ONGOING RESEARCH ON SPECIES OF *DINOPHYSIS*: ALTERNATIVES WITH UNCULTURABLE DINOFLAGELLATES

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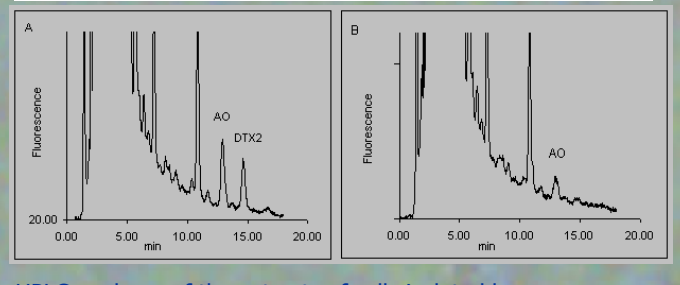
A traditional mussel raft from the Galician Rías

Diarrhetic shellfish toxins in bivalves (DSP) associated with proliferations of species of Dinophysis constitute the main risk factor to bivalve marketing in Galicia (NW Spain) in terms of days of market closure. Knowledge of basic biology and toxicology of the different species involved in the episodes has been hindered by the lack of cultures and the usually moderate field concentrations of *Dinophysis* spp. Ongoing studies apply a combination of in situ species-specific measurements, laboratory incubations of single cell isolations, and advanced techniques that allow toxin determination, genetic analysis and life history observations with very small samples.

Toxin analyses by conventional liquid chromatography methods of single cell isolations have confirmed the toxicity of *Dinophysis acuminata* (OA), *D. acuta* (OA:DTX2 3:2) and *D. caudata* (OA), and the large variability (up to two orders of magnitude) of toxin content per cell. These results together with the patchy distribution of *Dinophysis* spp. add further arguments to the unreliability of the use of a "*Dinophysis* index" as an early warning tool that triggers mouse bioassays in DSP monitoring. Ongoing efforts include the application of phosphatase inhibition assays to estimate toxicity per cell, and new derivatization procedures to improve sensitivity and resolution to obtain the toxin profiles.

Toxin content (pg · cel^{-1}) in single cell isolates from natural populations of different species of *Dinophysis* from the Galician Southern Rías and some multispecific net-hauls (20-100) where *D. acuta* was the dominant *Dinophysis*.

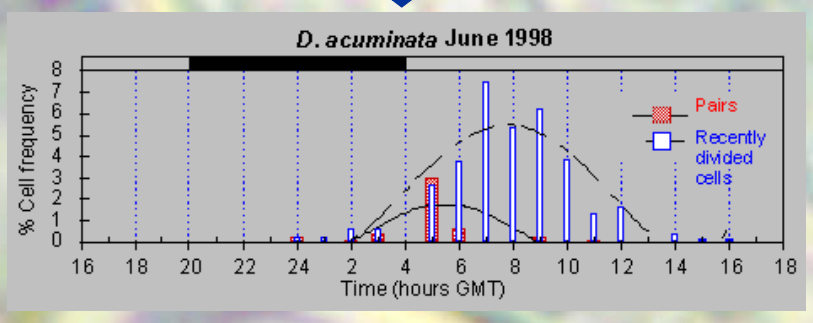
Species	Sampling Date	Isolation Date	Number of cells isolated	Number of cells injected	Toxicity (pg · cel^{-1})
D. acuminata	06.98	06.98	461 491	201 199	**9.9** OA **7.9** OA
	8.09.98	9.09.98	857	246	**21.7** OA
D. acuta	9.10.97	10.10.97	1250	308	**10.7** 6.3 OA 1. DTX2 **OA: DTX2 = 3:2**
	9.10.97	14.10.97	1214	412	**55.1** 33.1 OA 22.0 DTX2 **OA: DTX2 = 3:2**
	9.10.97	16.10.97	534	175	**24.1** 18.4 OA 5.7 DTX2 **OA:DTX2 = 3:1**
D. acuta (net-hauls)	507934 cells *D.acuta*, 75044 *D. acuminata*, 5300 *D.caudata* (28.08.90)				**2.6** OA:DTX = 1.6
	26928 *D.acuta*, 528 *D.caudata* (2.10.90)				**4.0** OA: DTX2 = 1.5
	45425 *D. acuta*, 7820 *D. caudata* and 115 *D. rotundata* (22.10.90)				**0.6** OA: DTX2 = 1.5
D. caudata	9.10.97	10.10.97	1388	342	**0.73** OA



HPLC analyses of the extracts of cells isolated by micromanipulation: a) *D. acuta*; b) *D. acuminata*.

a) *Dinophysis acuminata*, and b) *Dinophysis sacculus* from the Galician Rías.

Intensive *in situ* sampling of the cell cycle and laboratory incubations of *Dinophysis* isolates have led to new observations on common patterns in the complex life history of *Dinophysis* species, and their large intraspecific morphological variability associated with environmental conditions, division rates, feeding behaviour, and small and intermediate cell formation as part of a putative sexual cycle.



Frequency distribution of paired cells (joined by their dorsal margins) and recently divided cells (uncomplete development of the left sulcal list) of *Dinophysis acuminata* in Ría de Pontevedra (Galicia, Spain) during a 24 h *in situ* sampling for cell cycle studies and division rate measurements.

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A new technique has been developed that allows genetic analyses of *Dinophysis* species, and studies on phylogenetic relationships are underway. Clean DNA of *Dinophysis* have been obtained from samples as small as one cell. These promising results will enable rapid progress in the solution of old taxonomic and biological questions concerning *Dinophysis*.

