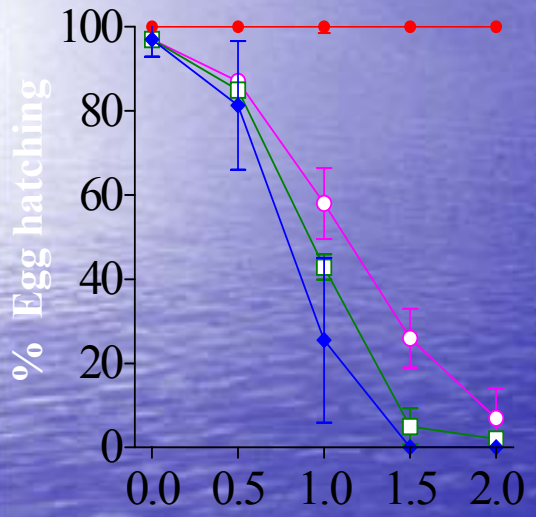
The effect of diatom secondary metabolites on other phytoplankton

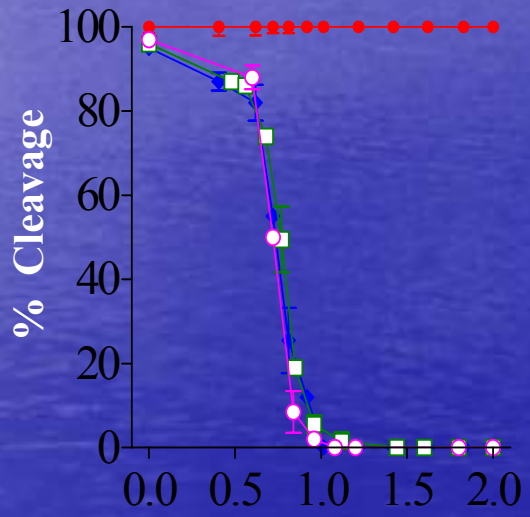
Raffaella Casotti
François Ribalet

Sabina Mazza

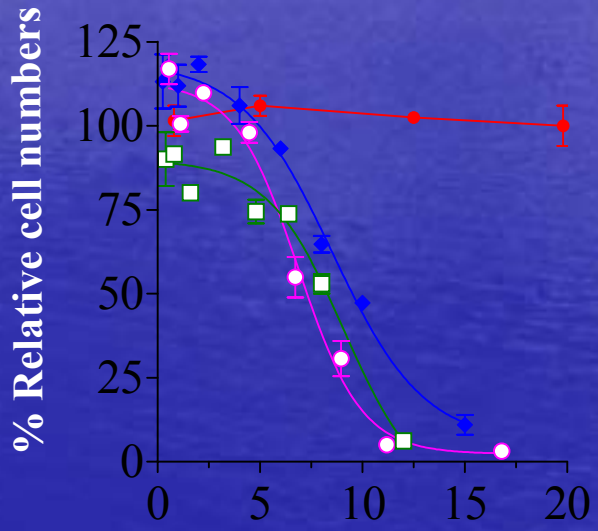
Effect on model animals



Copepod



Sea urchin



human

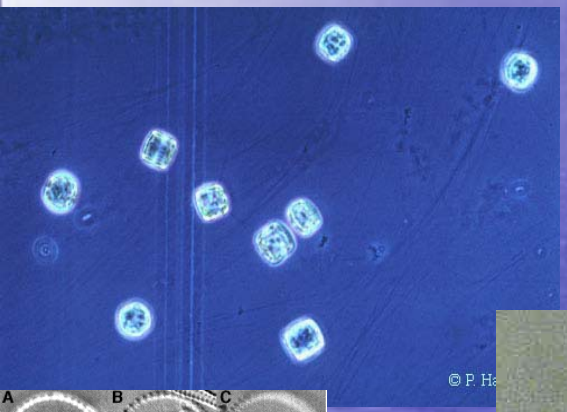
Aims

- **Test the effect of different aldehydes on different phytoplankton species**
  - Diatoms/non-diatoms
  - Describe the cellular effects and localization
  - Investigate the action as infochemicals
  - Modulation by environmental factors (light, nutrients)

