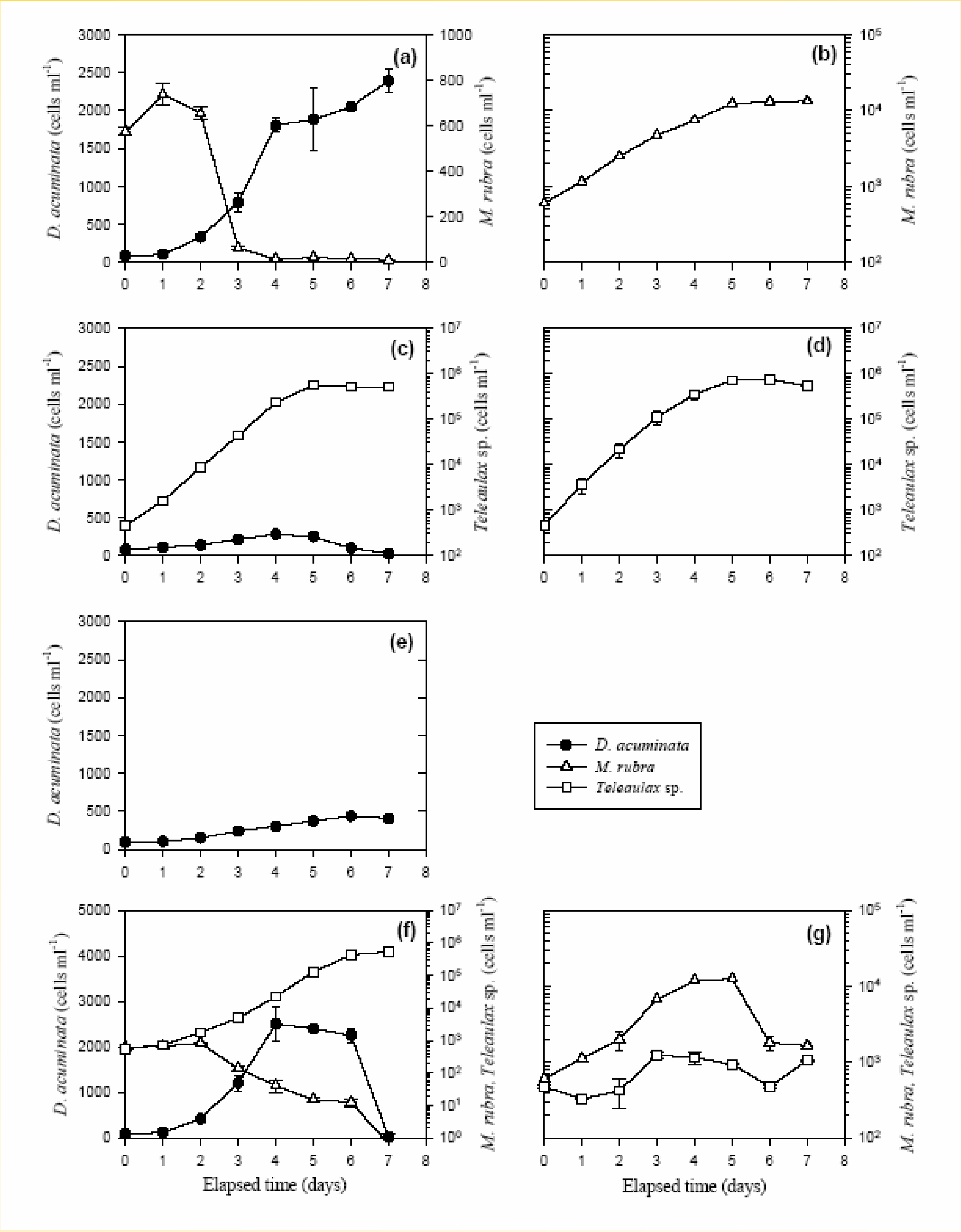


Dinophysis acuminata feeding on Mesodinium rubrum

Mechanism: Myzocytosis

Park et al.
XII HAB Conference

Aquat. Microb. Ecol. in press



Park et al.
in press

letters to nature

Cryptophyte algae are robbed of their organelles by the marine ciliate *Mesodinium rubrum*

