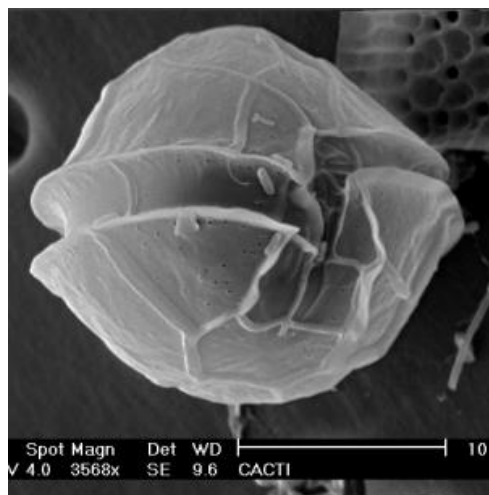


# Monitorización de condiciones oceanográficas y fitoplancton en zonas de producción

SEMINARIO INTERNACIONAL DE SALUD PUBLICA Y MAREA ROJA  
Puerto Varas, Chile. 23-24 de agosto de 2017



Jefa de Unidad de Oceanografía y Fitoplancton

Instituto Tecnológico para el Control del Medio Marino de Galicia. INTECMAR

**Yolanda Pazos**



Galicia:  
1195 km de costa  
29,575 km<sup>2</sup>  
2,737,370 habitantes

Galicia



Portugal

Image © 2005 EarthSat

© 2005 Google



Pointer 42°21'34.33" N 8°46'25.48" W

Streaming ||||| 100%

Eye alt 204.13 mi



# Rias Baixas

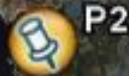


Image © 2005 EarthSat

© 2005 Google



Pointer 42°21'34.33" N 8°46'25.48" W

Streaming ||||| 100%

Eye alt 135.84 mi

Muros

Arousa

Pontevedra  P2

Vigo  Vigo



Image © 2005 EarthSat  
Image © 2005 DigitalGlobe

© 2005 Google™

Pointer 42°21'34.33" N 8°46'25.48" W

Streaming ||||| 100%

Eye alt 70.69 mi



# Ría de Arousa

## Ría de Pontevedra P2

## Ría de Vigo

Pontevedra

Vigo

Image © 2005 EarthSat  
Image © 2005 DigitalGlobe

© 2005 Google



Pointer 42°21'34.33" N 8°46'25.48" W

Streaming ||||| 100%

Eye alt 24.27 mi

# Ría de Pontevedra



Image © 2005 EarthSat  
Image © 2005 DigitalGlobe

© 2005 Google™

Pointer 42°21'34.33" N 8°46'25.48" W

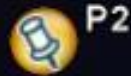
Streaming ||||| 100%

Eye alt 13.72 mi





# Estación de Bueu



**Bueu**

Image © 2005 DigitalGlobe

© 2005 Google™

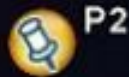


Pointer 42°21'34.33" N 8°46'25.48" W

Streaming ||||| 100%

Eye alt 31004 ft

# Polígono de bateas



P2



Image © 2005 DigitalGlobe

© 2005 Google™

Pointer 42°21'34.33" N 8°46'25.48" W

Streaming ||||| 100%

Eye alt 21605 ft



# *RIA DE PONTEVEDRA*

 Estación oceanográfica P2

Bateas  
Cultivo de mejillón



Image © 2005 DigitalGlobe

© 2005 Google™

Pointer 42°21'34.33" N 8°46'25.48" W

Streaming ||||| 100%

Eye alt 3647 ft

# SECTOR DEL MAR EN GALICIA

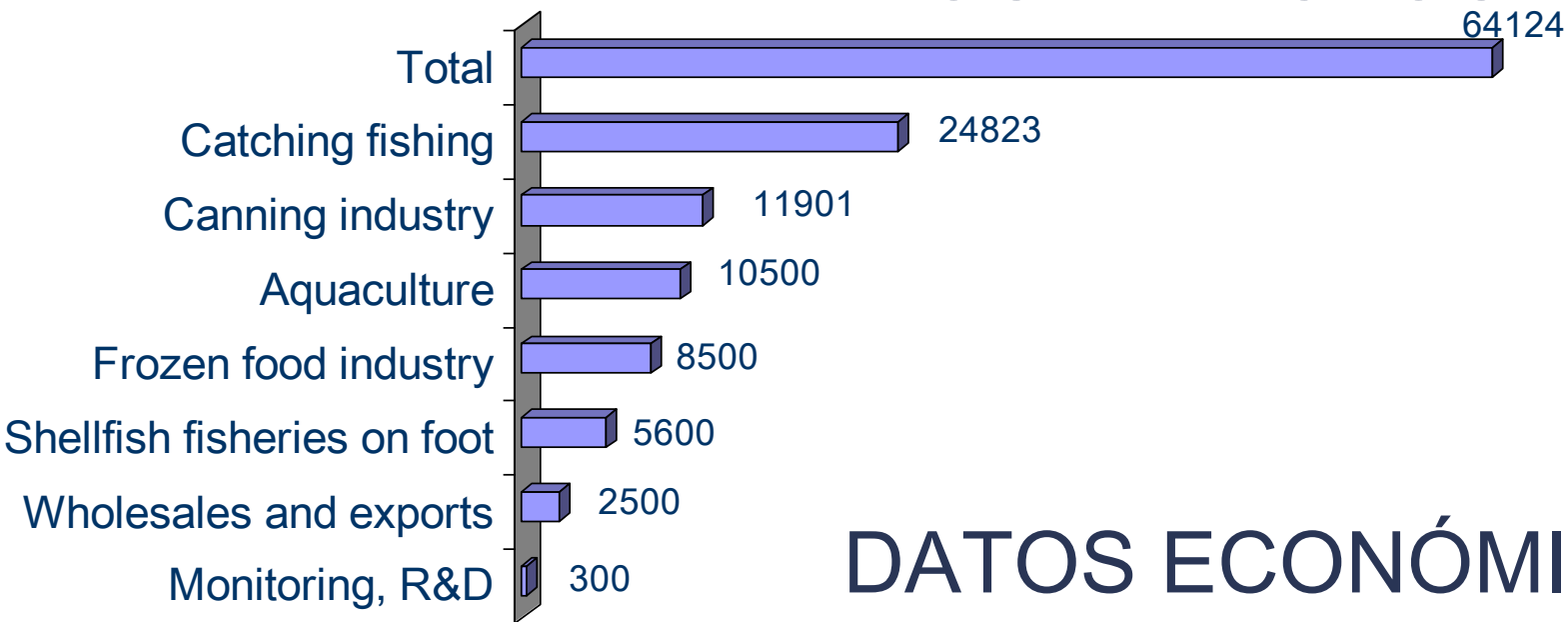




# SECTOR DEL MAR EN GALICIA

## Importancia social de la pesca

### EMPLEOS DIRECTOS



### DATOS ECONÓMICOS

Catching sector and fish processing industry 1,583 x 10<sup>6</sup> €

Galician G.D.P. 33,600 x 10<sup>6</sup> €

Amount of fishing in the G.D.P. 4.6 %

SECTOR DEL MAR EN GALICIA

# SECTOR MARISQUERO

## Mariscadoras a pie

5,600 shellfisherwomen  
(99% women)



## Mariscadores a flote

6,000 shellfishermen (mainly men)  
2,903 authorised boats





# SECTOR DEL MAR EN GALICIA

## marisqueo

La producción anual es sobre 9000 toneladas y unos 64 millones de €



### Clams:

Annual production 4,450,142 Kgs  
First sail income 45,203,639 euros



### Cockles:

Annual production 4,142,744 Kgs  
First sail income 15,564,754 euros



### Razor clams:

Annual production 155,129 Kgs  
First sail income 2,156,750 euros



### Pectinids:

Annual production 289,158 Kgs  
First sail income 1,247,944 euros

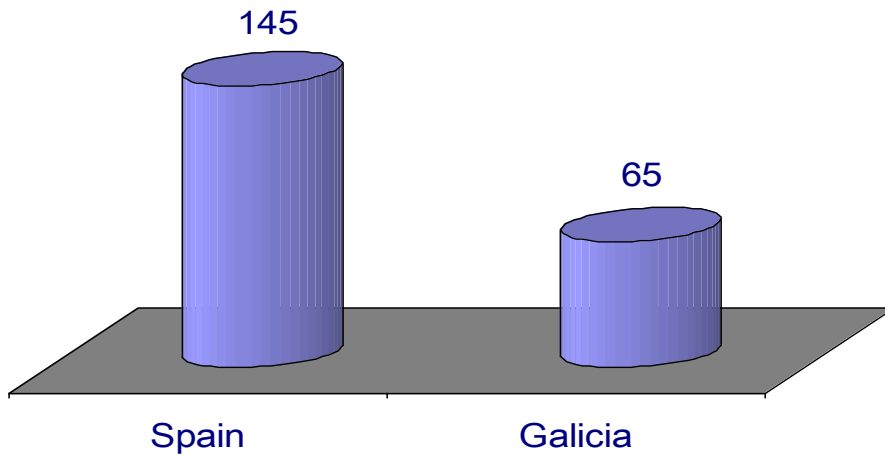


### Oysters:

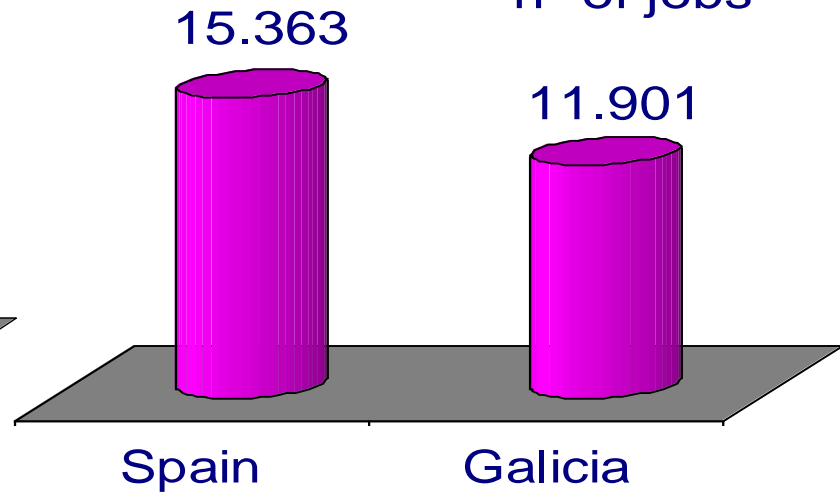
Annual production 25,709 Kgs  
First sail income 94,924 euros



nº of factories



nº of jobs



Source: ANFACO

Volume of Spanish exports	177,233 t	413 x 10 <sup>6</sup> €
Volume of Galician exports	90,386 t	284 x 10 <sup>6</sup> €

Source: ANFACO

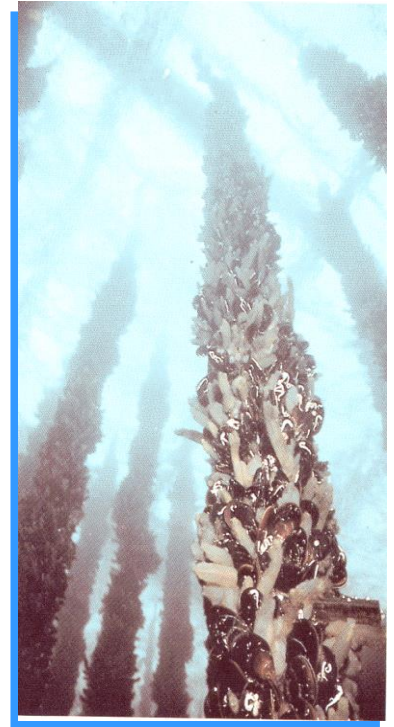
El mejillón es la especie base de la acuicultura en Galicia

La producción anual es de 250,000-275,000 toneladas año siendo

95% of de la producción española,

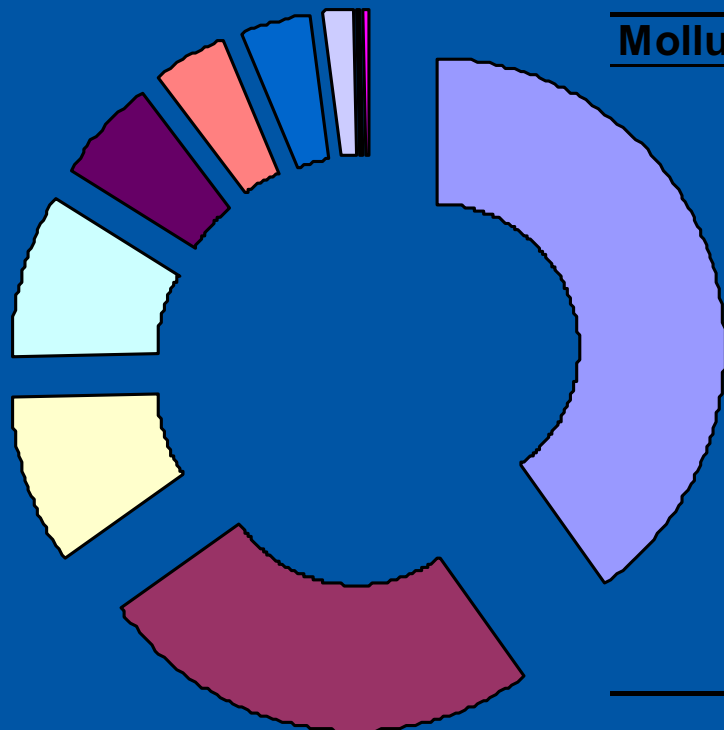
37% de la producción europea

21 % de la producción mundial





## Mollucs production in Europa in 2004 (tonnes live weight)

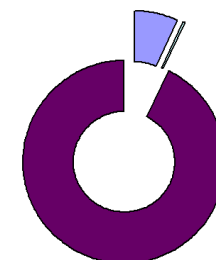
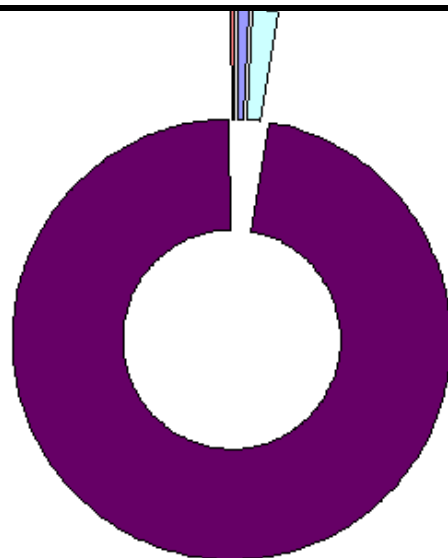


Spain	302181
France	191750
Netherlands	70400
Italy	70357
Ireland	43092
United Kingdom	32500
Greece	28803
Germany	12559
Portugal	2681
Sweden	1435
Slovenia	164
Denmark	55



# Galicia is the main producer of shellfish in Spain

	2013	2013	2013
	<i>Mytilus galloprovincialis</i>	<i>Ruditapes philippinarum</i>	10 <sup>3</sup> Tonnes
■ Andalucía	1601940	102280	1,70
■ Baleares	129800		0,13
■ Cantabria		650	0,00
■ Cataluña	3436280	5270	3,44
■ Galicia	183169490	1384910	184,55
■ Valencia	607430		0,61
<b>TOTAL Kg</b>	<b>188944940</b>	<b>1493110</b>	<b>190,44</b>





# Rafts for mussel farming

Inshore fishing boats

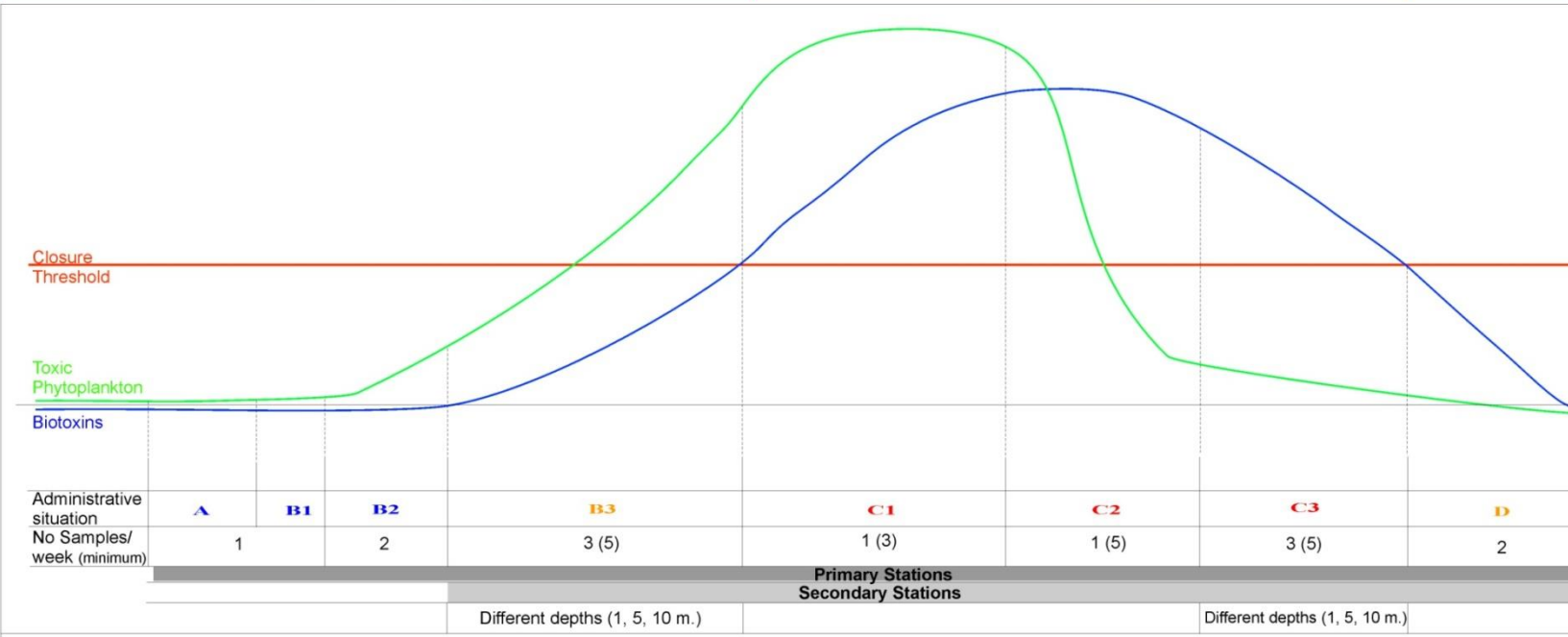
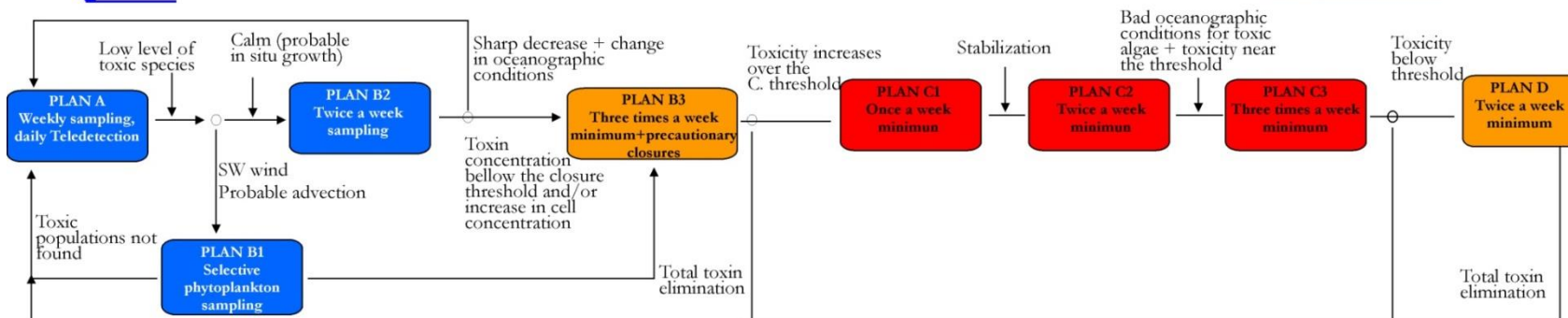
Ria de Arousa

[www.farodevigo.es](http://www.farodevigo.es)





[www.intecmar.gal](http://www.intecmar.gal)





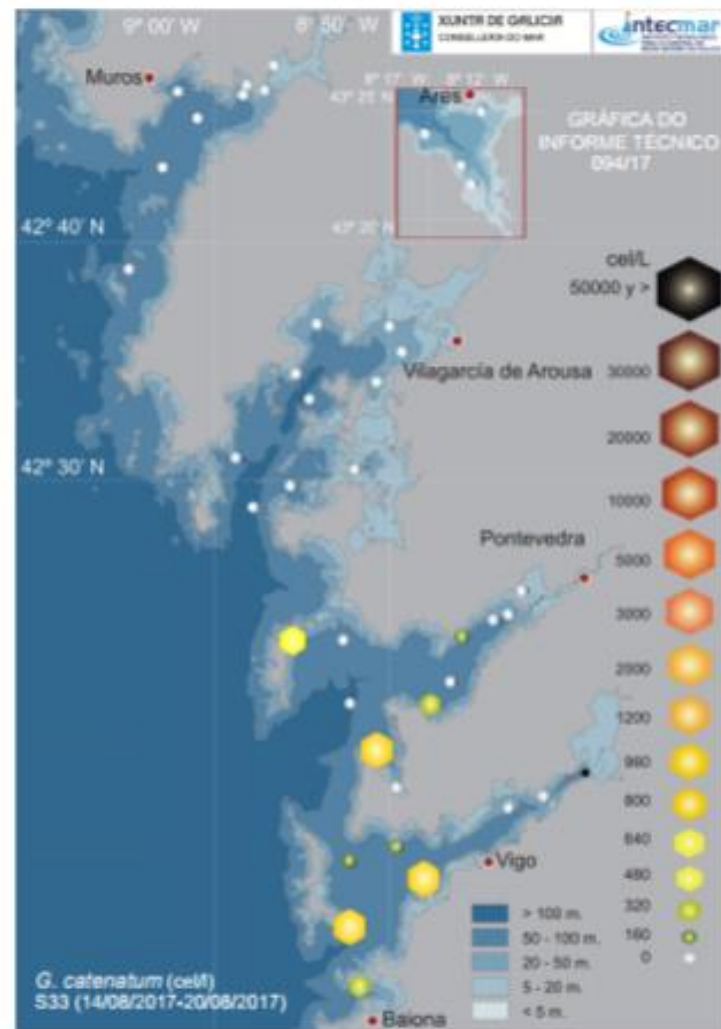
**SITUACION DAS ZONAS  
(Viveiros Flotantes)**

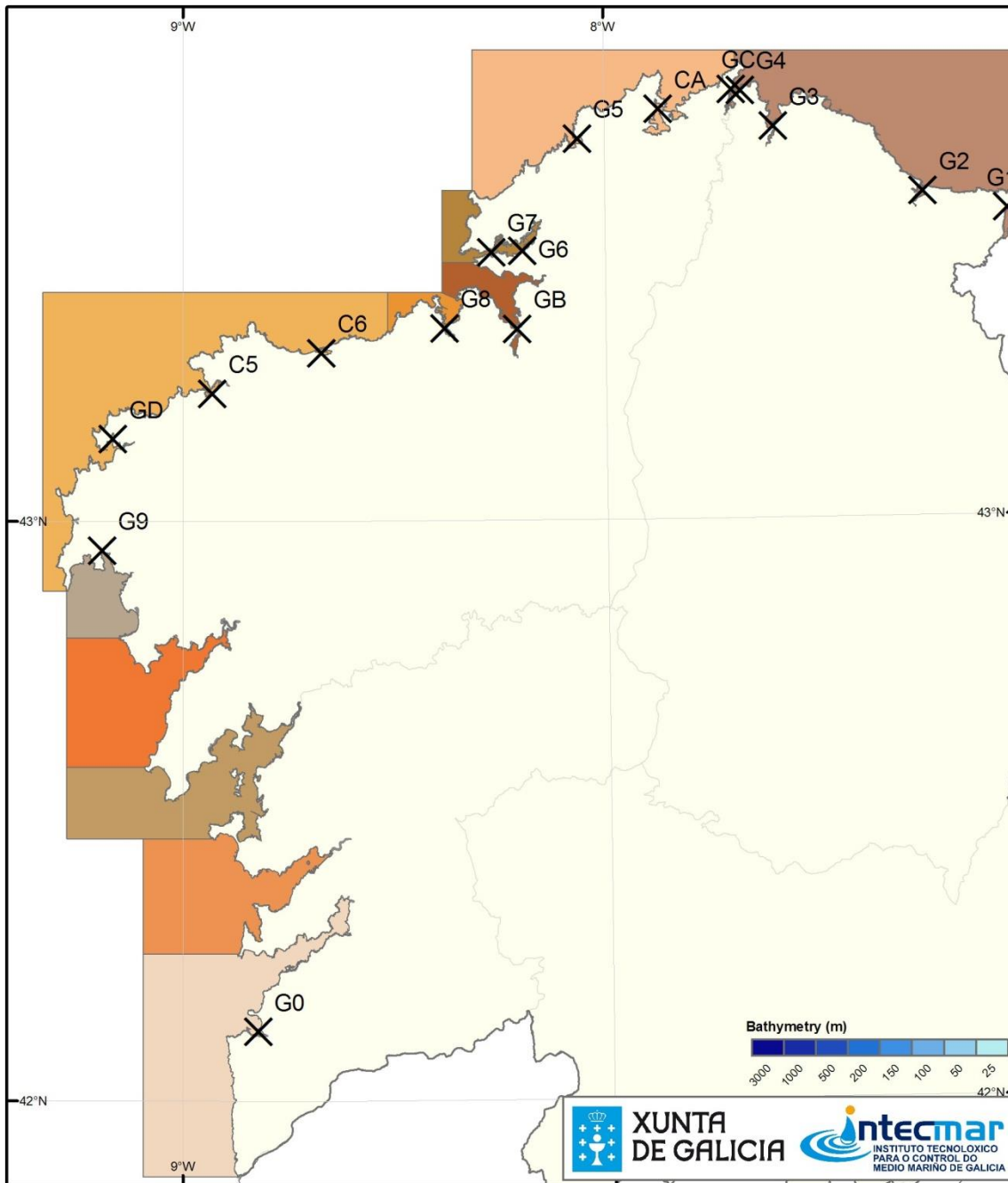
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INFORME Nº: SZ000337/17

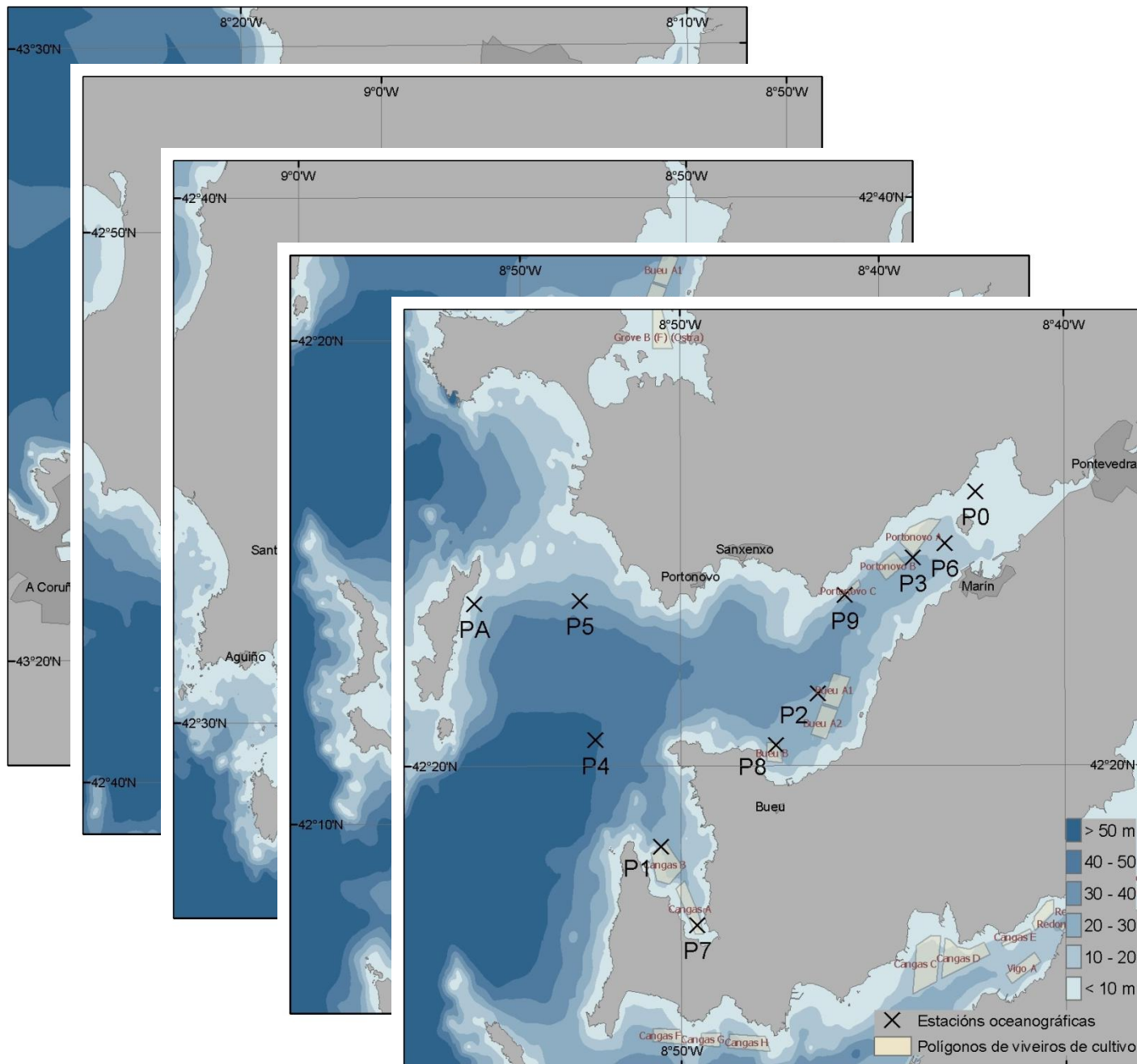
Folla 2 de 2

Orde do 14 de novembro de 1995 (DOG Nº 221 do 17/11/1995)  
Orde do 31 de outubro de 1989 (DOG Nº 235 do 11/12/1989)  
Orde do 28 de outubro de 1996 (DOG Nº 226 do 19/11/1996)  
Orde do 14 de marzo de 1997 (DOG Nº 64 do 04/04/1997)

RIA	ZONA	SUB-ZONA	POLIGONO	Plan de Actuación			Situación Administrativa
				PSP	Lipofílicas	ASP	
Muros-Noia	I	I	Muros B				Aberta (06/06/2017)
Muros-Noia	II	II	Muros A				Aberta (03/06/2017)
Muros-Noia	III	III	Noia A				Aberta (27/05/2017)
Muros-Noia	IV	IV	Muros C				Aberta (06/06/2017)
Pontevedra	I	I.1	Cangas A	✓	B3	B2 B1	Aberta (15/06/2017)
Pontevedra	I	I.2	Cangas B	✓	B3	B2 B1	Aberta (17/06/2017)
<b>Pontevedra</b>	<b>II</b>	<b>II.1</b>	Bueu B	✗	B3	<b>*B2</b> B1	Aberta (22/08/2017)
Pontevedra	II	II.2	Bueu A2	✗	C3	B3 B1	Pechada (11/08/2017)
Pontevedra	II	II.3	Bueu A1		C1	C1 B1	Pechada (07/08/2017)
Pontevedra	III	III.1	Portonovo A	✓	B3	B2 B1	Aberta (06/06/2017)
Pontevedra	III	III.2	Portonovo B	✓	B3	B3 B1	Aberta (06/06/2017)
Pontevedra	III	III.3	Portonovo C		C1	B3 B1	Pechada (22/08/2017)



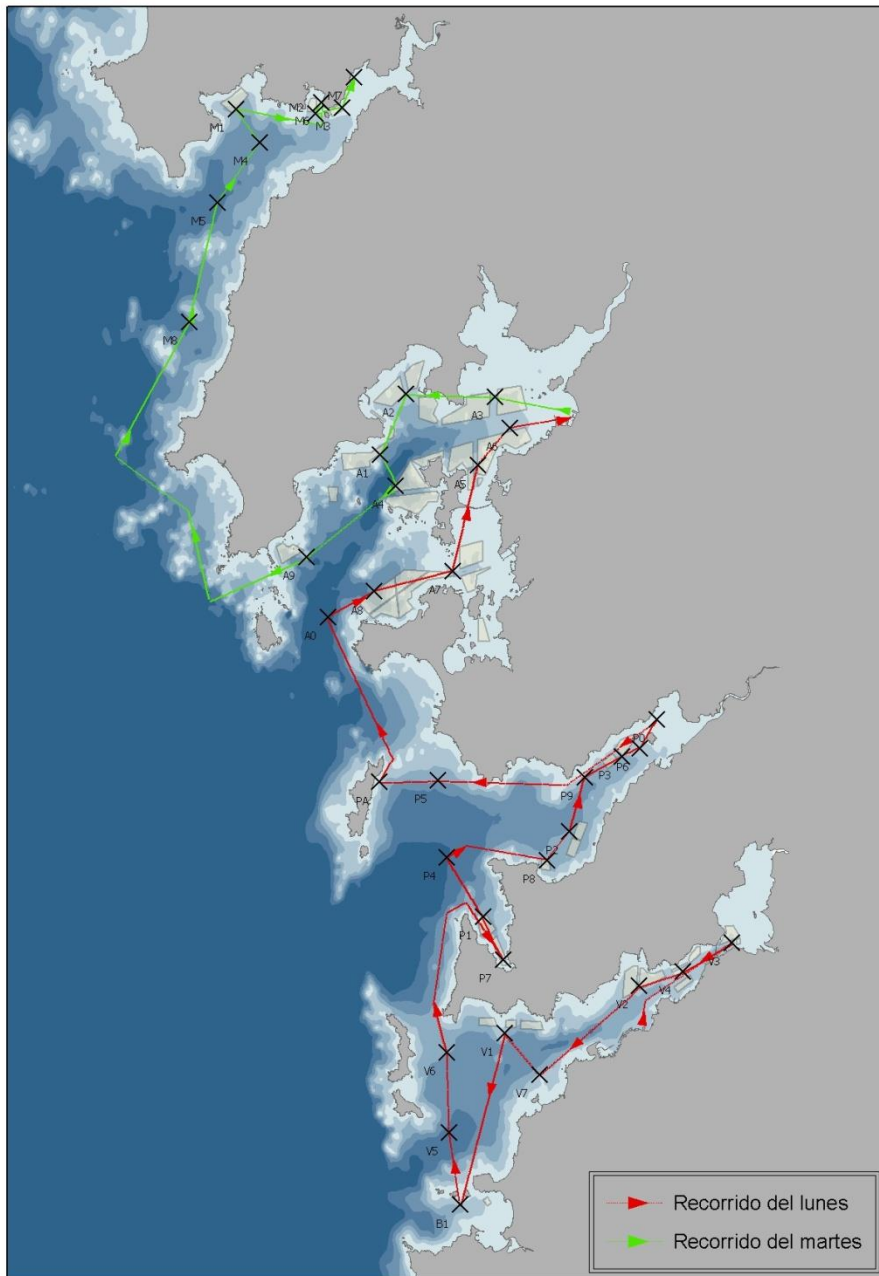






# B/O José María Navaz

## Instituto Español de Oceanografía











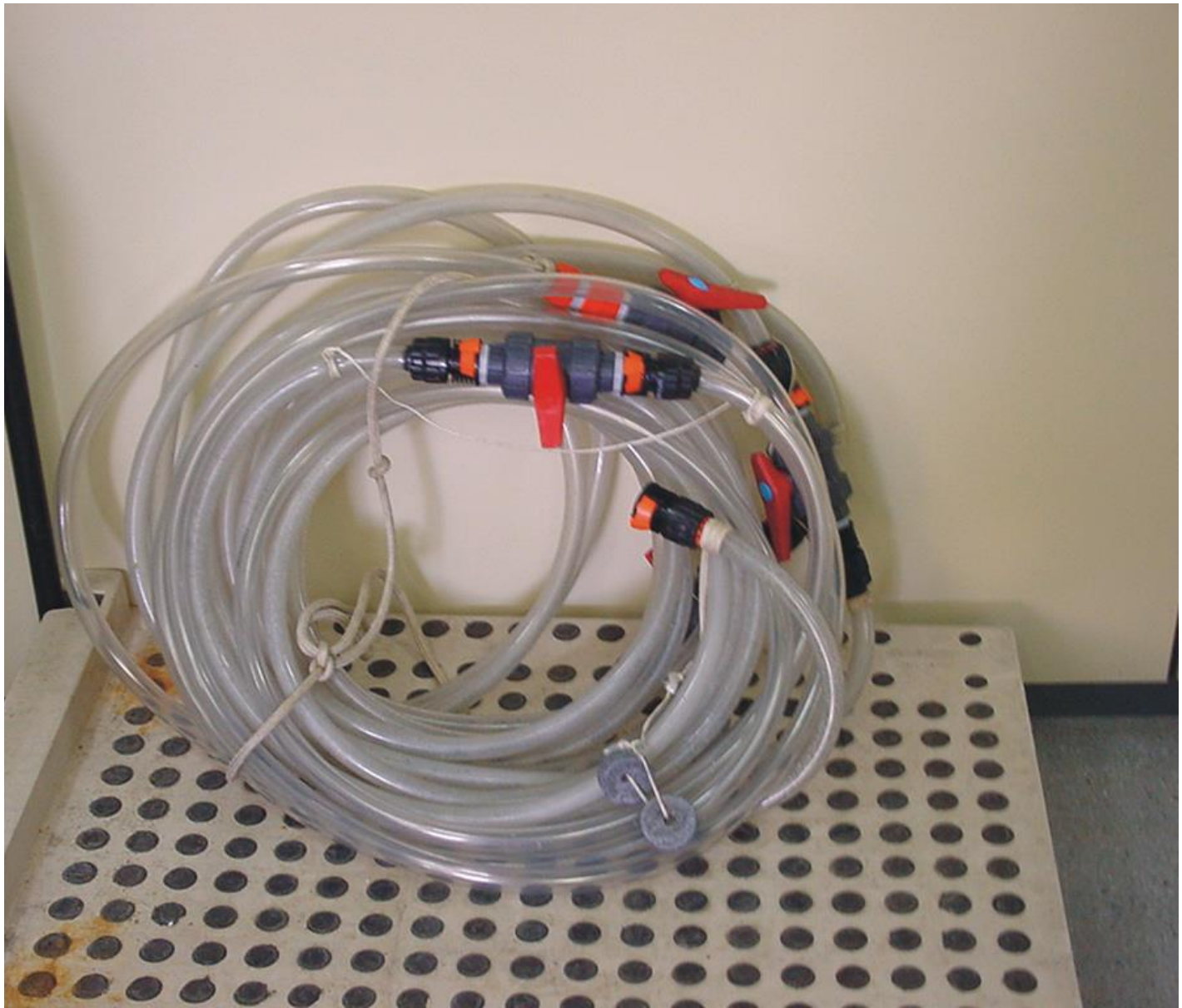








































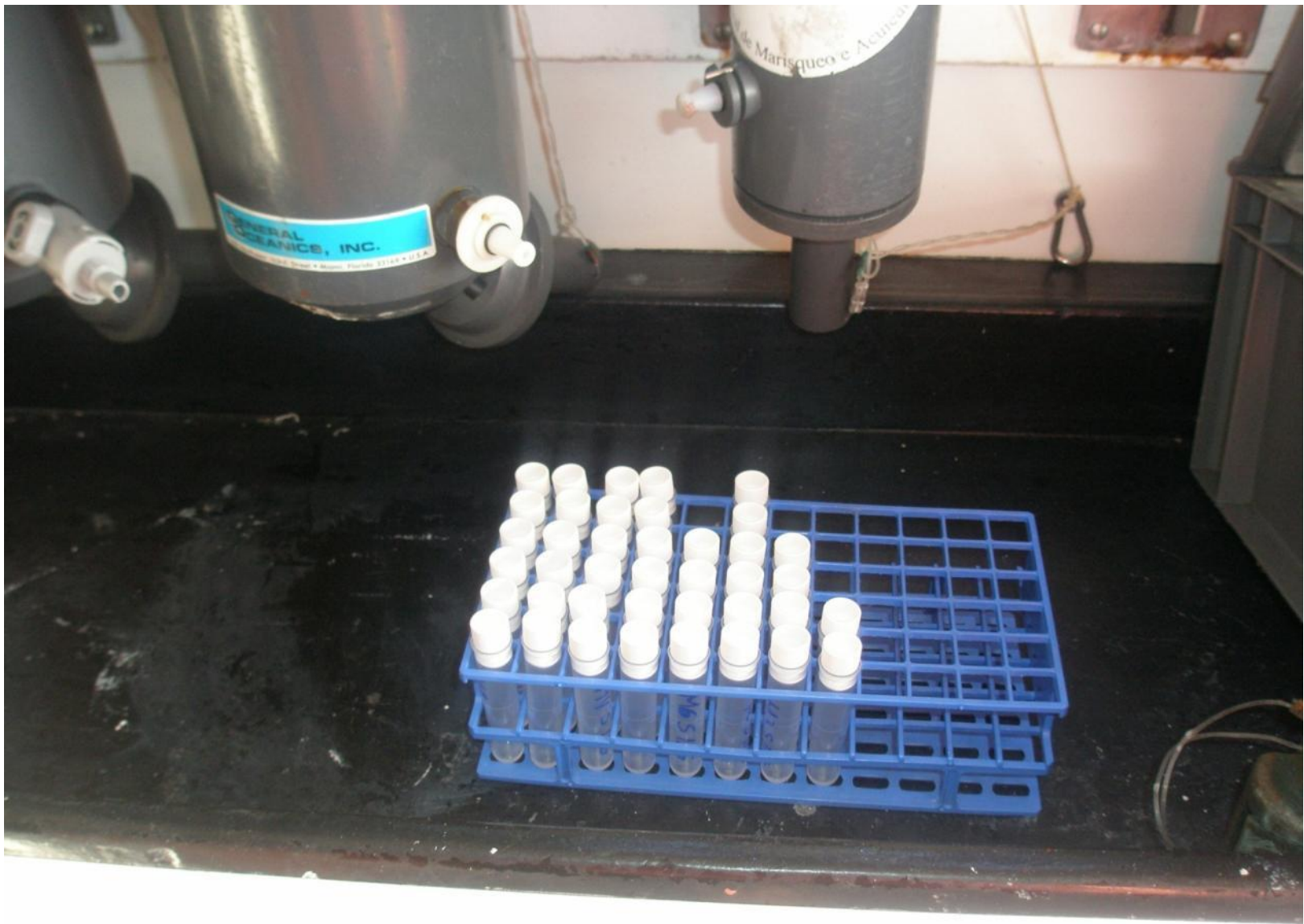


































Red tide off Moon Bay in Shenzhen, S China



# Sample Bottles



Edler and Elbrätcher in Karlson et al 2010

# Preservation agents

## Recipes for Lugol's iodine solution

Acidic	Alkaline	Neutral
20 g potassium iodide (KI)	20 g potassium iodide (KI)	20 g potassium iodide (KI)
10 g iodine (I <sub>2</sub> )	10 g iodine (I <sub>2</sub> )	10 g iodine (I <sub>2</sub> )
20 g conc. acetic acid	50 g sodium acetate	200 mL distilled water
200 mL distilled water	200 mL distilled water	

Utermöhl 1958, Willén 1962, Andersen and Thronsen, 2004

# Preservation agents

Recipe for neutral formaldehyde: Filter after one week to remove any precipitates.

## Neutral formaldehyde

500 mL 40% formaldehyde

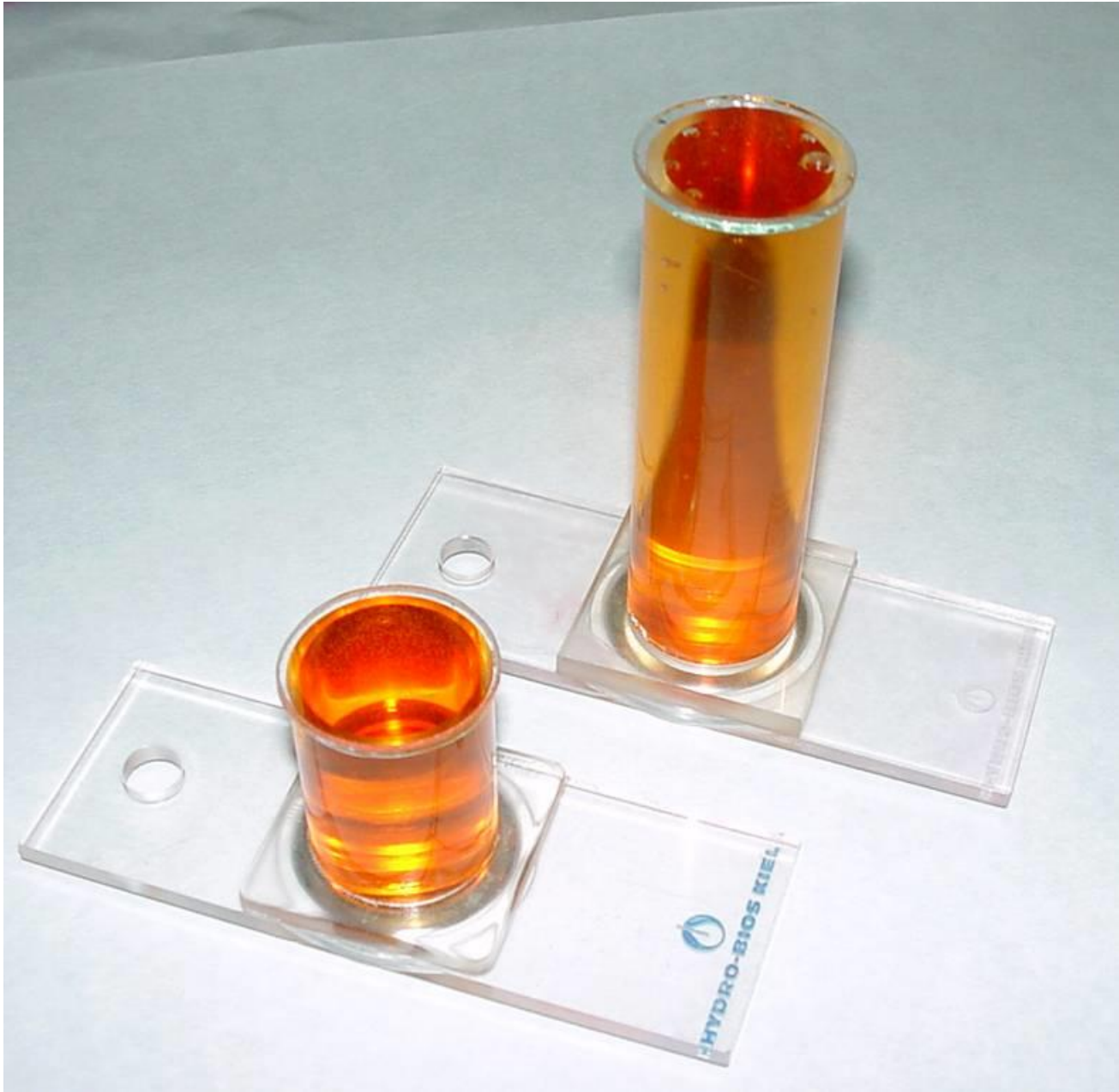
500 mL distilled water

100 g hexamethylenetetramid

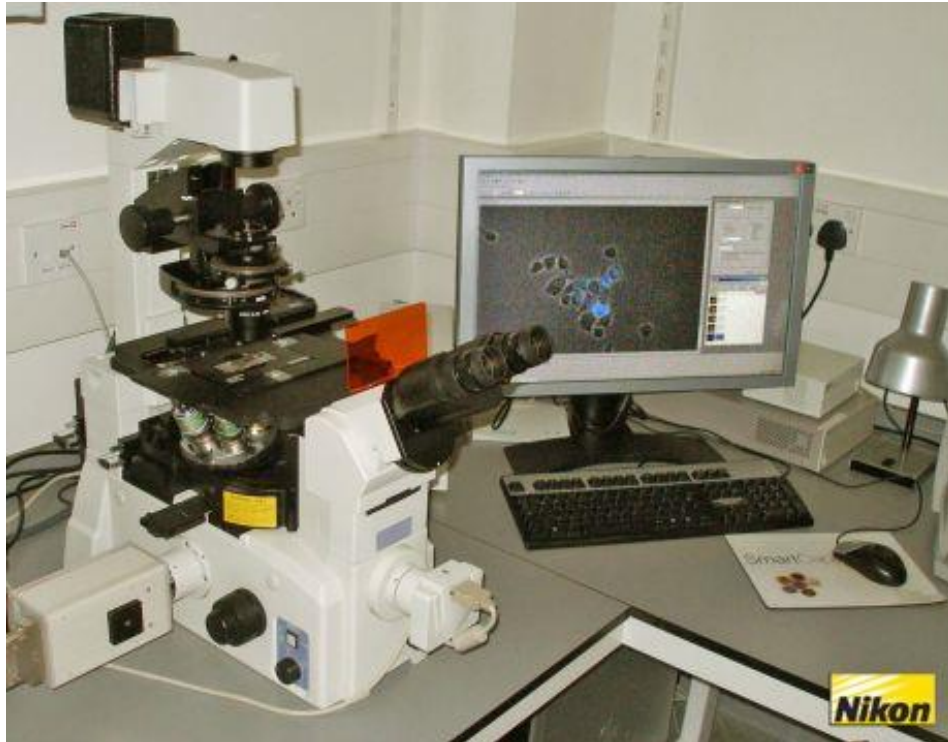
pH 7.3 – 7.9

Thronsdn 1978, Edler 1979, Andersen and Thronsdn 2004





# The inverted microscope



Phase- and/or differential interference-contrast for general phytoplankton

Bright-field for coccolithophorids

Epifluorescence for samples stained

Autofluorescence in live samples (chlorophyll)

Fixed sample fluorescence (calcofluor for plates of dinoflagellates) DAPI (staining of nucleic acids)...



