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.)
Reported distribution of DSP toxins detected in shellfish

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*
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 asynchronos 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 (t₁ - t₀).
**MITOTIC INDEX APPROACH** (McDuff & Chisholm, 1988)

\[
(T_c + T_r) = (t_0 - t_1)
\]

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

**Or the distance between two maxima**

(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)

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

(Model of Carpenter & Chang, 1988)

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

Frequency %

DNA quota
CELL FISSION IN
DINOPHYISIS SPP.

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.
Estimates of \textit{in situ} division rates of several species of \textit{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 downwelling (right) in Western Iberia, October 2005
Concentrated population of dinoflagellates from the Galician Rías (November 2005). Notice the differences in cell content in *D. acuta* cells
Life History
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 numbers IS NOT ENOUGH
Morphological variability of *Dinophysis caudata*, in one sample from Delta del Ebro, shows a continuum 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 hypothetical cysts is an unusual event (Moita & Sampayo 19933; Reguera et 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 retículación, similar a la de las células vegetativas. (Reguera, Bravo & Fraga 1995)
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 13° 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).
Results

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*
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

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


*University of Maryland, Center for Environmental Science, Horn Point Laboratory, PO Box 775, Cambridge, Maryland 21613, USA*

First succes to cultivate *Mesodinium rubrum* fed with *Teleaulax acuta*
The bloom forming ciliate *Mesodinium rubrum* harbours a single permanent endosymbiont