Daniel E. Gustafson Jr, Diane K. Stoecker, Matthew D. Johnson, William F. Van Heukelem & Kerri Sneider

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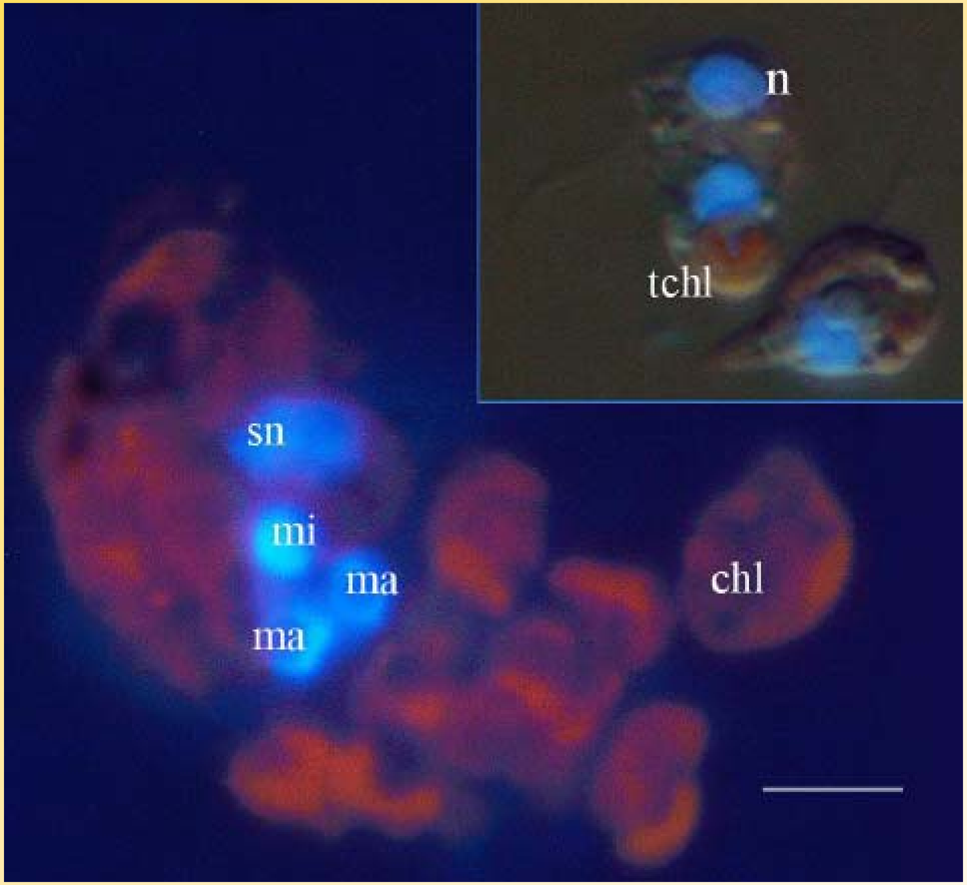
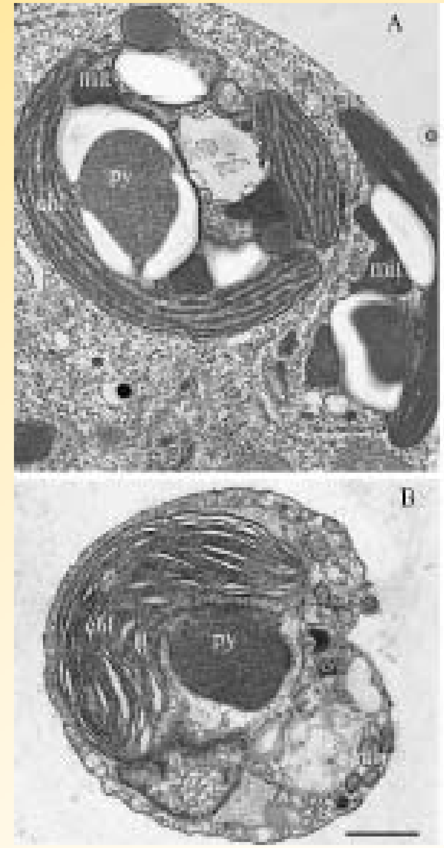
NATURE | VOL 405 | 29 JUNE 2000