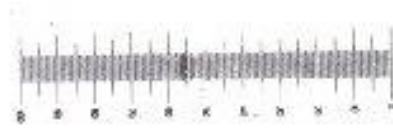
[institutobiomedicina.unileon.es](http://institutobiomedicina.unileon.es)



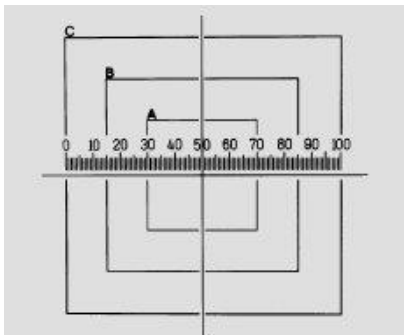
Basic Objectives: 10x, 20x, 40x, 100x oil

# The inverted microscope

One eyepiece equipped with a calibrated ocular micrometer



One eyepiece equipped with a graticule® such as a square field or grids



# Laboratory facilities



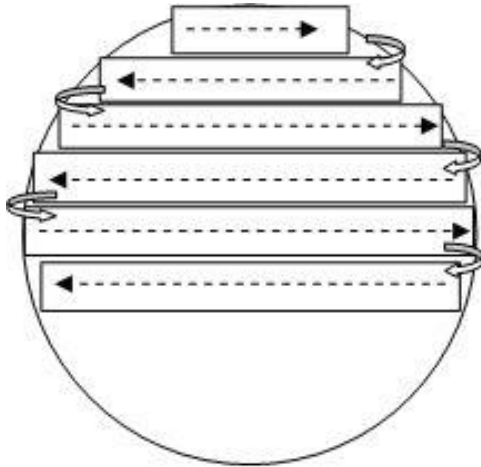
[www.marine.ie](http://www.marine.ie)

Amenities for storing, handling, mixing and pouring samples  
For washing sedimentation chambers

Facilities to stored samples in cool and dark contidions

During sedimentation should be placed horizontal and solid surfact to prevent any non random accumulaton of phytoplankton cells

# Counting procedure

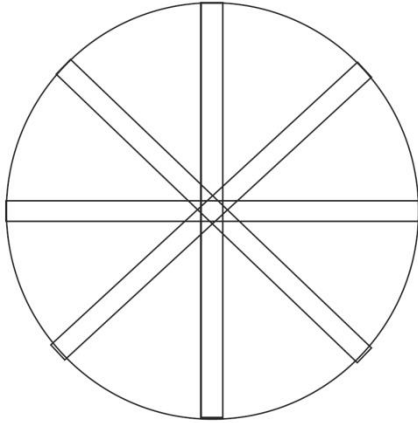


Counting of the whole chamber bottom with the parallel eyepiece threads indicating the counted area.

Organisms should be identified to the lowest taxonomic level that time and skill permits (Hasle 1978)



# Counting procedure



Counting of diameter transects

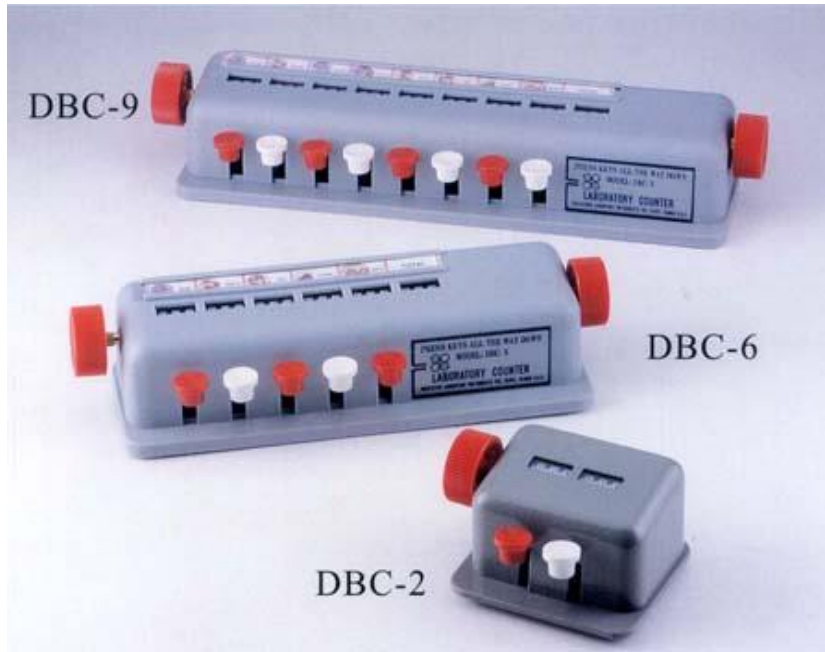
Recommended magnification for counting of different size classes of phytoplankton  
(Edler, 1979, Andersen and Thronsen 2004)

# Counting procedure

Size class	Magnification
0.2-2.0 $\mu\text{m}$ (picoplankton)	1000 x
2.0 – 20.0 $\mu\text{m}$ (nanoplankton)	100 – 400 x
>20 $\mu\text{m}$ (microplankton)	100 x

Recommended magnification for counting of different size classes of phytoplankton (Edler, 1979, Andersen and Throndsen 2004)

# Medical blood cell counter







IN THE GALICIAN RIAS MONITORING BY  
INTECMAR

25 mL

100 X

The whole chamber bottom

Photo Pazos. INTECMAR

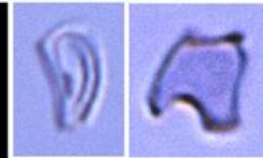
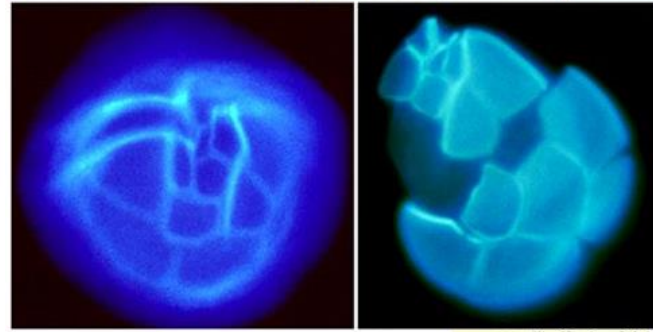
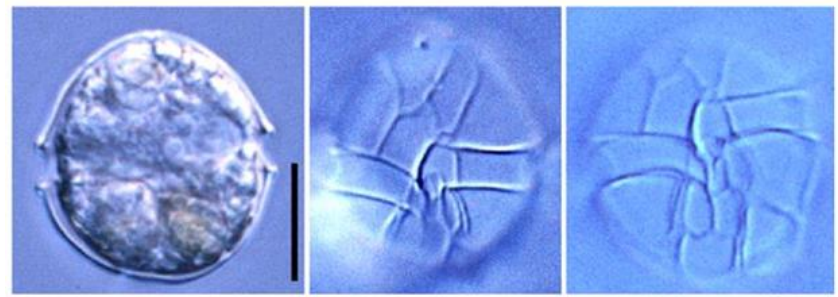
**PSP causing *Gymnodinium* and DSP causing *Dinophysis*  
in Galician waters**

Photomicrograph offered by the Marine Environmental  
Quality Control Center (CCCMM) of Galicia, NW Spain

WESTPAC-HAB  
IOC Harmful Algal Bloom Programme

**R0014**





*Alexandrium minutum*



*Gymnodinium catenatum*

Photomicrographs by Sadaaki Yoshimatsu  
Tomotoshi Okaichi and Haruyoshi Takayam.

IN THE GALICIAN RIAS  
MONITORING BY INTECMAR:

*Alexandrium minutum*

25 mL 200 X

2 diametral transects

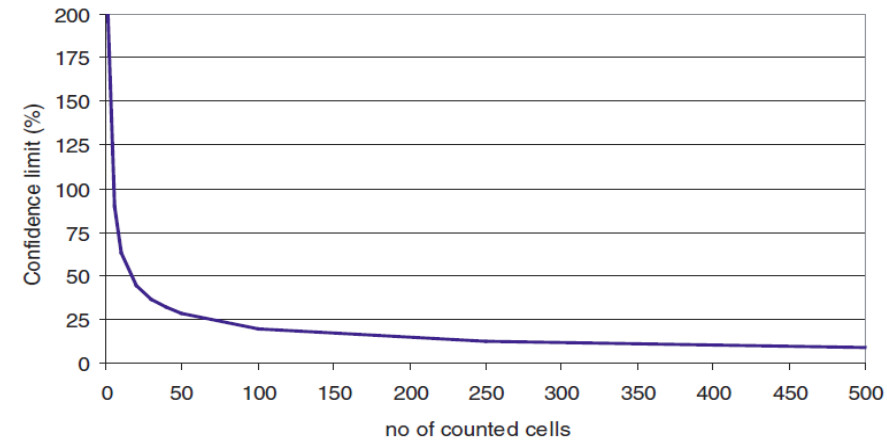
*Gymnodinium catenatum*

25 mL 100 X

The whole chamber bottom

# How many cells to count?

No of counted cells	Confidence limit +/- (%)	Absolute limit if cell density is estimated at 500 cells L <sup>-1</sup>
1	200	500 ± 1000
2	141	500 ± 705
3	116	500 ± 580
4	100	500 ± 500
5	89	500 ± 445
6	82	500 ± 410
7	78	500 ± 380
8	71	500 ± 355
9	67	500 ± 335
10	63	500 ± 315
15	52	500 ± 260
20	45	500 ± 225
25	40	500 ± 200
50	28	500 ± 140
100	20	500 ± 100
200	14	500 ± 70
400	10	500 ± 50
500	9	500 ± 45
1000	6	500 ± 30



Relationship between number of cells counted and confidence limit at the 95% significance level

$$Precision\ \% = \frac{2 * 100}{\sqrt{\text{number of cells counted}}}$$

Relationship between number of cells counted and confidence limit at 95% significance level (Edler 1979, Andersen and Thronsen 2004)



## Final calculations

$$\text{Cells } L^{-1} = N * \left( \frac{A_t}{A_c} \right) * \frac{1000}{V}$$

$$\text{Cells } mL^{-1} = N * \left( \frac{A_t}{A_c} \right) * \frac{1}{V}$$

V: volume of counting chamber (mL)

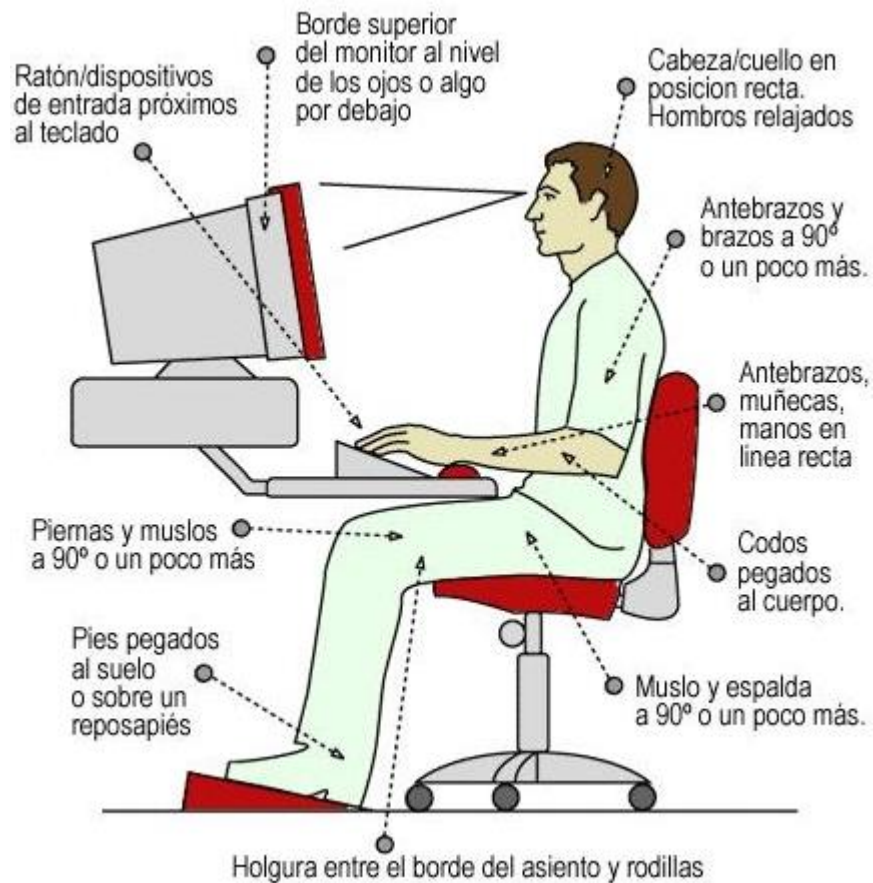
At: total area of the counting chamber (mm<sup>2</sup>)

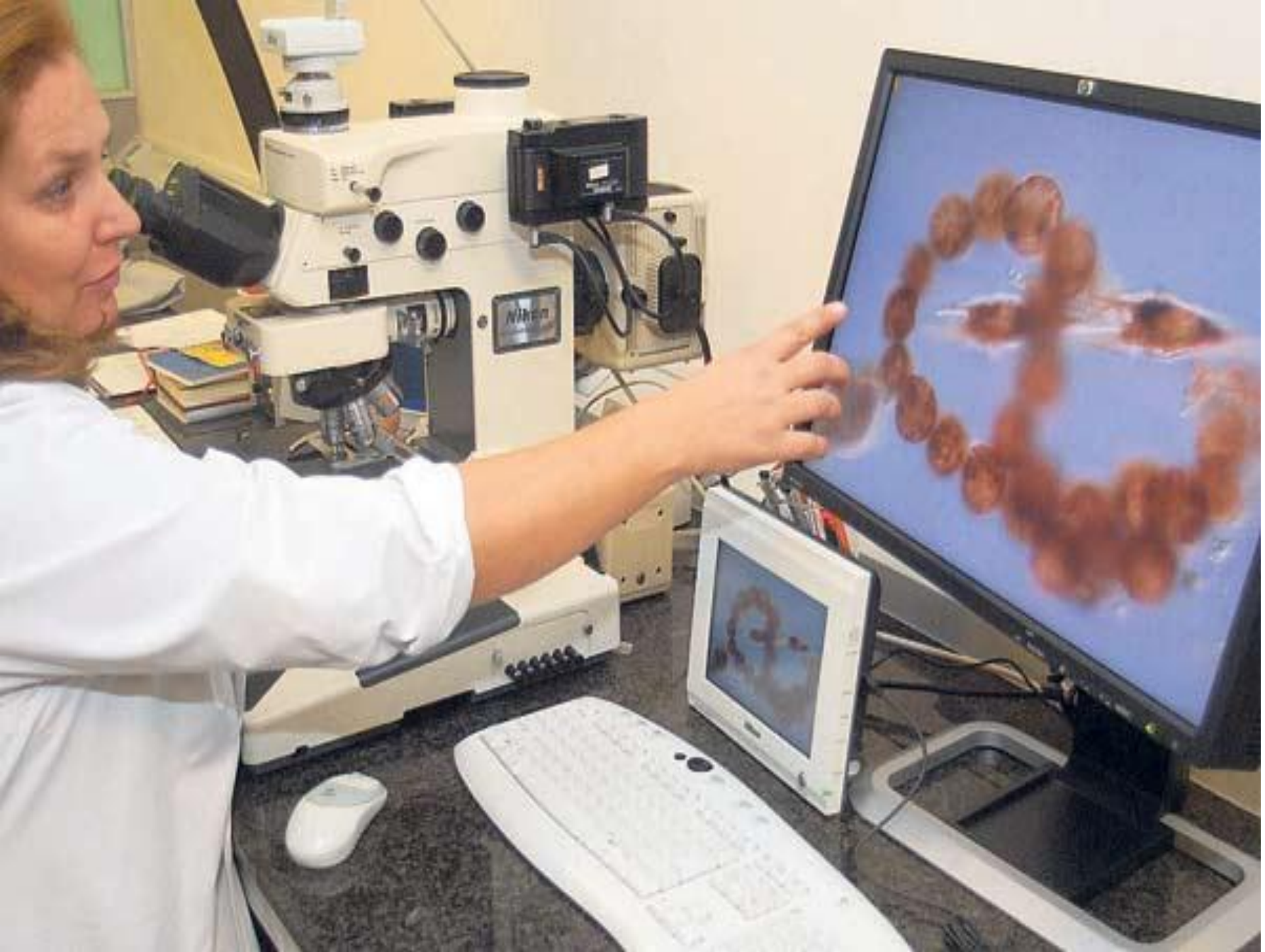
Ac: counted area of the counting chamber (mm<sup>2</sup>)

N: number of units (cells) of specific species counted

C: concentration (density) of the specific species

## Cuidar la posición y la óptica





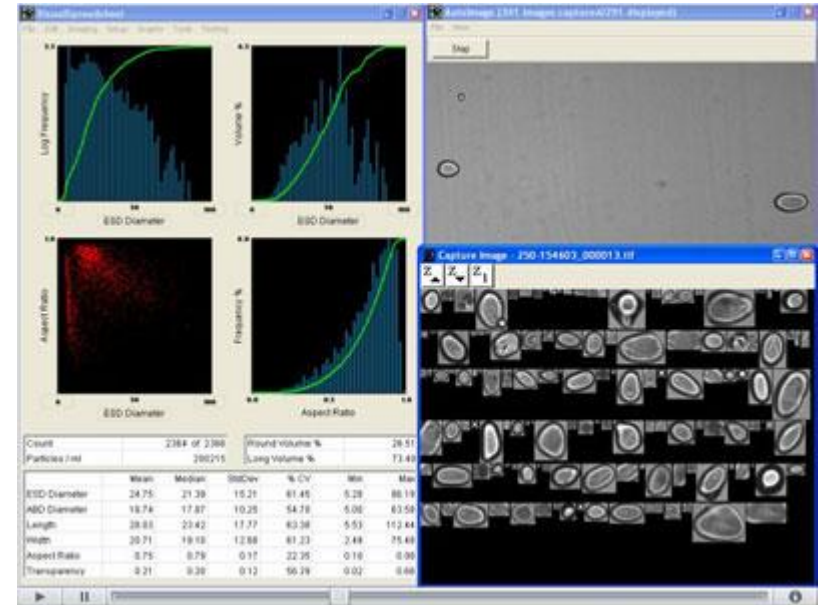
Yolanda Pazos, *Gymnodinium catenatum* and two *Ceratium furca*

Iñaki Abella. FARO DE VIGO

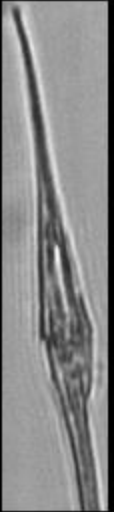
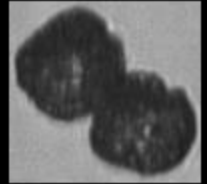
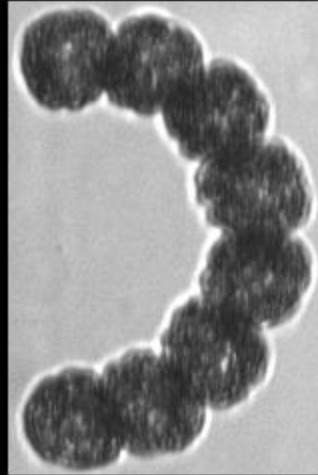
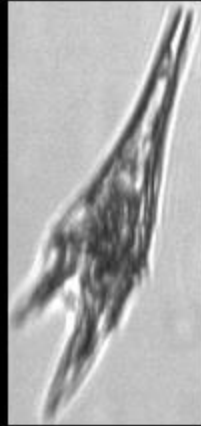
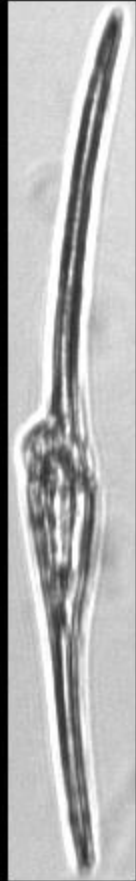
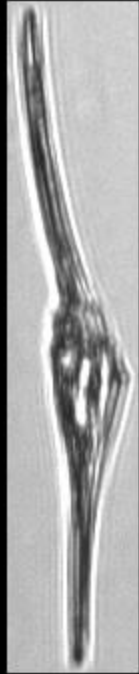
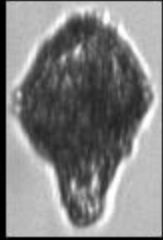
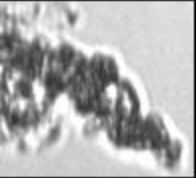
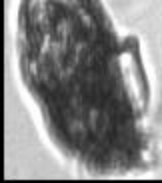
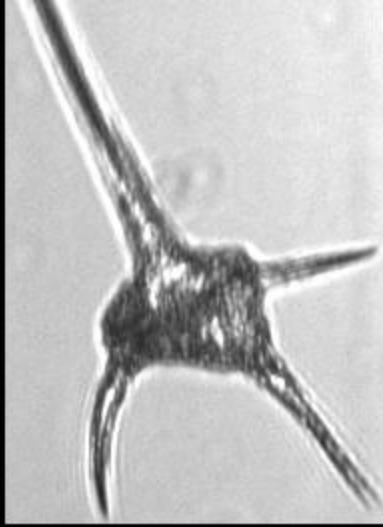
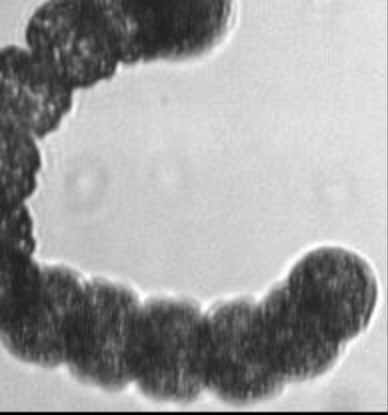


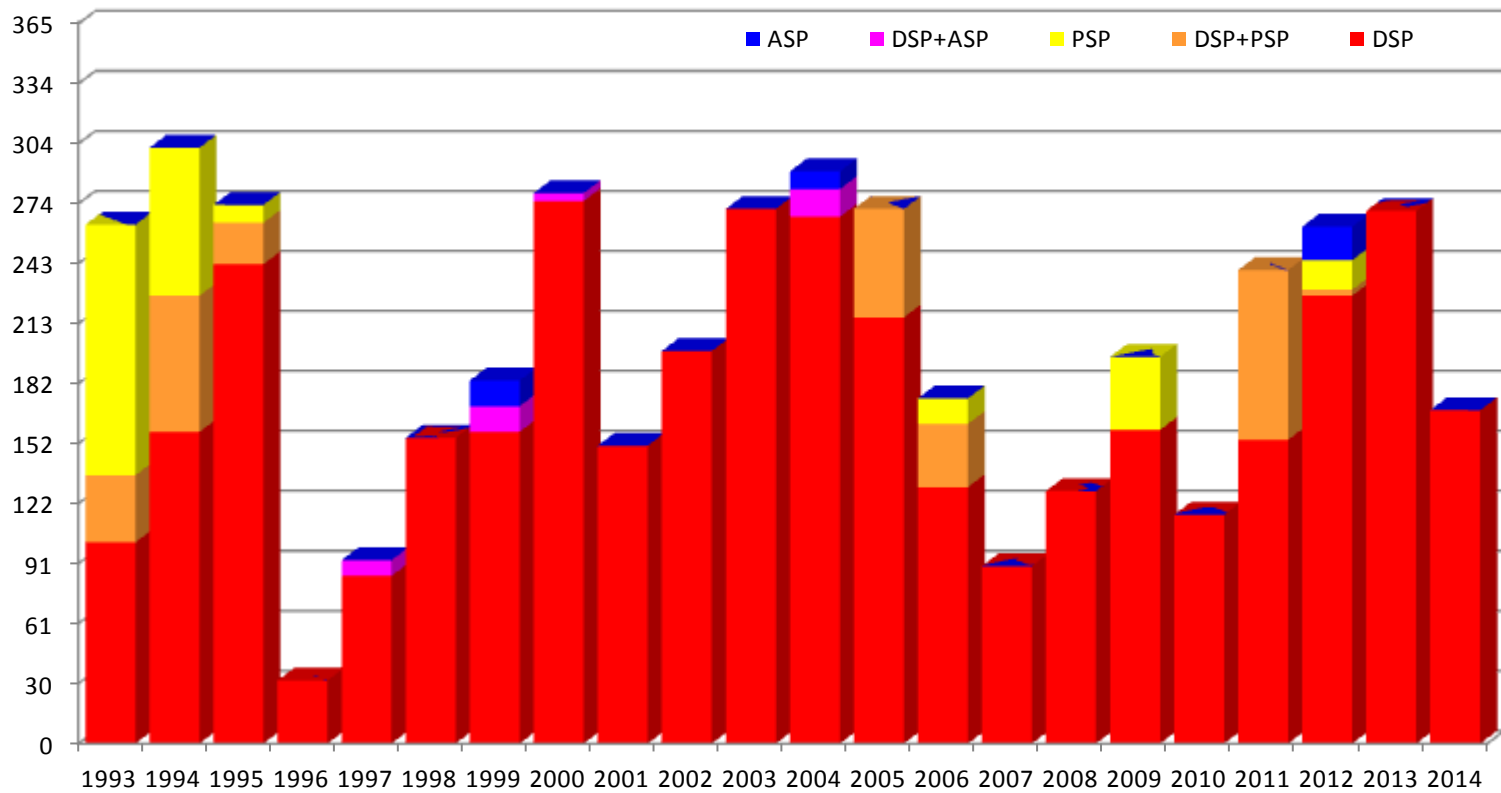
# FlowCAM®

Gathers and stores all passing data, including particle size, particle images, and time of collection



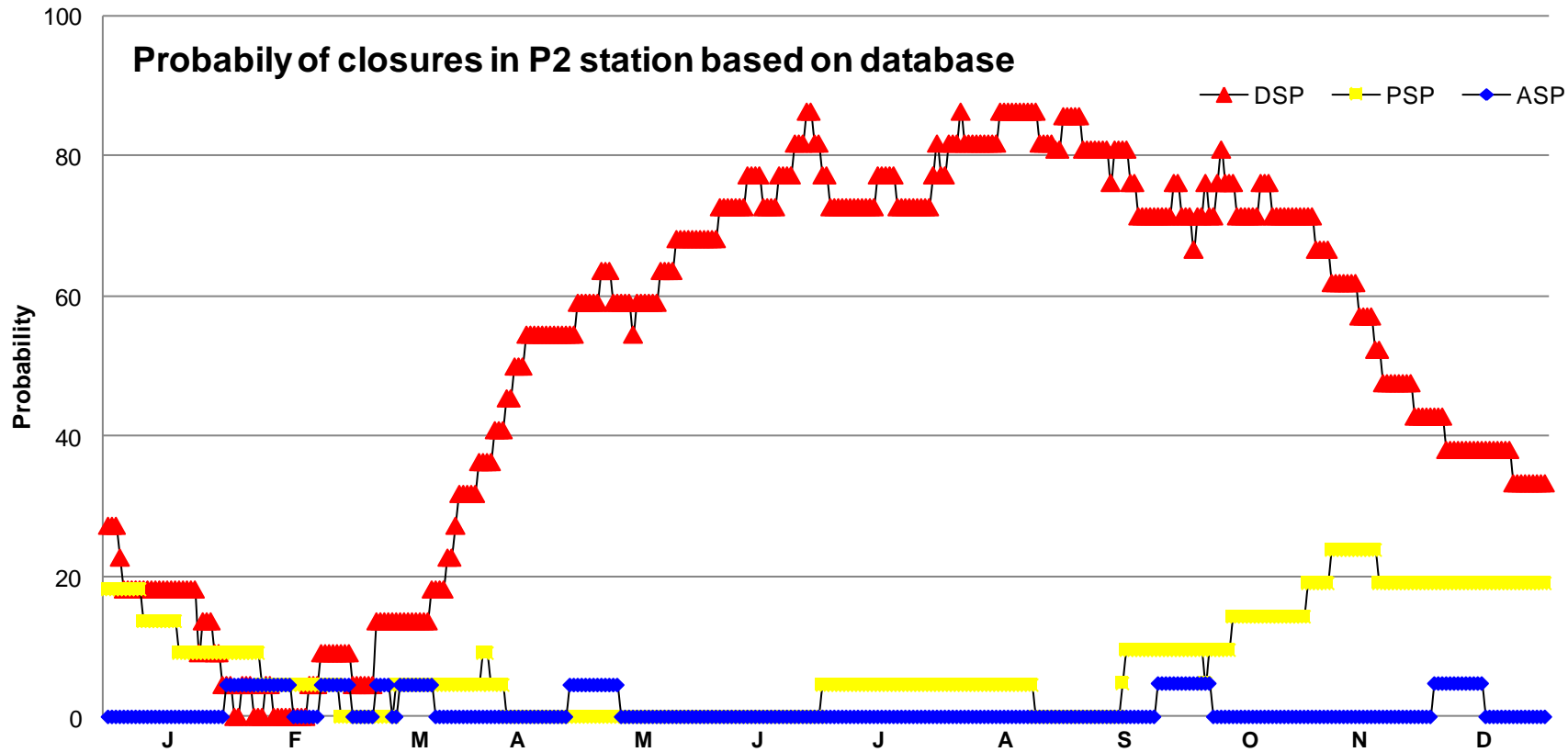
[blogs.oregonstate.edu/](http://blogs.oregonstate.edu/)







1993- 2017



Positive bioassay +      Negative bioassay -

*Dinophysis acuminata* + *D. acuta* (cellL<sup>-1</sup>)

# Trigger levels?

1-The number of cases analysed for both variables is very high: 7606

2-The mouse bioassay analysis for the mussels and *Dinophysis acuminata* + *D. acuta* cell counts in seawater are analysis completely independent

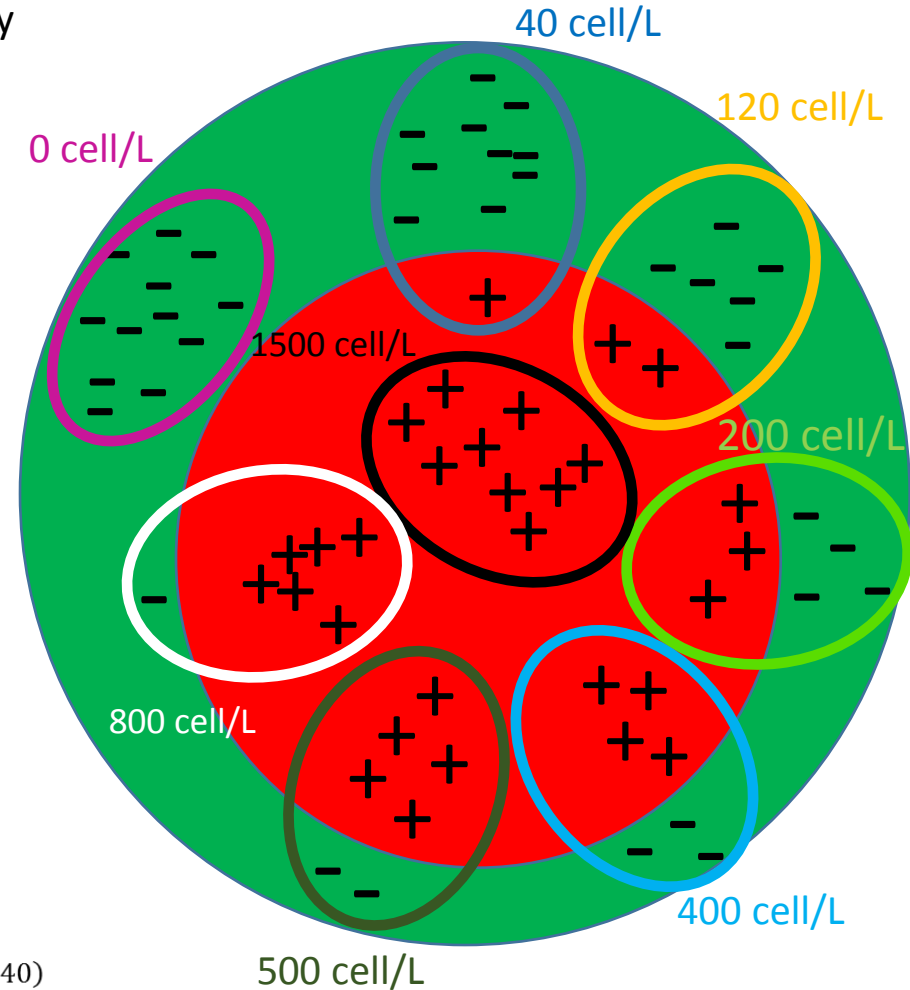
## CONDITIONAL PROBABILITY

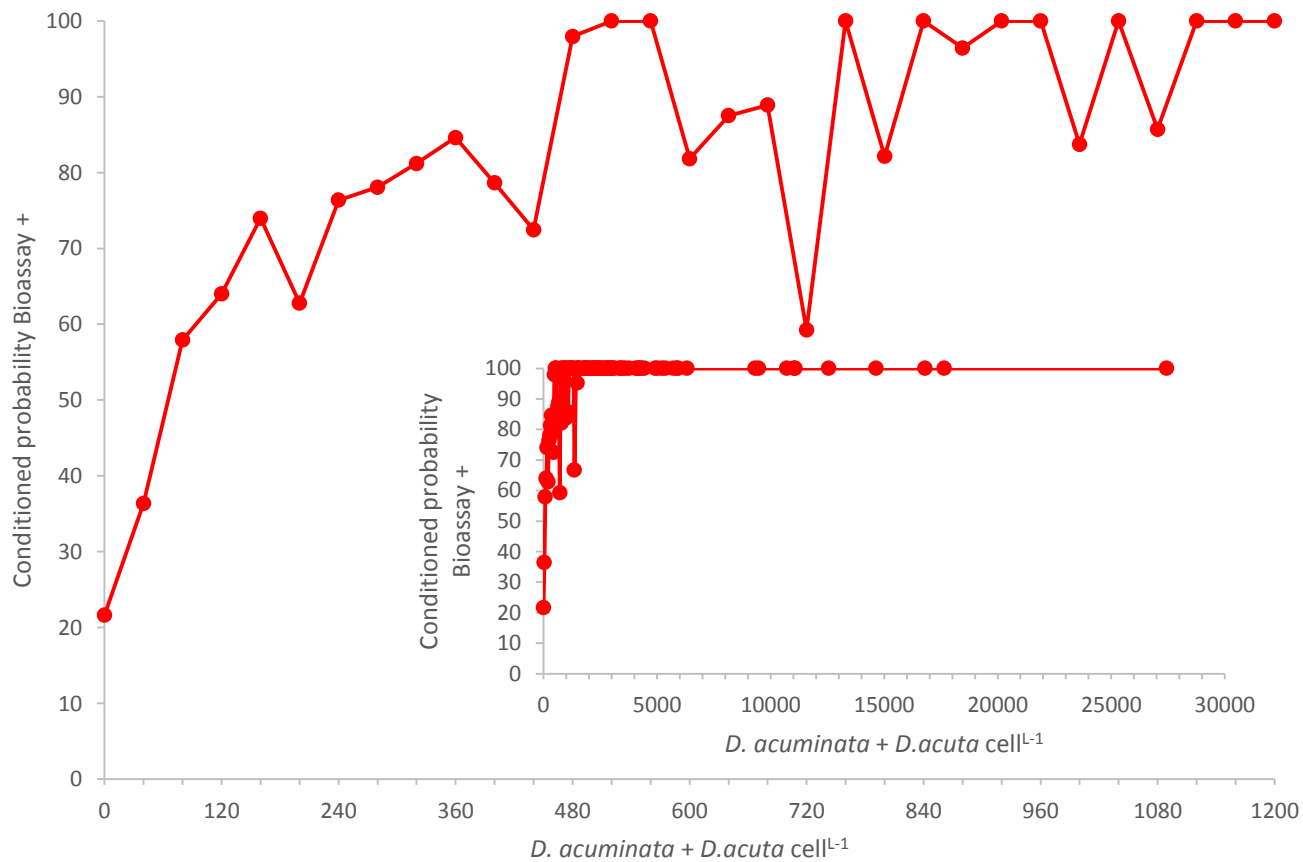
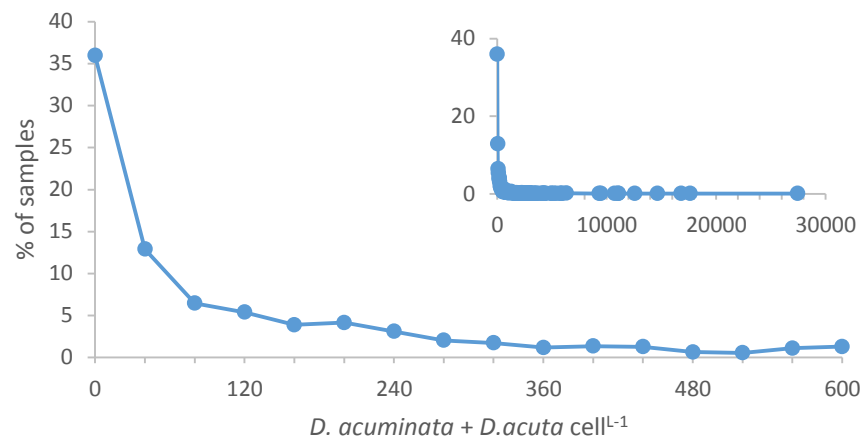
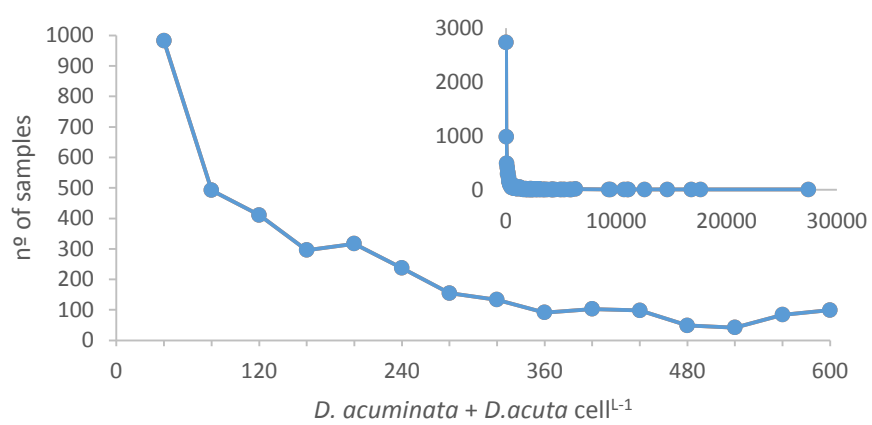
$$P(\text{Bioassay}^+ / \text{Dinophysis } 40) = \frac{P(\text{Bioassay}^+ \cap \text{Dinophysis } 40)}{P(\text{Dinophysis } 40)}$$

$$P(\text{Bioassay}^+ / \text{Dinophysis } 120) = \frac{P(\text{Bioassay}^+ \cap \text{Dinophysis } 120)}{P(\text{Dinophysis } 120)}$$

- 
- 
- 

$$P(\text{Bioassay}^+ / \text{Dinophysis } 27440) = \frac{P(\text{Bioassay}^+ \cap \text{Dinophysis } 27440)}{P(\text{Dinophysis } 27440)}$$

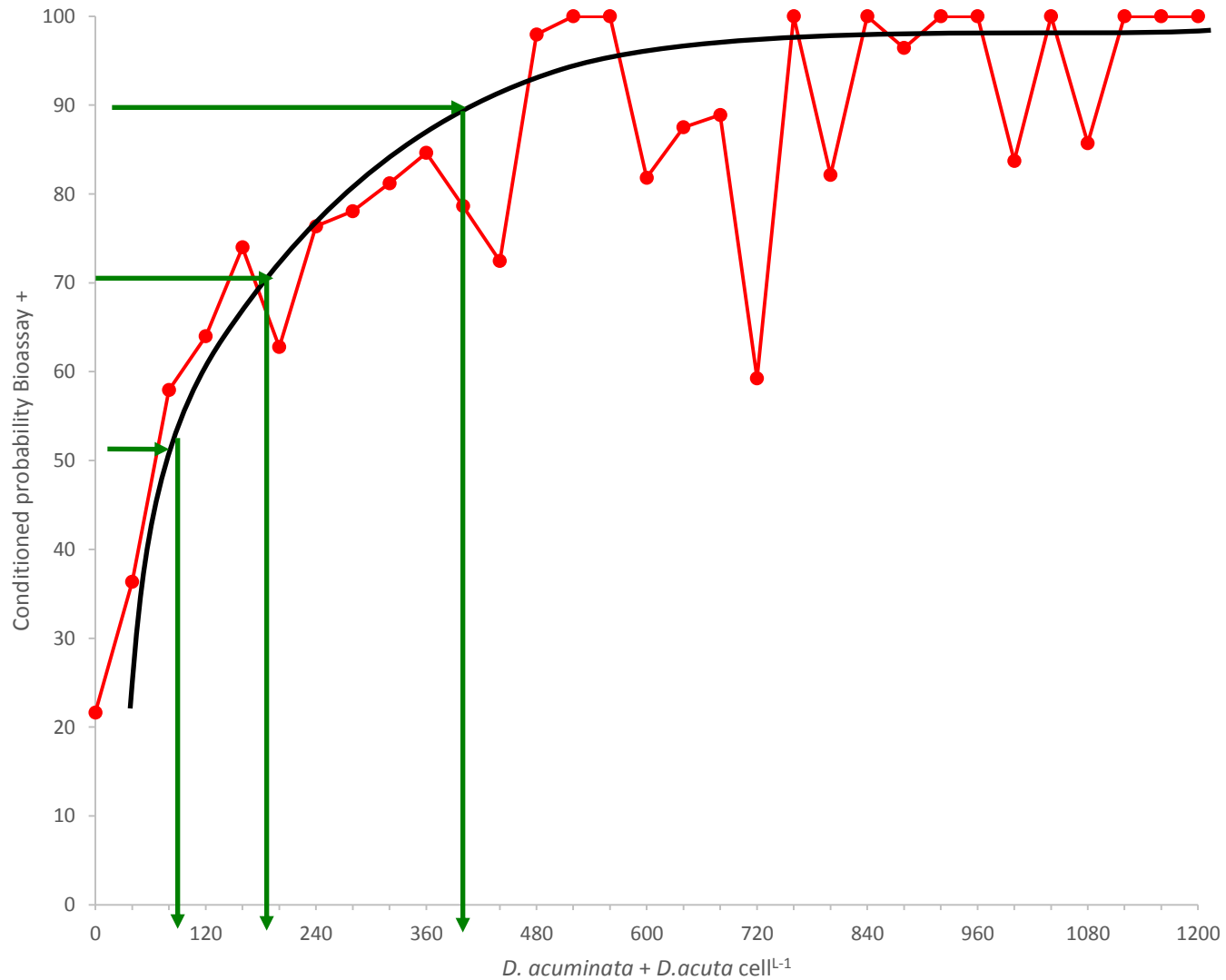






Probability of Bioassay + given  
*Dinophysis acuminata* + *acuta* cell concentration  
based on cases in real monitoring

# Trigger levels?



# E-EN 15204:2007

Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)

## Traceability: certified equipment



micrometer



precision scale



Caliper

## Qualification of workers: continuous training and evaluation



# Quality assurance

The method must be validate :

- . Repeteability and reproducibility
- . The distribution in the camber tottom
- . The homogenisation of sample
- . The setimentation
- . blank samples...

Accuracy (mean) versus precision (standard deviation)



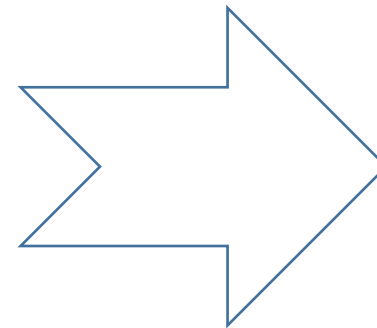
Accurate but not precise



Precise but not accurate



Precise and accurate

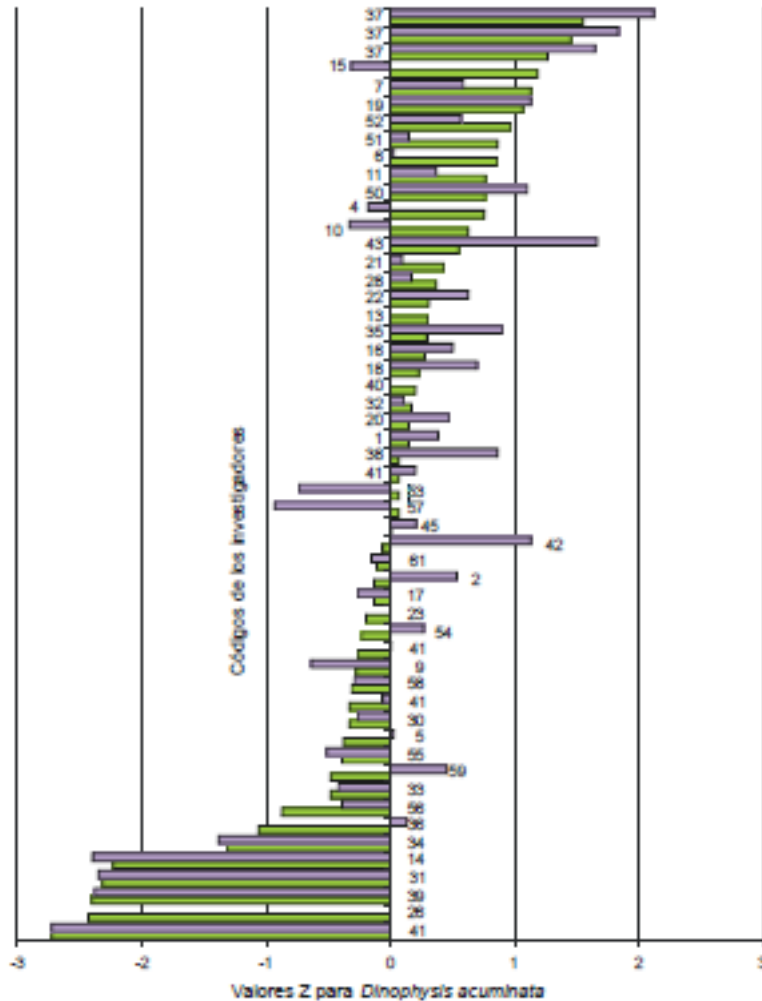


INTERLABORATORY  
INTERCALIBRATION  
EXERCICES.