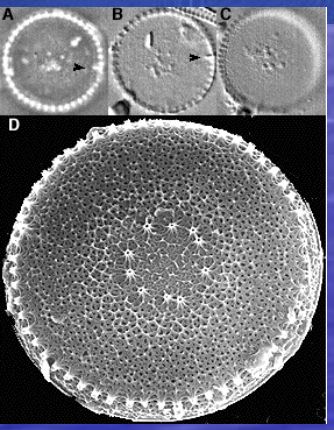
- **Bloom dynamics *in situ***

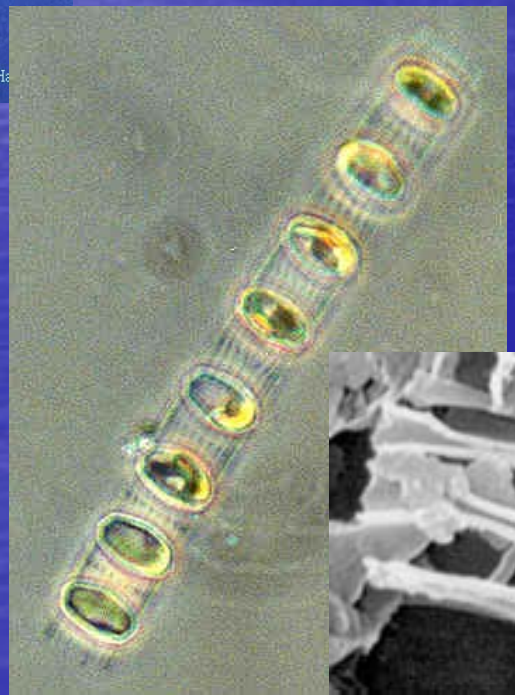
Phytoplankton models