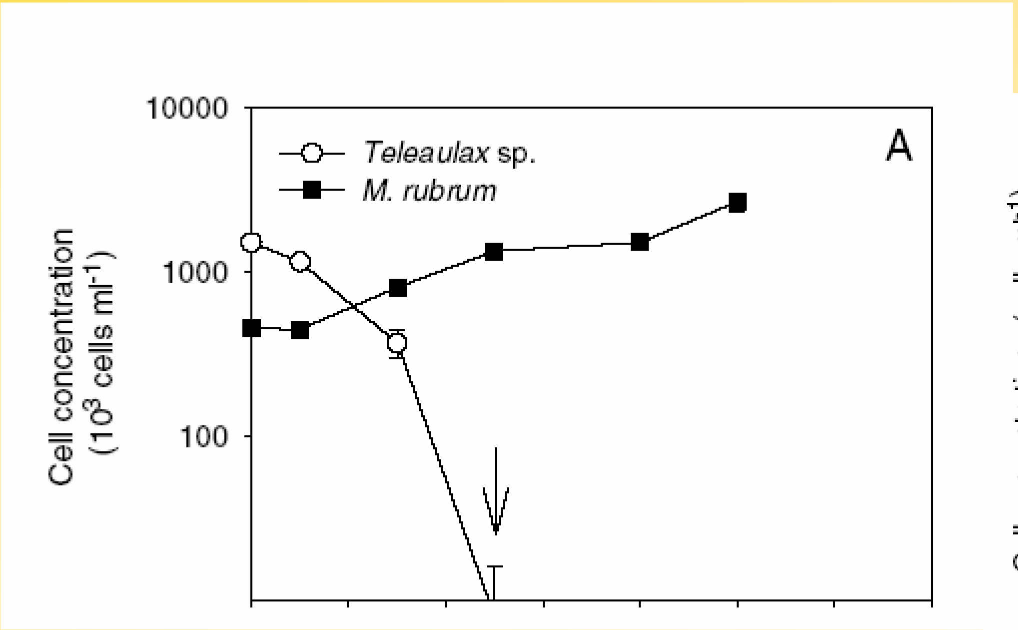
First succes to cultivate *Mesodinium rubrum* fed with *Teleaulax acuta*

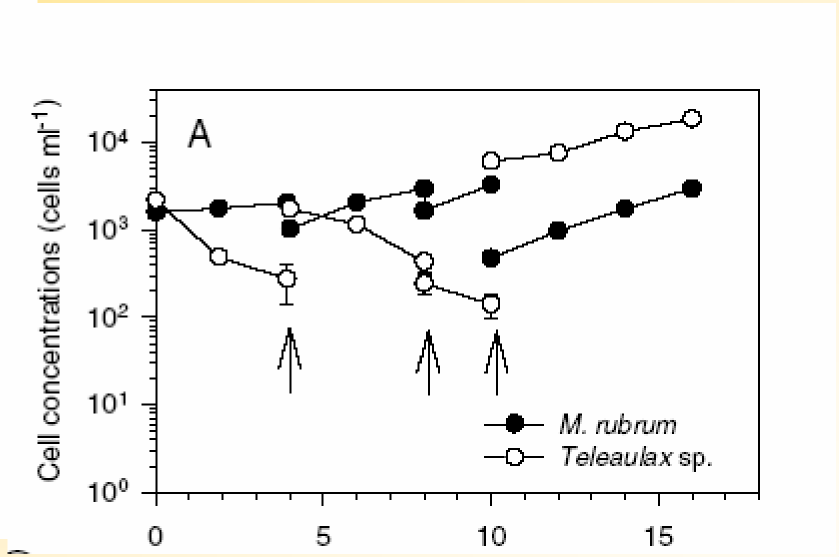
The bloom forming ciliate *Mesodinium rubrum* harbours a single permanent endosymbiont





Hansen & Fenchel, *Mar.Biol.Res.* 2006)





Hansen & Fenchel 2006