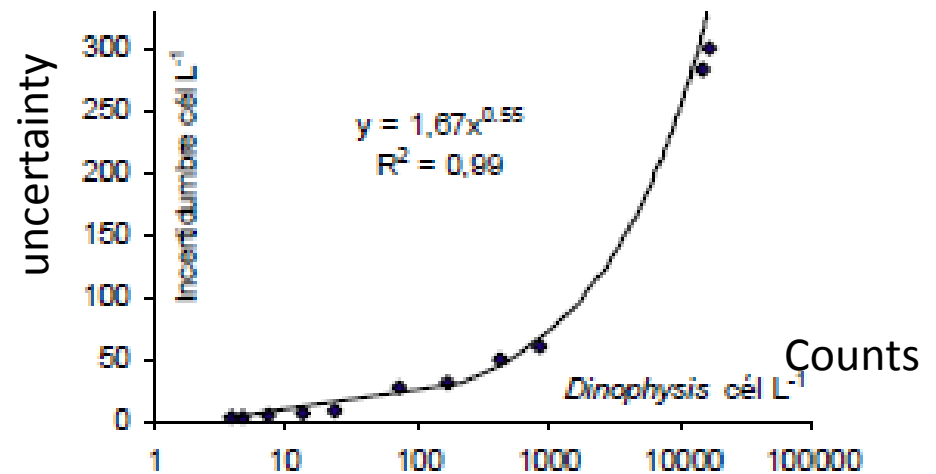
Example: Pazos et al



Z scores for *Dinophysis acuminata* counts of the 62 researchers from 13 countries



	Promedio (cel L <sup>-1</sup> )	DESVEST	C.V	Nº muestras	Incertidumbre (cel L <sup>-1</sup> )	I relativa %
<i>D. acuminata</i>	14612	2867	20	102	284	2
<i>D. acuta</i>	5	35	742	102	3	73
<i>D. caudata</i>	173	314	182	102	31	18
<i>D. diegensis</i>	24	88	369	102	9	37
<i>D. fortii</i>	7	53	711	102	5	70
<i>D. parvula</i>	4	28	707	102	3	70
<i>D. rotundata</i>	832	614	74	102	61	7
<i>D. sacculus</i>	13	69	513	102	7	51
<i>D. skagii</i>	429	507	118	102	50	12
<i>Dinophysis</i> spp	72	286	396	102	28	39
Total	16170	3042	19	102	301	2



Estimación de incertidumbre de recuentos celulares de *Dinophysis acuminata* por el método Uthermöhl: Resultado de un ejercicio de intercalibración interlaboratorio

Yolanda Pazos<sup>1</sup>, Luz Mamán<sup>2</sup> y Maximino Delgado<sup>3</sup>

(1) Instituto Tecnológico para o Control do Medio Mariño de Galicia (INTECMAR) Vilagarcía de Arousa.

(2) Laboratorio de Control de Calidad de los Recursos Pesqueros (L.C.C.RR.PP.). Huelva.

(3) Institut de Recerca i Tecnologia Agroalimentaries (IRTA). Tarragona.

# MIDTAL

MICROARRAYS FOR THE DETECTION OF TOXIC ALGAE

Now: 2010-09-27 08:11:22

Last update: 09/04/2010

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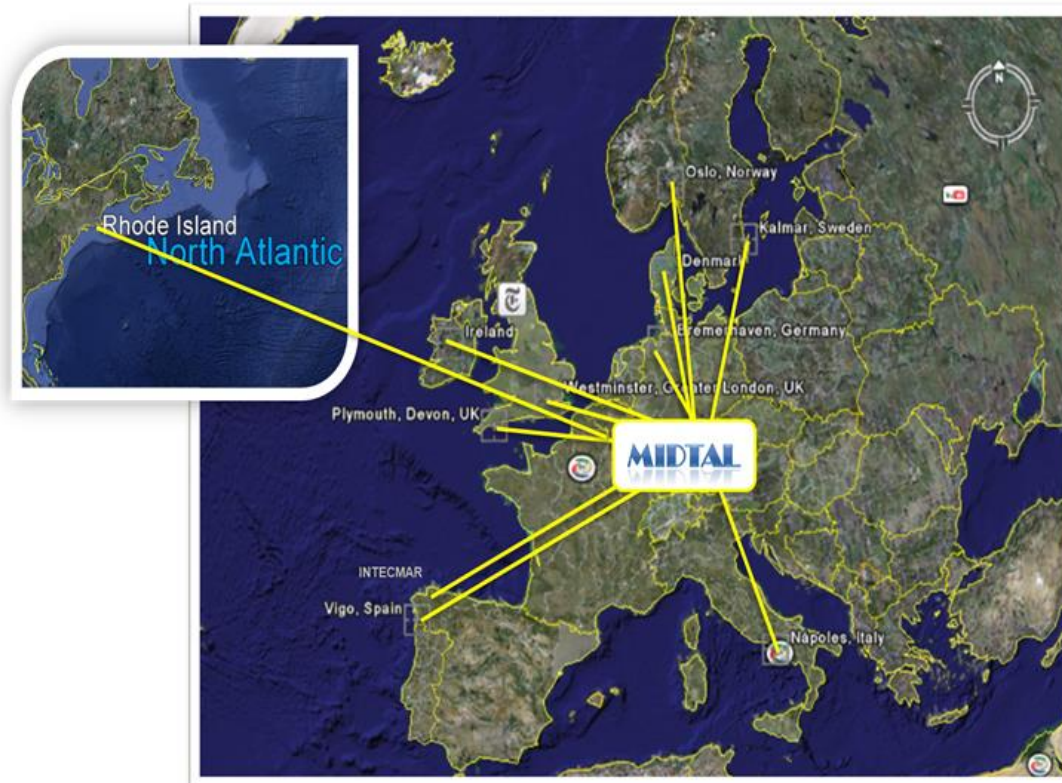
005609

Project acronym: MIDTAL

Project full title: [MICROARRAYS FOR THE DETECTION OF TOXIC ALGAE](#)

Grant agreement no.: 201724

MIDTAL is a cooperated project covering several institutes over Europe coastal seas. Ten partners make up the consortium and include scientists from 7 European countries and the USA.



## NEWS AND UPDATES

**NEW** Vigo meeting reports are uploaded (2010-03-31)

**NEW** 15 months report is uploaded in Internal page (2010-03-31)

**NEW** Culture list is updated (2010-03-31)

**NEW** Probes and primers list is updated (2010-03-08)

**NEW** **Periodical meeting was held in Vigo, Spain during 1-3, March (2010-03-08)**

**NEW**

[More](#)

# Midtal Objectives



- To **test and optimise existing rRNA probes** for toxic species and antibodies for toxins for their application to a microarray
- To **design** and test the specificity of any **new probe** needed
- To construct **a universal microarray** from the probes tested and optimized by all of the partners for the detection of harmful algae and their toxins
- To provide national monitoring agencies with **a rapid molecular tool** to monitor toxic algae to validate or replace traditional methods for monitoring for toxic algae
- To **integrate** European efforts to monitor coastal waters for toxic algal species

Dittami, Pazos, Laspra, & Medlin **Environ Sci Pollut Res**, 2012

Microarray testing for the presence of toxic algae monitoring programme in Galicia (NW Spain)



# PSP

## SPECIES

*Alexandrium andersonii* Balech, 1990

*Alexandrium catenella* (Whedon & Kofoid) Balech, 1985

*Alexandrium minutum* Halim, 1960

*Alexandrium ostenfeldii* (Paulsen) Balech & Tangen, 1985

*Alexandrium tamarense* complex (Lebour) Balech, emended U. John

*Gymnodinium catenatum* H.W.Graham, 1943

OPTICAL MICROSCOPE: XUNTA DE GALICIA

ELECTRONIC MICROSCOPE: XUNTA DE GALICIA

MOLECULAR TOOLS: XUNTA DE GALICIA

OPTICAL MICROSCOPE: JUNTA DE ANDALUCÍA

ELECTRONIC MICROSCOPE: JUNTA DE ANDALUCÍA

MOLECULAR TOOLS: JUNTA DE ANDALUCÍA

OPTICAL MICROSCOPE: GENERALITAT DE CATALUNYA

ELECTRONIC MICROSCOPE: GENERALITAT DE CATALUNYA

MOLECULAR TOOLS: GENERALITAT DE CATALUNYA

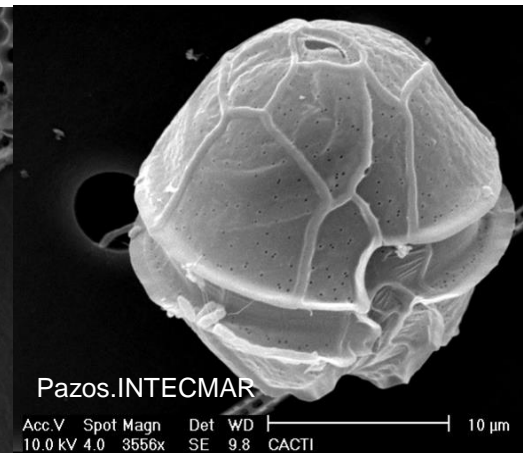
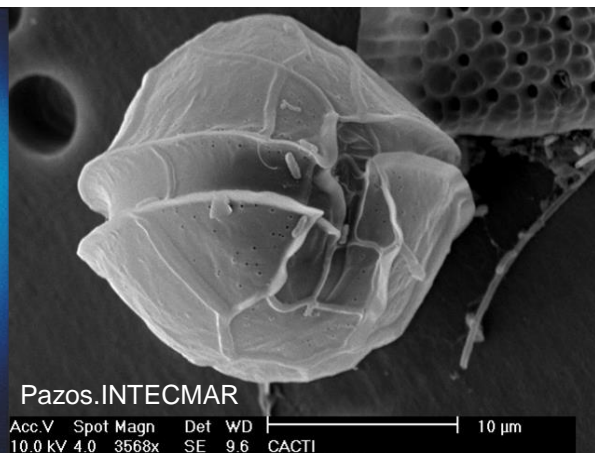
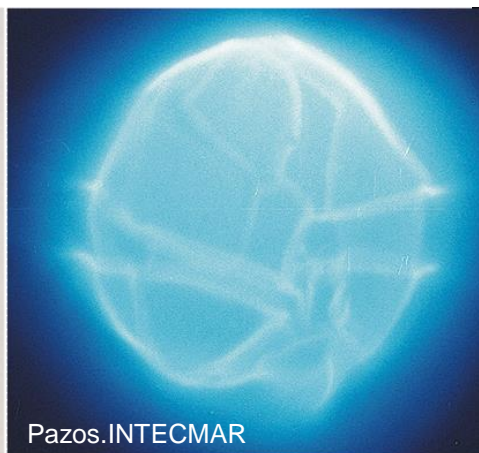
OPTICAL MICROSCOPE: BASQUE COUNTRY

ELECTRONIC MICROSCOPE: BASQUE COUNTRY

MOLECULAR TOOLS: BASQUE COUNTRY

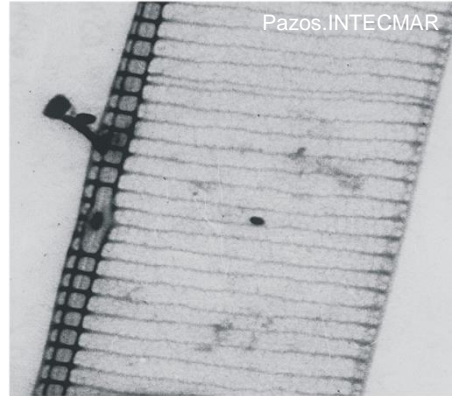
			yes						
				yes					
yes	SEM	microarray	yes					yes	
yes		microarray		yes					
yes		microarray	yes					yes	
yes	SEM	microarray	yes		qPCR				

## *Alexandrium minutum* Halim, 1960





Pazos.INTECMAR



Pazos.INTECMAR

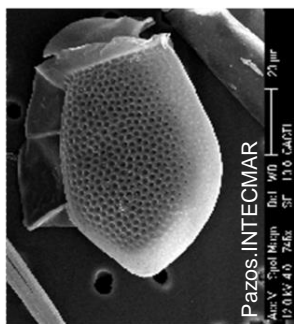
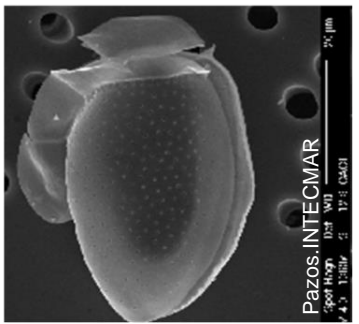
*Pseudo-nitzschia* sp.  
Peragallo in Peragallo & Peragallo, 1900

*Pseudo-nitzschia australis*  
Frenguelli, 1939

SPECIES

ASP

SPECIES	ASP	OPTICAL MICROSCOPE. XUNTA DE GALICIA	ELECTRONIC MICROSCOPE. XUNTA DE GALICIA	MOLECULAR TOOLS. XUNTA DE GALICIA	OPTICAL MICROSCOPE. JUNTA DE ANDALUCÍA	ELECTRONIC MICROSCOPE. JUNTA DE ANDALUCÍA	MOLECULAR TOOLS. JUNTA DE ANDALUCÍA	OPTICAL MICROSCOPE. GENERALITAT DE CATALUNYA	ELECTRONIC MICROSCOPE. GENERALITAT DE CATALUNYA	MOLECULAR TOOLS. GENERALITAT DE CATALUNYA	OPTICAL MICROSCOPE. BASQUE COUNTRY	ELECTRONIC MICROSCOPE. BASQUE COUNTRY	MOLECULAR TOOLS. BASQUE COUNTRY
<i>Pseudo-nitzschia australis</i> Frenguelli, 1939	yes	TEM	microarray	yes	SEM						TEM	yes	
<i>Pseudo-nitzschia calliantha</i> Lundholm, Moestrup & Hasle, 2003			microarray					SEM	qPCR				
<i>Pseudo-nitzschia cuspidata</i> (Hasle) Hasle, 1993			microarray									TEM	yes
<i>Pseudo-nitzschia delicatissima</i> (Cleve) Heiden, 1928			microarray									TEM	yes
<i>Pseudo-nitzschia multiseriata</i> (Hasle) Hasle, 1995			TEM	microarray									
<i>Pseudo-nitzschia multistriata</i> (Takano) Takano, 1995	yes		microarray	yes	SEM			TEM	qPCR			TEM	yes
<i>Pseudo-nitzschia plurisecta</i> Orive & Pérez-Aicua, 2013												TEM	yes
<i>Pseudo-nitzschia pseudodelicatissima</i> (Hasle) Hasle, 1993			microarray									TEM	yes



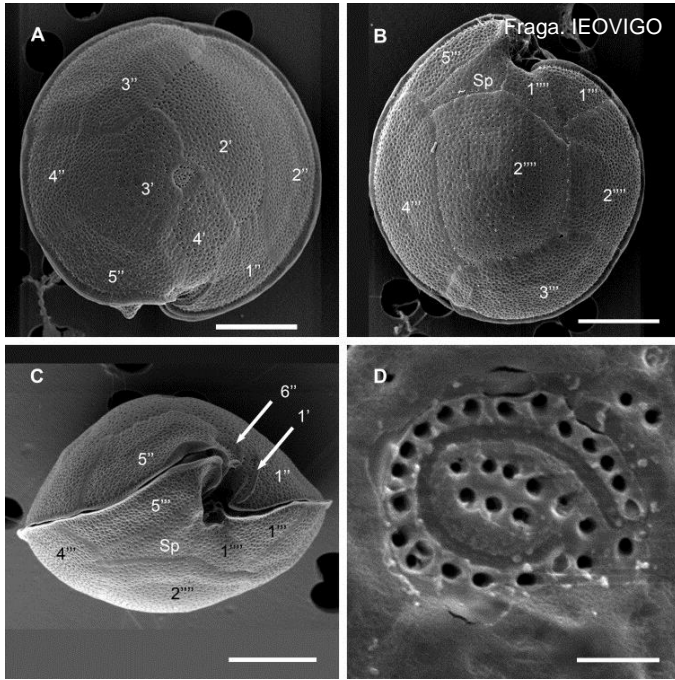
*Dinophysis acuminata*  
Claparède & Lachmann,  
1859

*Dinophysis acuta*  
Ehrenberg, 1839

*Dinophysis caudata*  
Saville-Kent, 1839

	OPTICAL MICROSCOPE: XUNTA DE GALICIA	ELECTRONIC MICROSCOPE: XUNTA DE GALICIA	MOLECULAR TOOLS: XUNTA DE GALICIA	OPTICAL MICROSCOPE: JUNTA DE ANDALUCÍA	ELECTRONIC MICROSCOPE: JUNTA DE ANDALUCÍA	MOLECULAR TOOLS: JUNTA DE ANDALUCÍA	OPTICAL MICROSCOPE: GENERALITAT DE CATALUNYA	ELECTRONIC MICROSCOPE: GENERALITAT DE CATALUNYA	MOLECULAR TOOLS: GENERALITAT DE CATALUNYA	OPTICAL MICROSCOPE: BASQUE COUNTRY	ELECTRONIC MICROSCOPE: BASQUE COUNTRY	MOLECULAR TOOLS: BASQUE COUNTRY
Lipohilic (OA+derivates+AZA+Yesso+Palitoxins)												
<i>Amphidoma languida</i> Tillmann et al., 2012					yes	SEM	yes					
<i>Azadinium poporum</i> Tillmann & Elbrächter, 2011			microarray									
<i>Azadinium spinosum</i> Elbrächter & Tillmann, 2009												
<i>Coolia tropicalis</i> M.A.Faust, 1995												
<i>Dinophysis acuminata</i> Claparède & Lachmann, 1859	yes	SEM	microarray	yes			yes			yes		
<i>Dinophysis acuta</i> Ehrenberg, 1839	yes	SEM	microarray	yes			yes			yes		
<i>Dinophysis caudata</i> Saville-Kent, 1881	yes	SEM		yes			yes			yes		
<i>Dinophysis fortii</i> Pavillard, 1923	yes		microarray	yes			yes			yes		
<i>Dinophysis infundibula</i> J.Schiller, 1928	yes						yes					
<i>Dinophysis miles</i> Cleve, 1900												
<i>Dinophysis norvegica</i> Claparède & Lachmann, 1859												
<i>Dinophysis ovum</i> (F.Schütt) T.H.Abé	yes						yes					
<i>Dinophysis sacculus</i> Stein, 1883	yes						yes					
<i>Dinophysis tripos</i> Gourret, 1883	yes	SEM		yes			yes		yes			
<i>Gonyaulax spinifera</i> (Claparède & Lachmann) Diesing, 1866	yes			yes			yes		yes			
<i>Lingulodinium polyedrum</i> (F.Stein) J.D.Dodge, 1989 †	yes	SEM	microarray	yes			yes		yes			
<i>Ostreopsis ovata</i> Fukuyo, 1981				yes			yes			qPCR		
<i>Ostreopsis siamensis</i> Schmidt, 1901	yes			yes			qPCR	yes		yes	SEM	yes
<i>Phalacroma mitra</i> F.Schütt, 1895	yes			yes			yes		yes			
<i>Phalacroma rotundatum</i> (Claparède & Lachmann) Kofoid & Michener, 1911	yes	SEM	microarray	yes			yes		yes			
<i>Prorocentrum lima</i> (Ehrenberg) F.Stein, 1878	yes		microarray	yes			yes		yes	SEM	yes	
<i>Prorocentrum maculosum</i> M.A.Faust, 1993				yes								
<i>Prorocentrum rhathymum</i> Loeblich, Sherley & Schmidt, 1979	yes		microarray	yes			yes		yes	SEM	yes	
<i>Protoceratium reticulatum</i> (Claparède & Lachmann) Butschli, 1885	yes	SEM		yes			yes		yes			





Ciguatera

*Gambierdiscus australes* M.Chinian & M.A.Faust, 1999

*Gambierdiscus excentricus* S.Fraga, 2011

*Gambierdiscus silvae* Fraga S. & F. Rodríguez, 2014

ELECTRONIC MICROSCOPE: CANARY ISLANI

SEM

SEM

SEM

MOLECULAR TOOLS: CANARY ISLANDS

yes

yes

yes

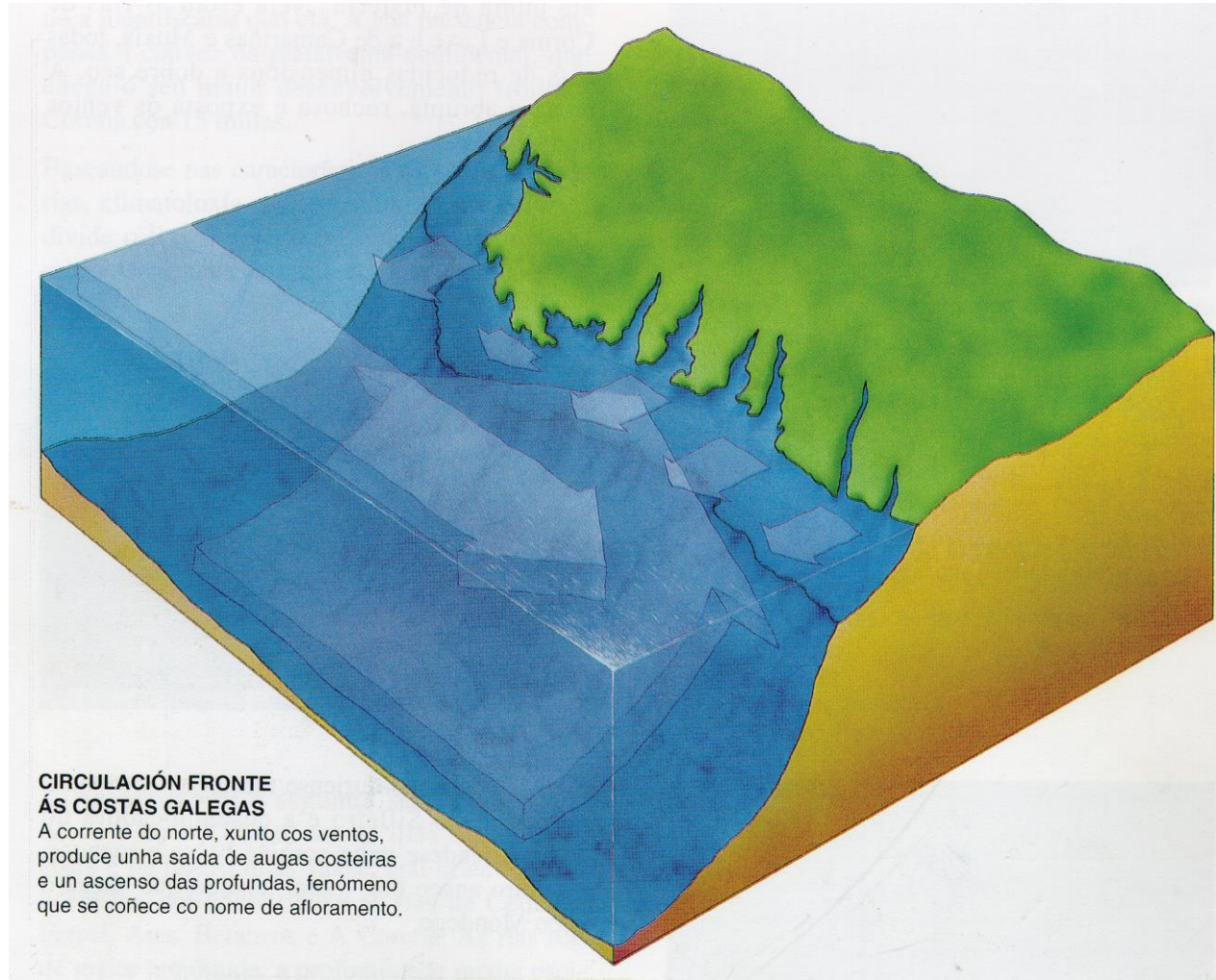


## Marine cyanotoxins Galicia

*Nodularia spumigena* Mertens ex Bornet & Flahault, 1886

*Phormidium formosum* (Bory de Saint-Vincent ex Gomont) Anagnostidis & Komárek, 1988

# UPWELLING SYSTEM



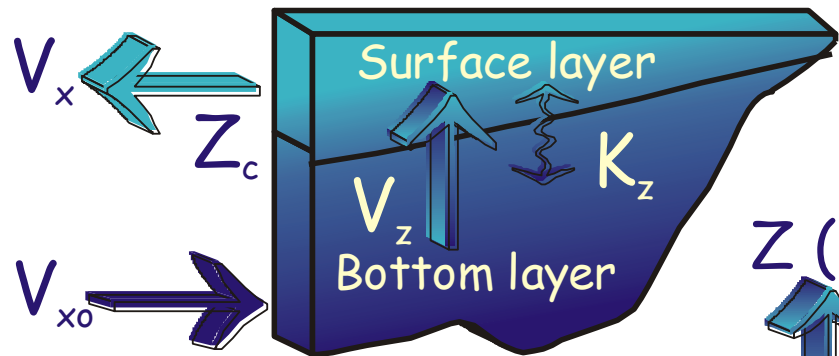
## **CIRCULACIÓN FRONTE ÁS COSTAS GALEGAS**

A corrente do norte, xunto cos ventos, produce unha saída de augas costeiras e un ascenso das profundas, fenómeno que se coñece co nome de afloramento.



# UPWELLING SYSTEM

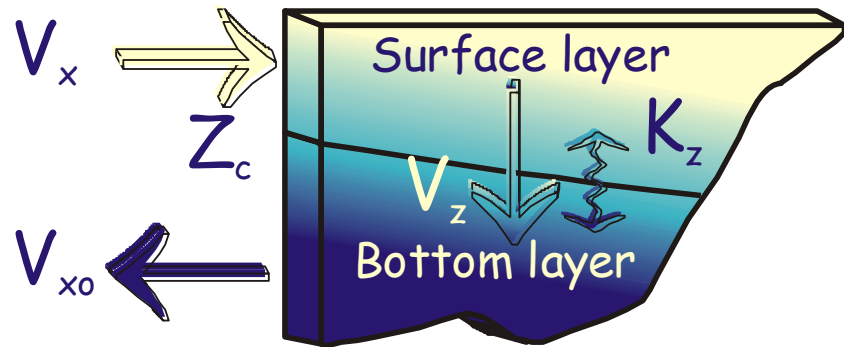
Upwelling



$Z$  (m)

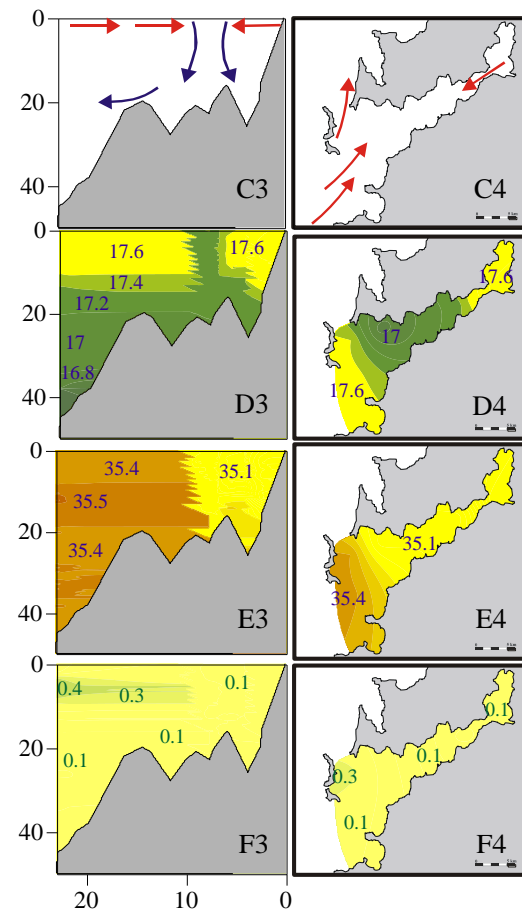
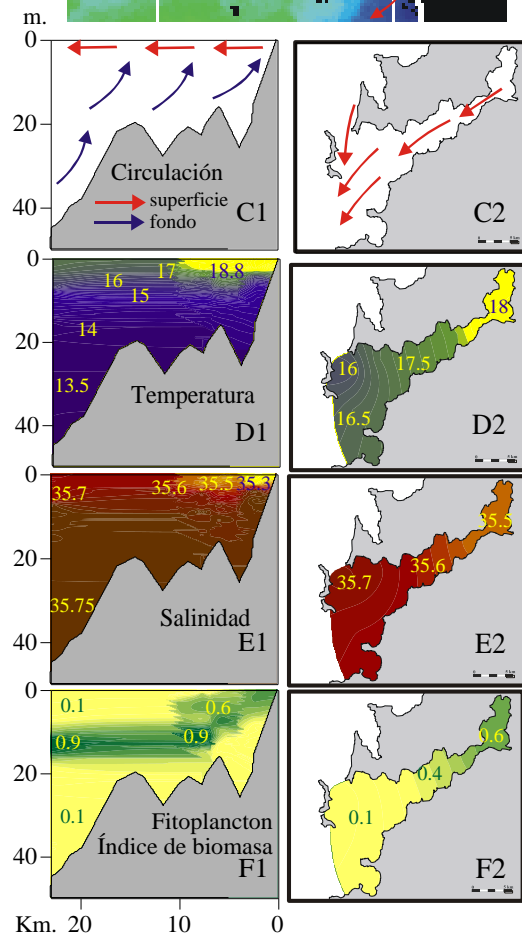
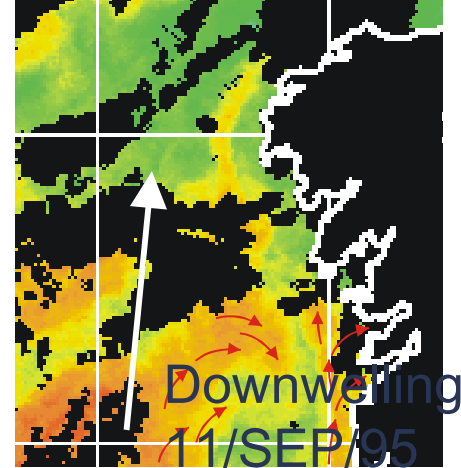
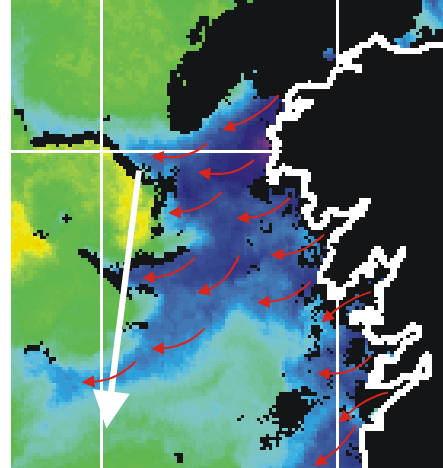
$X$  (Km)

Downwelling

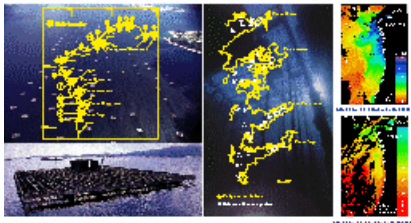


# UPWELLING SYSTEM

Upwelling  
26/AUG/95

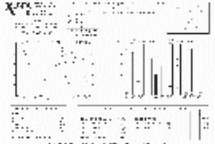


# Condições oceanográficas e microalgas tóxicas e/ou nocivas en Galicia



As variables oceanográficas: temperatura, salinidade, oxixeiro, pH, fluorescencia e transmitancia, mideza e a toda a profundidade da auga empregaándose as CTD. A salinidade e a temperatura indican movementos e estabilidade das augas. O pH e o oxixeiro indican fotosíntese e polo tanto abundancia de fitoplacton. A fluorescencia e a transmitancia indican a biomasa fitoplanctónica e a abundancia de todo tipo de partículas e a súa estabilidade.

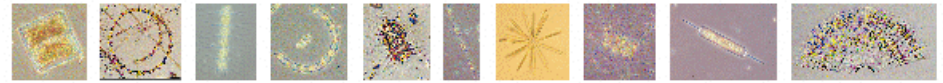
As análises de sales nutrientes e de carbono orgánico disolto indican alimento disponible para o fitoplankton e procesos de renovación e principais pigmentos foto sintéticos (clorofila a, b e c) así como nun espectrofotómetro. Indican abundancia e diversidade de fitoplankton.



O fitoplankton identifícase e análíase en microscopios ópticos (verdes) e tamén as especies tóxicas ou potencialmente dañinas con os que non o son.

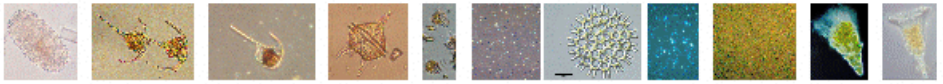
## Floracións de microalgas

O fitoplankton das Rías Galegas inclúe un grupo variado de microorganismos que, inflexiblemente, aparecen en todas as partes das rías que nos rodean. O grupo máis común son as diatomeas (*Skeletonema*) e os dinofitocitos (*Dinoflagellata*). Non microplankton tamén aparecen outros fitozoaes moi comúns como os copilípticos, as radiolarias, as silicoflageladas, o bacterioplankton e o microzooplankton, representado polos diatomeas, infusórios, larvas e ovos de outros organismos.



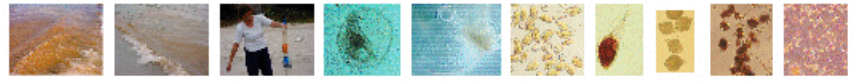
## Inócuas

A maior parte do fitoplankton está formado por especies inofensivas. O primeiro e o primeiro nivel da rede trófica do ecosistema das Rías sendo o principal alimento dos moluscos bivalvos (meixillón, améixas, ultras...). A elevada produtividade marxeira das Rías, debido á floración de fitoplankton que medra en base de sales nutrientes, e por tanto, fan que tamén se alimenten polo alimento do coqueiro.



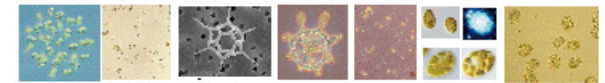
## Mareas vermellas

En determinadas condicións oceanográficas, o fitoplankton pode proliferar masivamente, ata producir coloracións visibles a simple vista na auga, debido ás pigmentos xenosintéticos que contíñen. Na maioría dos casos, a "purga de mar", é debido a organismos que non producen ningunha toxina, sen embargo poden ser nocivos pola súa sordía e a súa incidencia no turismo.



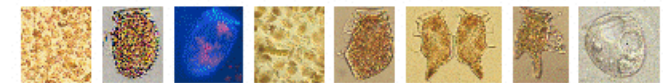
## Ictiotóxicas

Os mecanismos de acción das especies que afectan os cultivos de peixe son variados. Algunhas especies producen toxinas hemolíticas (*Pseudo-nitzschia*), outras producen toxinas que afectan ao sistema nervioso (*Dinoflagellata*). As toxinas producidas por algunhas especies producen un dano físico nas vísceras e nos órganos do sistema circulatorio. A elevada taxa de renovación da auga nas rías que non secan facen que os problemas de toxicidade.



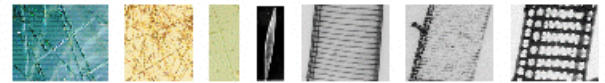
## Lipofílicas (DSP)

As especies relacionadas coa produción de toxinas nocivas nos moluscos das Rías son, basicamente, as do xénero *Dinoflagellata*. Non é necesario que actúen en concentracións máis altas para que supoñan un risco para a saúde pública. Algunhas especies son as que causan máis danos, e máis perigosas, do período de activación de mareas en Galicia.



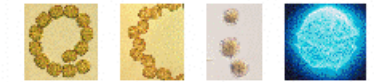
## Amnésicas (ASP)

As especies relacionadas coa produción de toxinas nocivas nos moluscos das Rías Galegas é debido sobre todo a dúas especies: *Cyrodinium choisyi* e *Cyrodinium choisyi*. A principal especie causante das prolongadas prohibicións de extracción de ústias de Galicia, *Pseudo-nitzschia australis*.



## Paralizantes (PSP)

A acumulación de toxinas paralizantes nos moluscos das Rías Galegas é debido sobre todo a dúas especies: *Cyrodinium choisyi* e *Cyrodinium choisyi*. A parte da súa gravidade nos últimos anos estas especies tamén nos causan.



## Novas toxinas (AZP, YTX, PTX)

Síndromes que afectan ao sistema nervioso dos consumidores, tamén asociado a especies de fitoplankton que exhiben características similares. A Unión Europea e a publicacións de decisións neste senso.

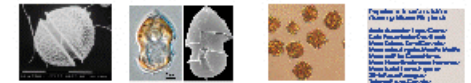


Figura 1. Especies de fitoplankton tóxicas e/ou nocivas en Galicia. (Munier et al., 2010)

1. *Pseudo-nitzschia australis*  
2. *Dinoflagellata*  
3. *Cyrodinium choisyi*  
4. *Dinoflagellata*  
5. *Dinoflagellata*  
6. *Dinoflagellata*  
7. *Dinoflagellata*  
8. *Dinoflagellata*  
9. *Dinoflagellata*  
10. *Dinoflagellata*  
11. *Dinoflagellata*  
12. *Dinoflagellata*  
13. *Dinoflagellata*  
14. *Dinoflagellata*  
15. *Dinoflagellata*  
16. *Dinoflagellata*  
17. *Dinoflagellata*  
18. *Dinoflagellata*  
19. *Dinoflagellata*  
20. *Dinoflagellata*



## MAREAS ROJAS

En determinadas condiciones oceanográficas, el fitoplancton puede proliferar masivamente hasta producir coloración visible a simple vista en el agua, debido a los pigmentos fotosintéticos que contienen. En la mayoría de los casos esta “purga del mar” es debida a organismos que no producen ninguna toxina pero pueden ser nocivas por la alarma social y su incidencia en el turismo.

Foto Iñaki Abella. Faro de Vigo

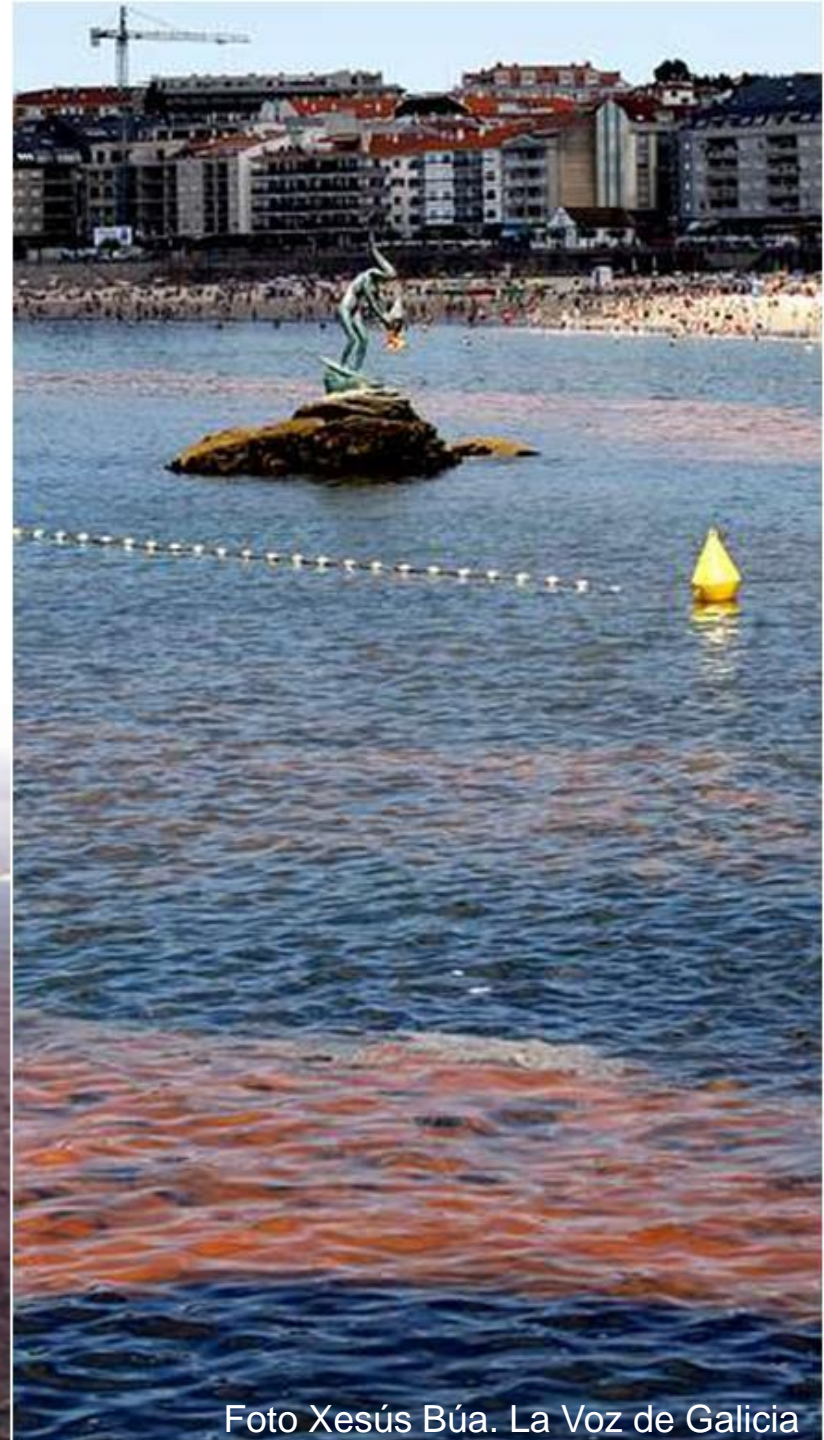


Foto Xesús Búa. La Voz de Galicia

# Clase Noctilucaeae



*Noctiluca scintillans* (Mcartney) Kofoid & Swezy, 1921

Dinoflagelado?

Marea roja





(B)

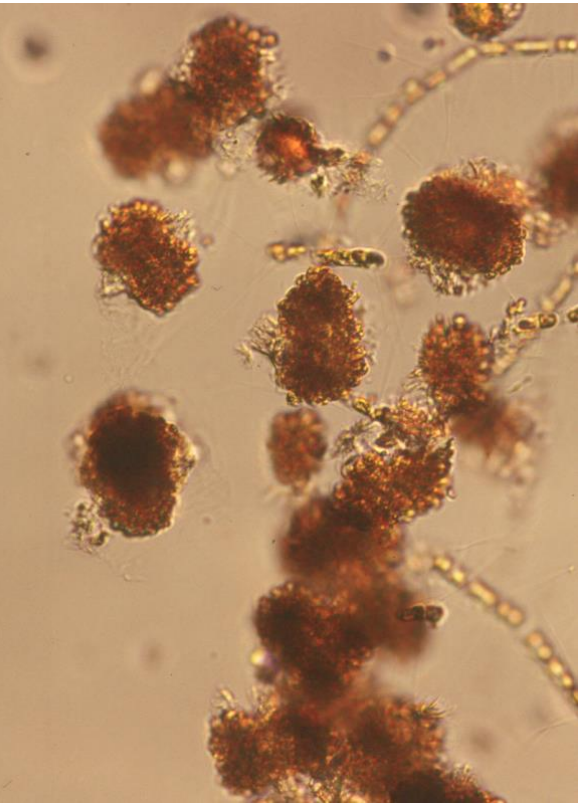


(C)

*Noctiluca scintillans* may act as a vector of toxigenic microalgae

Laura Escalera<sup>a,\*</sup>, Yolanda Pazos<sup>b</sup>, Ángeles Morono<sup>b</sup>, Beatriz Reguera<sup>a</sup>

Harmful Algae, 2007



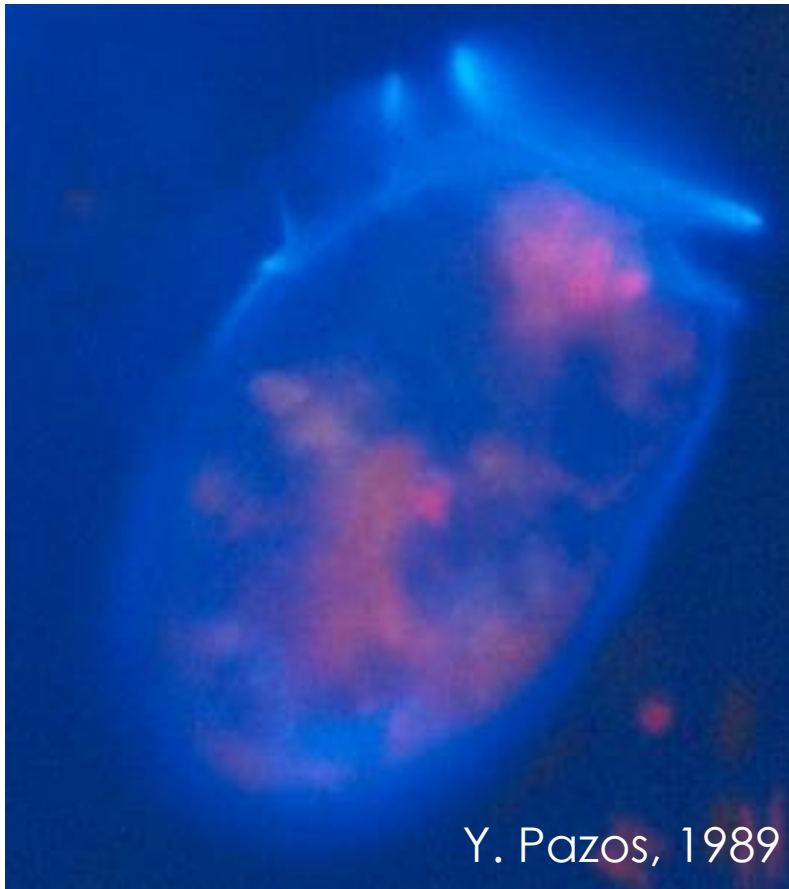
*Mesodinium rubrum* (Lohmann 1908) Jankowski 1976

## Ciliado



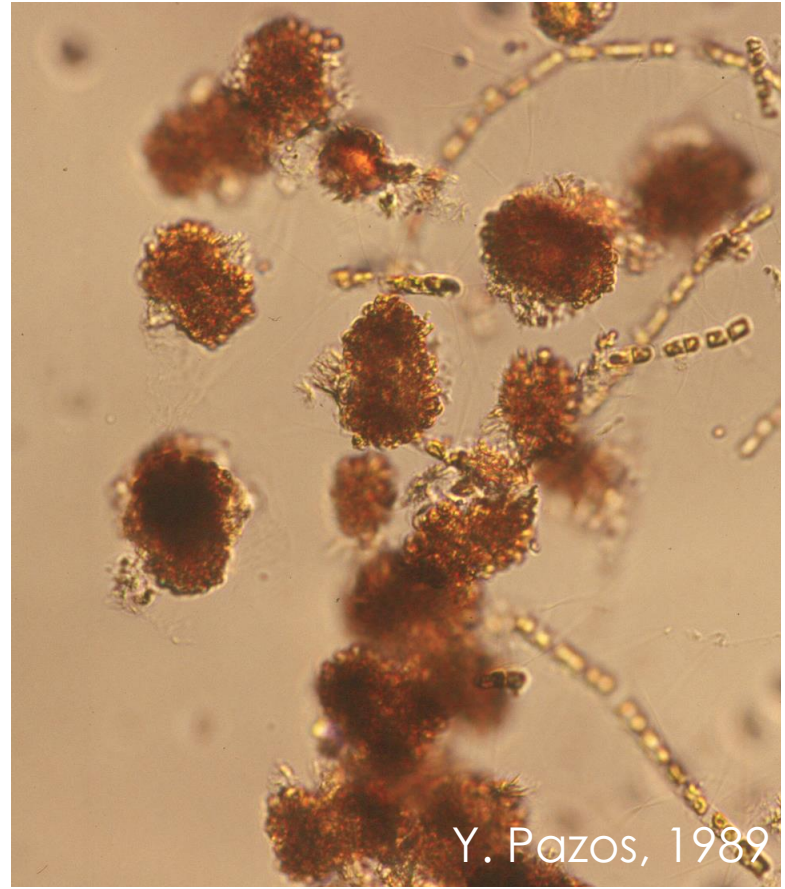
Foto Iñaki Abella. Faro de Vigo





Y. Pazos, 1989

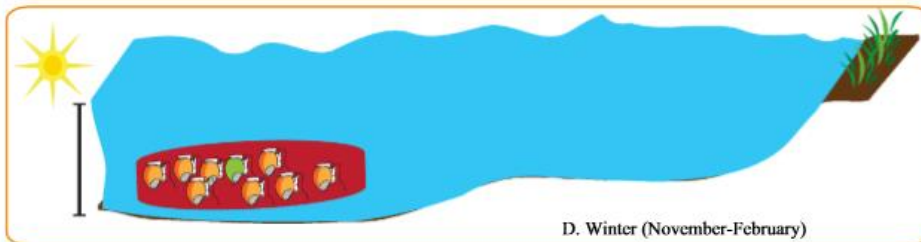
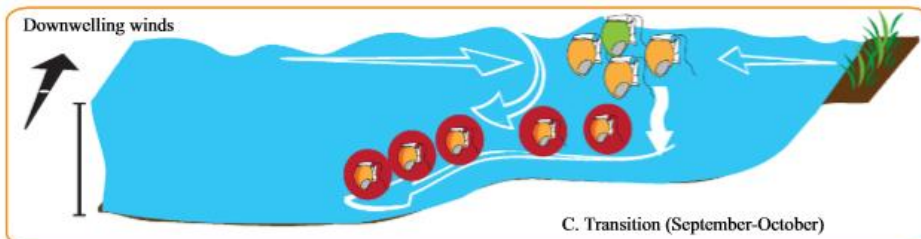
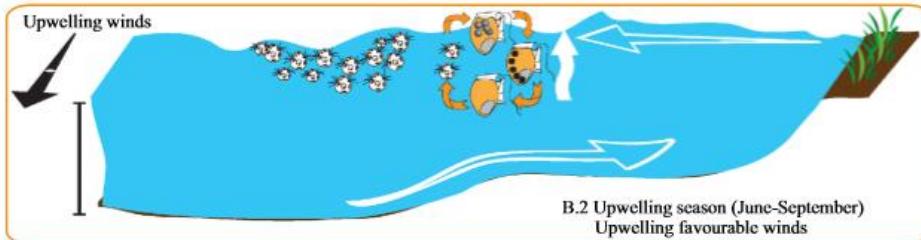
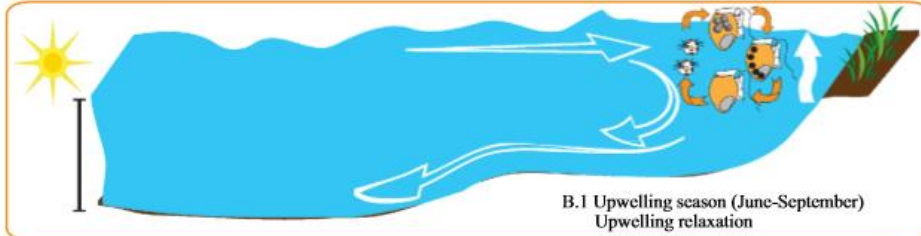
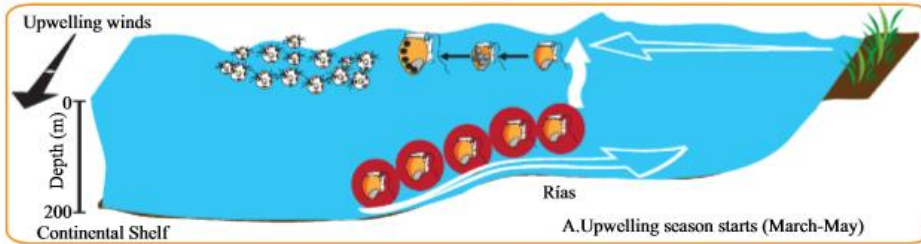
*Dinophysis acuminata*  
Epifluorescence B2



Y. Pazos, 1989

*Messodinium rubrum*  
Lugol





Velo, González-Gil , Pazos, Reguera  
Deep Sea Research, 2014





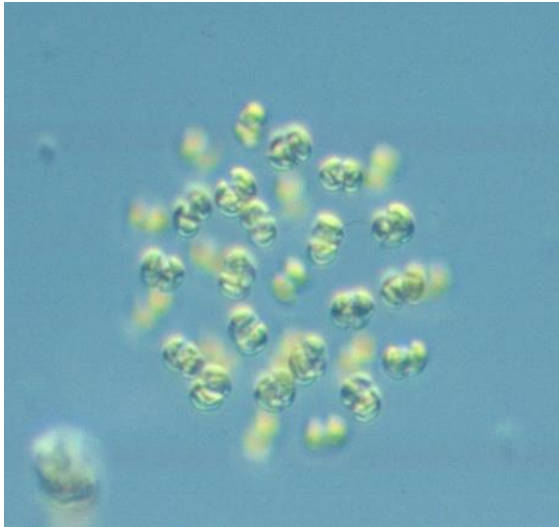
[youtube.com/watch?v=GZo5dMVYTS4](https://www.youtube.com/watch?v=GZo5dMVYTS4)



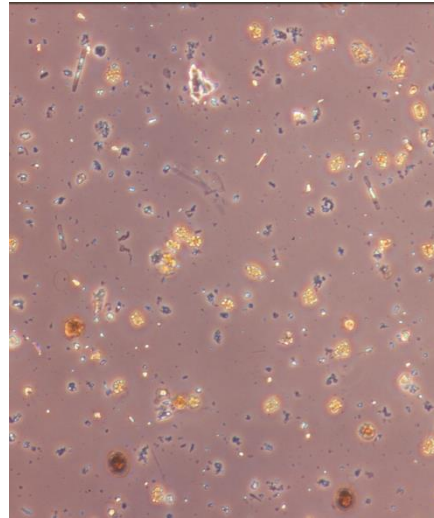
## Ictiotóxicas

Los mecanismos de acción de las especies que afectan a los cultivos de peces son variados. *Heterosigma akashiwo* produce toxinas hemoíticas, *Phaeocystis pouechetii* produce mucus que obtura las branquias llevándolos a la asfixia. Los silicoflagelados del género *Dictyocha* producen un daño físico en las vías respiratorias de los peces haciéndolos susceptibles de infecciones. La elevada tasa de renovación del agua en las rías hace que no sean frecuentes.