- *Thalassiosira weissflogii*

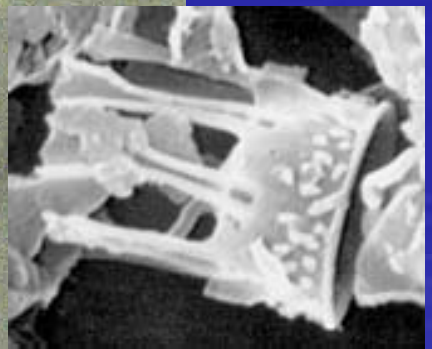


Phaeodactylum tricornutu

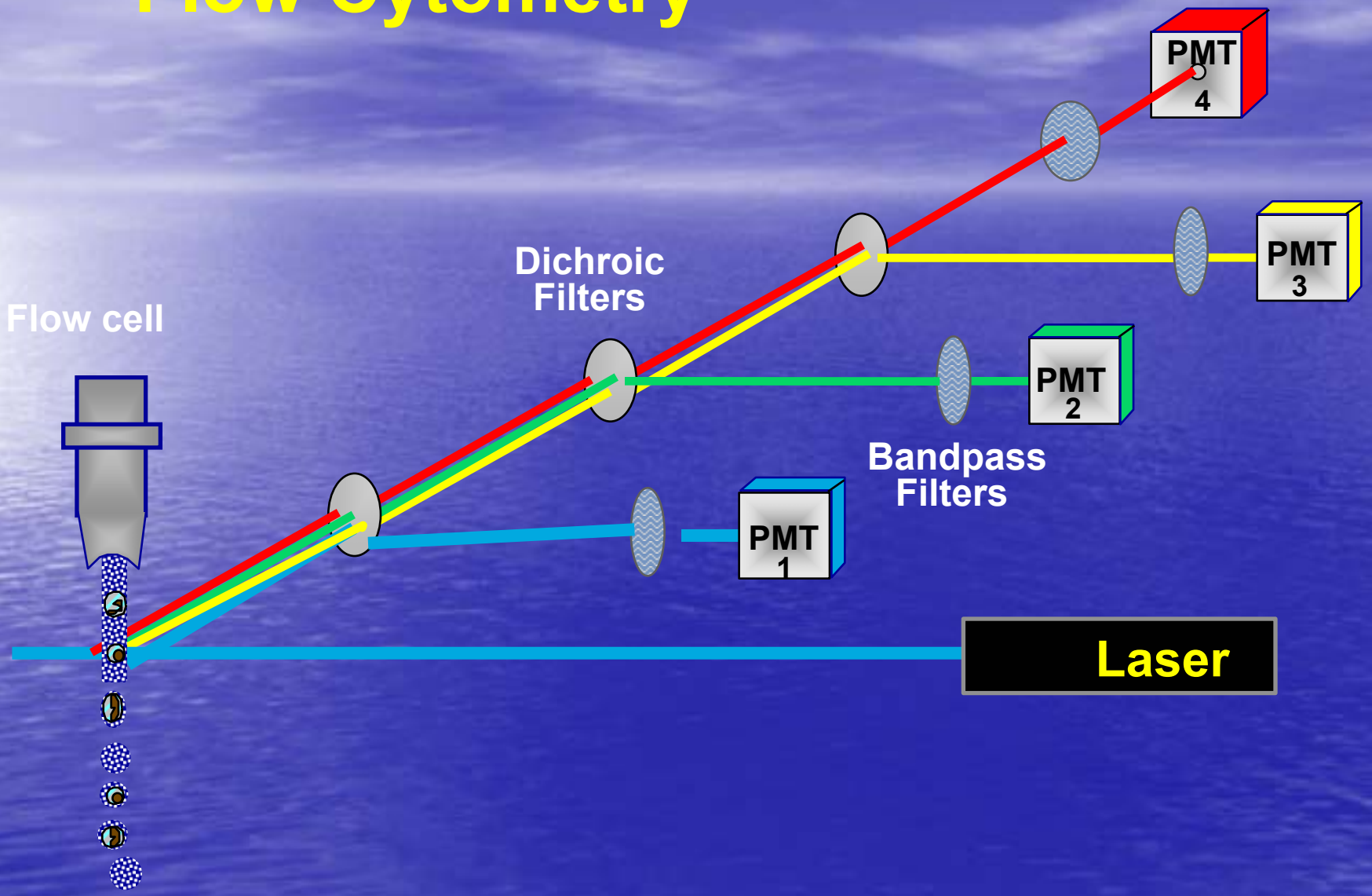