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                          1                    ↓ITS1                50
D. acuminata  GAACCTGCGG AAGGATCATT --CGCACGCA TCCAATATCC ATAACTTGAA
D. sacculus   .......... .......... --......... .......... ..........
D. tripos     C..G..T..- ...CG-..A. AAG..T.--T .-..G.CC.. ..........

                         51                                       100
D. acuminata  ATTT-CTTGT GTGAGCTTCT GGGTGAGGTT GAACAAAGTG TTGCCTTCAT
D. sacculus   ....-..... .......... .......... .......... ..........
D. tripos     ....T..-.. .......... .......... .......... .......-..

                        101                                       150
D. acuminata  GTGGAAGCTC GAGGGTAGAT GAACTGAAGC AGTGTGGTCT TGCTGTTTCG
D. sacculus   .......... .......... .......... .......... ..........
D. tripos     .......... .......... .......... ....CT.C.. .....C....

                        151                                       200
D. acuminata  TGGGCGCTAC CGTCTGCTTG GCTCACACTG CCTTGCGGTT GAACCTTTAT
D. sacculus   .......... .......... .......... .......... ..........
D. tripos     .......... .......... ...A...T.. ......T... .GG.......

                        201                ↓5,8S rRNA             250
D. acuminata  TT--GTTTGT ATGTGCATGC TGTATGTATC ACAATTTTCA GCGATGGATG
D. sacculus   ..--...... .......... .......... .......... ..........
D. tripos     G.AA..... G.......AT ...C..CTC. .......... ..........

                        251                                       300
D. acuminata  CCTCGGCTCG AACAACGATG AAGGGCGCAC CGAAGTGTGA TAATCTTTGT
D. sacculus   .......... .......... .........G .......... ..........
D. tripos     .......... .......... .......... ....C..... ..........

                        301                                       350
D. acuminata  GAATTGCAGA ATTCCGTGAA TCAATAGAGC TTTGAACGTG TCTTGCGCTT
D. sacculus   .......... .......... .......... .......... ..........
D. tripos     .......... .......... .......... .......... .T........

                        351                ↓ITS2                  400
D. acuminata  TCGGGCTATA CCTGAAAGCA TGCCTGCGTT GGTGTCTGTA TGGCCTCATT
D. sacculus   .......... .......... .......... .......... ..........
D. tripos     .......... .......... .TTT...... .......... ...T......

                        401                                       450
D. acuminata  CAGTCAGCAA CACTAAACCT CATTGGTAGT GTGATGTCTG TGTGTCGGTG
D. sacculus   .......... .......... .......... .......... ..........
D. tripos     .......... .......... T..G...... ...C..AT.. ......AA..

                        451                                       500
D. acuminata  TGTGC-AAGG TTCACCCTTG TTACACAGAA -TGCATGTTT ATTATCGTTG
D. sacculus   .....-.... .......... .......... -......... ..........
D. tripos     A..T.A.... G......... .....AC... A......C.. T..G......

                        501                                       550
D. acuminata  CCCAACAGCT TGCTGTGCCT CTGTATAGTG GTTAACCTTC TGTTGGCACG
D. sacculus   .......... .......... .......... .......... ..........
D. tripos     -......... .........-- ....G..A.. ......T... ..........

                        551                 572
D. acuminata  AAATCCAAAG CACATGCCCC -A
D. sacculus   ......-... .......... C.
D. tripos     ..C...-... ......T.A. -.
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Alignment of ITS1-5.8S rRNA gene-ITS2 sequences for isolates of *Dinophysis acuminata*; *D. sacculus* and *D. tripos.*

REFERENCES:

Delgado, M., Garcés, E., and Camp, J. (1996). Growth and behaviour of Dinophysis sacculus from NW Mediterranean Sea. In: Yasumoto, T., Oshima, Y., and Fukuyo, Y. (Eds.) Harmful and Toxic Algal Blooms. IOC of UNESCO Publisher pp. 261-264.

Garcés, E., Delgado, M. and Camp, J. (1997). Phased cell division in natural population of *Dinophysis sacculus* and the *in situ* measurement of potential growth rate. J. Plankton Res. 19: 2067-2077.

Marín, I. Aguilera, A., Reguera, B. and Abad, J.P. (in revision). A method for preparation of DNA suitable for molecular biology applications from single cell of dinoflagelates. Biotechniques.

Marín, I., Aguilera, A., Reguera, B., and Abad, J.P. (submitted). Genetic analysis of several species of *Dinophysis* causing diarrhetic shellfish outbreaks in Galicia (NW Spain). In: Harmful Algal Blooms. Hallegraeff, G., Blackburn, S., Lewis, R., and Bolch, C. (eds.) Intergovernmental Oceanographic Commission of UNESCO.

Fernández, M.L., Reguera, B., Ramilo, I., and Martínez, A. (submitted) Toxin content of *Dinophysis acuminata, D. acuta* and *D. caudata* from the Galician Rías Bajas. In: Harmful Algal Blooms. Hallegraeff, G., Blackburn, S., Lewis, R., and Bolch, C. (eds.) Intergovernmental Oceanographic Commission of UNESCO.

Reguera B., I. Bravo, H. McCall and M.I. Reyero, 1996. Phased cell division and other biological observations in field populations of *Dinophysis* spp during cell cycle studies. In: Yasumoto T. et al. (eds.) Harmful and toxic Marine Phytoplankton, Sendai (Japón), 12-16 Julio 1995. IOC of UNESCO, pp. 257-260.

Reguera, B., and González-Gil, S. (submitted) Small cell and intermediate cell formation in species of *Dinophysis* (Dinophyceae, Dinophysiales). J. Phycol.

Reguera, B., Garcés, E., Bravo, I., Pazos, Y., and Ramilo, I. (submitted). *In situ* division rates of several species of Dinophysis estimated by a postmitotic index. Mar. Ecol. Prog. Ser.