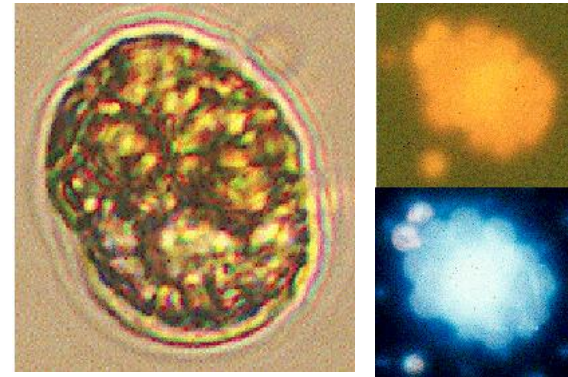




*Phaeocystis pouechetii* (Hariot) Lagerheim, 1893

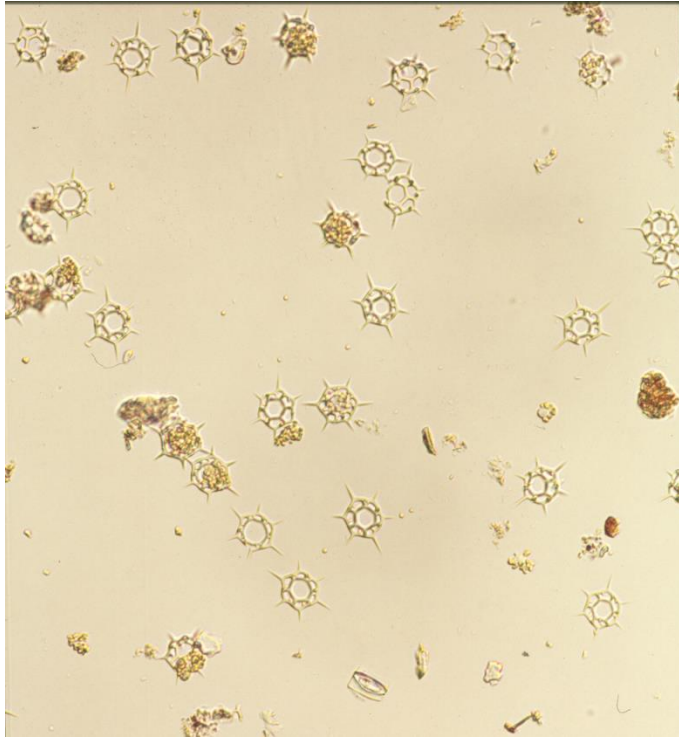


*Heterosigma akashiwo* (Hada) Hada, 1968 ex Sournia

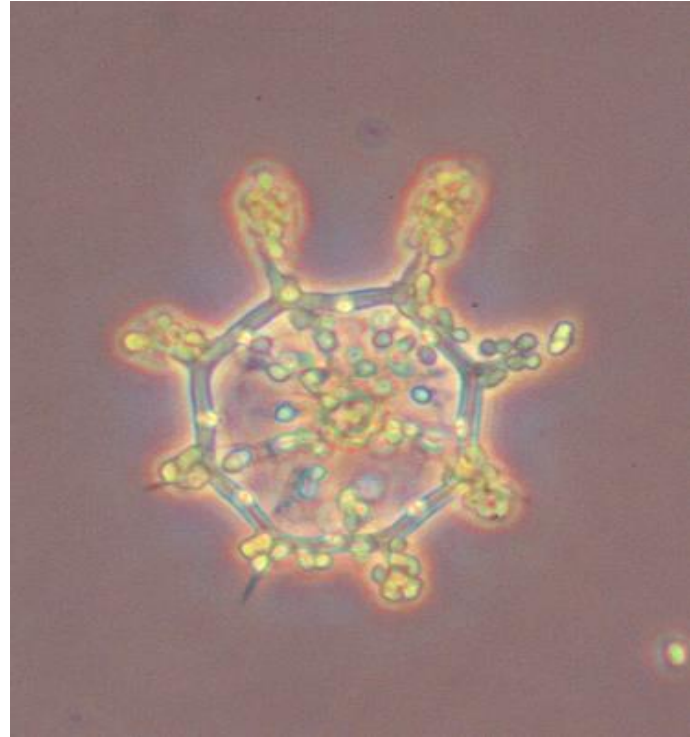


flageladas

Ictiotóxicas



*Dictyocha speculum* Ehrenberg, 1839



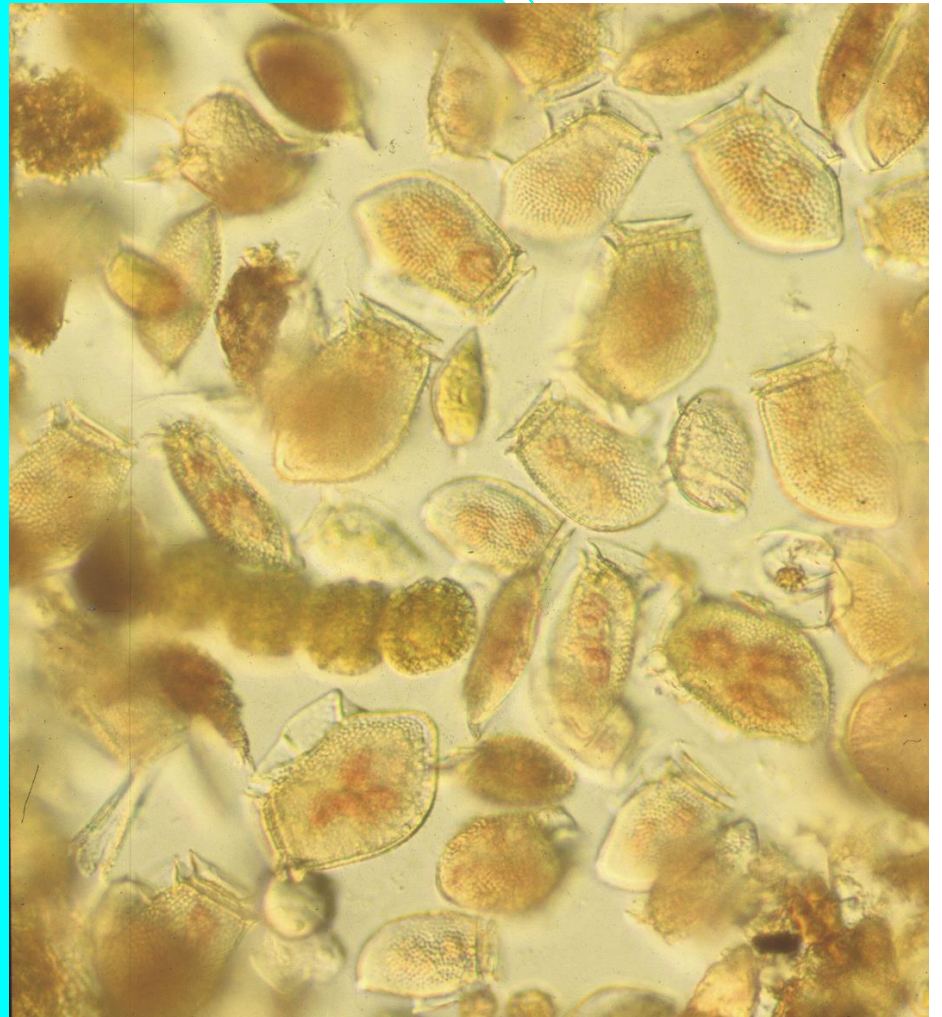
*Octatis octonaria* (Ehrenberg) Hovasse, 1946

Prego, Pazos, Varela 1997 Harmful Algae Blooms

silicoflageladas

Ictiotóxicas

The famous mother-in-law soup





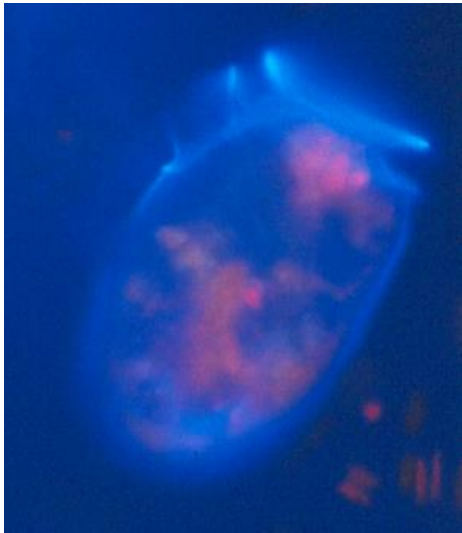


*Phalacroma rotundatum* (Claparède & Lachmann) Kofoid & Michener, 1911



*Dinophysis tripos* Gourret, 1883

# Clase Dinophyceae



*Dinophysis acuminata* Claparède & Lachmann, 1859



*Dinophysis acuta* Ehrenberg, 1839

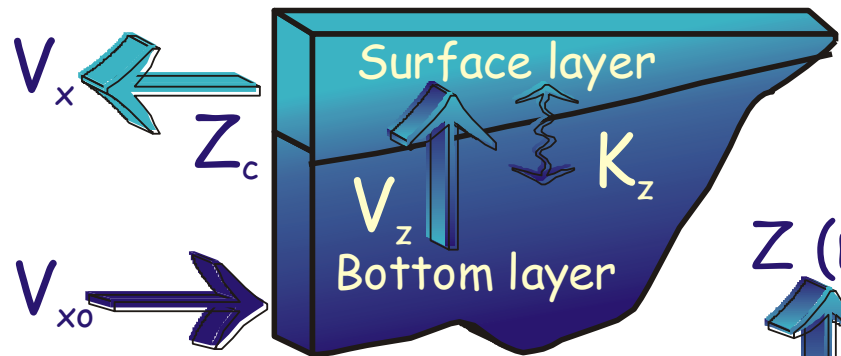


*Dinophysis caudata* Saville-Kent, 1881

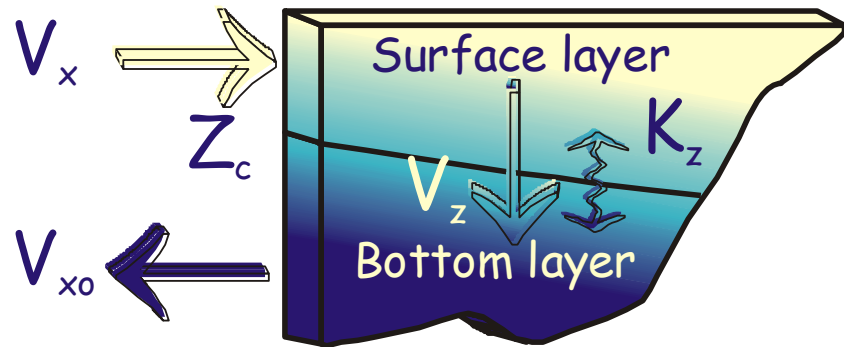
Dinoflagelados **Lipofílicas (DSP)**

# UPWELLING SYSTEM

Upwelling

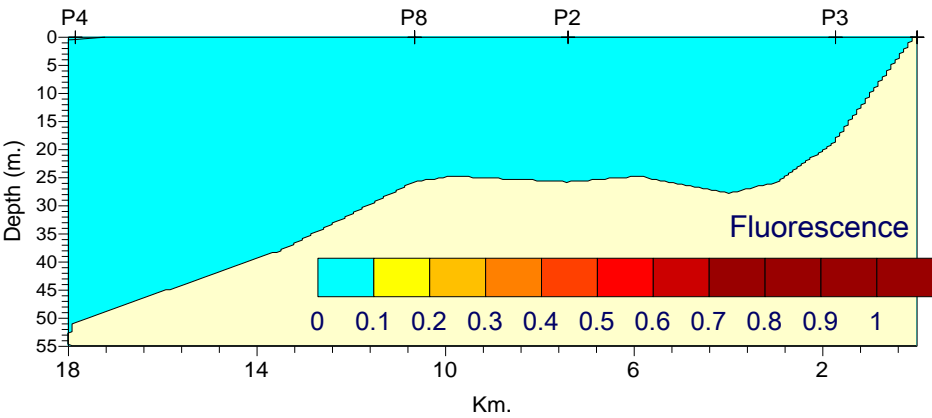
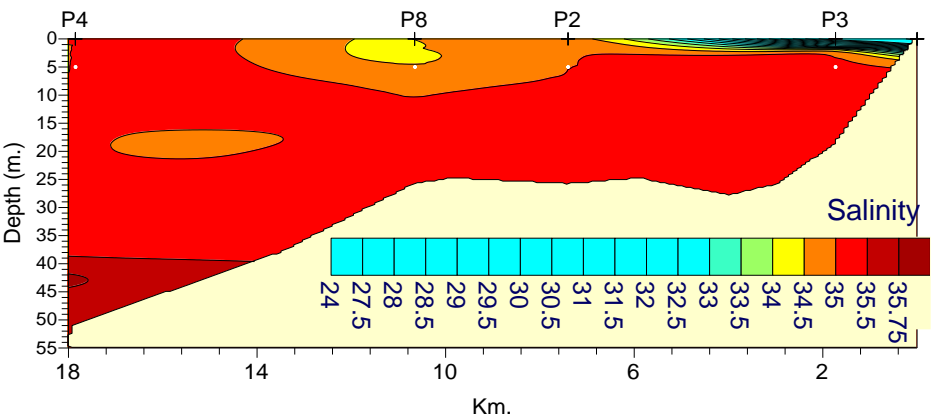
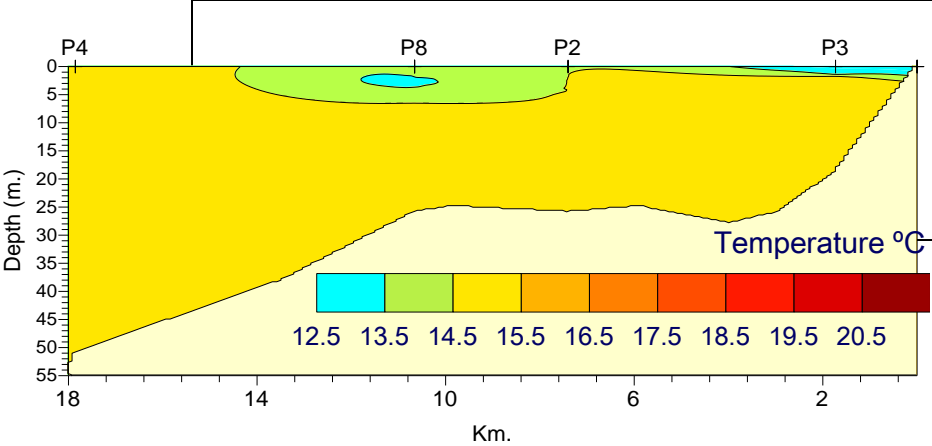


Downwelling



$Z$  (m)

$X$  (Km)

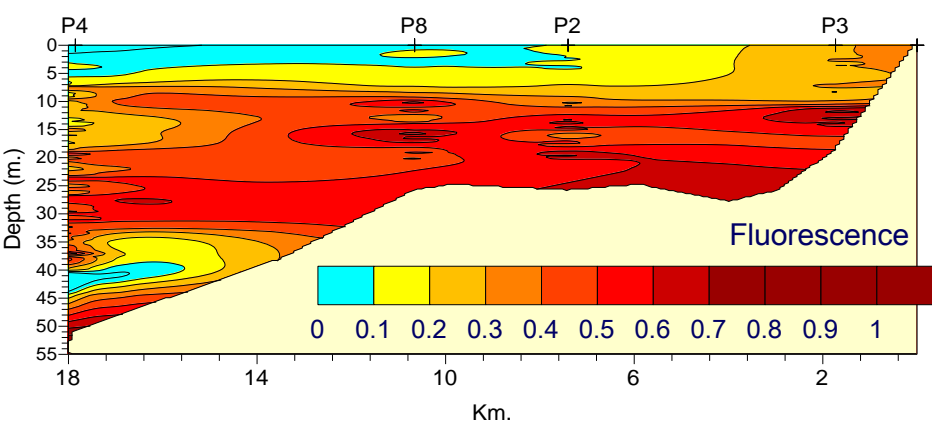
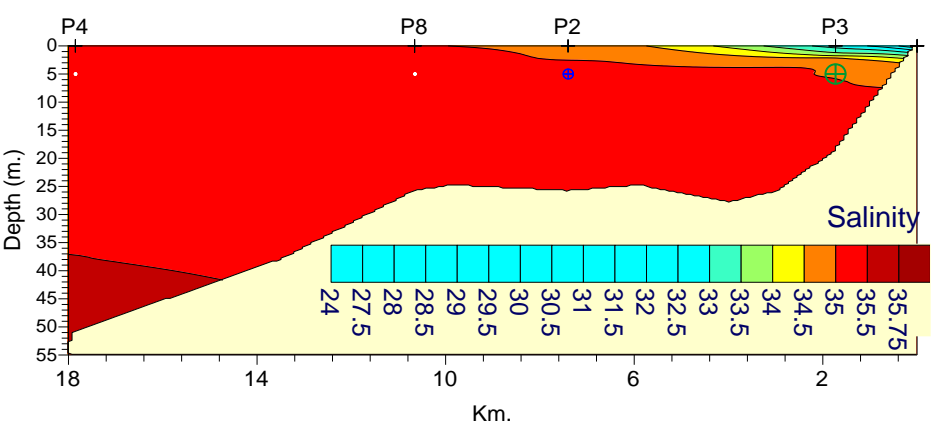
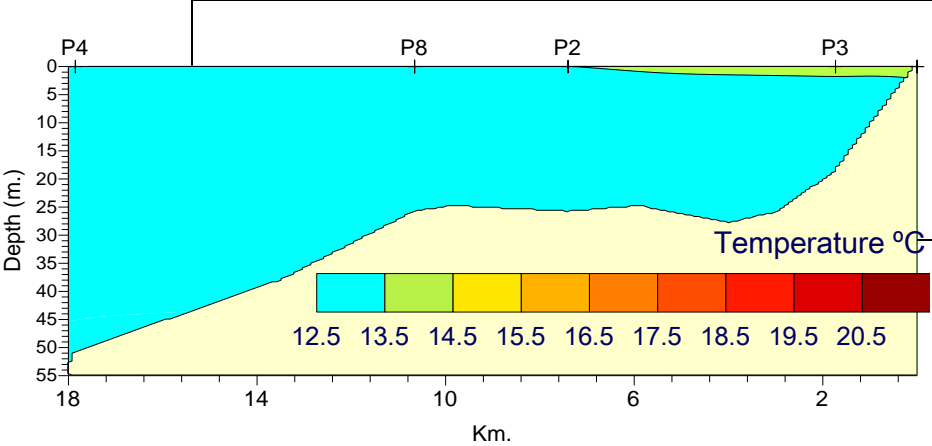


*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ 40 to 200
- ⊕ 200 to 1000
- ⊕ 1000 to 2000
- ⊕ 2000 to 5000
- ⊕ 5000 to 13000

WINTER SITUATION:  
 Vertical homogenization of  
 temperature and salinity  
 Phytoplankton VERY low  
*Dinophysis acuminata* N/D

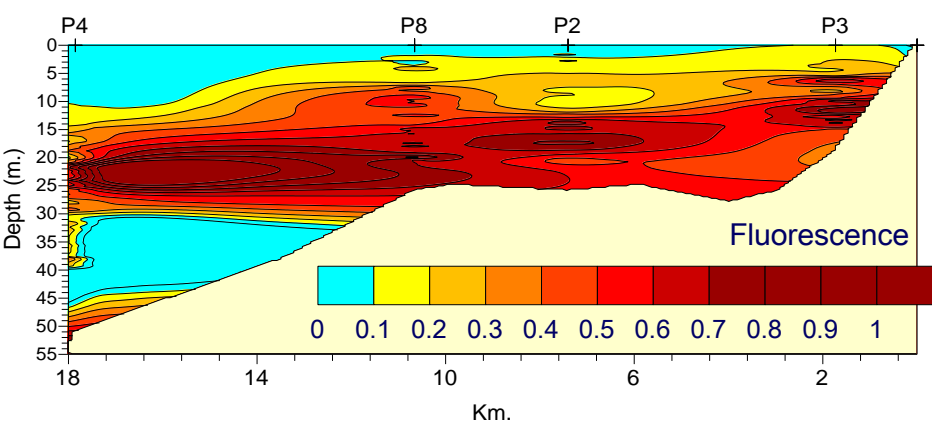
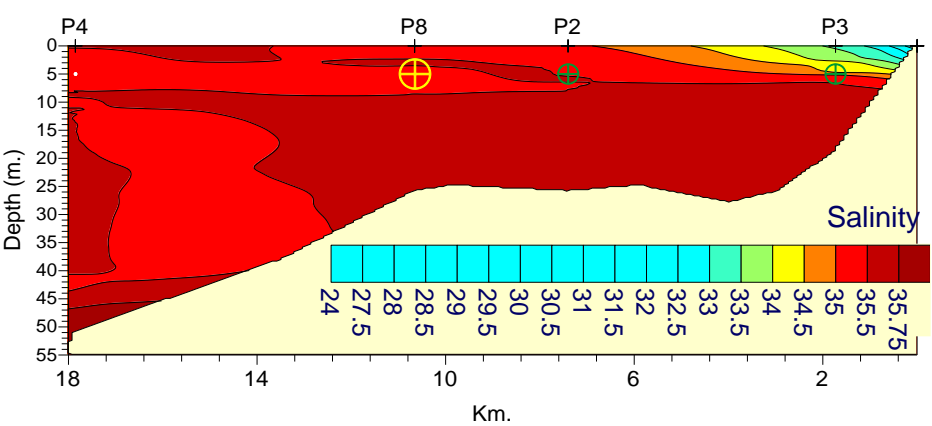
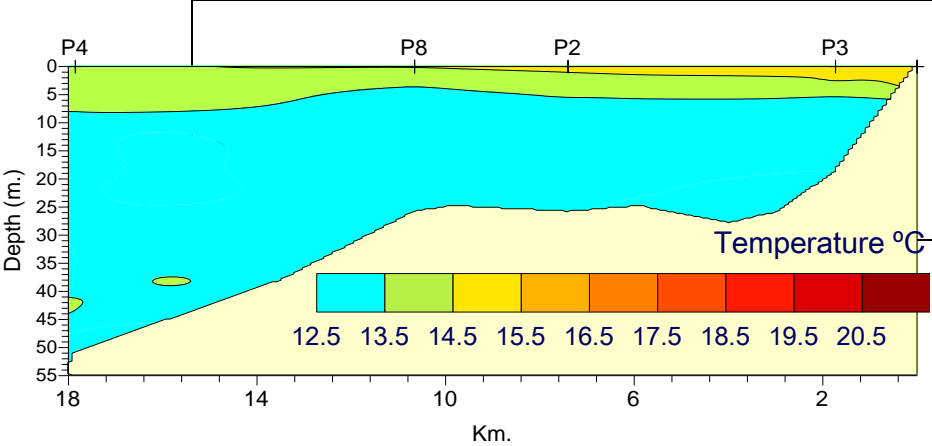




*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)

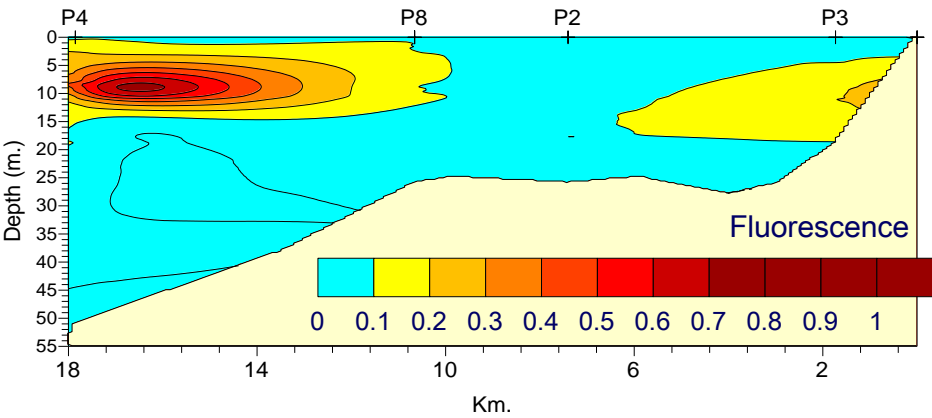
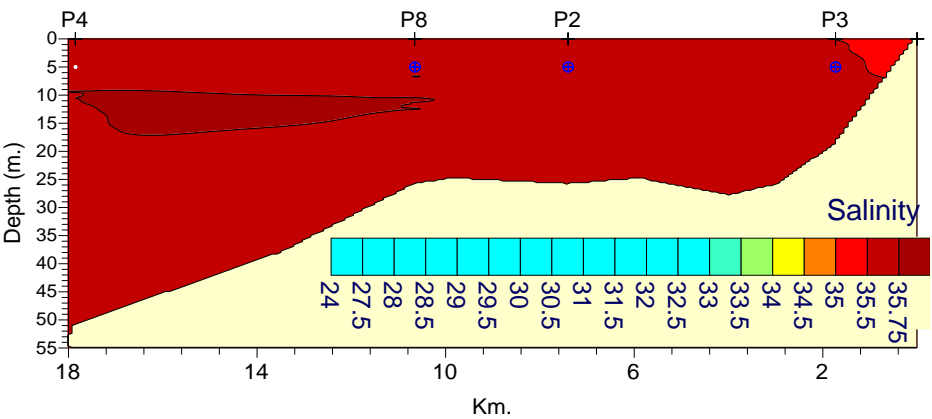
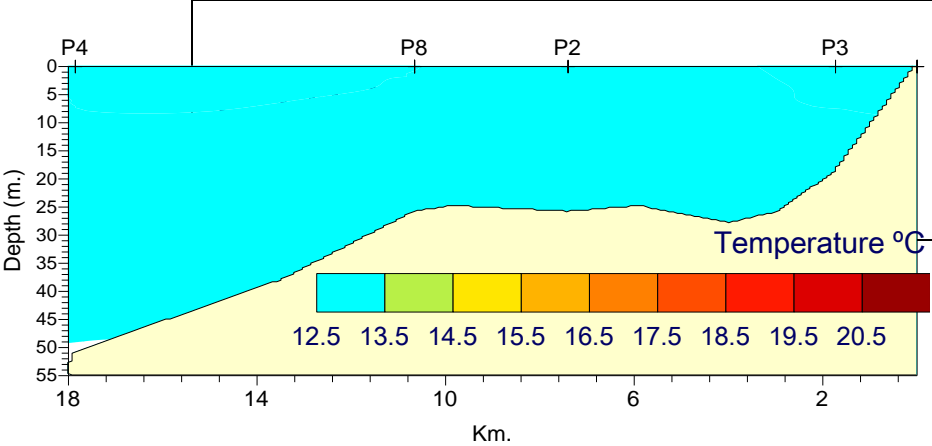
EARLY SPRING: Upwelling  
 Homogenization of temperature  
 First phytoplankton bloom  
 Halocline in the innermost station  
*Dinophysis acuminata* maximum in the  
 halocline spreads offshore



*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ (cyan)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)

SPRING: Upwelling  
 Light thermocline  
 Halocline in the inner part  
 Phytoplankton subsurface bloom  
*In situ* growth of *Dinophysis acuminata*

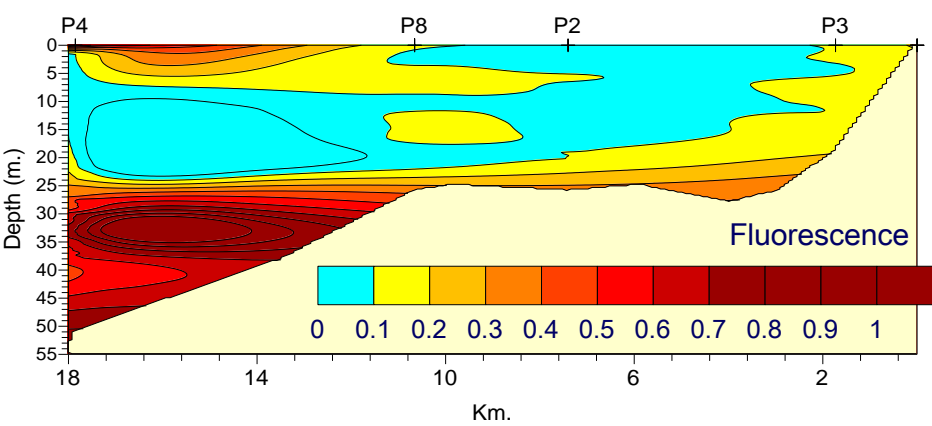
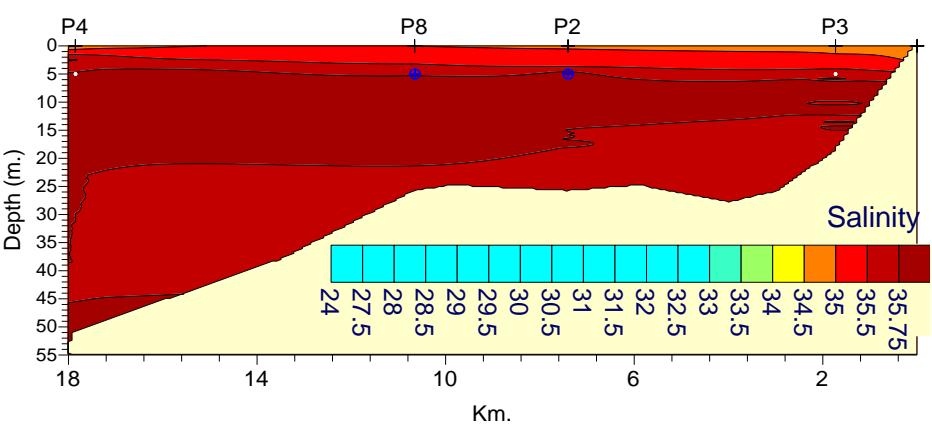
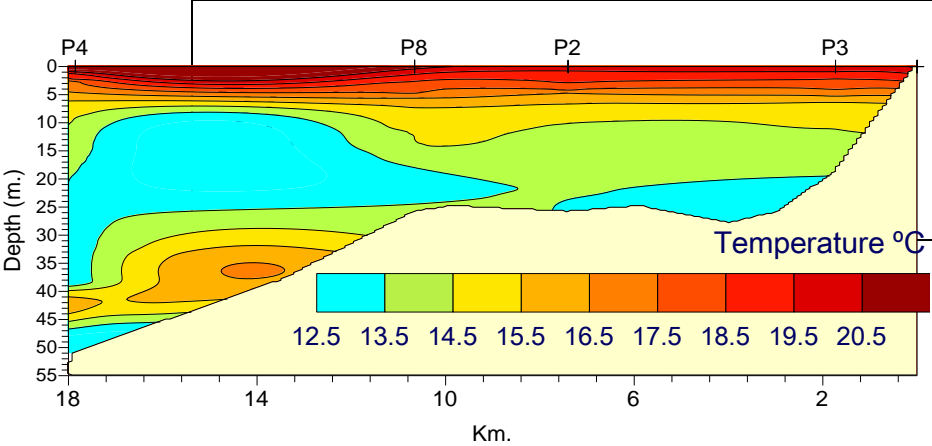


*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ 40 to 200
- ⊕ 200 to 1000
- ⊕ 1000 to 2000
- ⊕ 2000 to 5000
- ⊕ 5000 to 13000

**SPRING: Strong Upwelling**  
*Low levels of Dinophysis acuminata*  
 remain in the upper layers

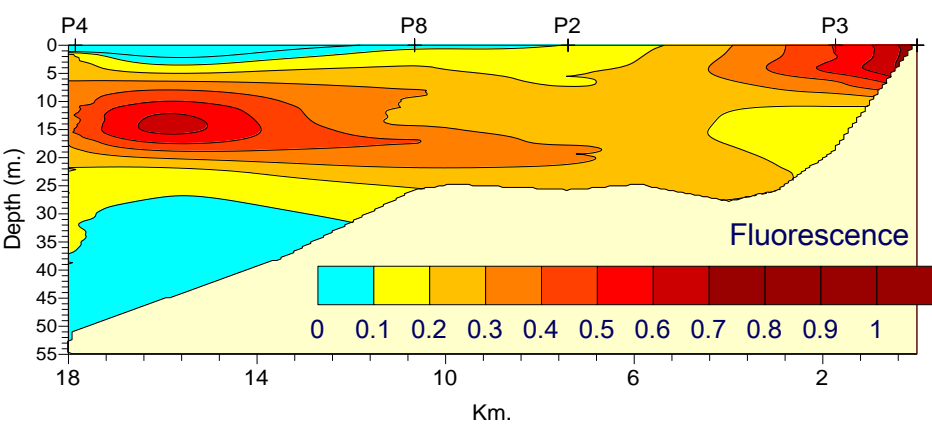
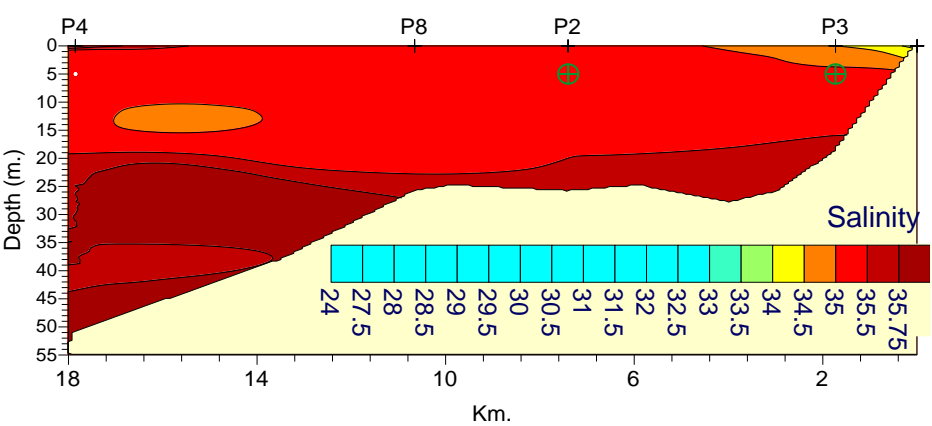
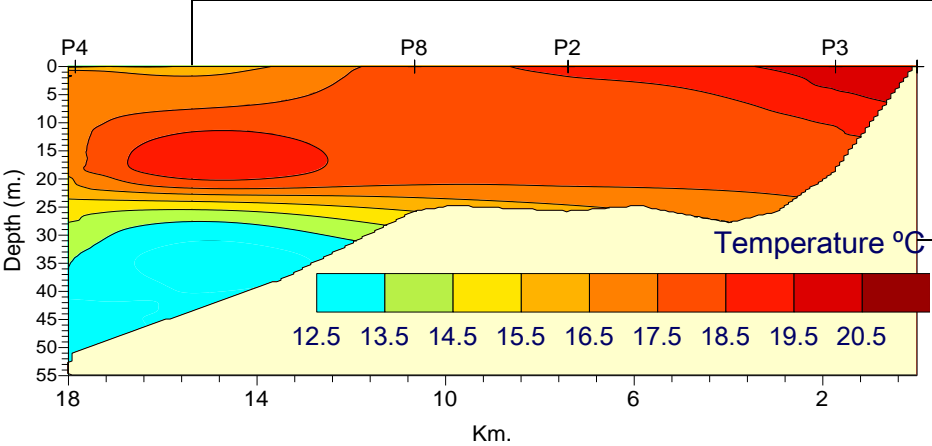




*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ 40 to 200
- ⊕ 200 to 1000
- ⊕ 1000 to 2000
- ⊕ 2000 to 5000
- ⊕ 5000 to 13000

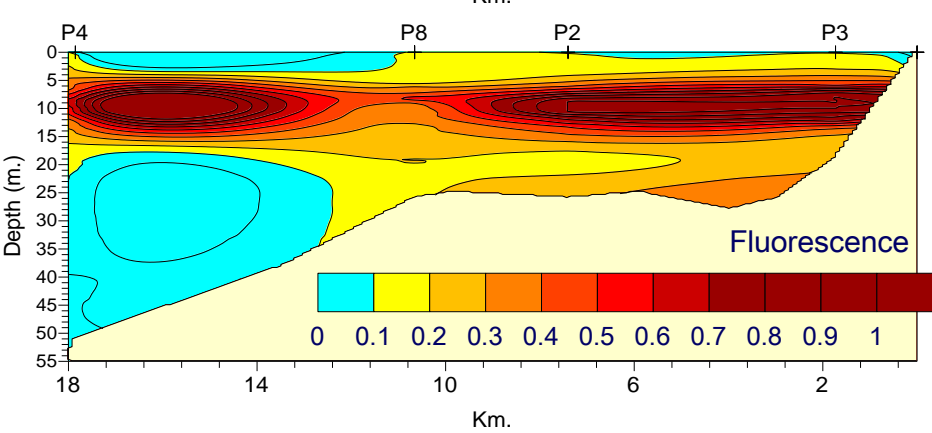
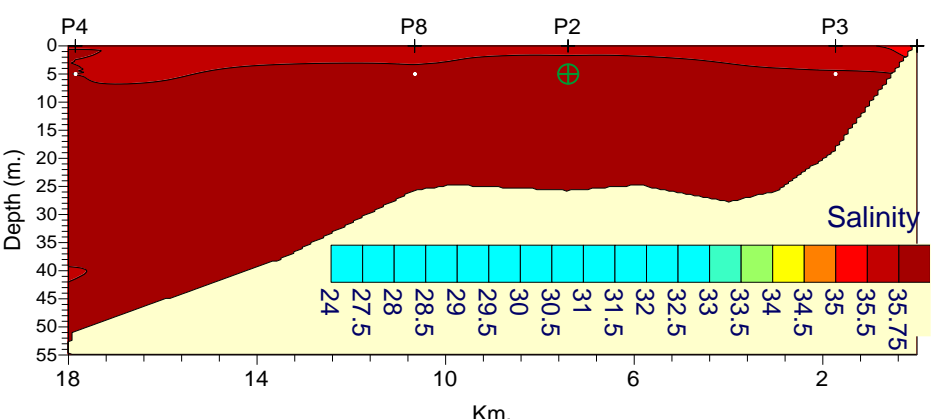
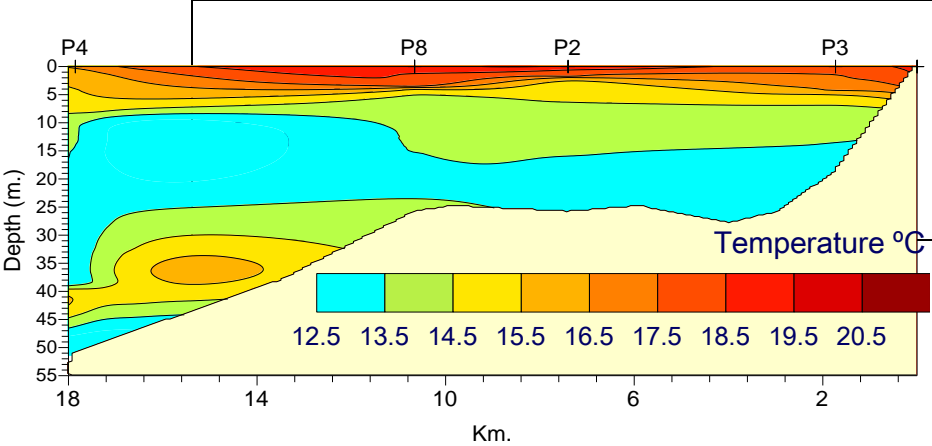
SUMMER: Upwelling relaxation  
 Strong thermocline  
 Intrusion of surface oceanic water  
 Phytoplankton surface patches  
*Dinophysis acuminata* *in situ* growth



*Dinophysis acuminata* Cell L<sup>-1</sup>

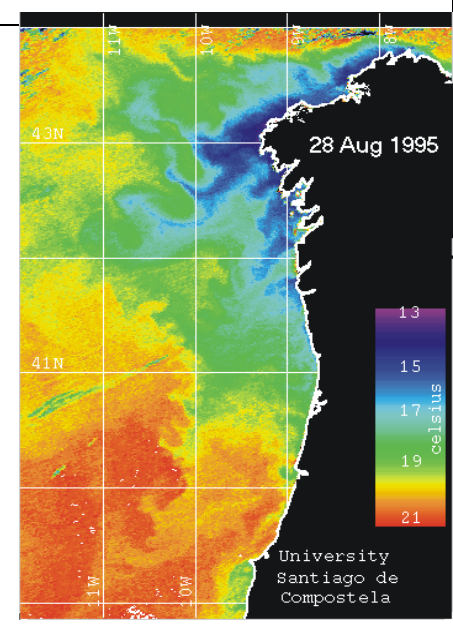
- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)

SUMMER: Stratification  
 Deepening of the thermocline  
 Phytoplankton patches  
*Dinophysis acuminata* growing

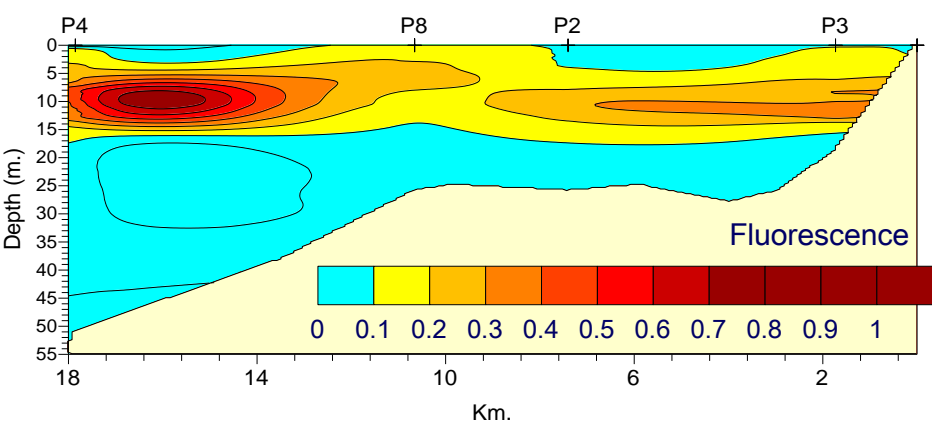
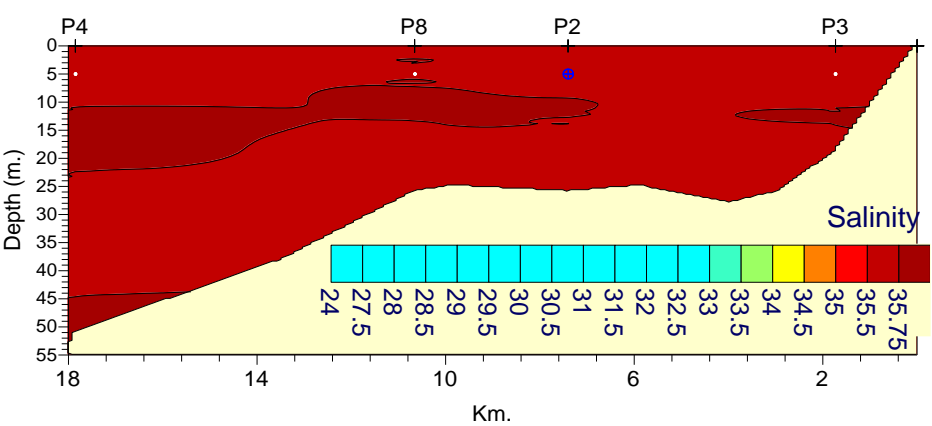
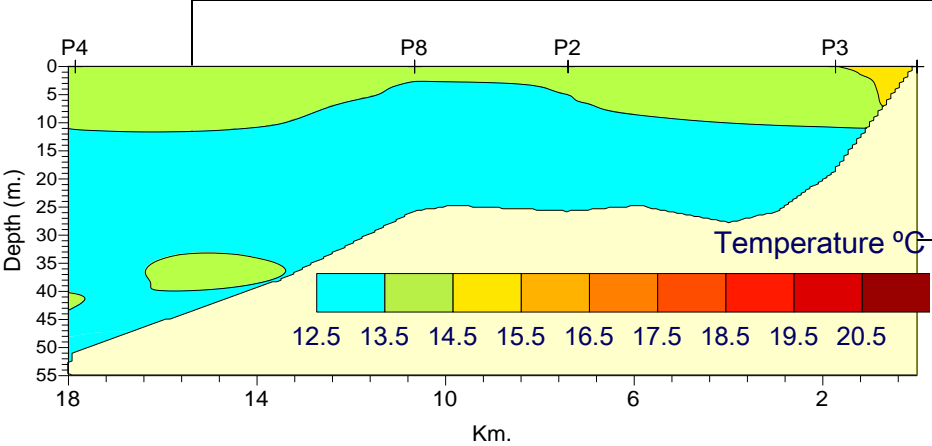