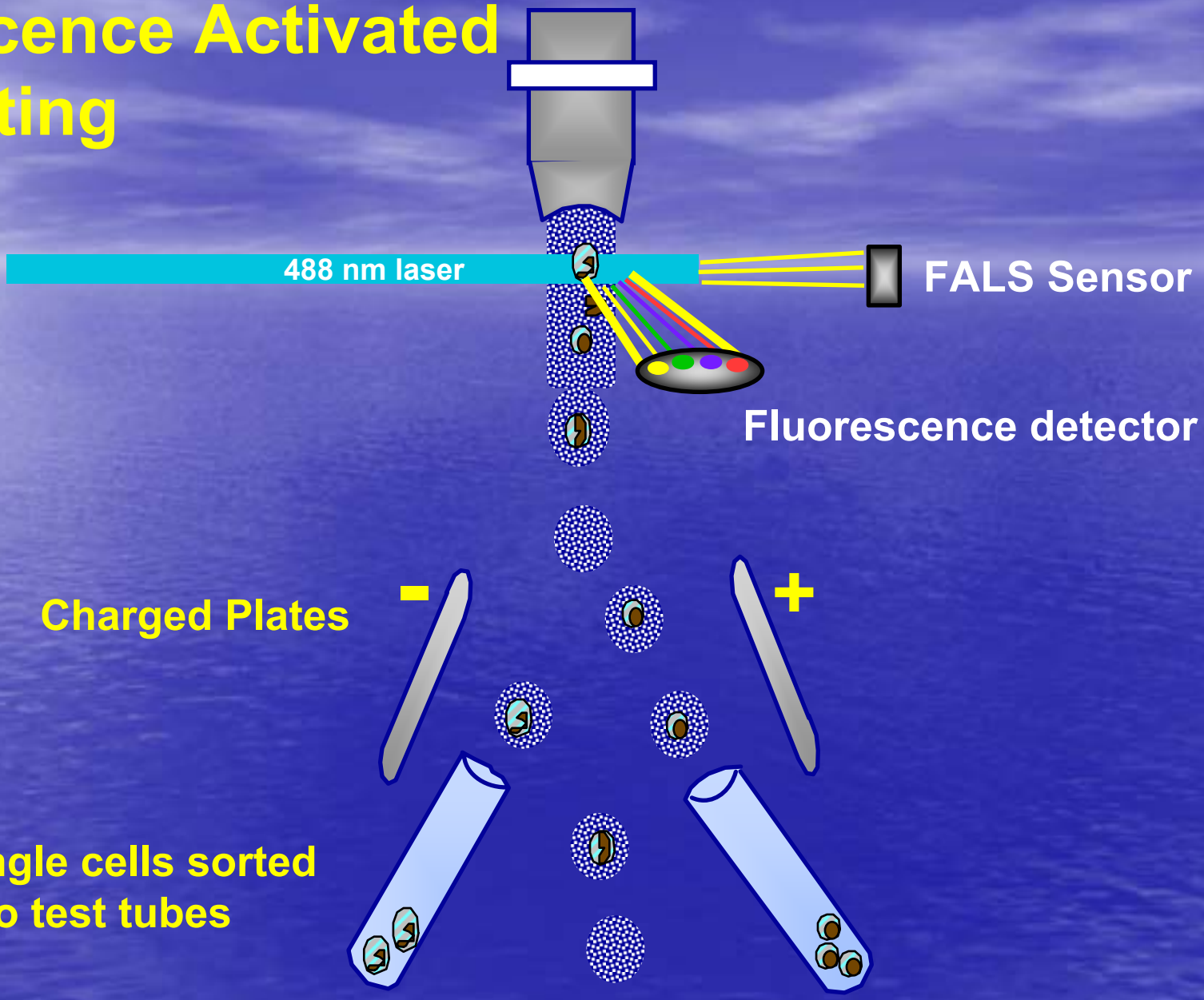
- *Skeletonema marinoi*



Flow Cytometry



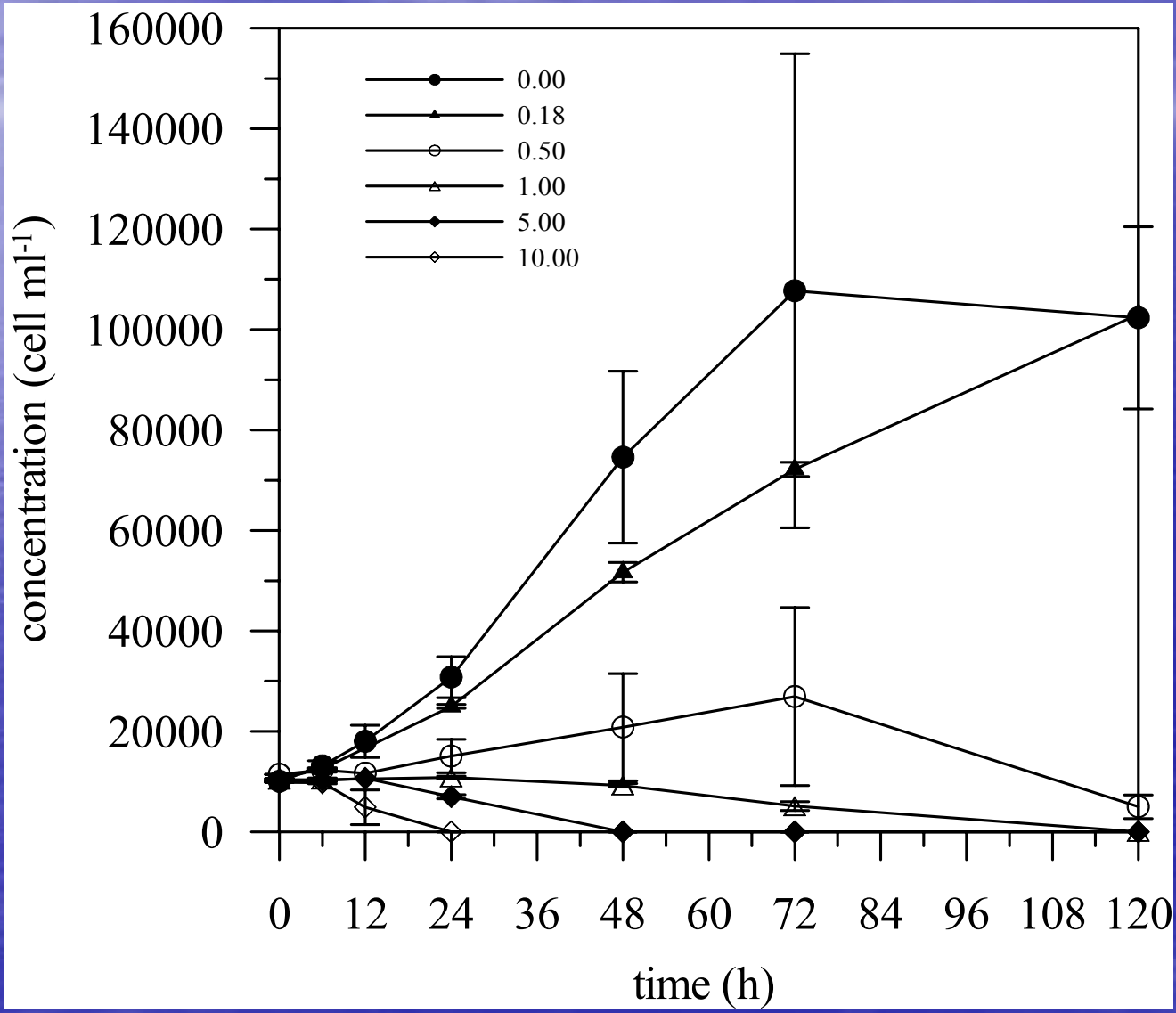
Fluorescence Activated Cell Sorting