*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)
- 40 to 200
- 200 to 1000
- 1000 to 2000
- 2000 to 5000
- 5000 to 13000



SUMMER: Upwelling  
 Strong compression of the thermocline  
 Phytoplankton subsurface bloom  
*Dinophysis acuminata* persistence

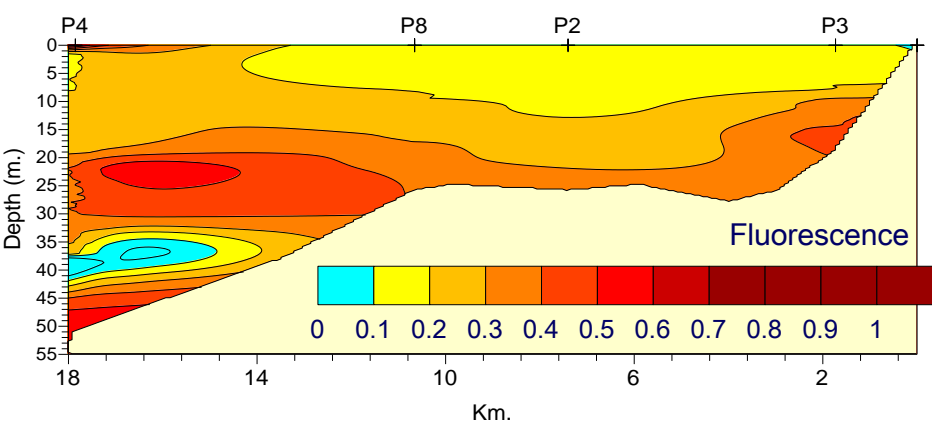
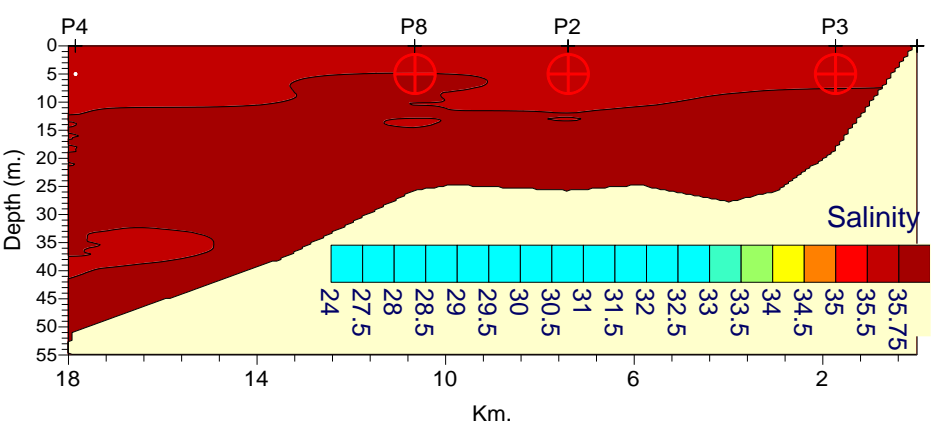
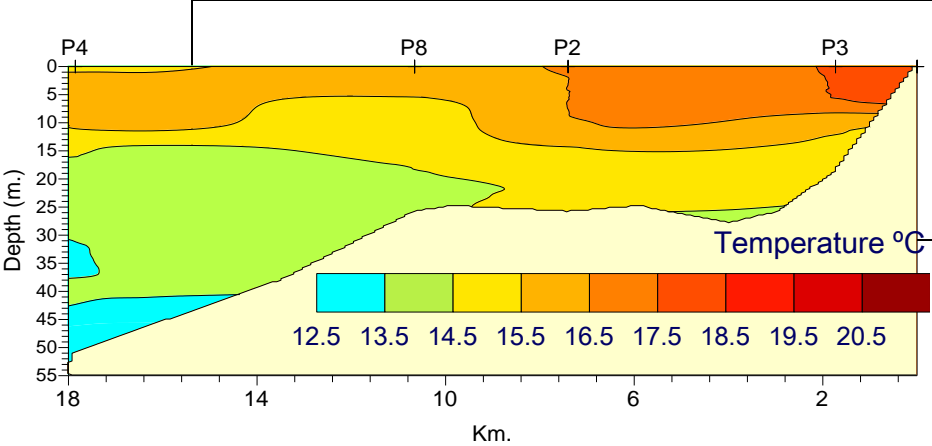


*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ 40 to 200
- ⊕ 200 to 1000
- ⊕ 1000 to 2000
- ⊕ 2000 to 5000
- ⊕ 5000 to 13000

SUMMER: Moderate downwelling  
 Intrusion of oceanic surface water  
 Phytoplankton patches  
*Dinophysis acuminata* presence

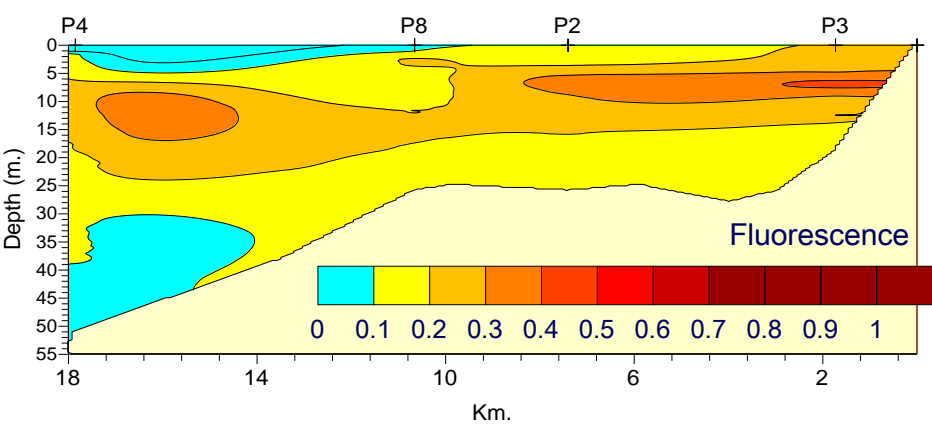
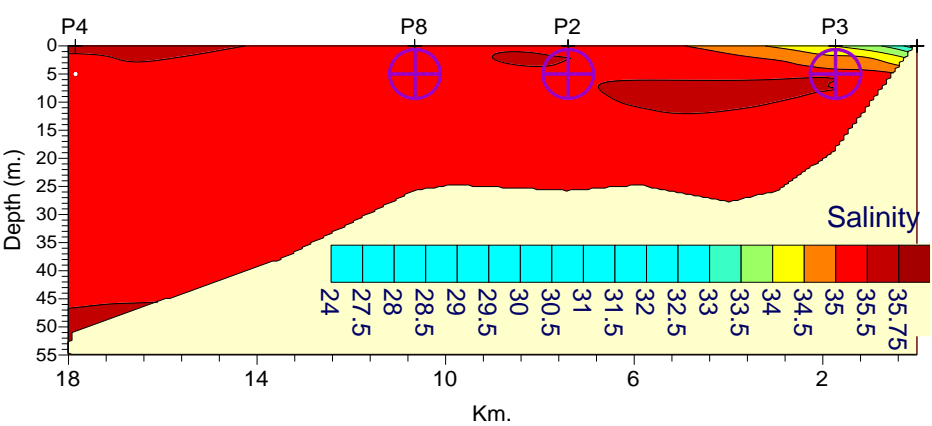
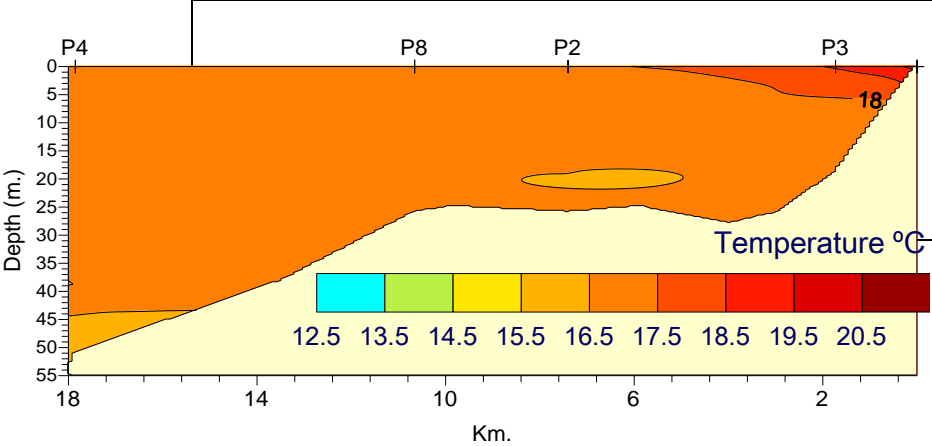




*Dinophysis acuminata* Cell L<sup>-1</sup>

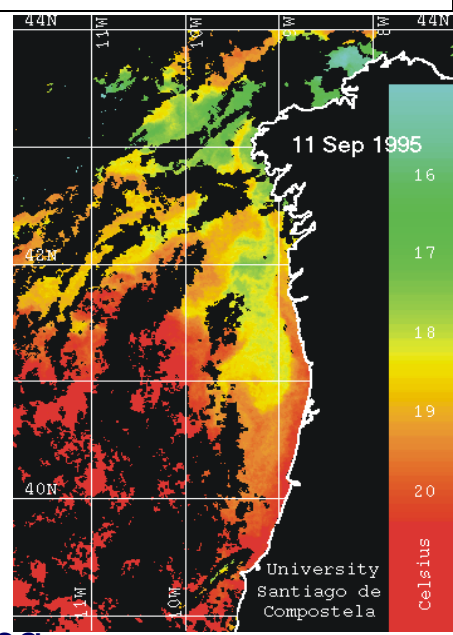
- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)

SUMMER: Moderate Downwelling  
 Intrusion of oceanic surface water  
*Dinophysis acuminata* accumulation  
 and growth

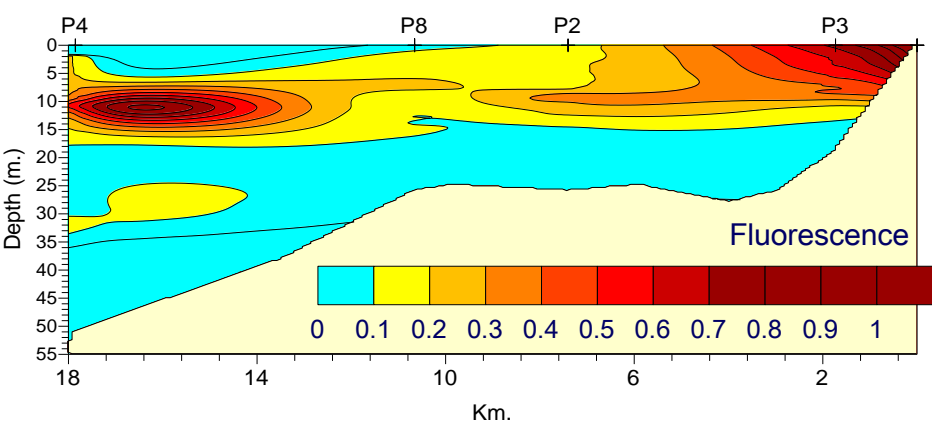
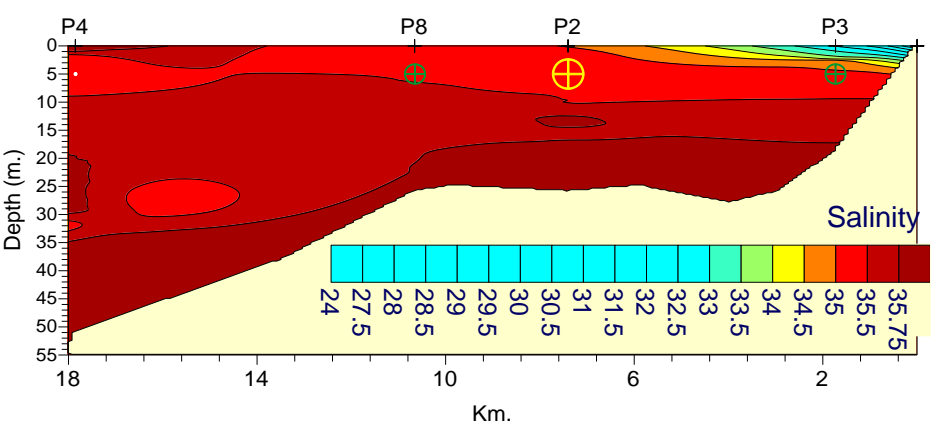
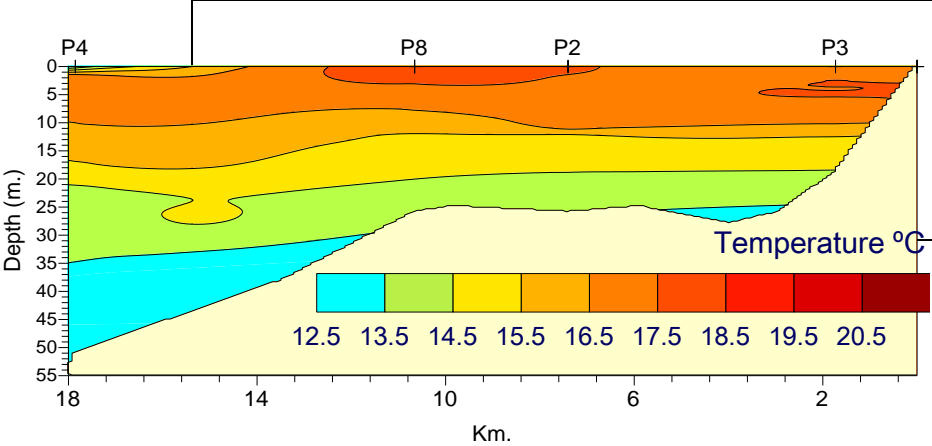


*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)
- 40 to 200
- 200 to 1000
- 1000 to 2000
- 2000 to 5000
- 5000 to 13000



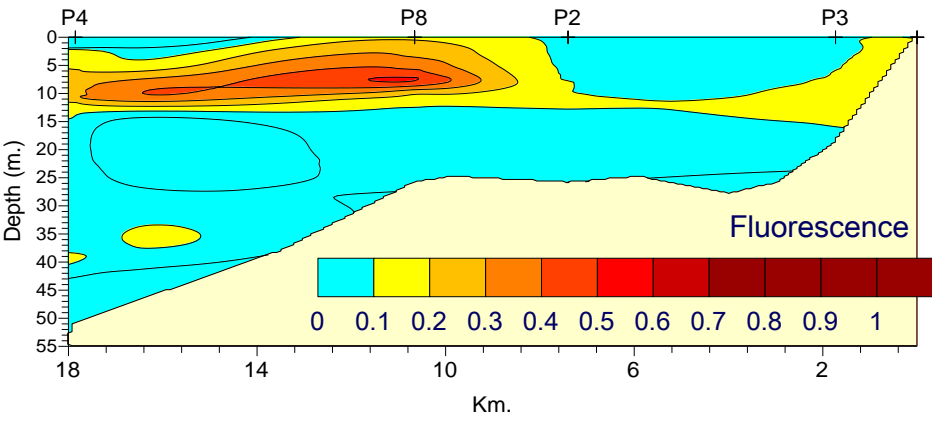
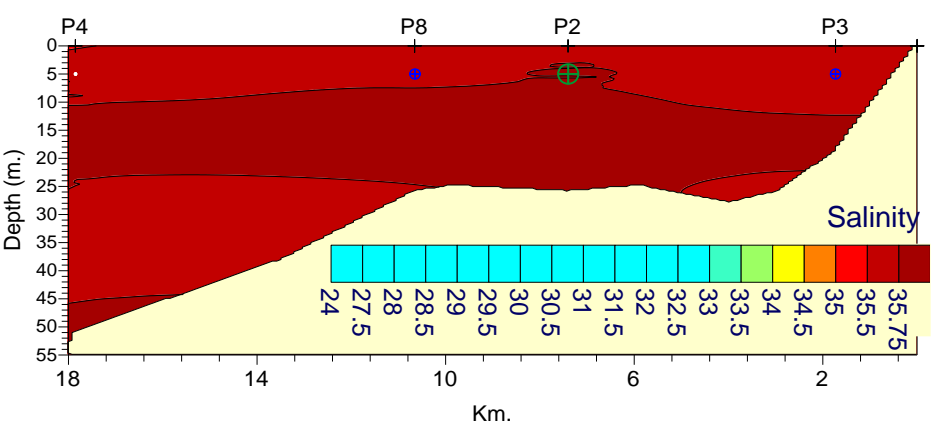
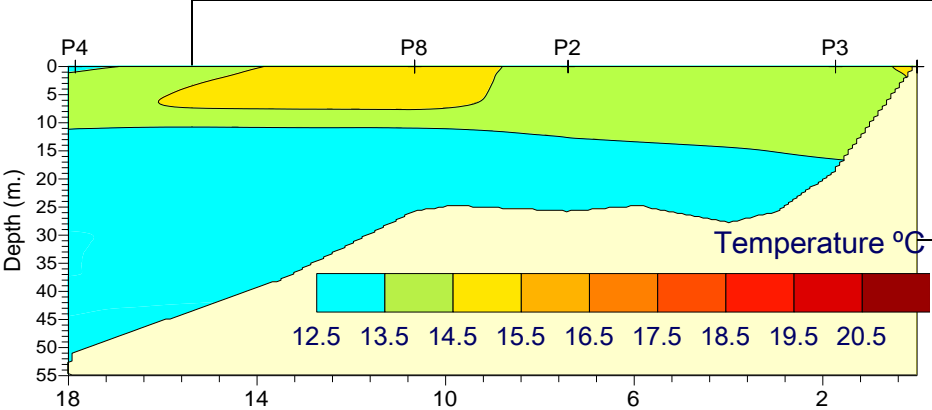
SUMMER: Downwelling  
 Intrusion of oceanic surface water  
 T= 17°C S= 35.0  
*Dinophysis acuminata* annual maximum



*Dinophysis acuminata* Cell L<sup>-1</sup>

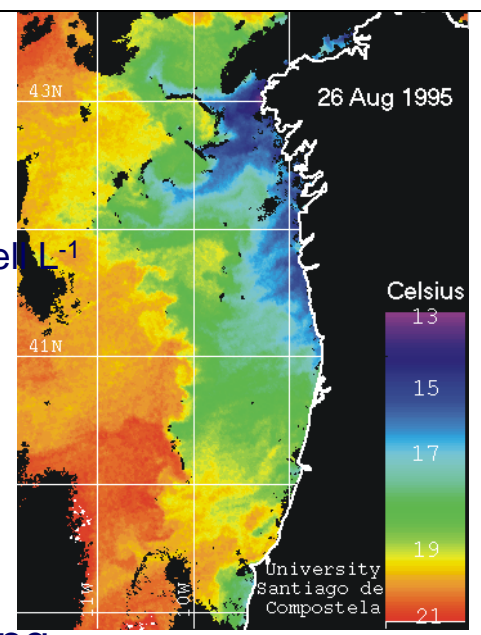
- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)
- 40 to 200
- 200 to 1000
- 1000 to 2000
- 2000 to 5000
- 5000 to 13000

SUMMER: Downwelling relaxation



*Dinophysis acuminata* Cells L<sup>-1</sup>

- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)
- 40 to 200
- 200 to 1000
- 1000 to 2000
- 2000 to 5000
- 5000 to 13000



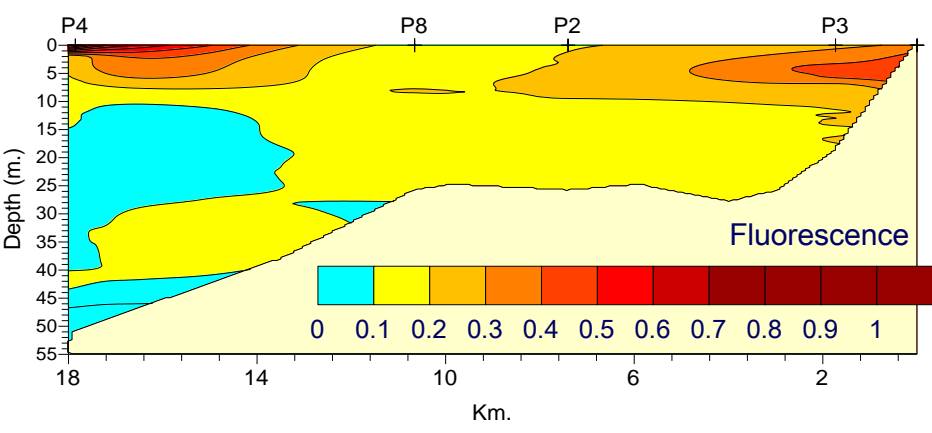
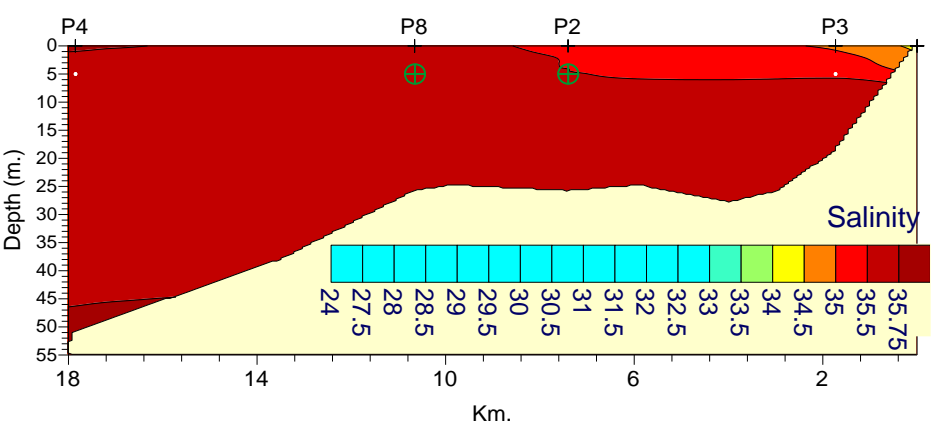
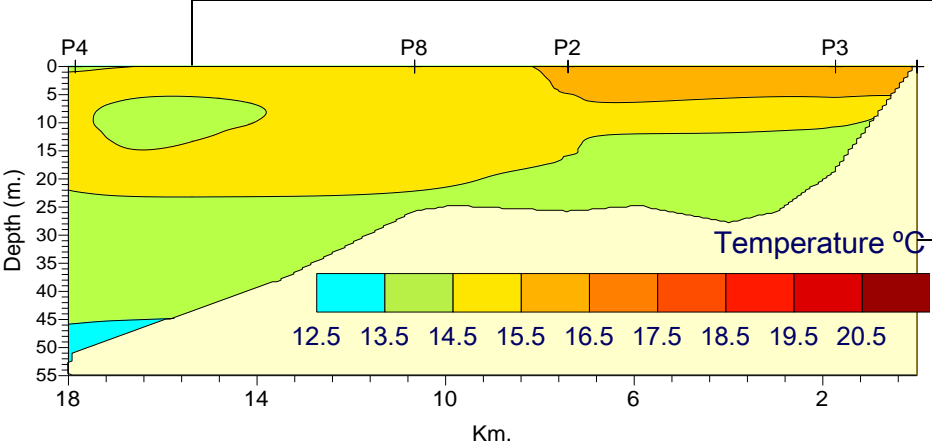
AUTUMN: Upwelling

Bottom intrusion of colder & saltier water

Phytoplankton exportation offshore

*Dinophysis acuminata* persistence

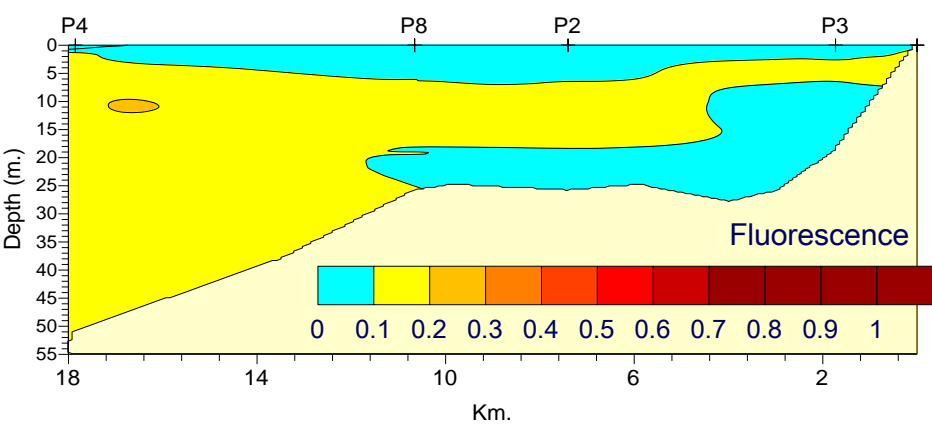
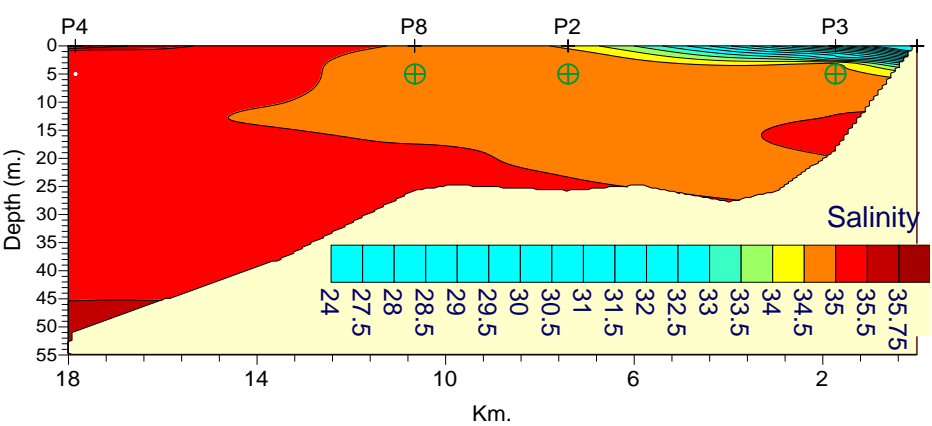
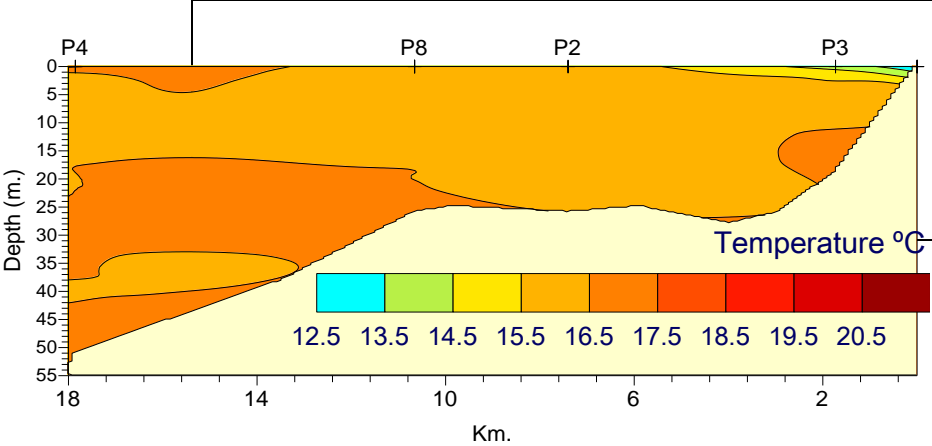




*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)

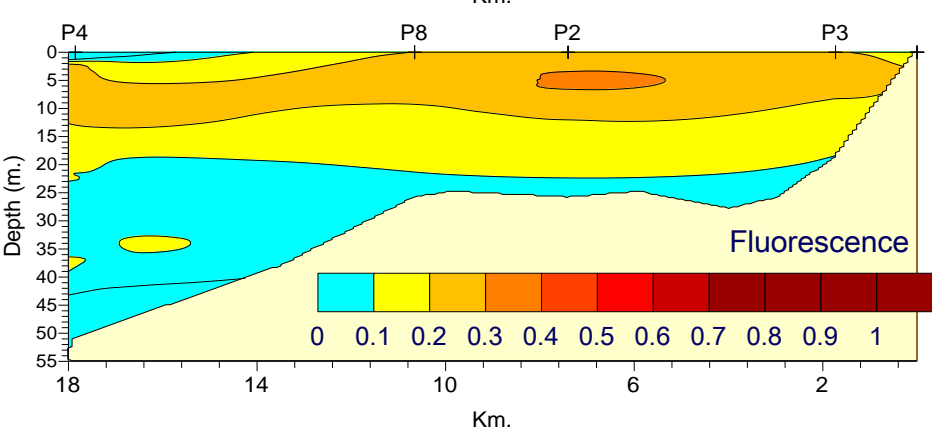
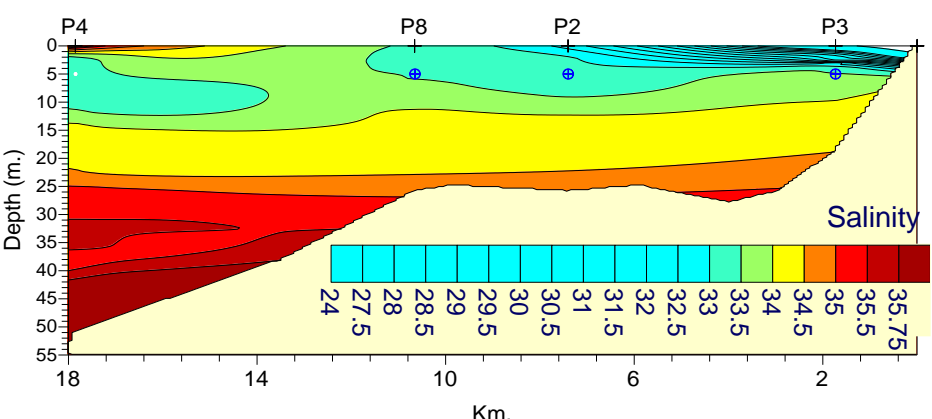
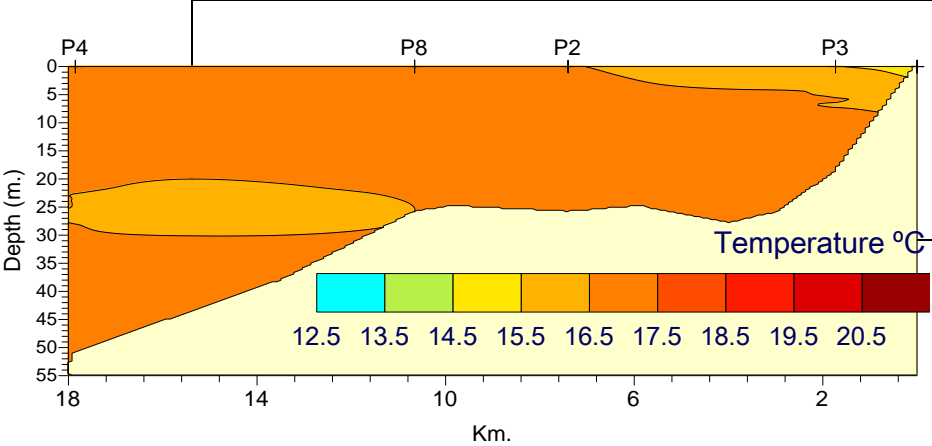
AUTUMN: Upwelling relaxation  
*Dinophysis acuminata* persistence



*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)

AUTUMN: Initiation of downwelling  
 Temperature and salinity  
 homogenization except in the innermost  
 part of the ría  
 Almost no phytoplankton  
*Dinophysis acuminata* advection

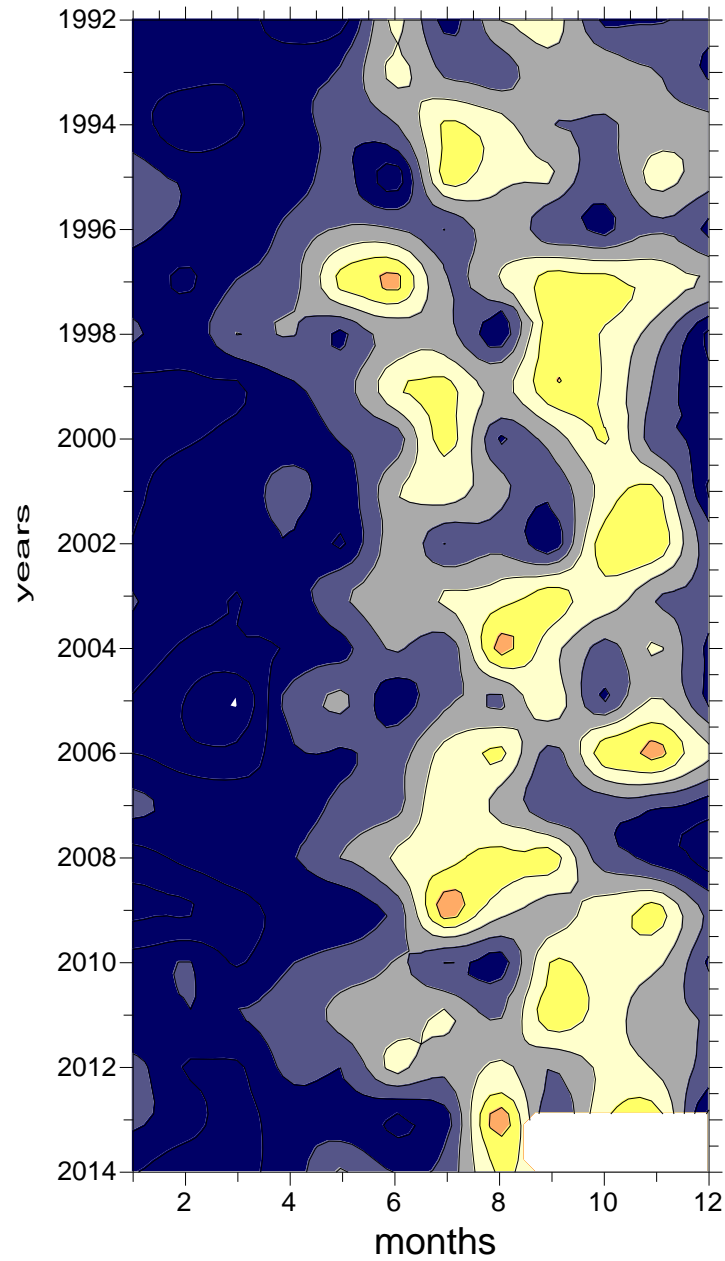


*Dinophysis acuminata* Cell L<sup>-1</sup>

- 0 to 40
- ⊕ (blue)
- ⊕ (green)
- ⊕ (yellow)
- ⊕ (red)
- ⊕ (purple)
- 40 to 200
- 200 to 1000
- 1000 to 2000
- 2000 to 5000
- 5000 to 13000

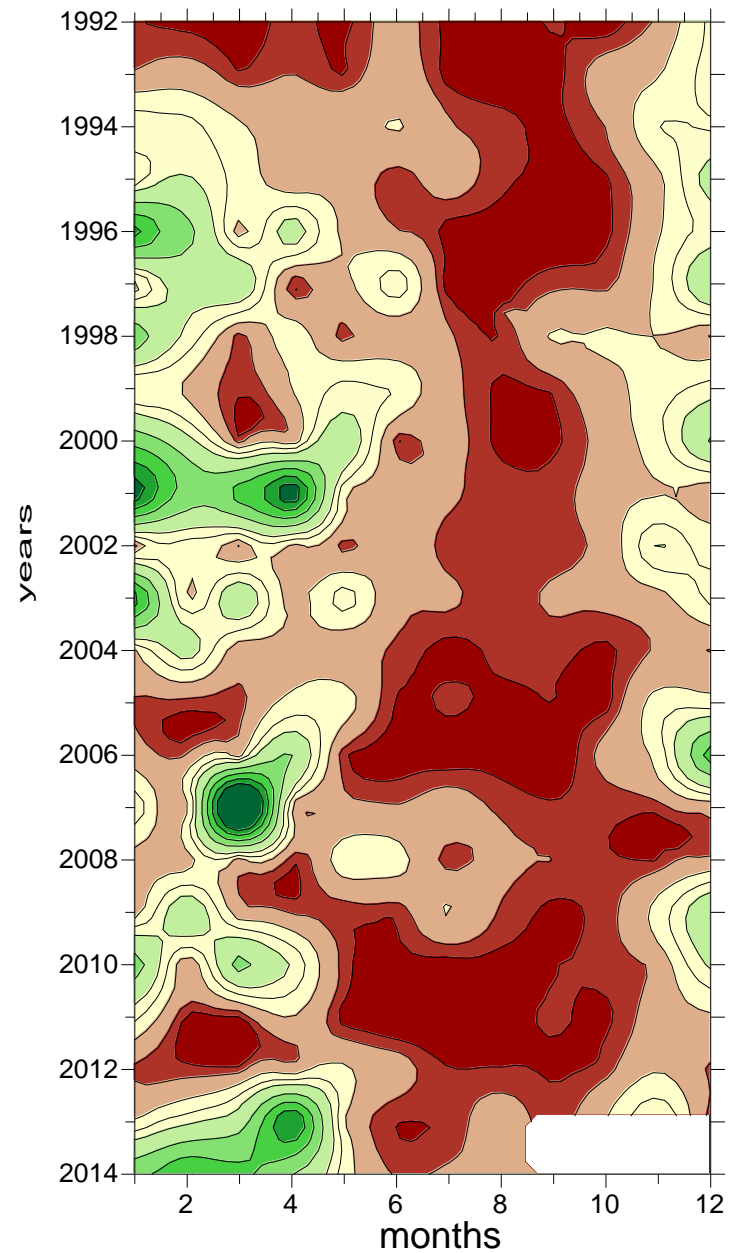
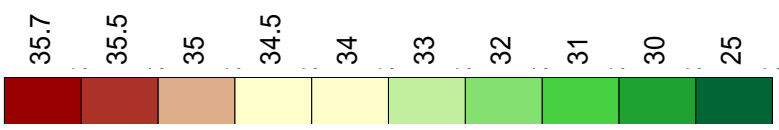
WINTER CONDITIONS: Downwelling & rainfall  
 Almost phytoplankton absence  
*Dinophysis acuminata* disappearing

Temperature (0-15 m.) °C

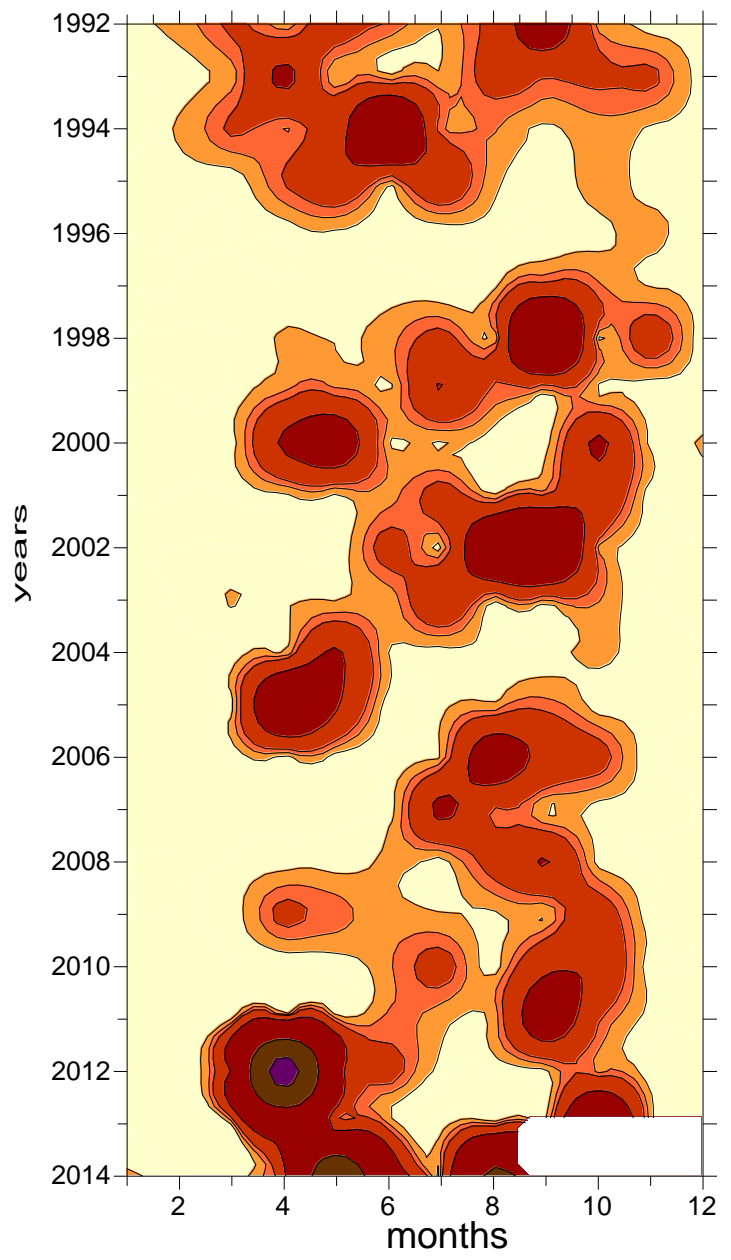
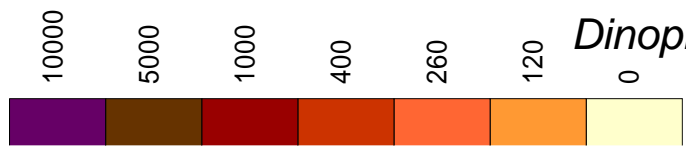




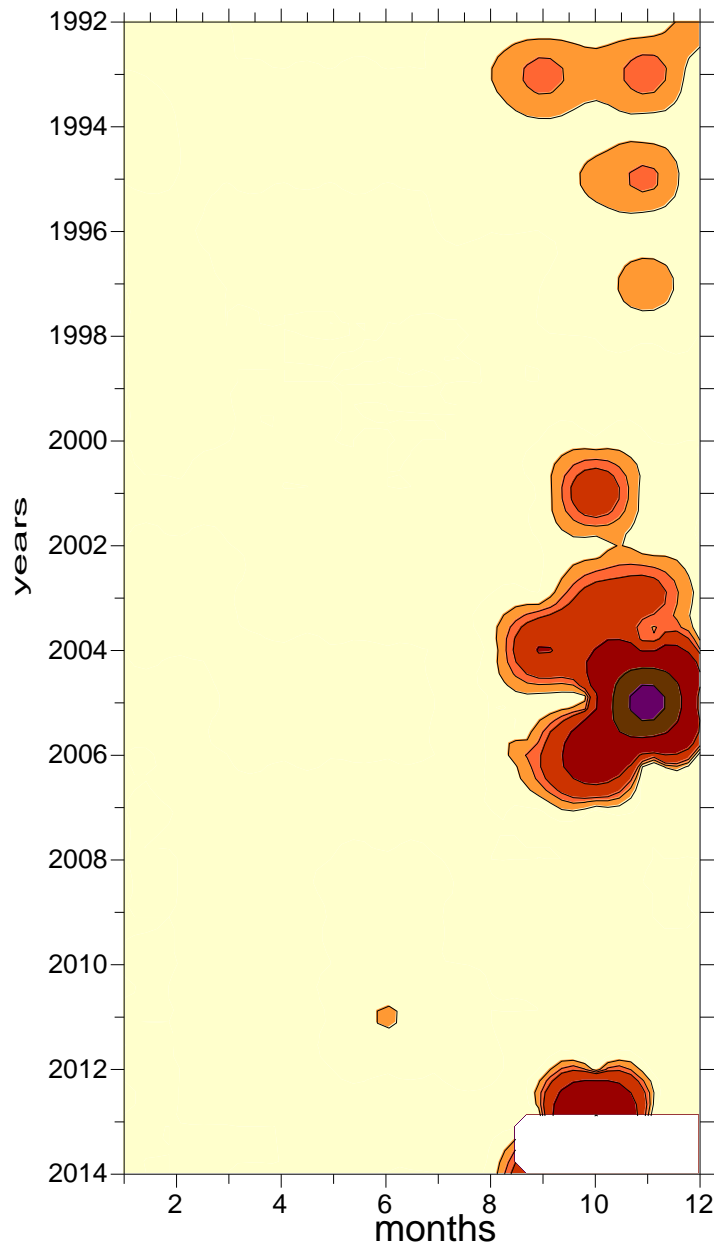
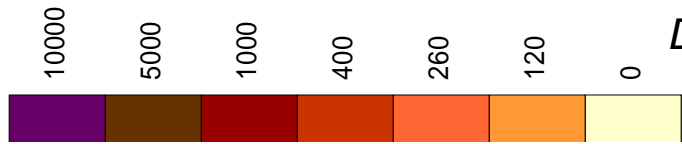
Salinity (0-15 m.) °C



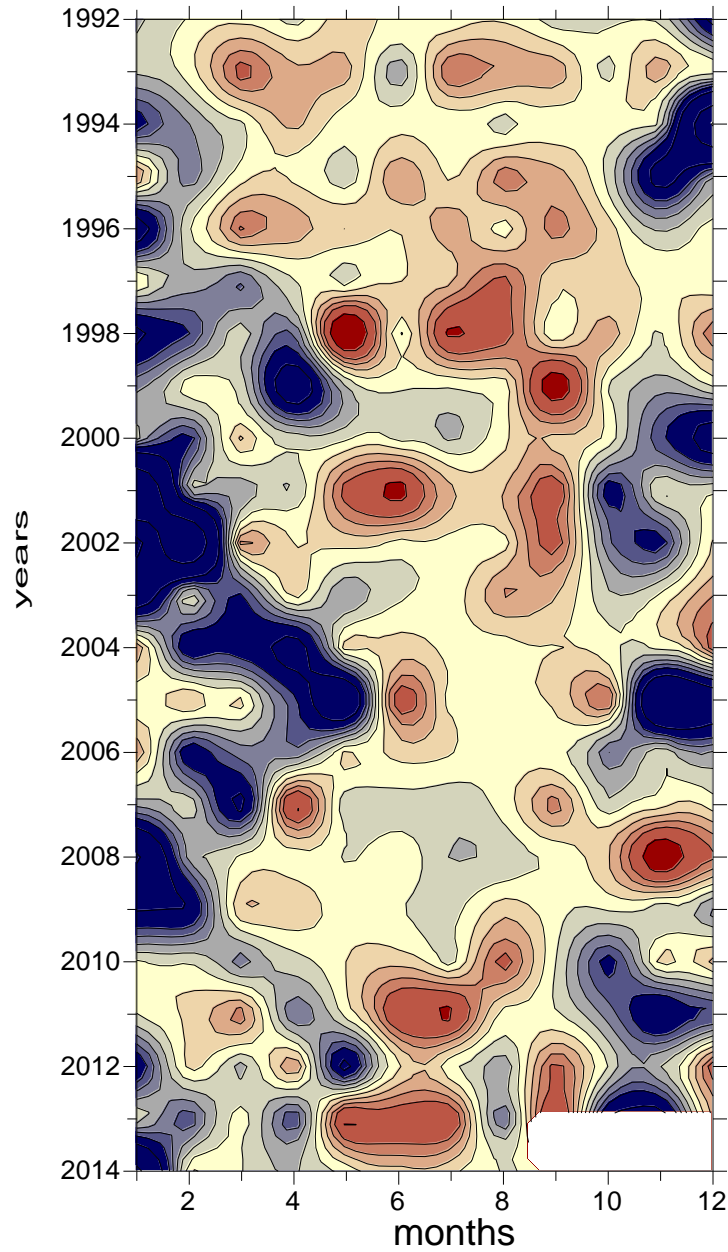
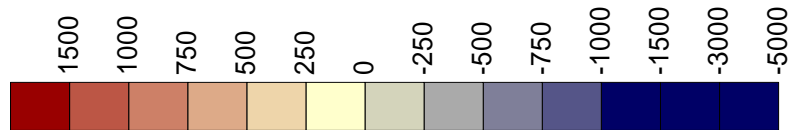
*Dinophysis acuminata* (0-15 m.) cellL<sup>-1</sup>



*Dinophysis acuta* (0-15 m.) cellL<sup>-1</sup>

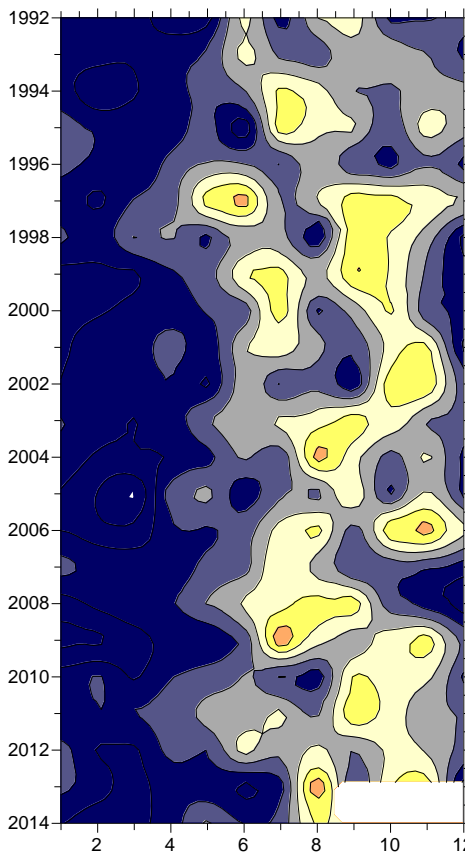
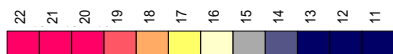


Upwelling index  $\text{m}^3/\text{s}/\text{Km}$  coastline

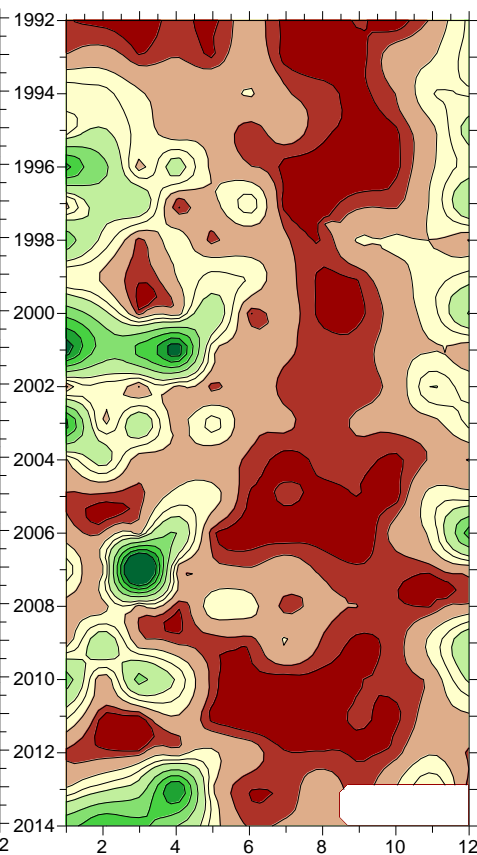
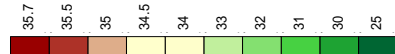




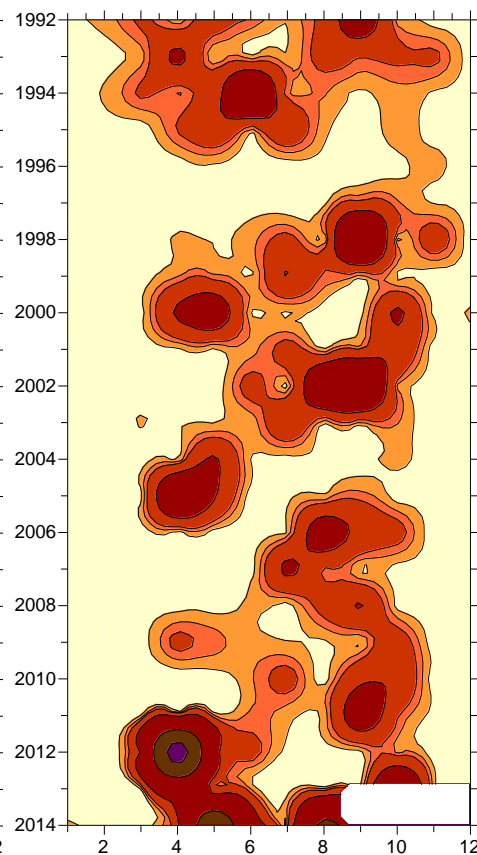
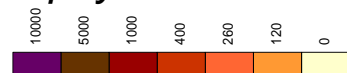
Temperature (0-15 m.) °C



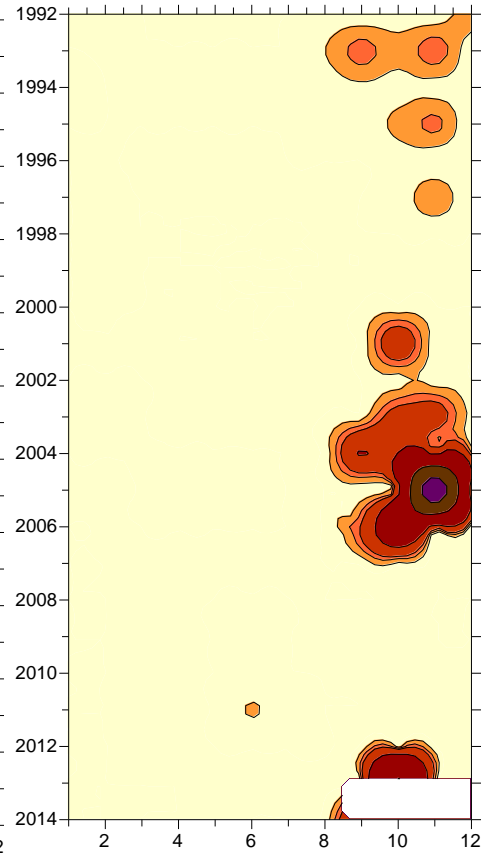
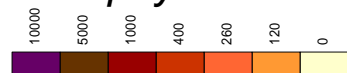
Salinity (0-15 m.) °C



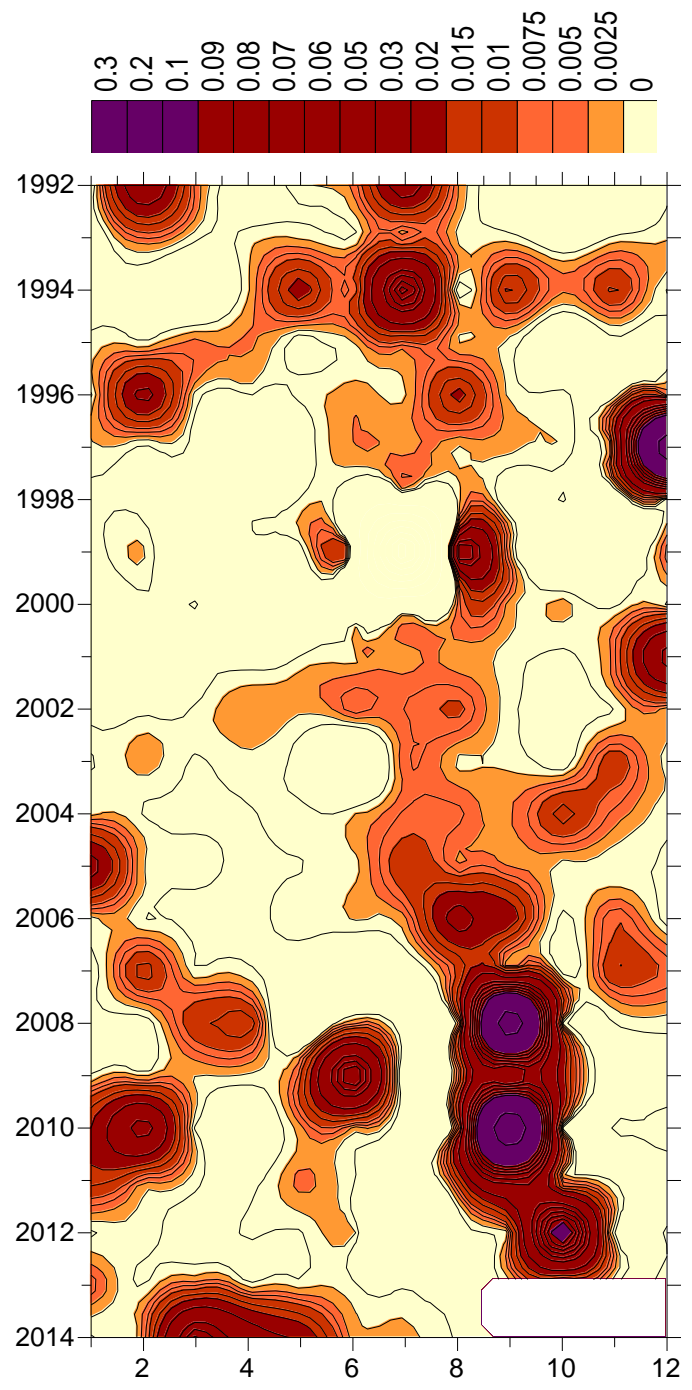
*Dinophysis acuminata*



*Dinophysis acuta*

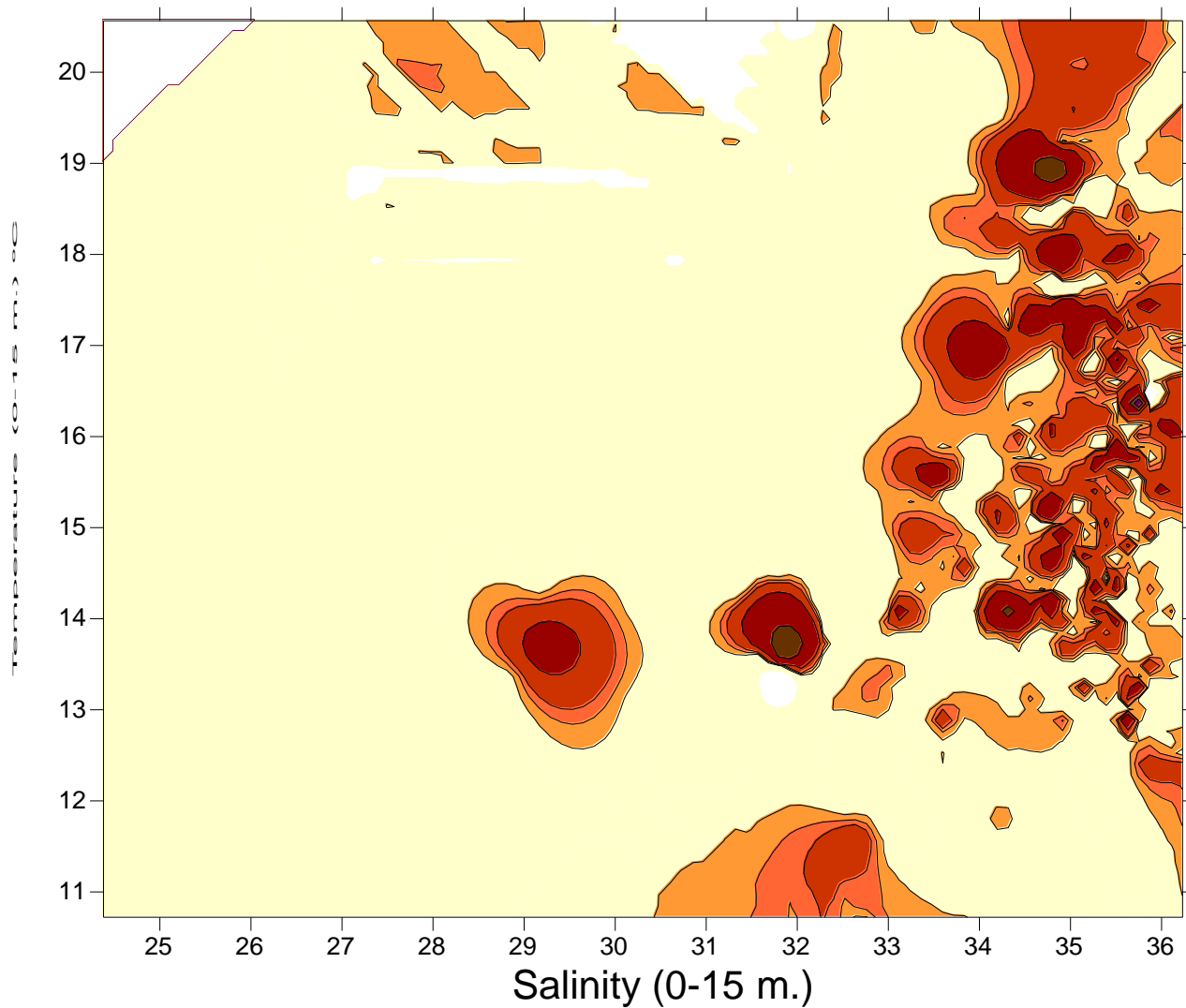


Renewal time (s)

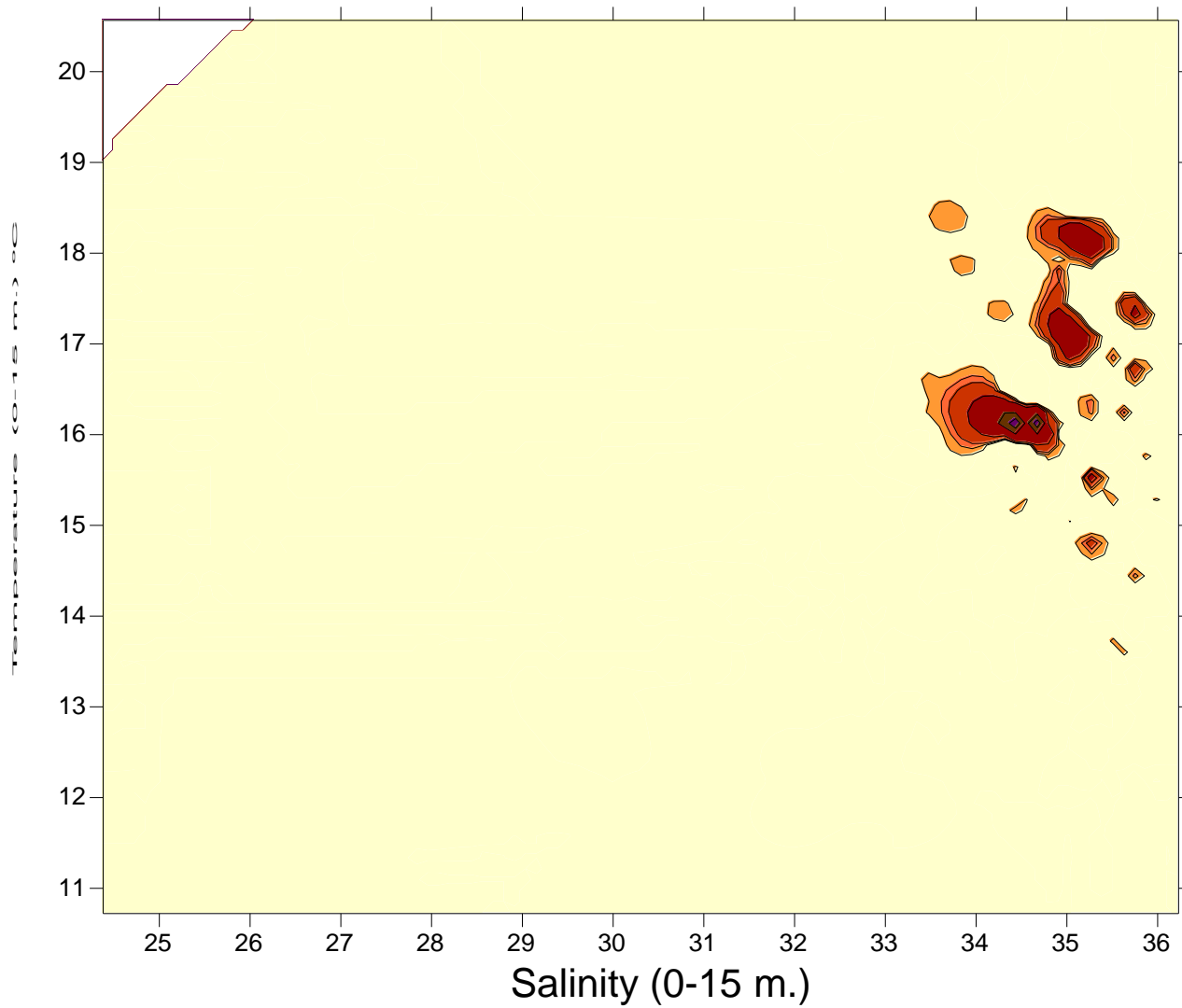
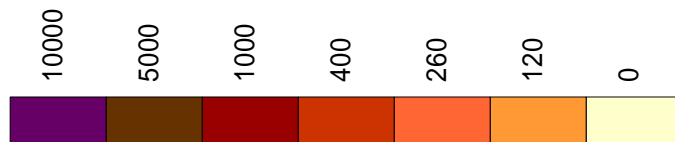


*Dinophysis acuminata* (0-15 m.) cellL<sup>-1</sup>

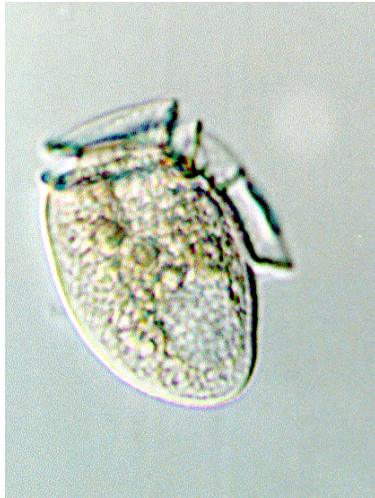
10000  
5000  
1000  
400  
260  
120  
0



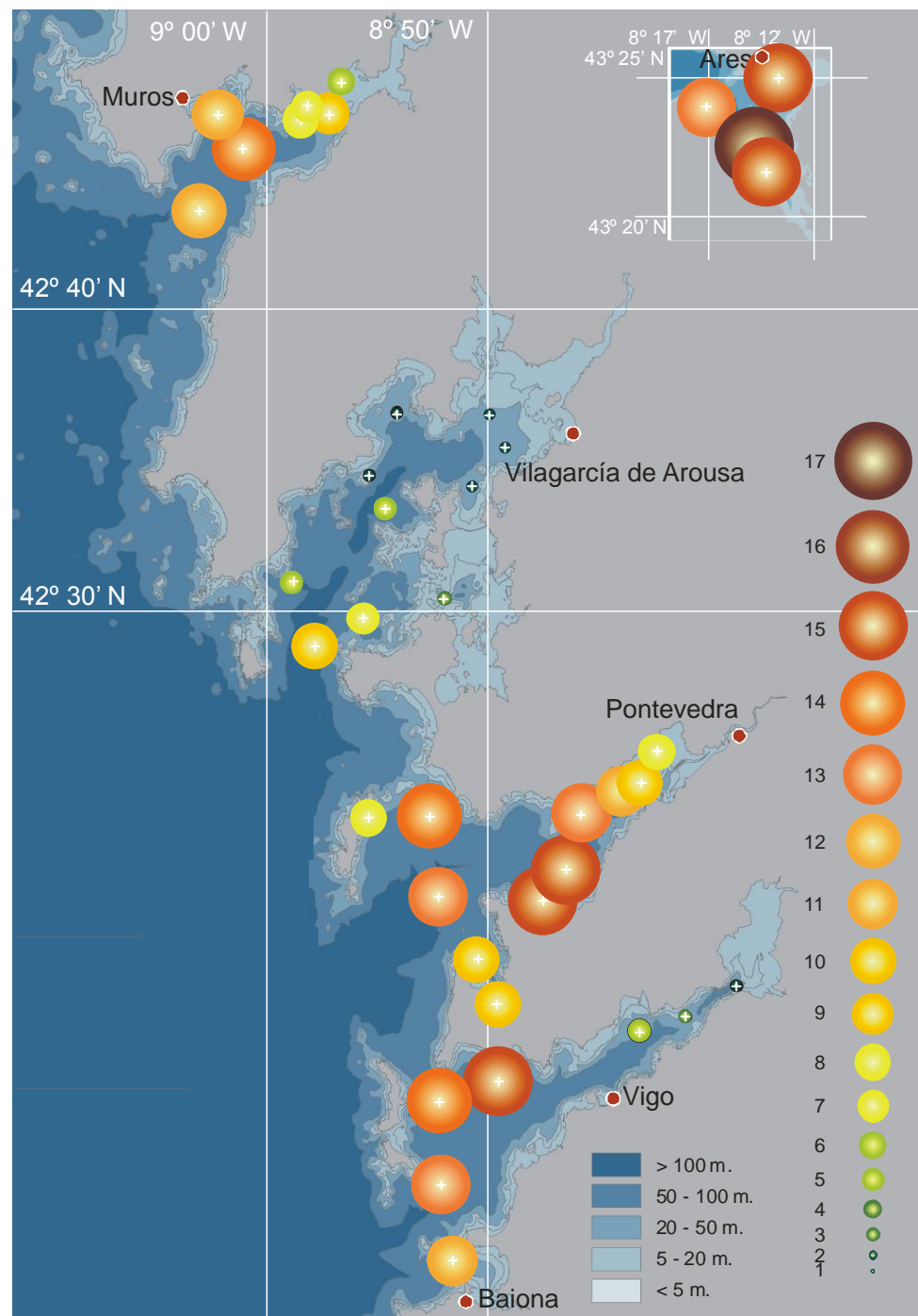
*Dinophysis acuta* (0-15 m.) cellL<sup>-1</sup>





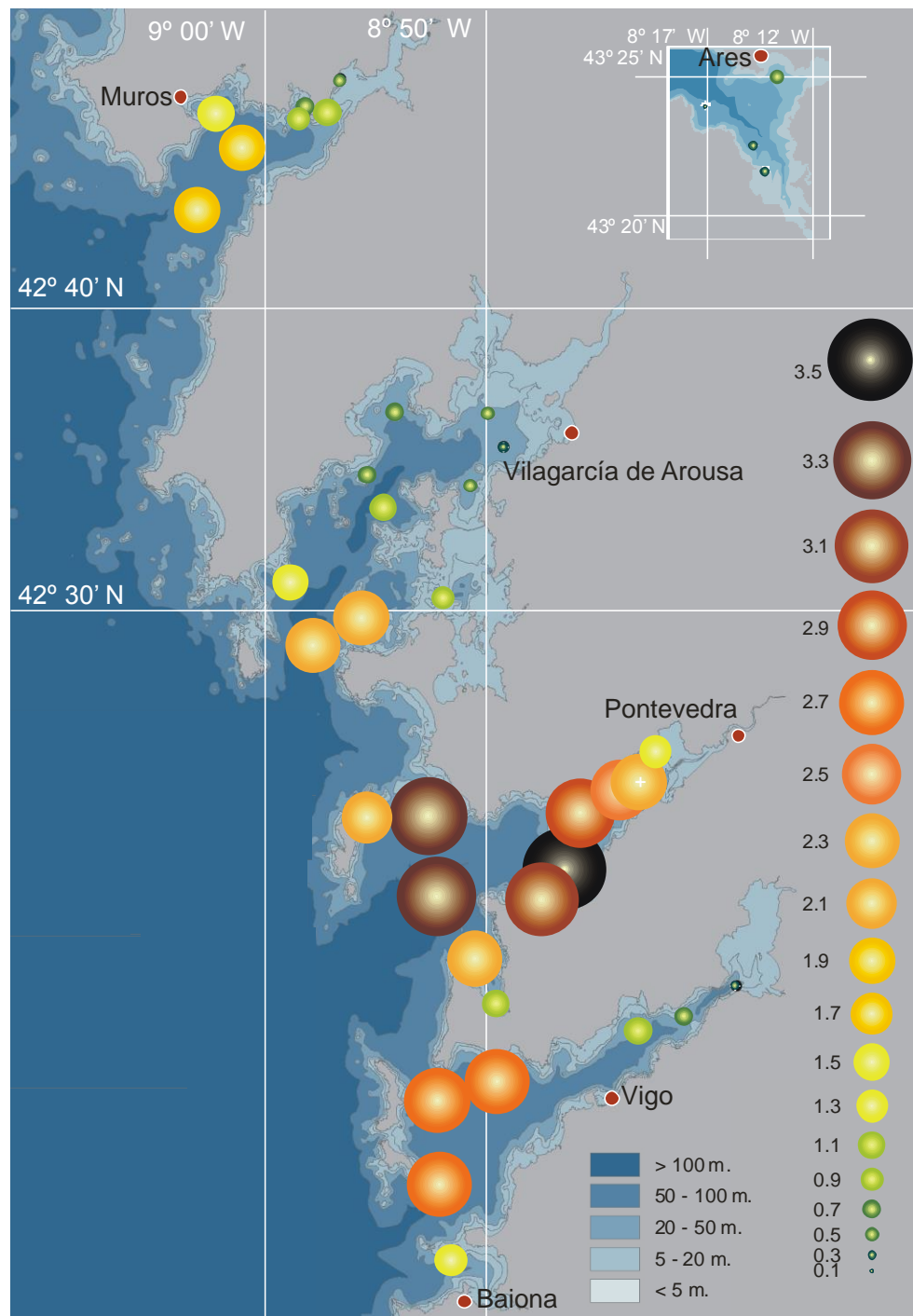


*Dinophysis acuminata*  
Persistence (weeks)  
>120 cel L<sup>-1</sup>  
1992-2014

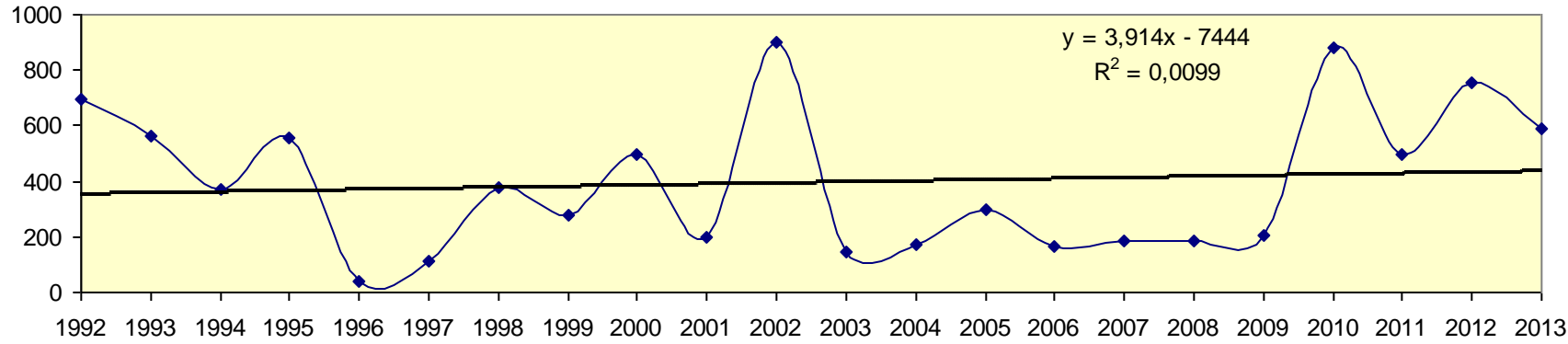




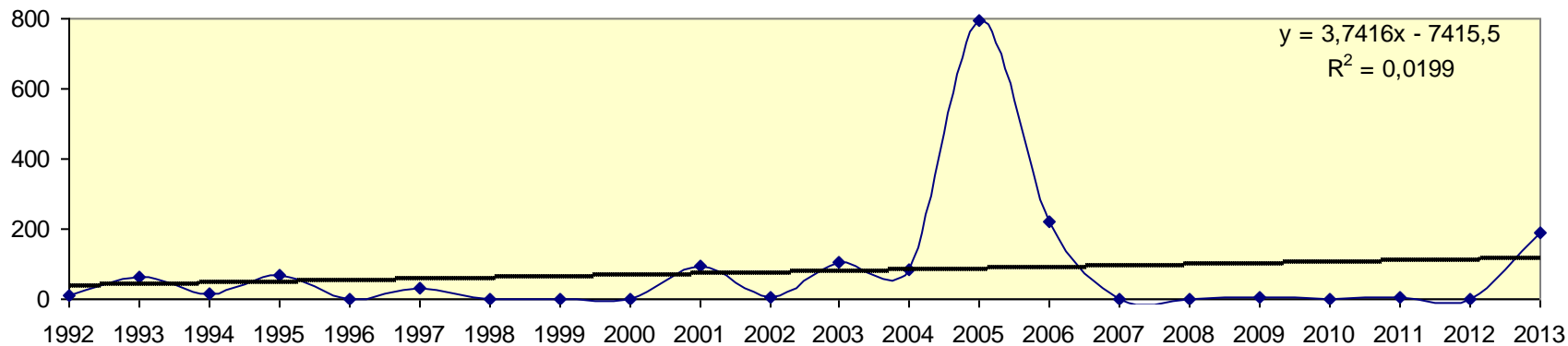
*Dinophysis acuta*  
 Persistence (weeks)  
 >120 cel L<sup>-1</sup>  
 1992-2014



### *Dinophysis acuminata* cellL<sup>-1</sup> (0-15 m) P2 St



### *Dinophysis acuta* cellL<sup>-1</sup> (0-15 m) P2 St

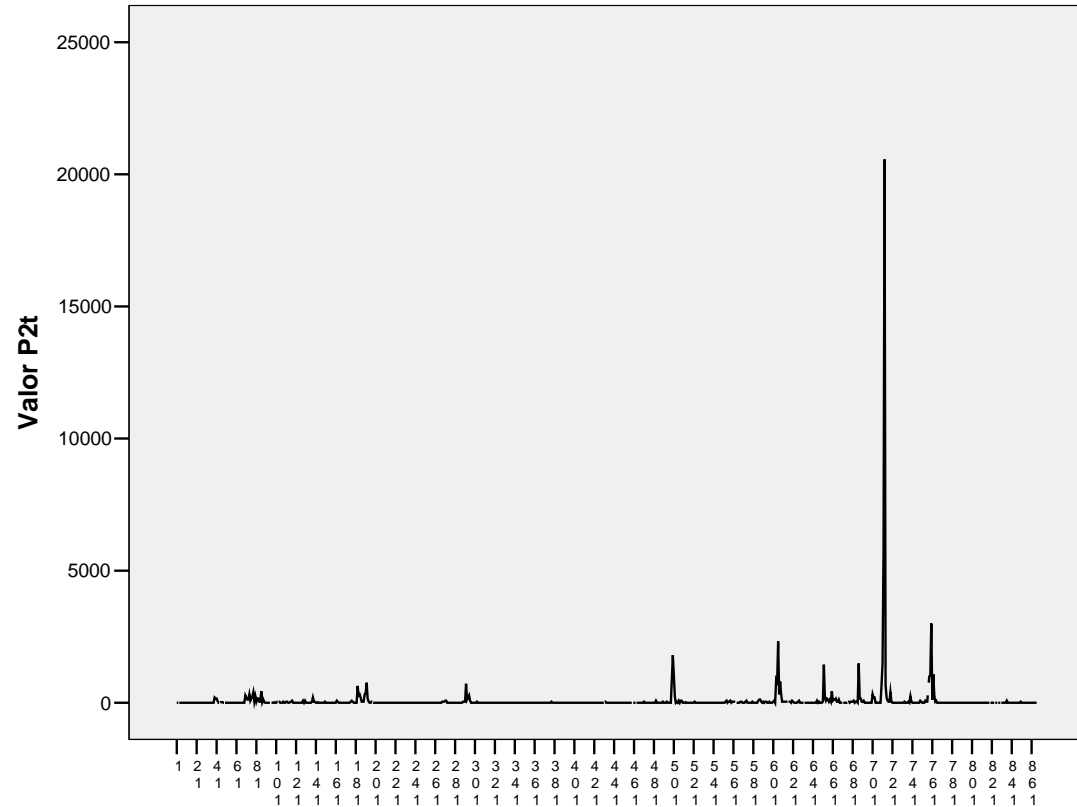




## *Dinophysis acuminata* (P2 st)

Model moving average  
MA (1) because the  
first two  
autocorrelations are  
partial and very high.

A trend is not observed





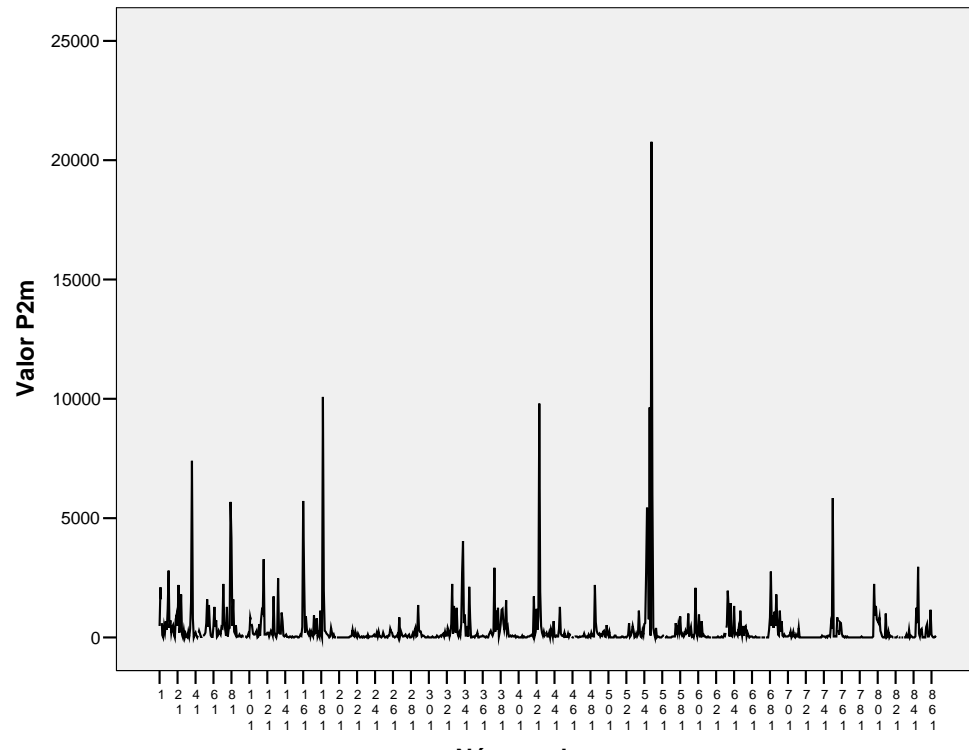


## *Dinophysis acuta* (P2 st)

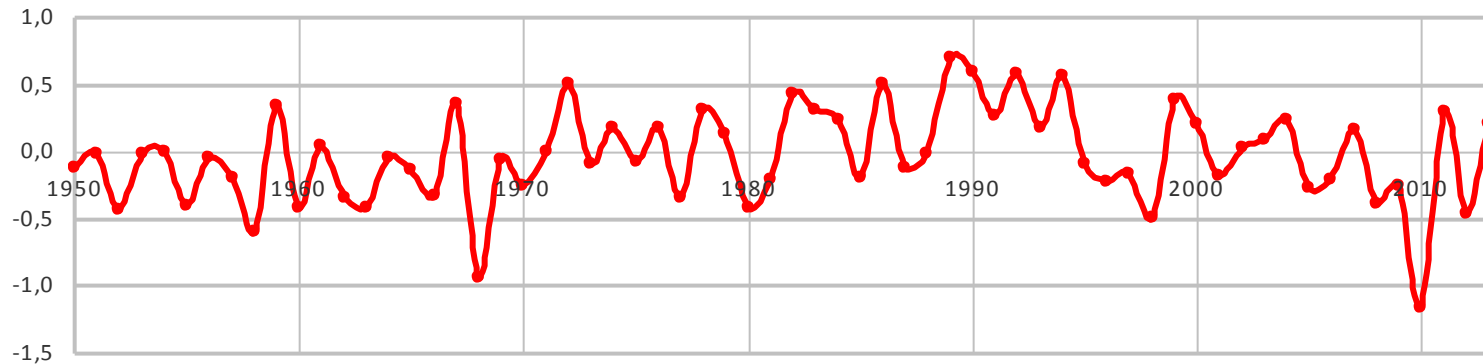
Autoregressive model AR (2).  
The first autocorrelation is high and the rest falls coup.

This series presents a seasonal cycle

There is not a long-term trend.

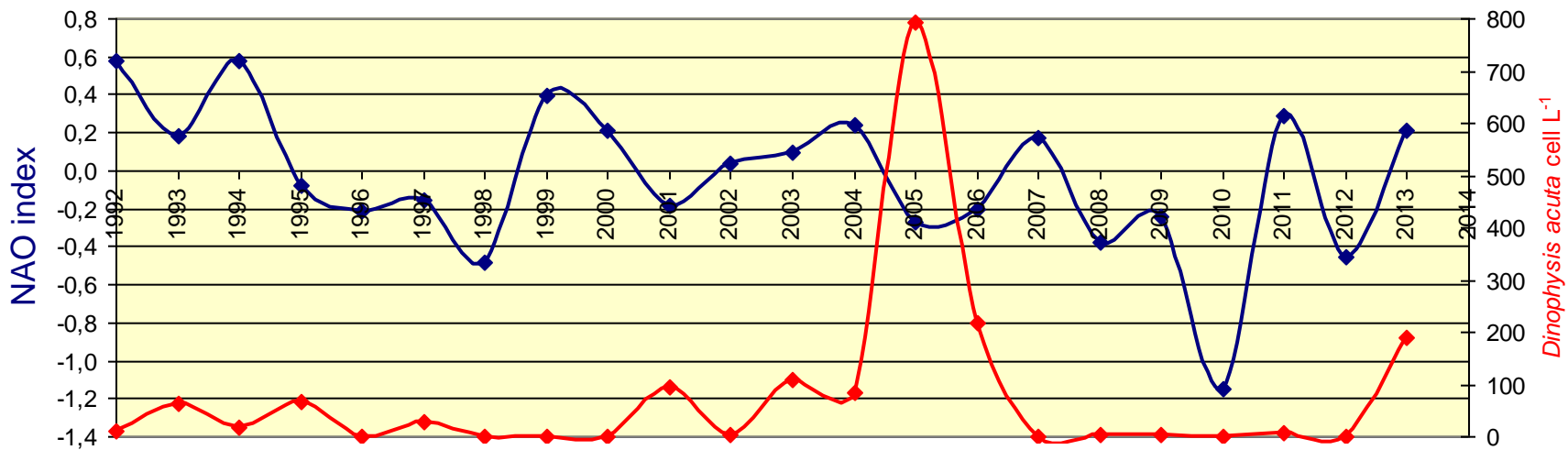


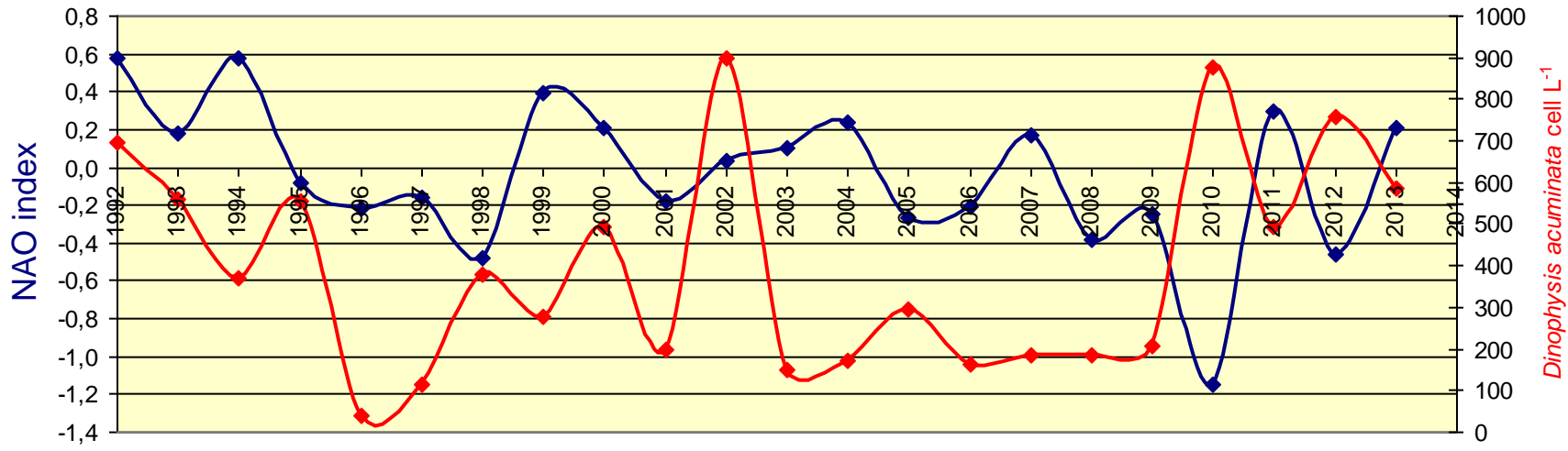
### NAO index



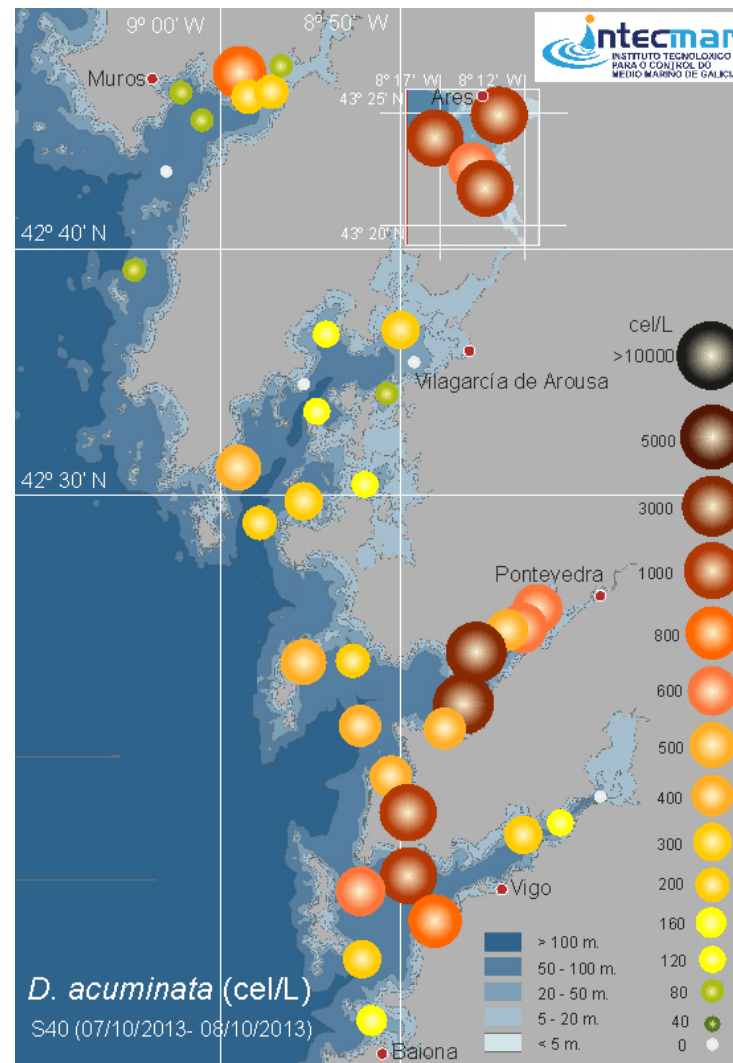
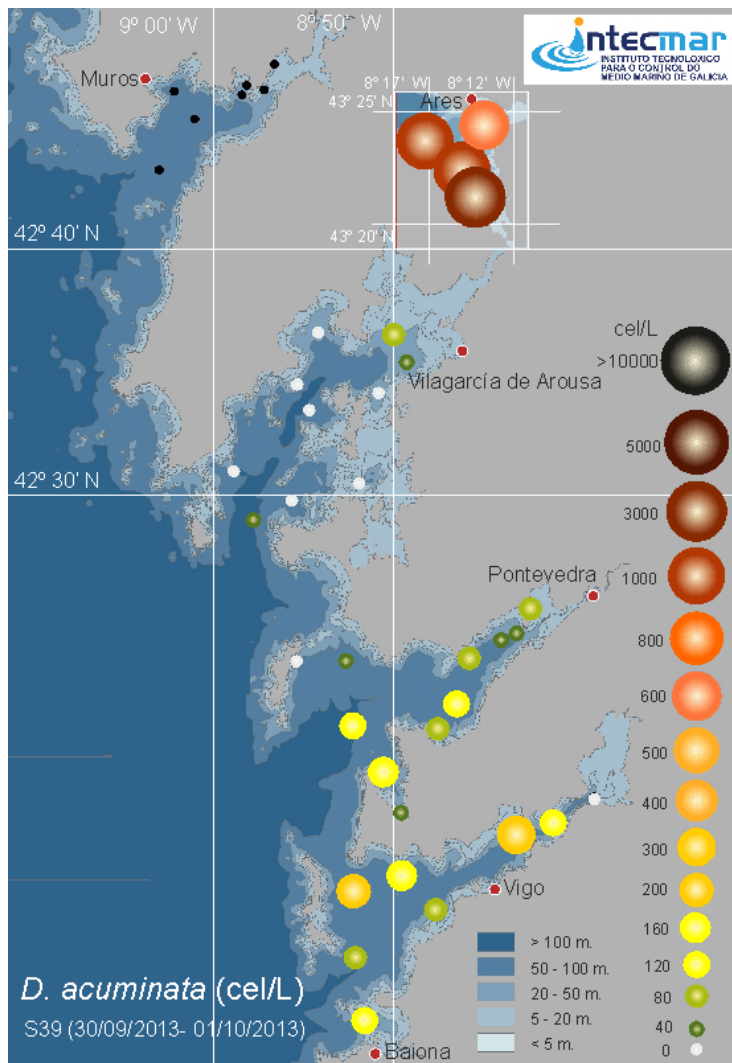
Jones PD, Jonsson T and Wheeler D (1997) Extension to the North Atlantic Oscillation using early instrumental pressure observations from Gibraltar and South-West Iceland. *Int. J. Climatol.* 17, 1433-1450.