Aldehydes tested

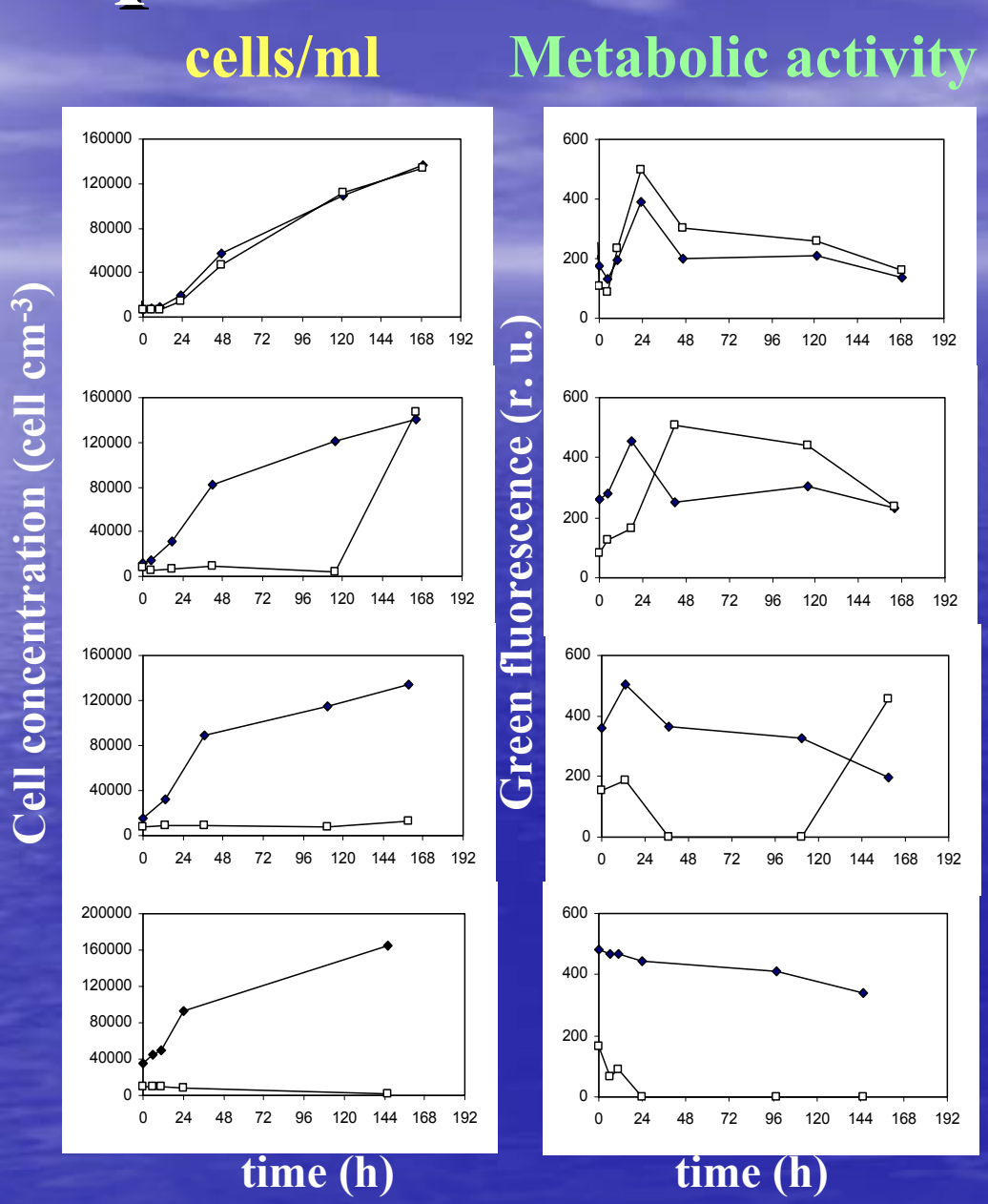
Decadienal (A3) (SIGMA)
Decatrienal (A1) (synthesized)
Octadienal (SIGMA)
Heptadienal (SIGMA)