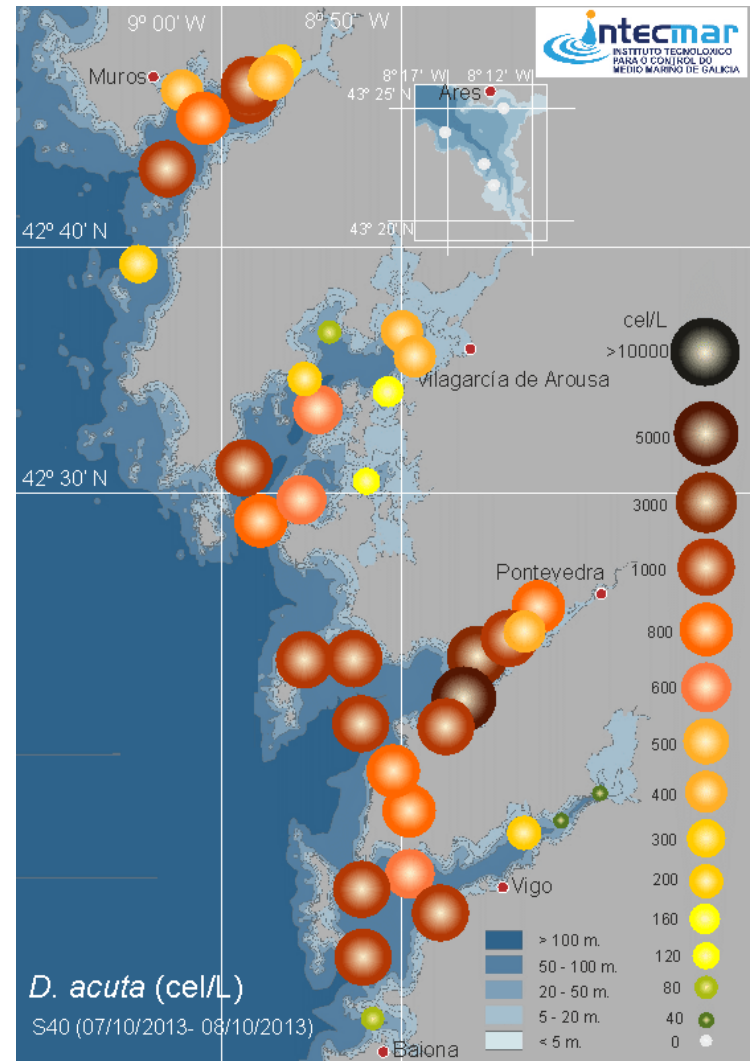
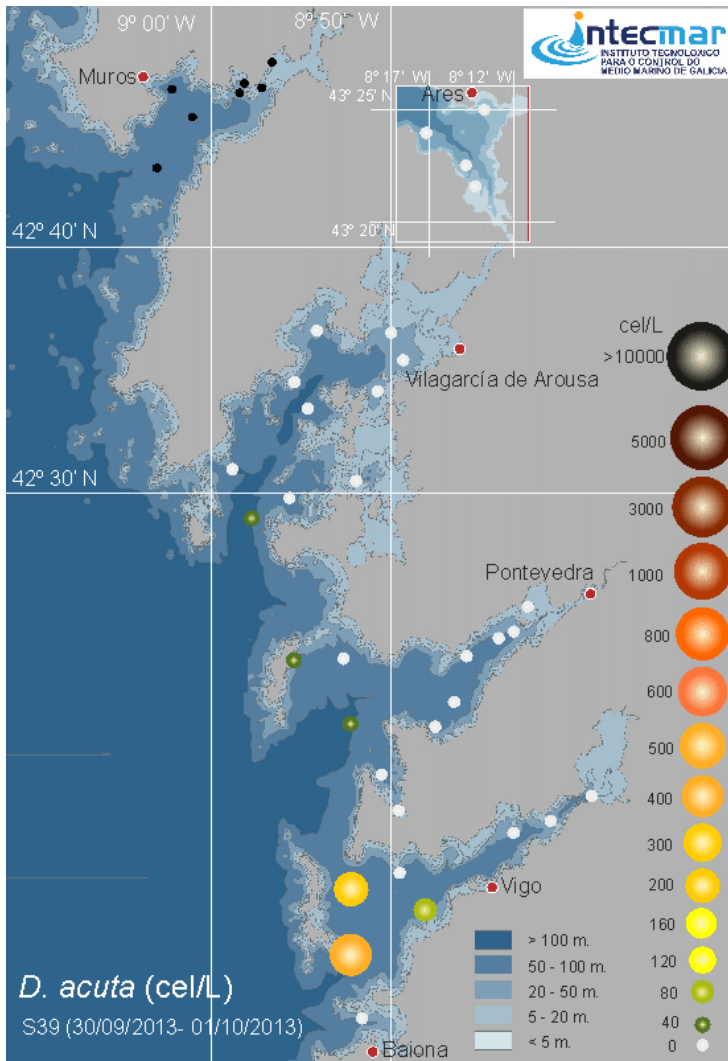
Osborn TJ (2004) Simulating the winter North Atlantic Oscillation: the roles of internal variability and greenhouse gas forcing. *Clim. Dyn.* 22, 605-623.











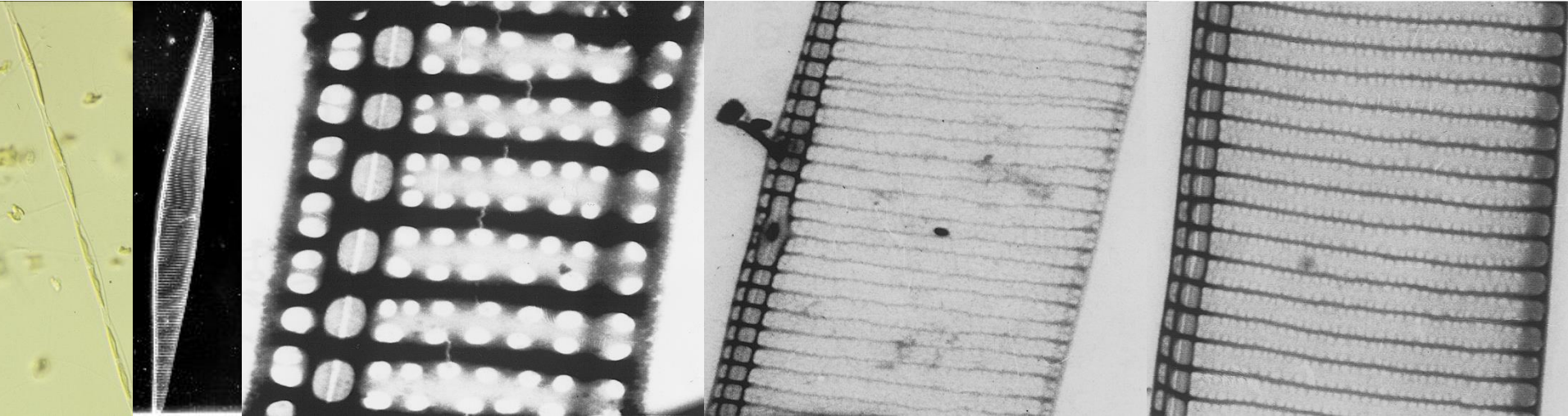
## Amnésicas (ASP)

Las especies relacionadas con la presencia de las toxinas descritas en 1987, toxinas amnésicas pertenecen al género *Pseudo-nitzschia*. La principal causante de las prolongadas prohibiciones de extracción de vieira en Galicia es *P. australis*



la sopa de nuera

# Clase Bacillariophyceae



*Pseudo-nitzschia* sp.

*Pseudo-nitzschia pungens* (Grunow ex Cleve) Hasle, 1993

*Pseudo-nitzschia fraudulentata* (Cleve) Hasle, 1993

*Pseudo-nitzschia australis* Frenguelli, 1939

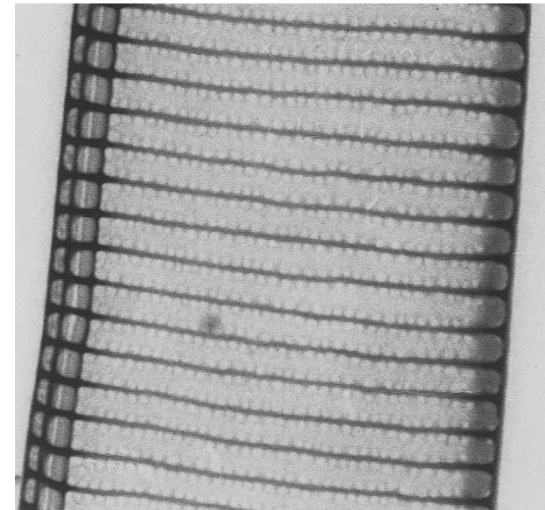
Diatomeas **Amnésicas (ASP)**



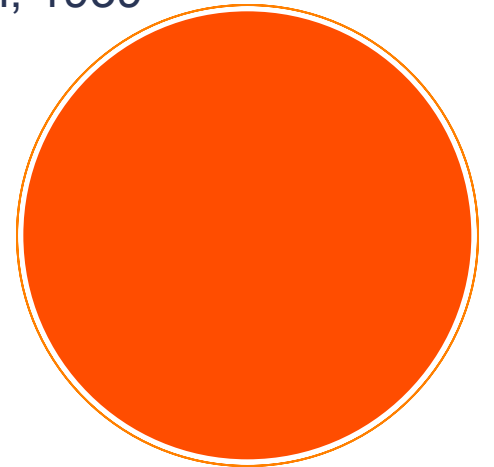
# AMNESIC SHELLFISH POISONING PRODUCER SPECIES



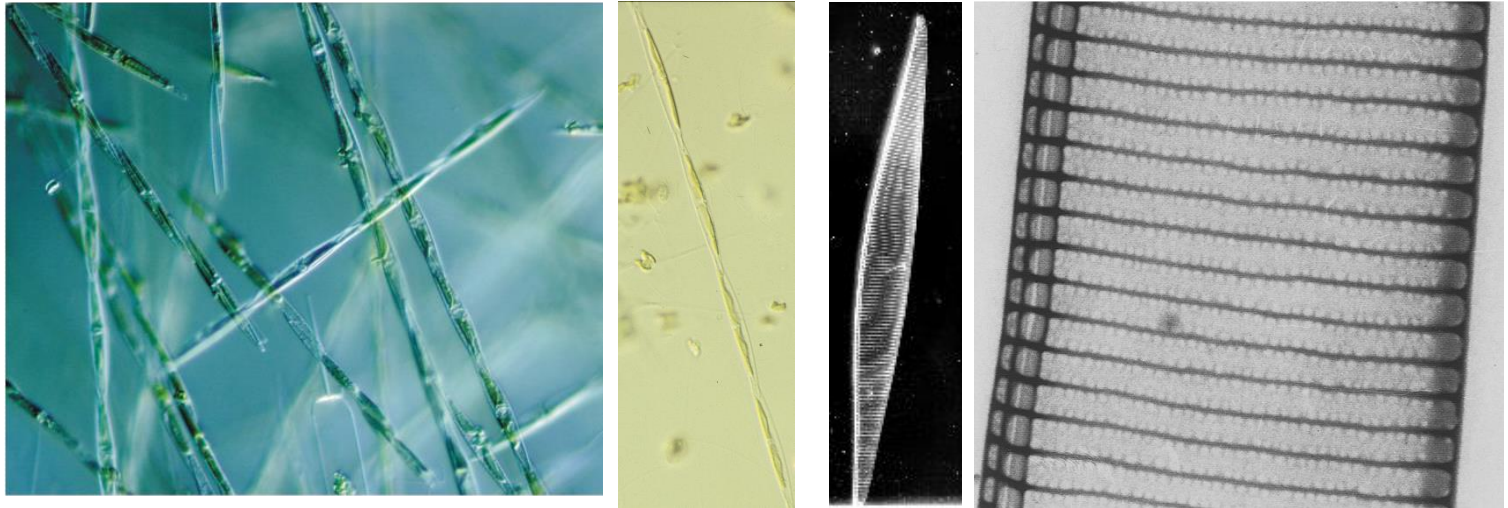
*Pseudo-nitzschia* sp.  
Peragallo in Peragallo & Peragallo, 1900



*Pseudo-nitzschia australis*  
Frenguelli, 1939

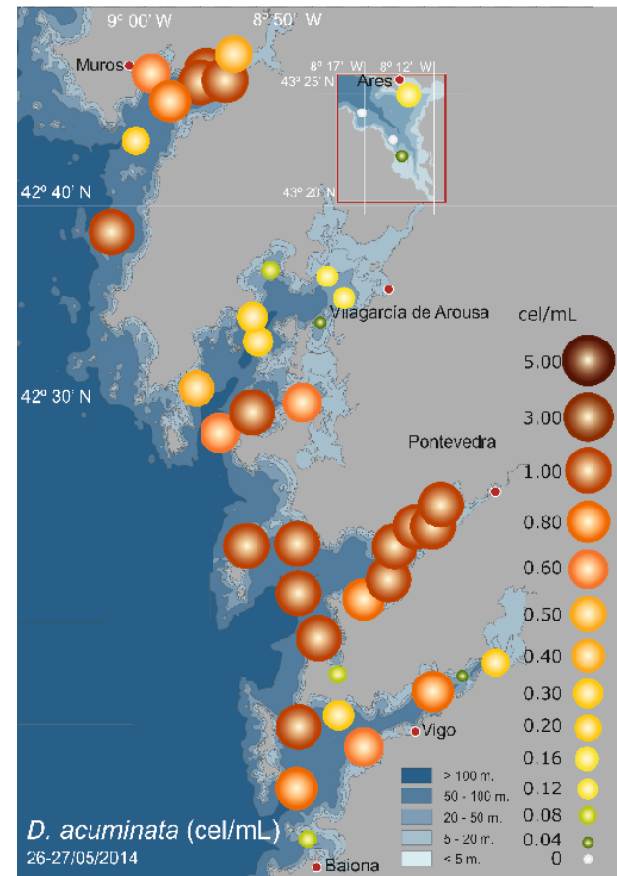
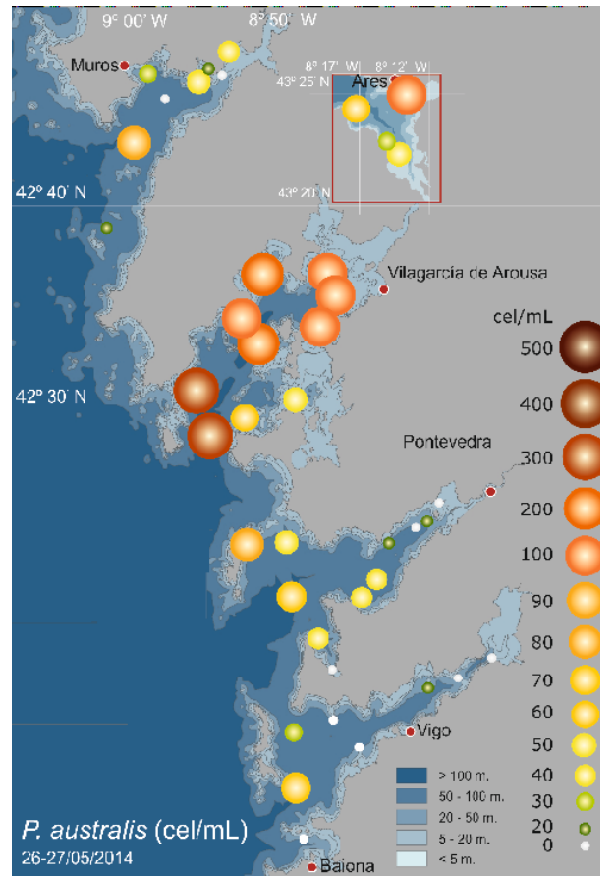
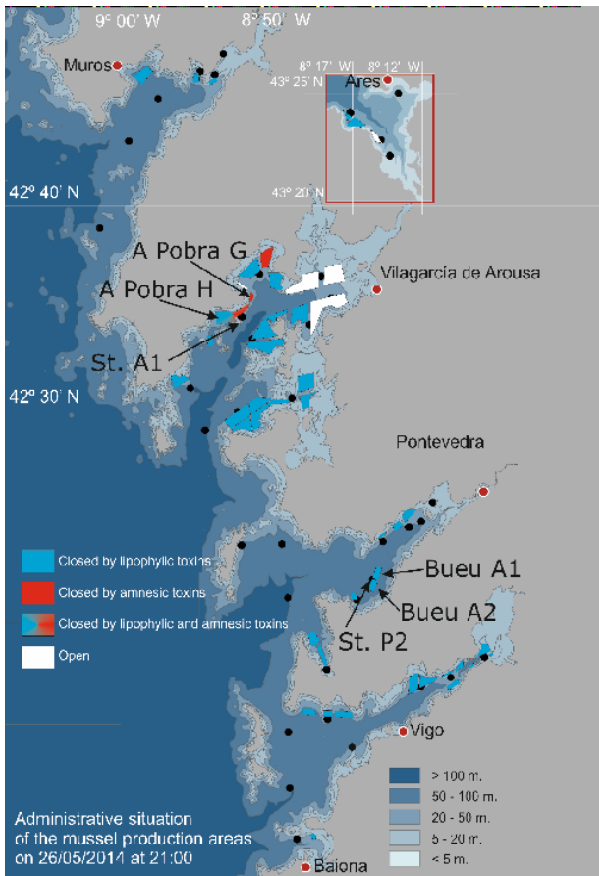


*Pseudo-nitzschia australis* Frenguelli, 1939



METHODOLOGY

- Biotoxins concentration in the mussels from the production areas (P.A) (0-15 m.)
  - Lipophilic toxins quantification by UPLC-MS/MS Sup. EURL-MB v.5
    - Domoic acid quantification by HPLC-UV-Diode
  - Lipophylic toxins by biological test. T. Yasumoto, 1984
    - Phytoplankton counts by Utermohl method
  - Vertical profiles of salinity and temperatura by CTD
    - *Pseudo-nitzschia australis* identification by TEM



According to the Epidemiological Bulletin of the Xunta de Galicia, and despite the ban of harvesting of mussels, two people were poisoned by ASP with symptoms of confusion and memory loss after consuming cooked paella prepared with frozen mussels acquired out of the legal channels of commercialization.

Pazos et al., 2016 Florianapolis ICHA conference

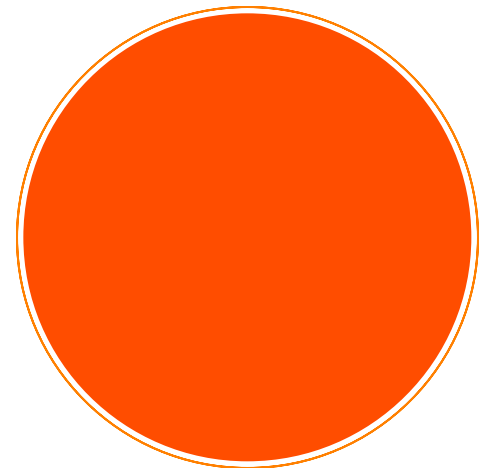


# Phytoplankton assemblages by their cellular shape and its ecological significance

Yolanda Pazos<sup>1</sup>, Juan Maneiro<sup>1</sup>, Ángeles Moroño<sup>1</sup>, Juan Blanco<sup>2</sup>

<sup>1</sup>INTECMAR. Vilagarcía de Arousa. Pontevedra. Spain.

ypazos@cccmm.cesga.es <sup>2</sup>CIMA. Vilanova de Arousa. Pontevedra. Spain. Xunta de Galicia



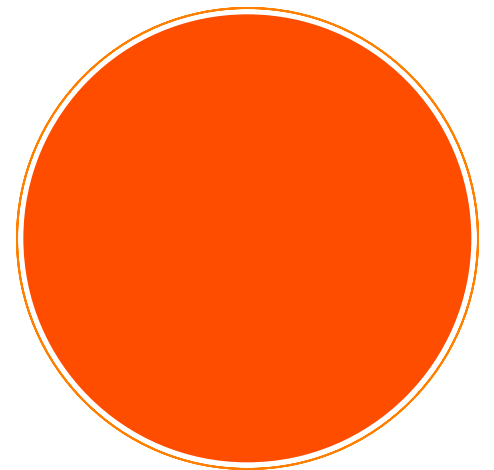




The cluster analyses, using abundance of the most frequently detected (50 % of presence) phytoplankton species, was carried out including 7288 samples weekly collected, from 1992 to 1995, in 33 stations located in the Rías Baixas (NW, Spain). Cluster was made by average linkage using correlation measure in SPSS 11.5 statistical package.



1 3 5 7 9 11 13 15 17 19 21 23 25



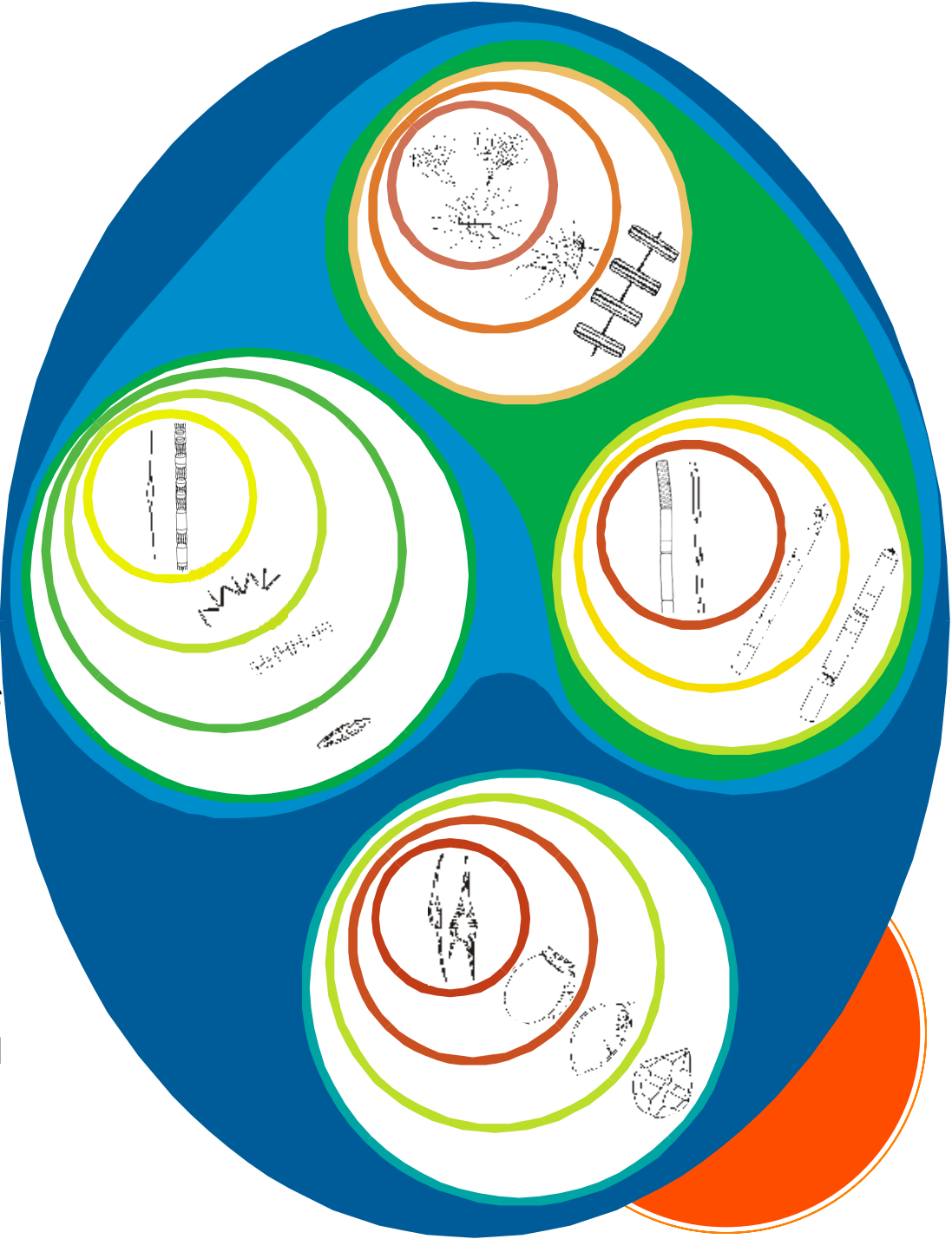
The first cluster included the dinoflagellate species that usually do not form chains, with low eccentricity and low surface/volume ratio: *Ceratium fusus*, *Ceratium furca*, *Dinophysis acuminata* and *Prorocentrum micans*.

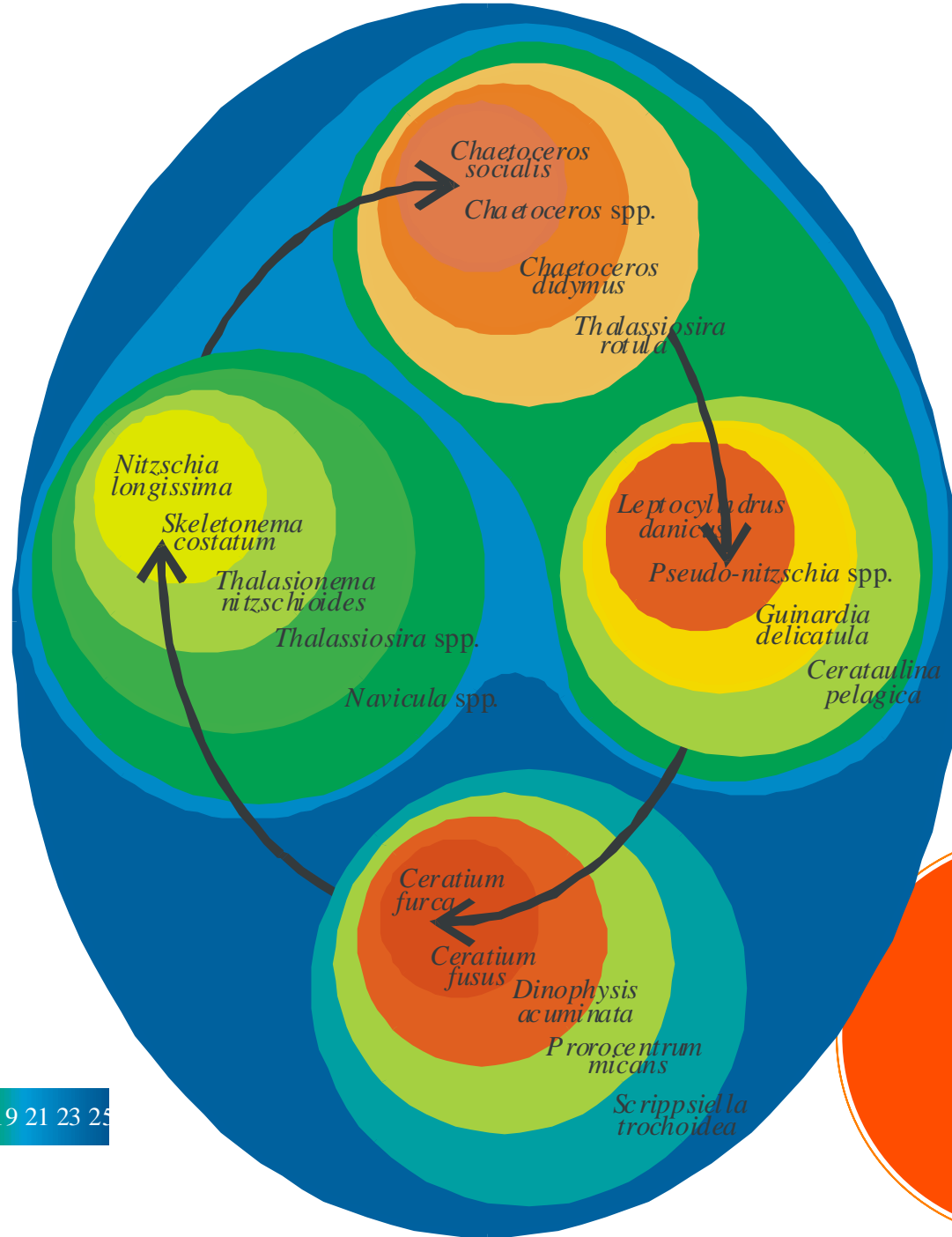
A second cluster associated all the remainder species, that is all the diatoms. Inside of this second cluster there are three clusters.

One of those clusters comprised diatom species, with setae and chain, or colony formers: *Chaetoceros sociale*, *Chaetoceros* spp., *Chaetoceros didymus* and *Thalassiosira rotula*.

The diatom species that form long chains as *Pseudo-nitzschia* spp., *Leptocylindrus danicus*, *Guinardia delicatula* and *Cerataulina pelagica* are included in another cluster.

To the last cluster, were associated small diatom species: *Nitzschia longissima*, *Skeletonema costatum*, *Thalassionema nitzschioides*, small *Thalassiosira* spp. and small *Navicula* sp.

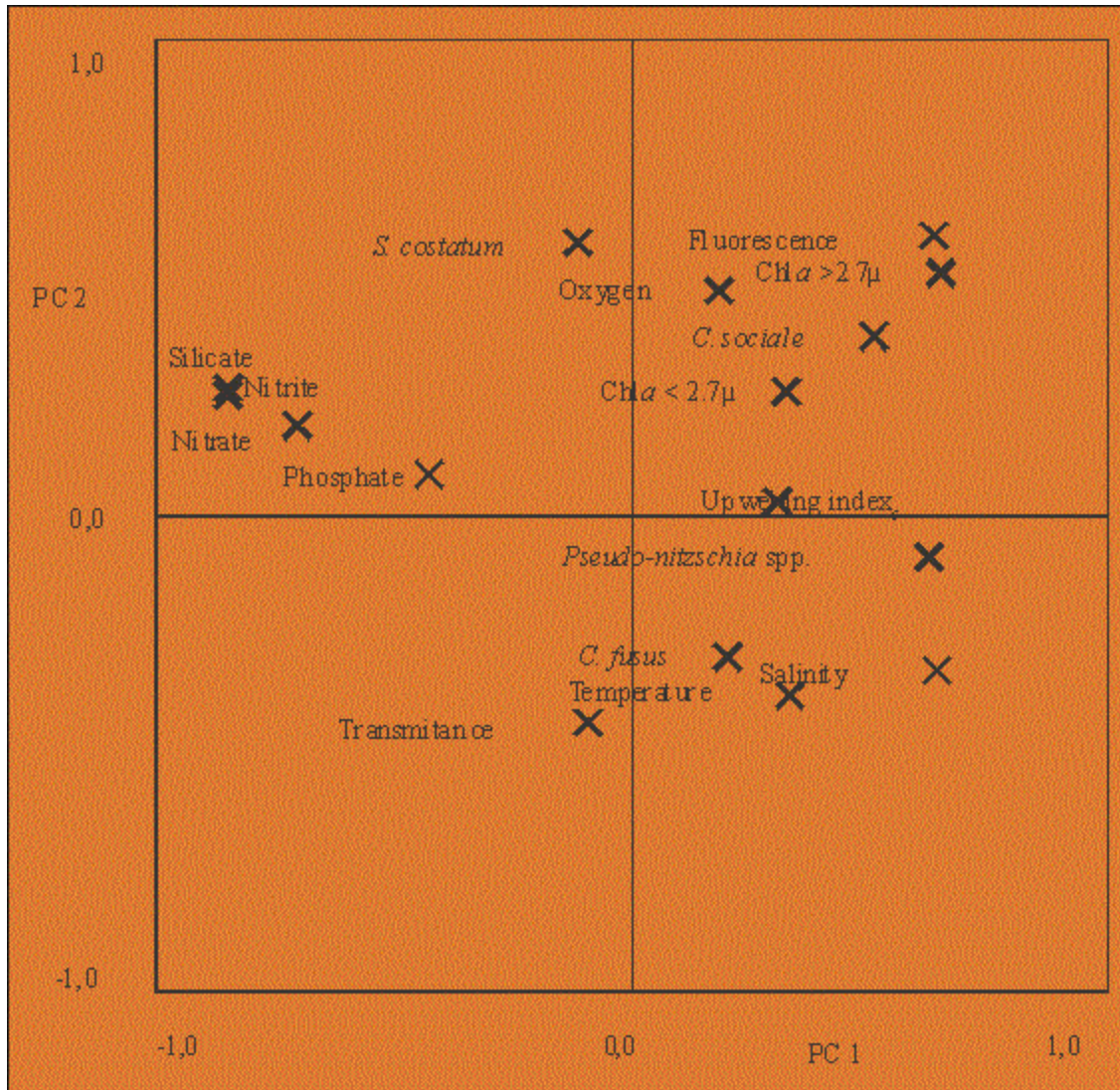




1 3 5 7 9 11 13 15 17 19 21 23 25



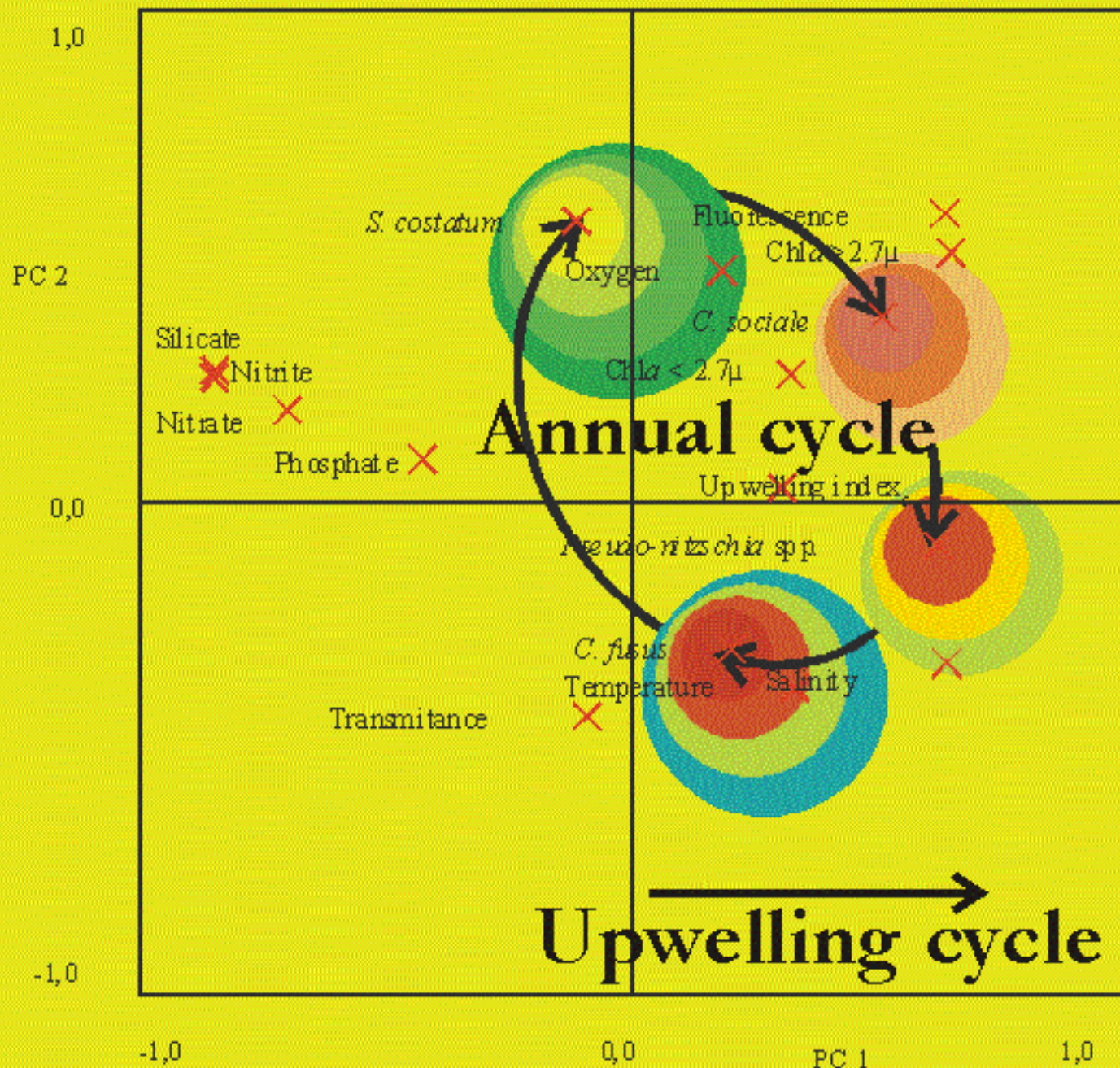


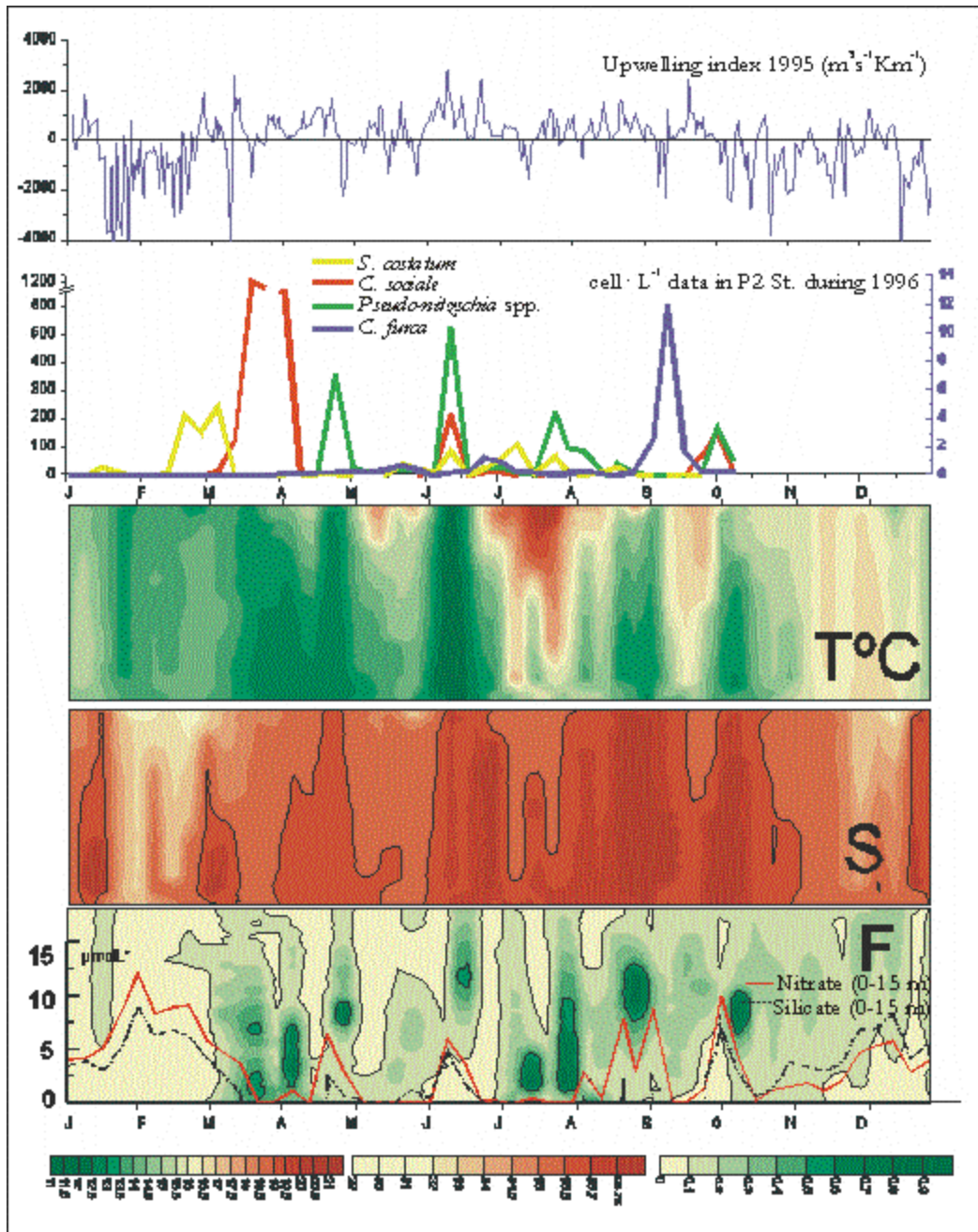


A principal component analysis (PCA) was carried out, including: upwelling index calculated from geostrophic winds (average of previous five days to sampling date); temperature, salinity, transmittance, fluorescence, oxygen from CTD measurements; nutrient salts (silicates, nitrates, nitrites, phosphates, ammonium) obtained by colorimetry; chlorophyll a by spectrofluorimetry (fractions higher and lower of  $2.7 \mu m$ ) and cellular concentration of phytoplankton species extracted from the previous cluster analyses. This PCA led to the extraction of two components with ecological significance: a first component that explained a 29% of variance and a second component that explained a 14% of the total variance.

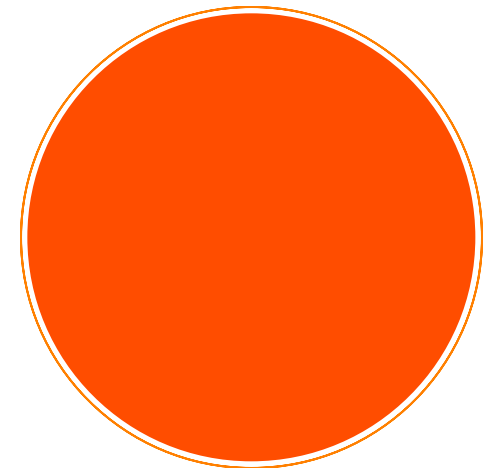


The first component of PCA is related to upwelling, primary production and decrease of nutrients. The second component showed a gradation in loadings from winter to autumn, and then associated to the annual cycle. Both components led to identify an annual cycle of species succession in winter, spring, summer and autumn to which are superimposed the following cycles: upwelling, upwelling relaxation and winter mixing.





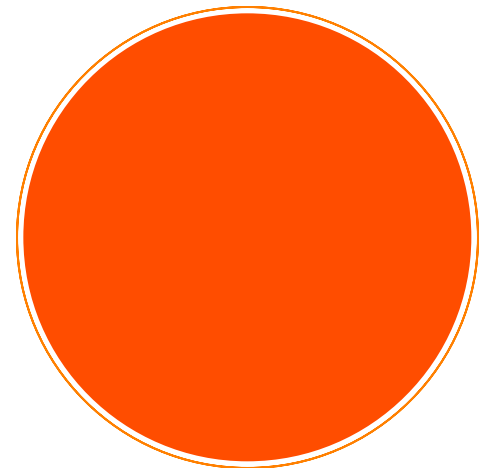
It is possible to see an example of this theory in the data obtained in P2 station throughout 1995.





The cellular shape of species of each cluster are related to with the oceanographic conditions inside the succession cycle.

The dinoflagellates of rounded forms (including the diarrhetic species *Dinophysis acuminata*) appear in summer and relaxation of upwelling, small diatoms are present in winter mixing situation adapted to turbulence, the setae of the spring diatoms are related to higher surface volume ratio to prevent sinking and finally the elongated chain-forming diatoms (including potentially amnesic species of the genus *Pseudo-nitzschia*) are related to strong upwelling in summer.





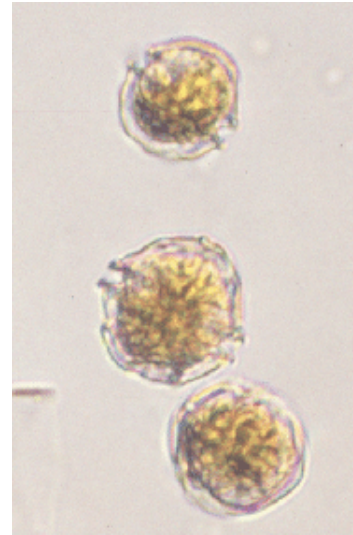
## Paralizantes (PSP)

La acumulación de toxinas paralizantes en los moluscos de las Rías Galegas es debida sobre todo a dos especies: *Gymnodinium catenatum* y *Alexandrium minutum*. A pesar de su gravedad en los últimos años estos episodios fueron muy escasos en Galicia.

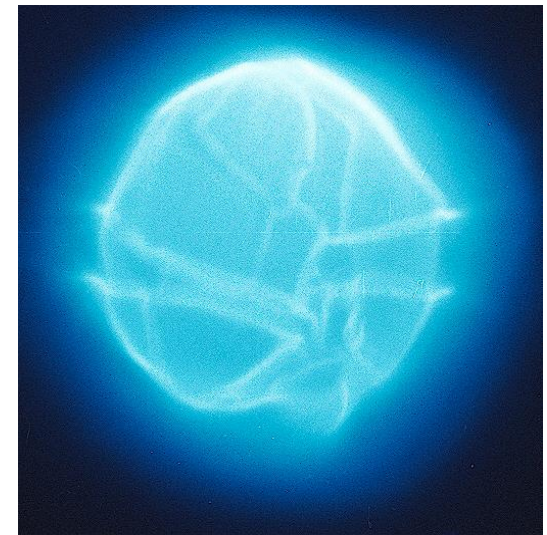
# Clase Dinophyceae



*Gymnodinium catenatum* Graham, 1943



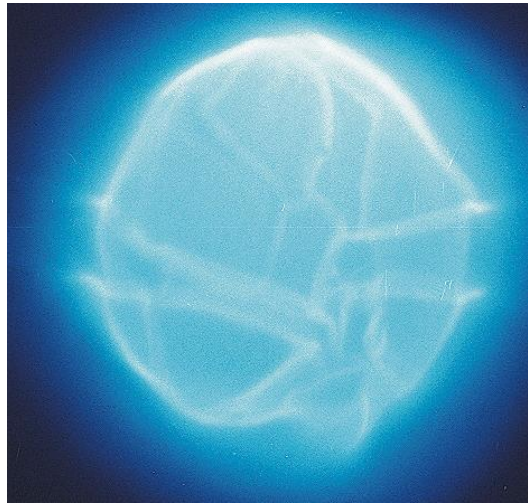
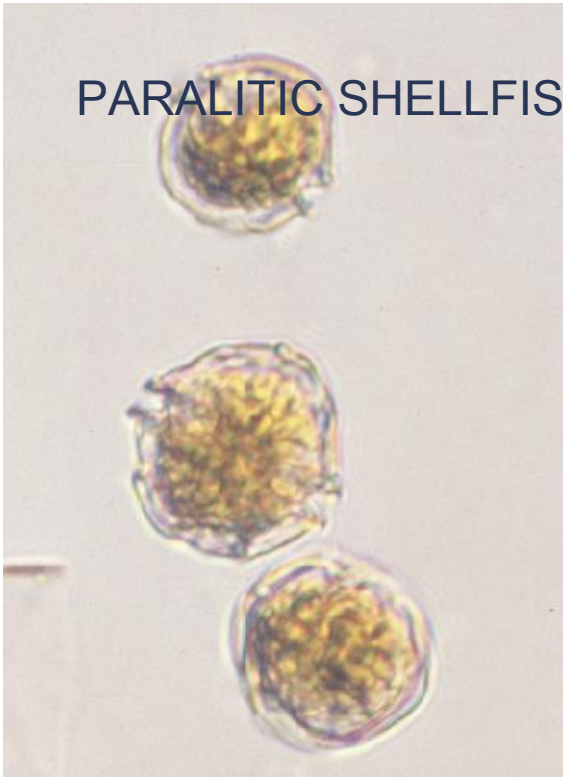
*Alexandrium minutum* Halim, 1960



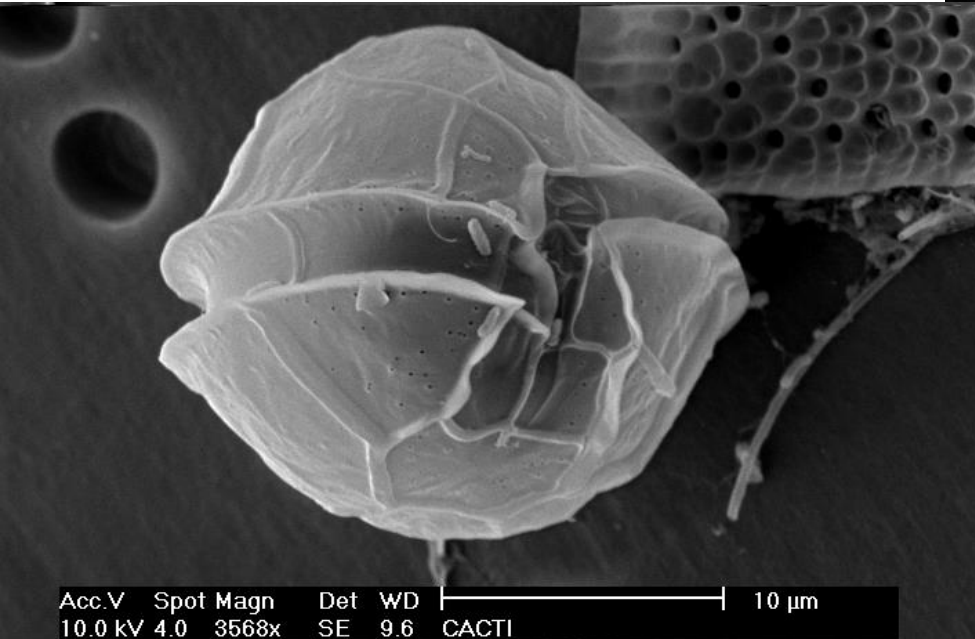
Dinoflagelados

Paralizantes (PSP)

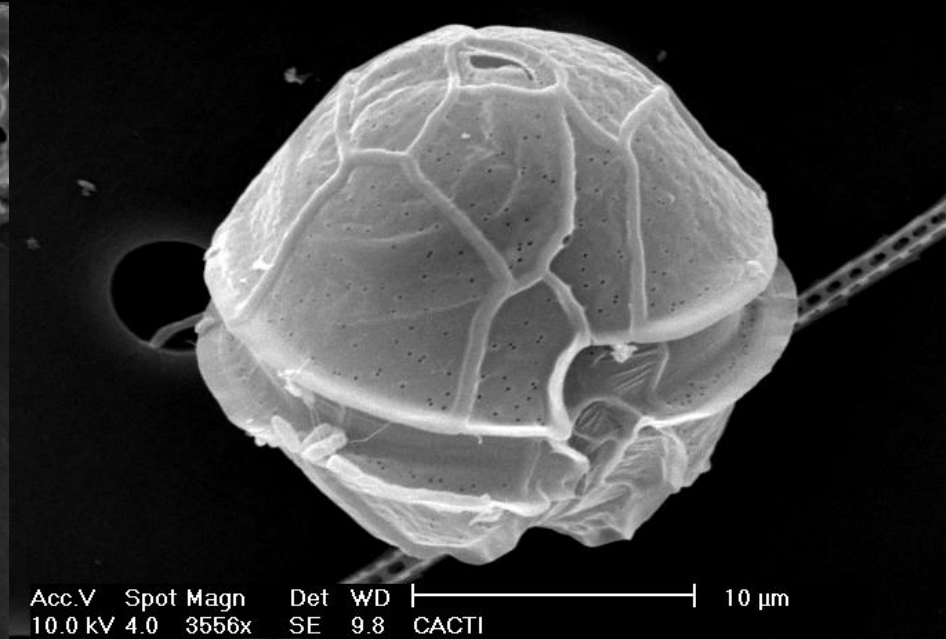
# PARALITIC SHELLFISH POISONING PRODUCER SPECIES



*Alexandrium minutum* Halim, 1960



Acc.V Spot Magn Det WD | 10 µm  
10.0 kV 4.0 3568x SE 9.6 CACTI

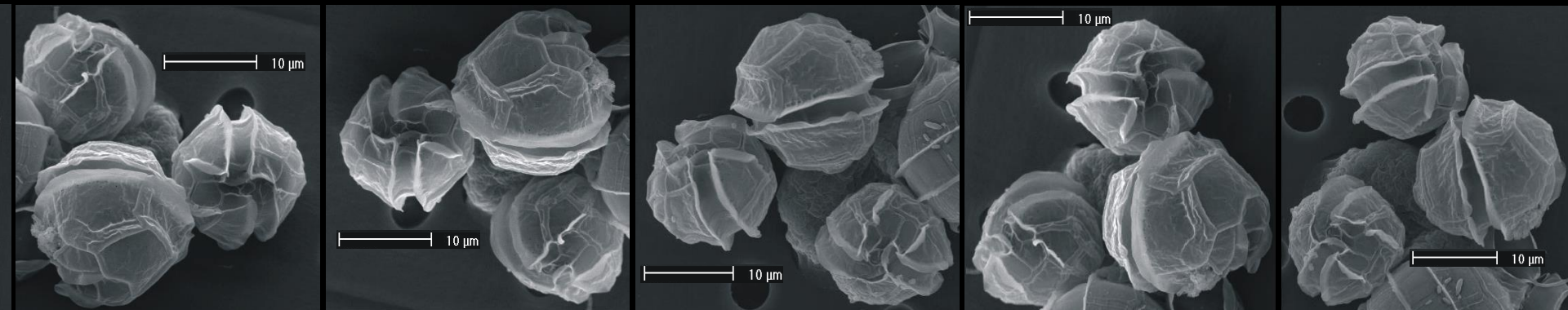


Acc.V Spot Magn Det WD | 10 µm  
10.0 kV 4.0 3556x SE 9.8 CACTI

# TIME SERIES ANALYSIS (1992-2003) OF *Alexandrium minutum* HALIM AND ITS OCEANOGRAPHIC CONDITIONS IN GALICIA (NW SPAIN)

Y Pazos<sup>1</sup>, J Maneiro<sup>1</sup>, Á Moroño<sup>1</sup>, M Doval<sup>1</sup> & J Blanco<sup>2</sup>

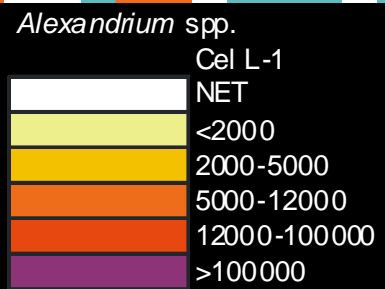
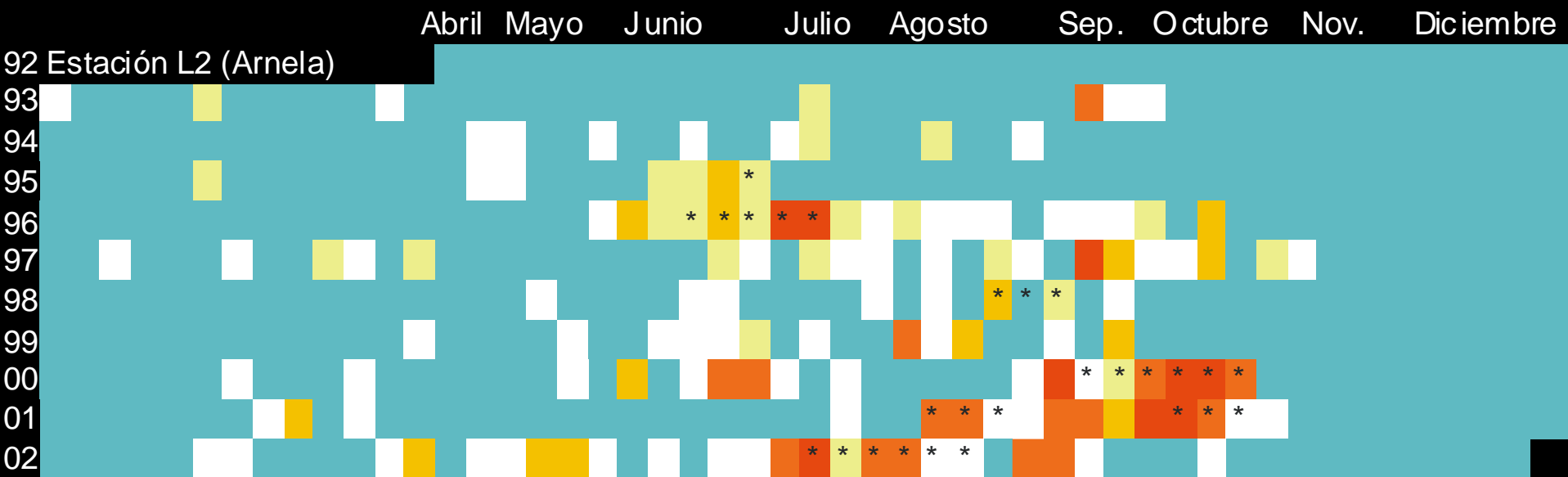
<sup>1</sup>INTECMAR. <sup>2</sup>Centro de Investigacións Mariñas. Consellería de Pesca e Asuntos. Xunta de Galicia







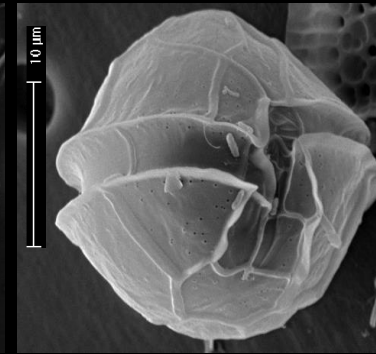
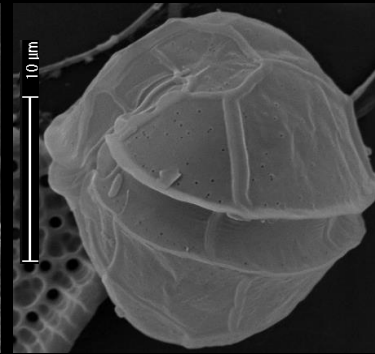
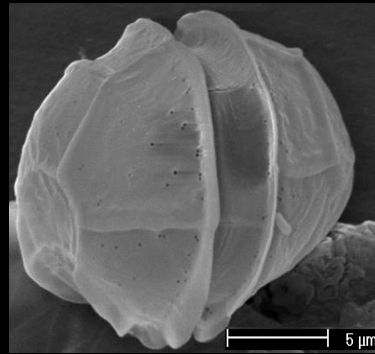
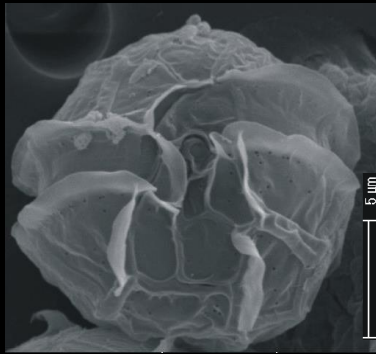
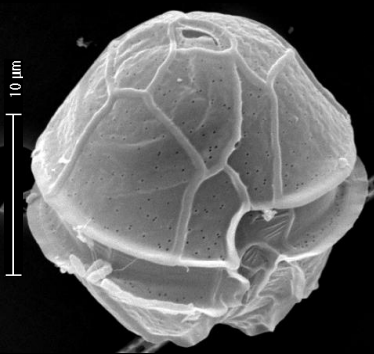
Distribution of mussel rafts production areas and position of oceanographic stations in the Ría de Ares

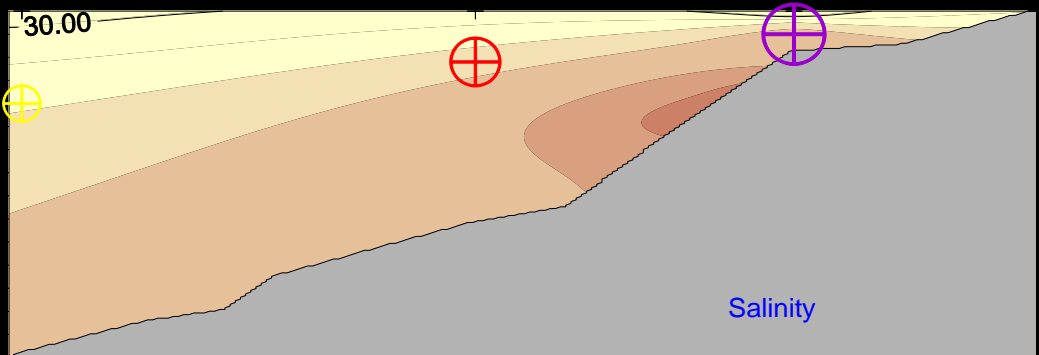
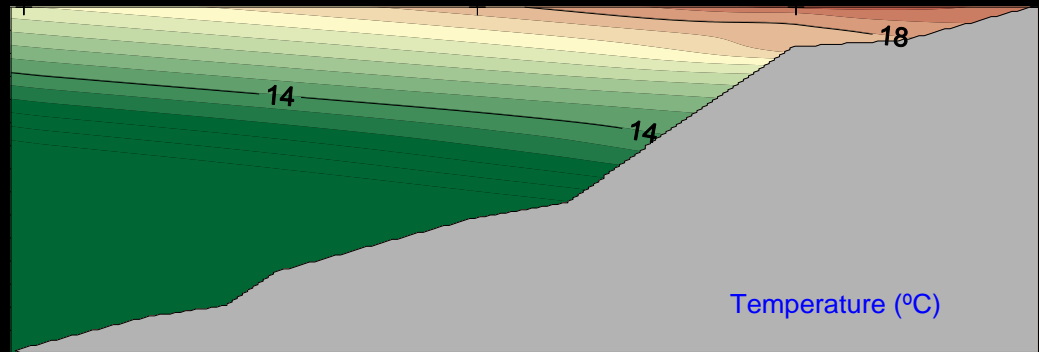
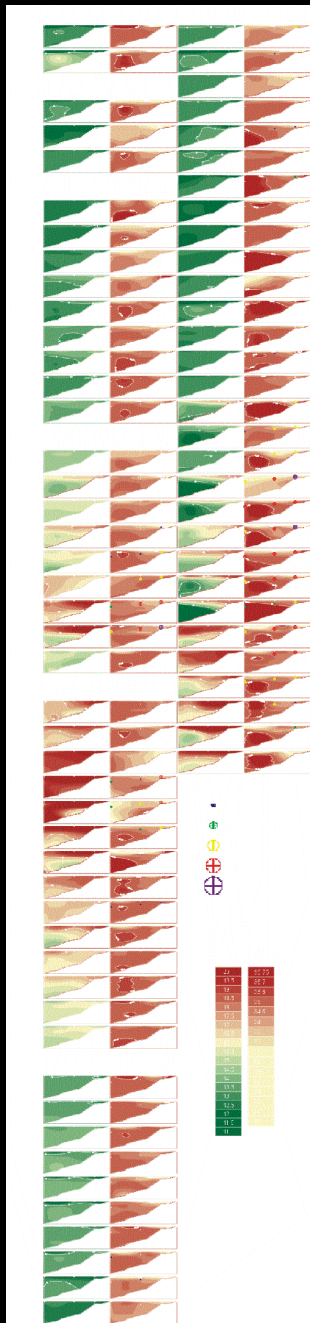


µg/100 g. STX Bioassay

\* <80

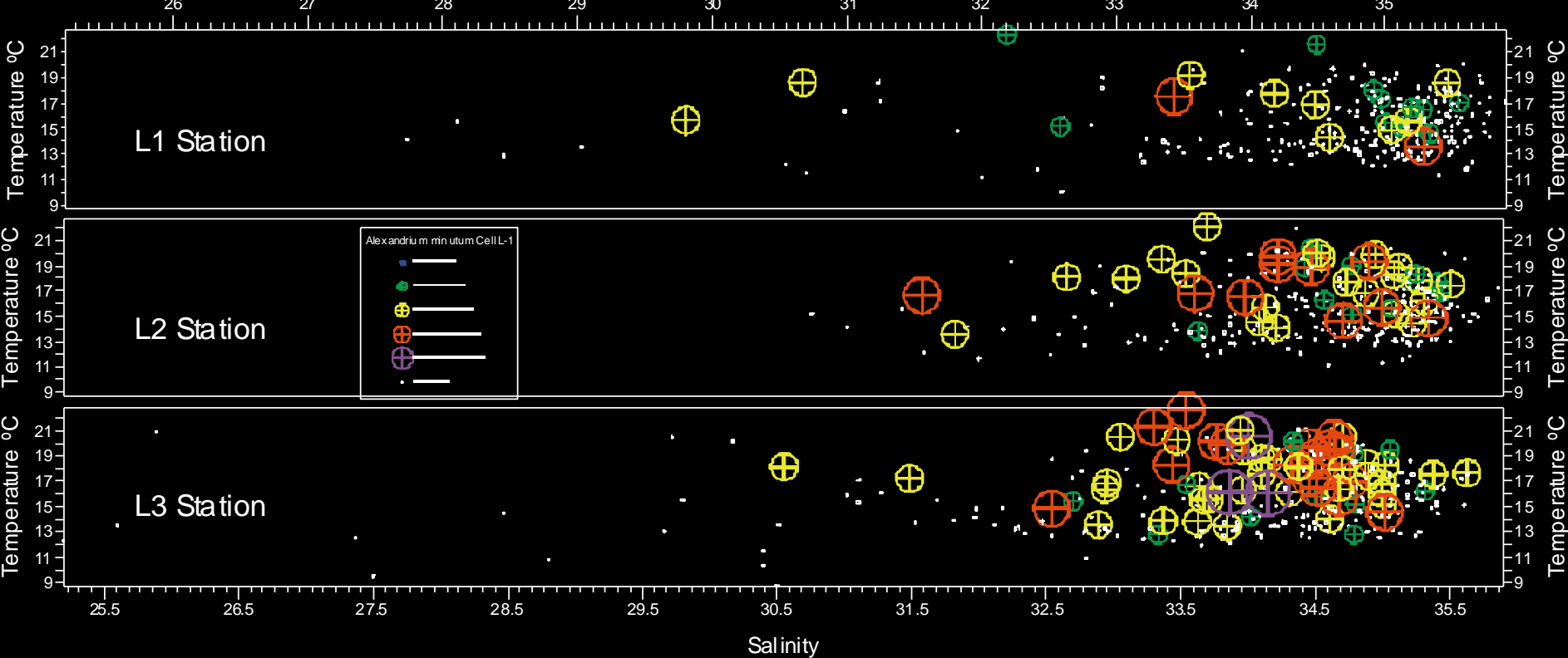
++ >80





The intensive analysis from three sampling points, located along the river-ocean axis, allow to conclude that the *A. minutum* populations develop by *in situ* grow in the inner part of the Ría with a dilution effect oceanwards.





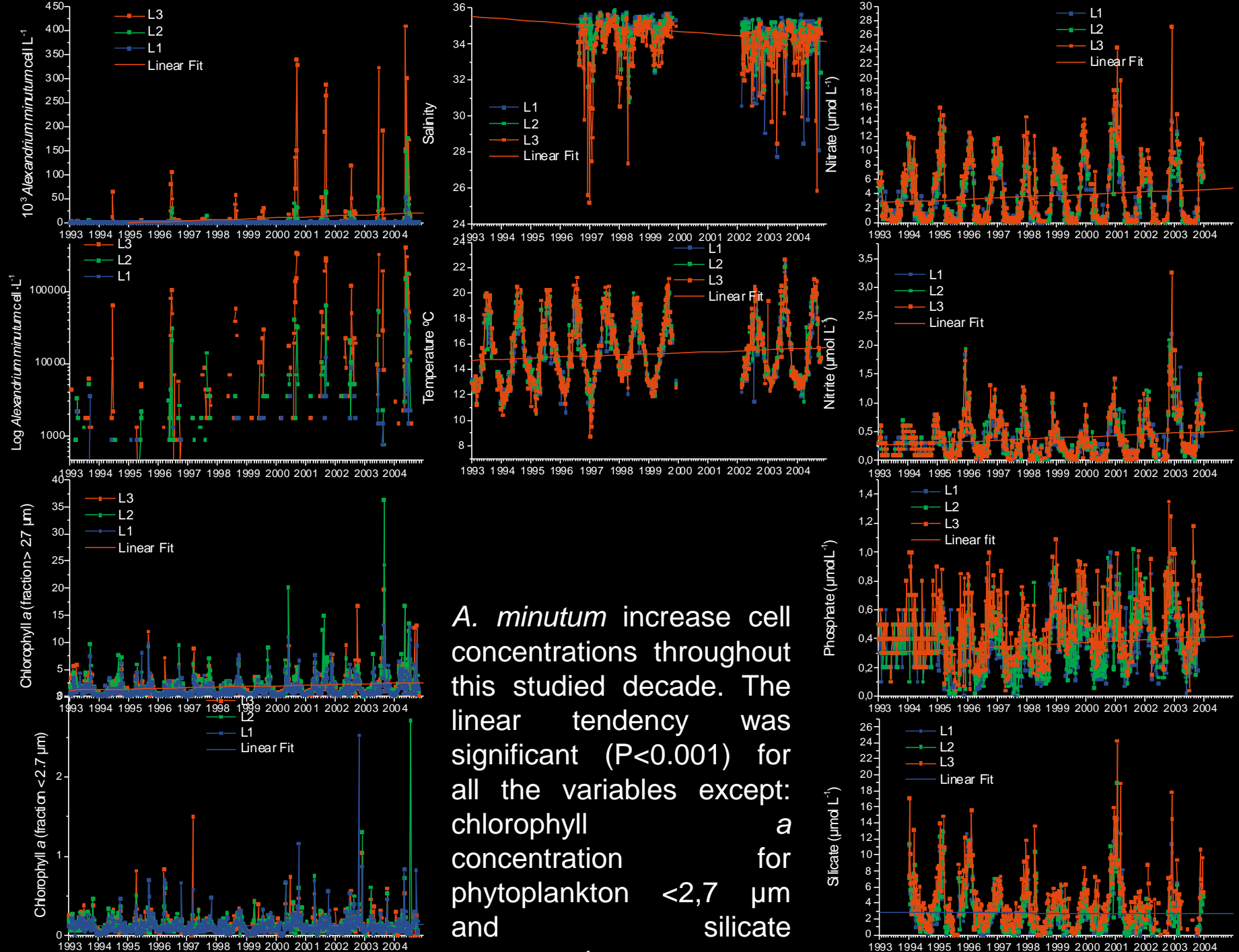
T/S diagrams allow to conclude that it's a brackish water organism.

*A. minutum* displays the higher cellular concentrations in a range of salinity of 33,5 and 35,0 and practically it doesn't appear with values higher than 35,5 and lower than 29.

Regarding temperature, the favourable range is wide, between 13,5 and 20°C. *A. minutum* doesn't appear related to upwelled waters.

It is also possible to observe that the inner station is the most affected by populations of *A. minutum* in agreement with its most brackish character.





*A. minutum* increase cell concentrations throughout this studied decade. The linear tendency was significant ( $P < 0.001$ ) for all the variables except: chlorophyll *a* concentration for phytoplankton < 2.7  $\mu m$  and silicate concentrations.

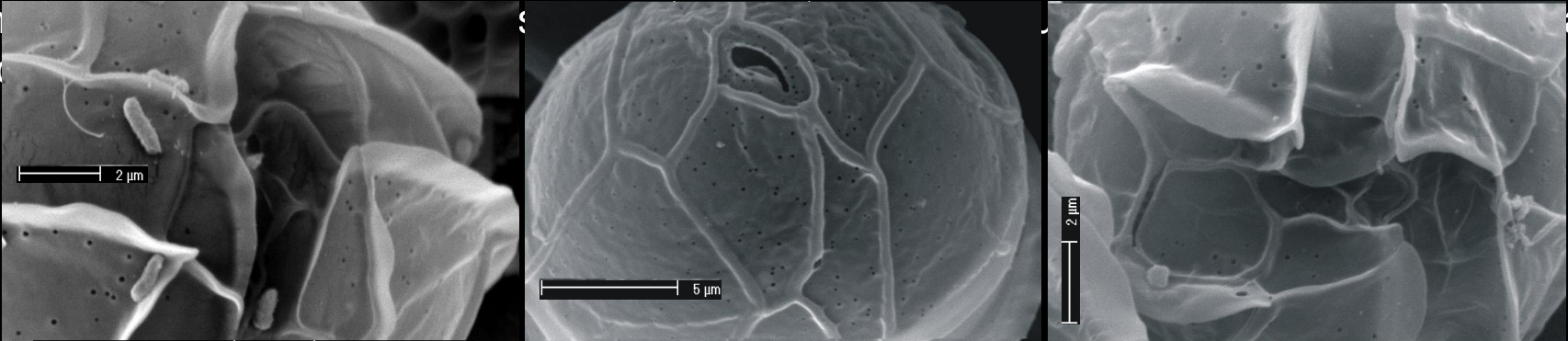
An apparent increase in toxic harmful algal blooms is detected by a weekly oceanographic conditions and phytoplankton monitoring, carried out since 1992, in Ría de Ares (NW Spain).

The small thecate dinoflagellate *Alexandrium minutum* Halim is the causative species of PSP toxins accumulation by rafts mussels and other molluscs cultivated in the area. PST detection was always predicted by previous detection of *A. minutum* cells in seawater.

The intensive analysis from three sampling points, located along the river-ocean axis, allow to conclude that the *A. minutum* populations develop by *in situ* grow in the inner part of the Ría with a dilution effect oceanwards.

Annual distribution shows a seasonal component with maximum cell around  $1 \cdot 10^5$  cell  $L^{-1}$  in June. Blooms of this species are detected in September (max.  $3 \cdot 10^5$  cell  $L^{-1}$ ) in the inner part of the Ría associated to an oceanographic front, in downwelling situations with blocking of estuarine circulation.

A temporal trend to increase was observed for *A. minutum*, nitrates, nitrites and



# Early detection and intensive monitoring during an unusual toxic bloom of *Gymnodinium catenatum* advected into the Galician Rías (NW, Spain)

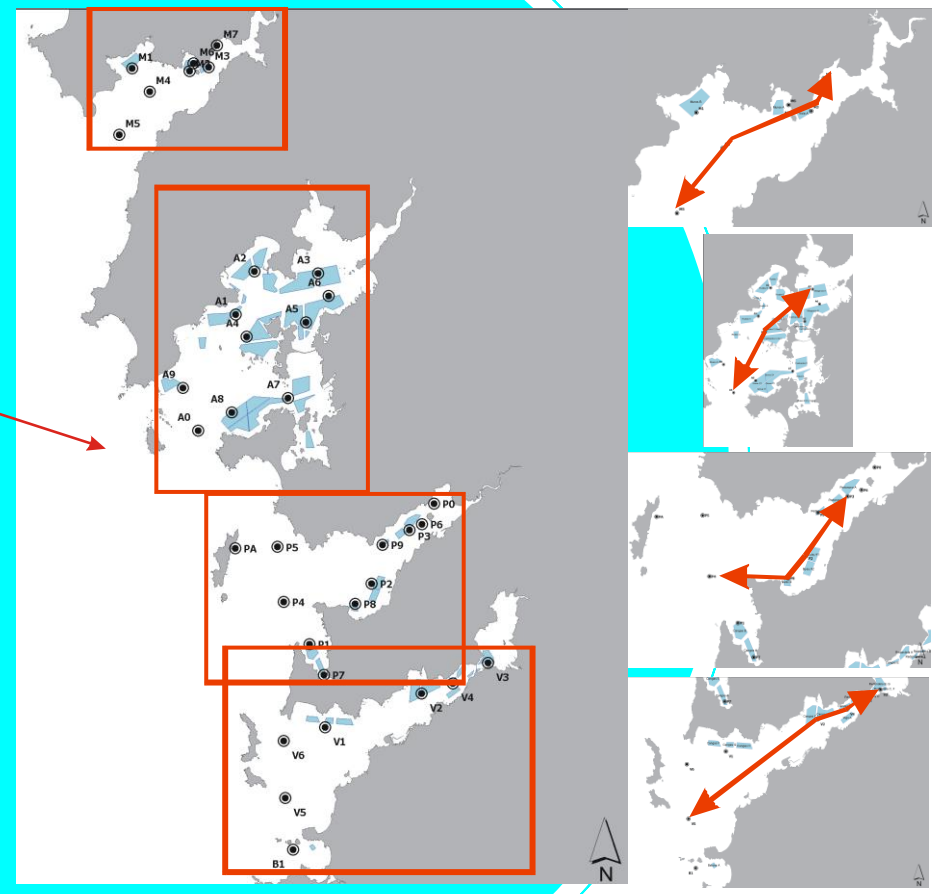
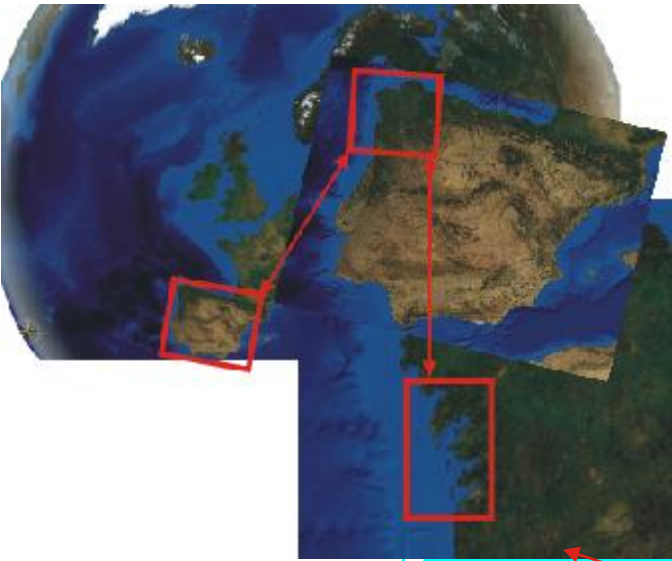
Yolanda Pazos<sup>1</sup>, Ángeles Moroño<sup>1</sup>, Joaquín Triñanes<sup>2</sup>, Mariló Doval<sup>1</sup>, Pedro Montero<sup>1</sup>, M. Graça Vilarinho<sup>3</sup> and M.Teresa Moita<sup>3</sup>

<sup>1</sup> Instituto Tecnolóxico de Control do Medio Mariño de Galicia. INTECMAR. Xunta de Galicia. Spain. Ypazos@intecmar.org. Amoronho@intecmar.org; Mdoval@intecmar.org; Pmontero@intecmar.org

<sup>2</sup> Universidad de Santiago de Compostela. Spain. Joaquín.Trinanes@noaa.gov; Trinanes@usc.es

<sup>3</sup> Instituto de Investigación das Pescas e do Mar. IPIMAR. Portugal. vilarinh@ipimar.pt; tmoita@ipimar.pt;





Location of oceanographic stations, weekly monitored by INTECMAR in the Galician Rías, since 1992. Blue polygons represents the position of the raft mussel culture areas.

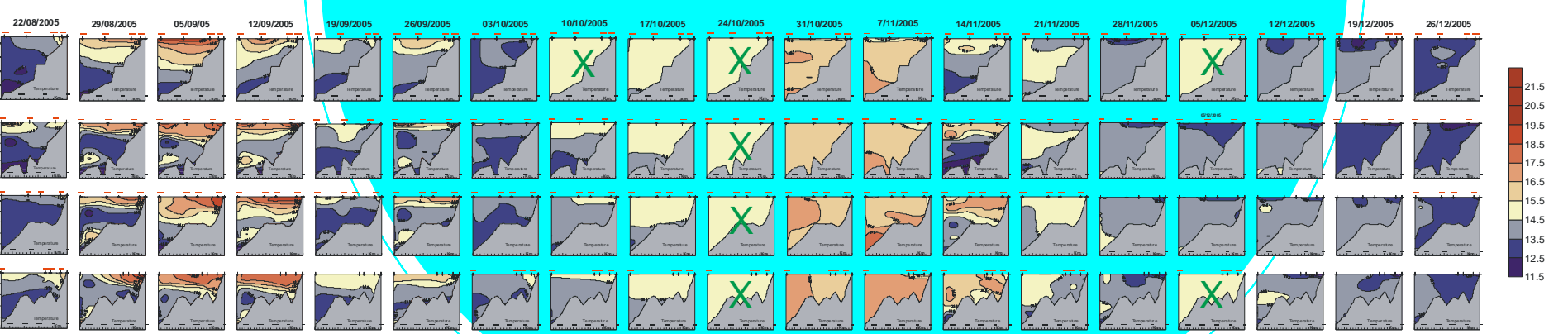
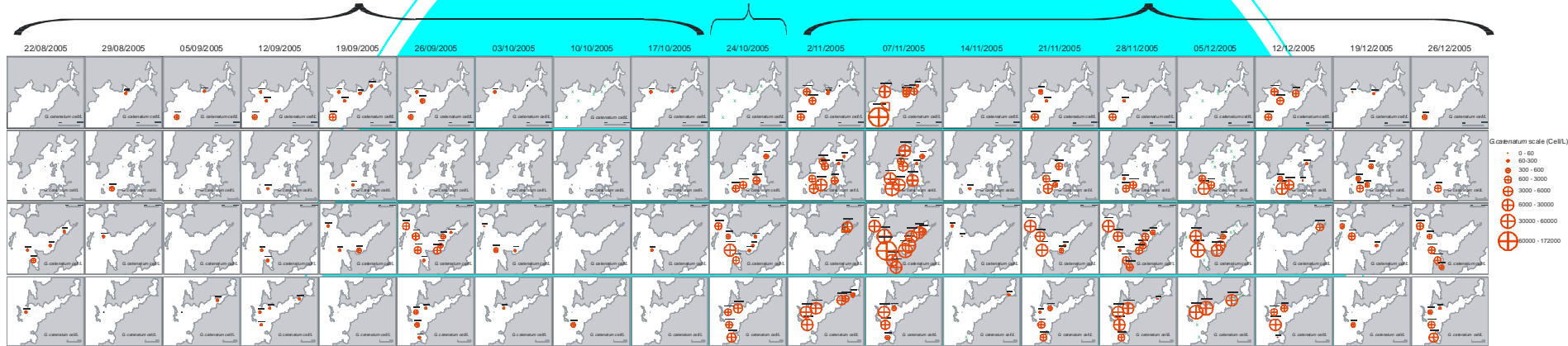


All the mussel production areas were closed due to PSP until spring 2006.  
 The ban caused serious economic losses but no human intoxications were reported.

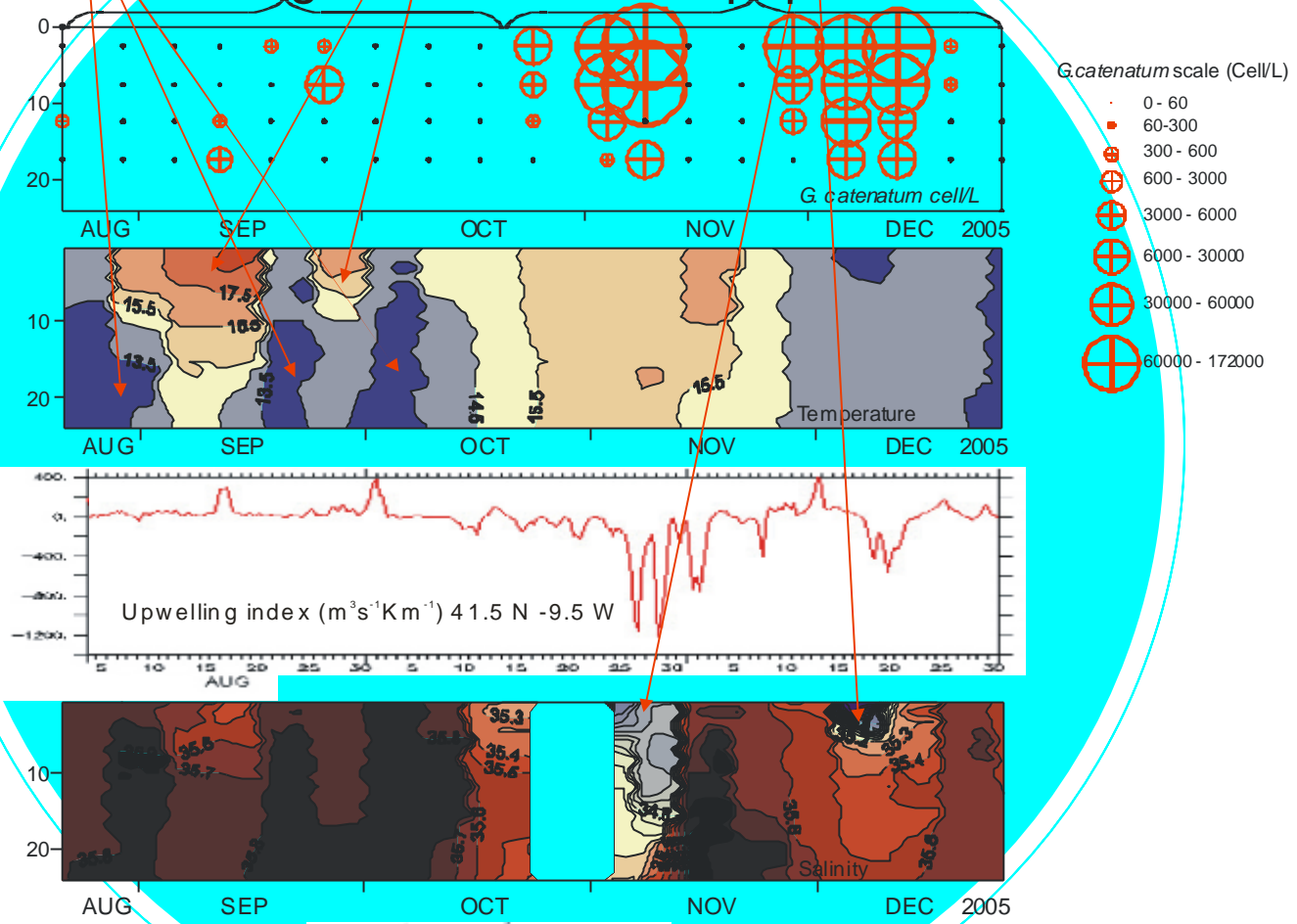
*In situ* growth of *Gymnodinium catenatum* population. Upwelling exported cells offshore from the Rías. Upwelling relaxation induced cell accumulation inside the Rías.

Downwelling introduced *Gymnodinium catenatum* bloom into the Rías below the surface layer. Water column temperature and salinity show clearly the position of these surface ocean waters.

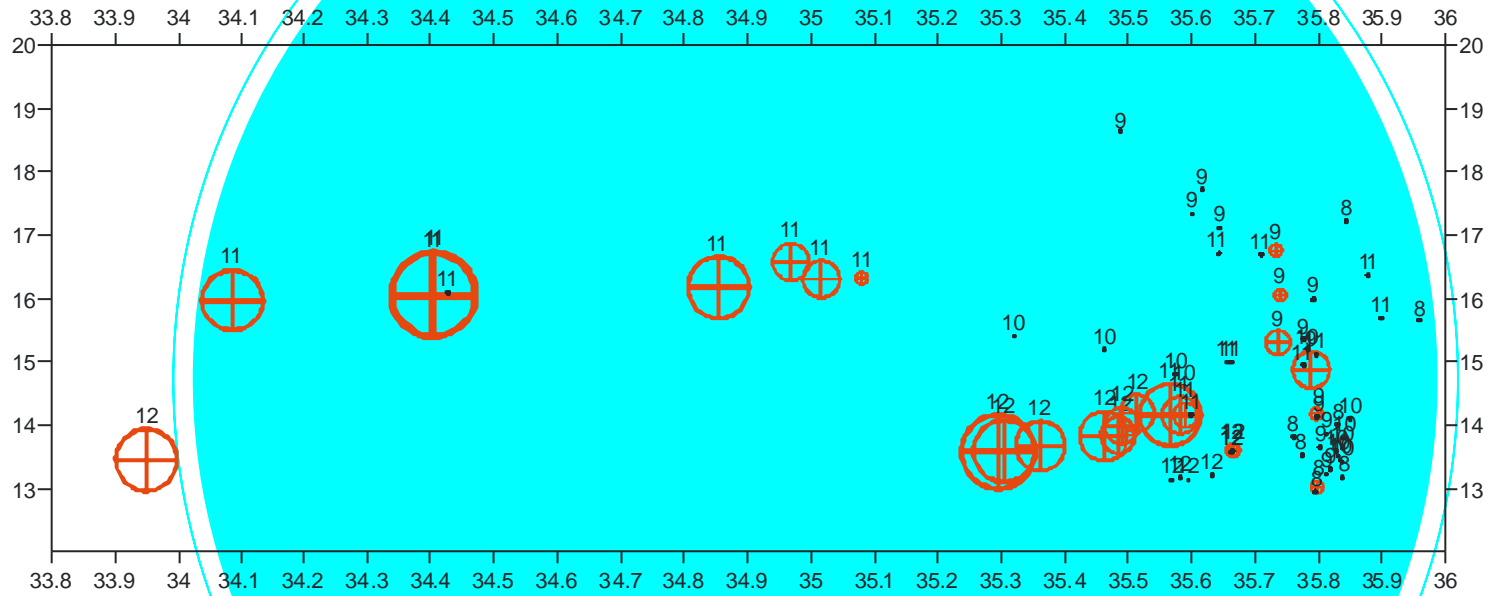
Strong gale



Upwelling  
 Upwelling relaxation  
 Downwelling  
 In situ growth  
 Advected population



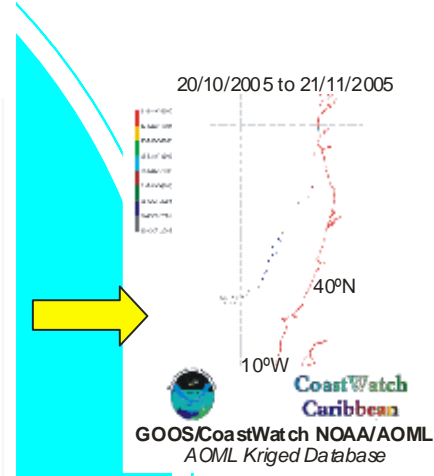
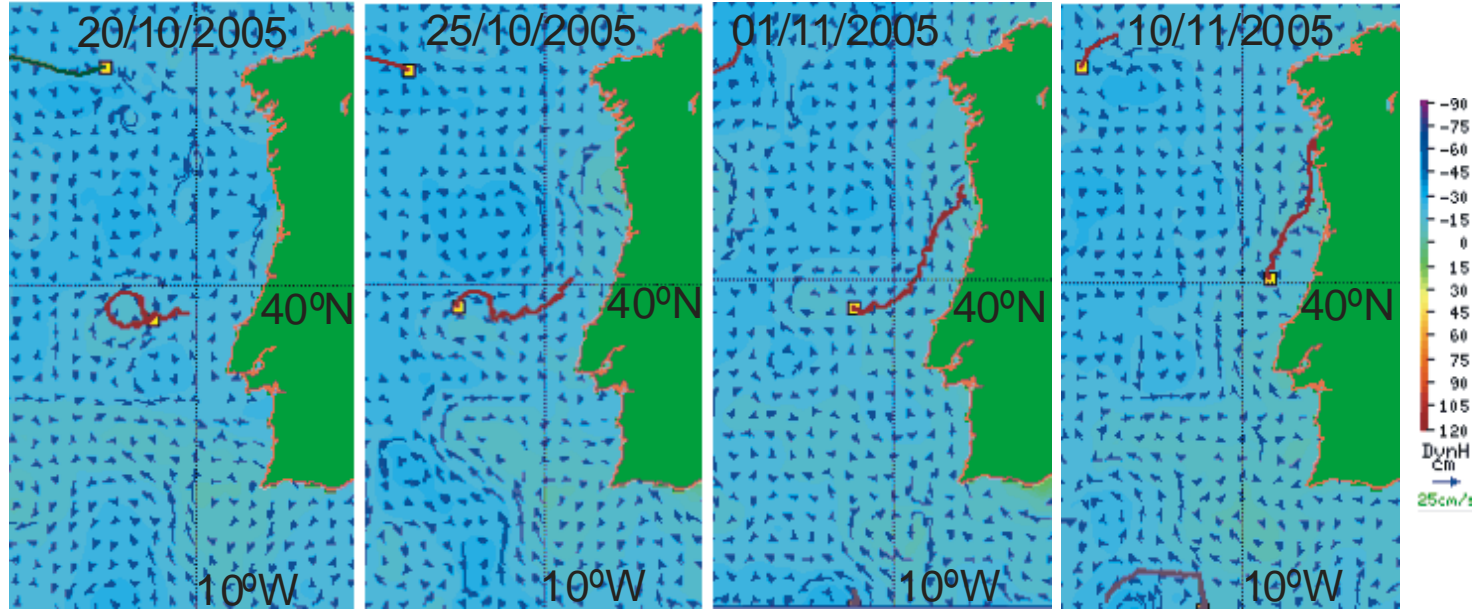
Vertical distribution of *G. catenatum*, temperature, upwelling index, and salinity in P2 station.



T-S diagram and *G. catenatum* cell concentration in P2 station. Numbers indicate the month.



CoastWatch NOAA/AOML  
Altimeter/GTS Interface



Data from a lagrangian drifter (NOAA/CoastWatch) confirmed the presence of a northward coastal current ( $Vel \sim 0.2-0.6 \text{ m}\cdot\text{s}^{-1}$ ) that appears to have advected the bloom northwards.





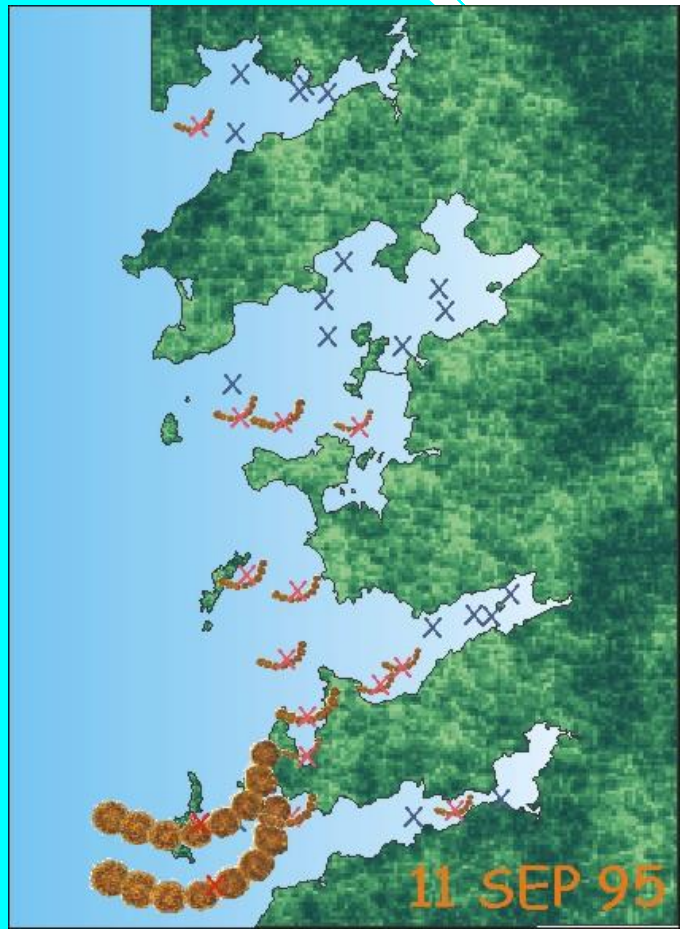
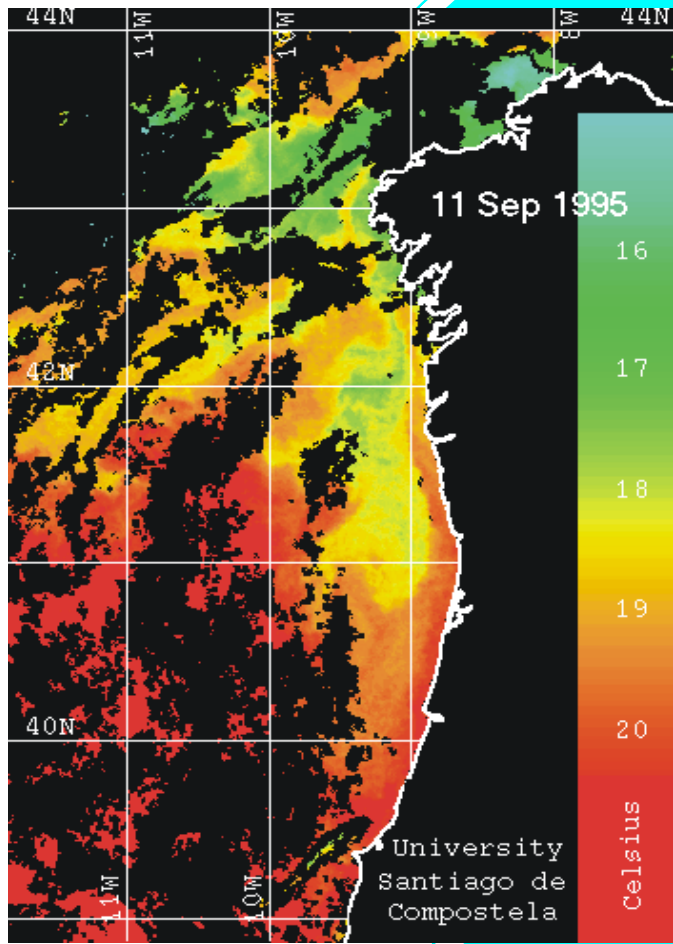
Cooperation between Galician and Portuguese monitoring programs allowed an early alert of a *Gymnodinium catenatum* bloom in the Galician Rías a month before the PSP toxins accumulation in mussels.

This intense and persistent episode caused bans on mussel culture areas (average production  $>2 \cdot 10^5$  Tons $\cdot$ y $^{-1}$ ) until mid February, having an important social and economic impact.

From the near real-time sea surface temperature fields, it was inferred that a northward coastal current was present during this period.

Data from a lagrangian drifter (NOAA/CoastWatch) confirmed the presence of a northward coastal current (Vel  $\sim 0.2$ - $0.6$  m $\cdot$ s $^{-1}$ ) on these dates.





## UNIDAD DE OCEANOGRAFÍA Y FITOPLANCTON

Yolanda Pazos, Silvia Calvo, Silvia Roura,  
Pilar García, Florentina Amoedo, Melchor  
Pérez, Isabel Lemos, Adriana Gil