dose-dependent effect



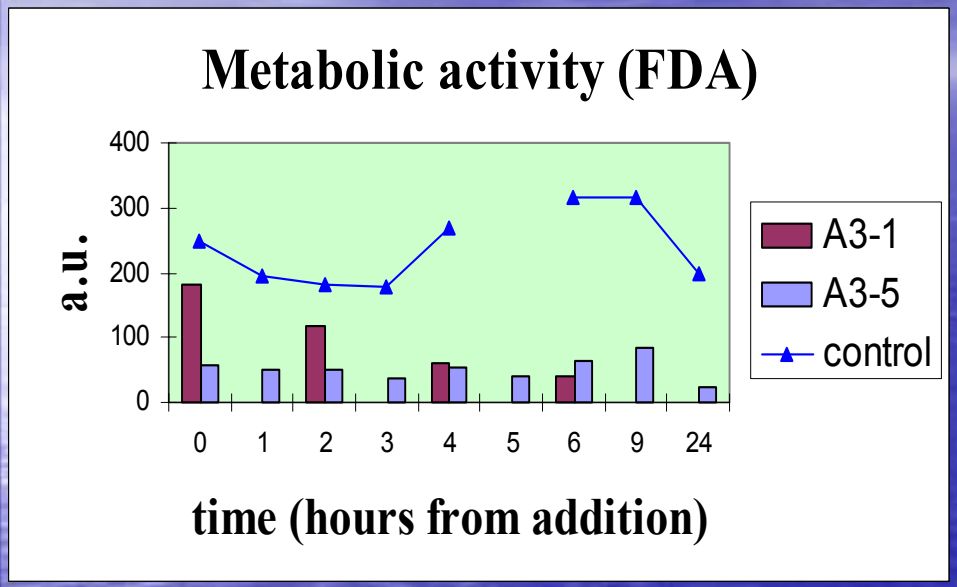
Casotti et al. 2005

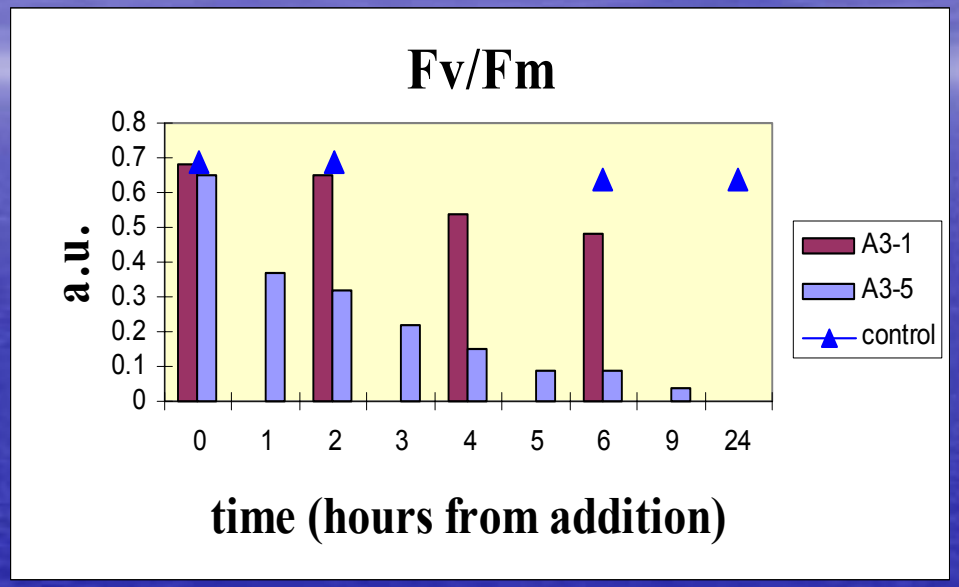
Time-dependent



Casotti et al. 2005

Effects on metabolic activity

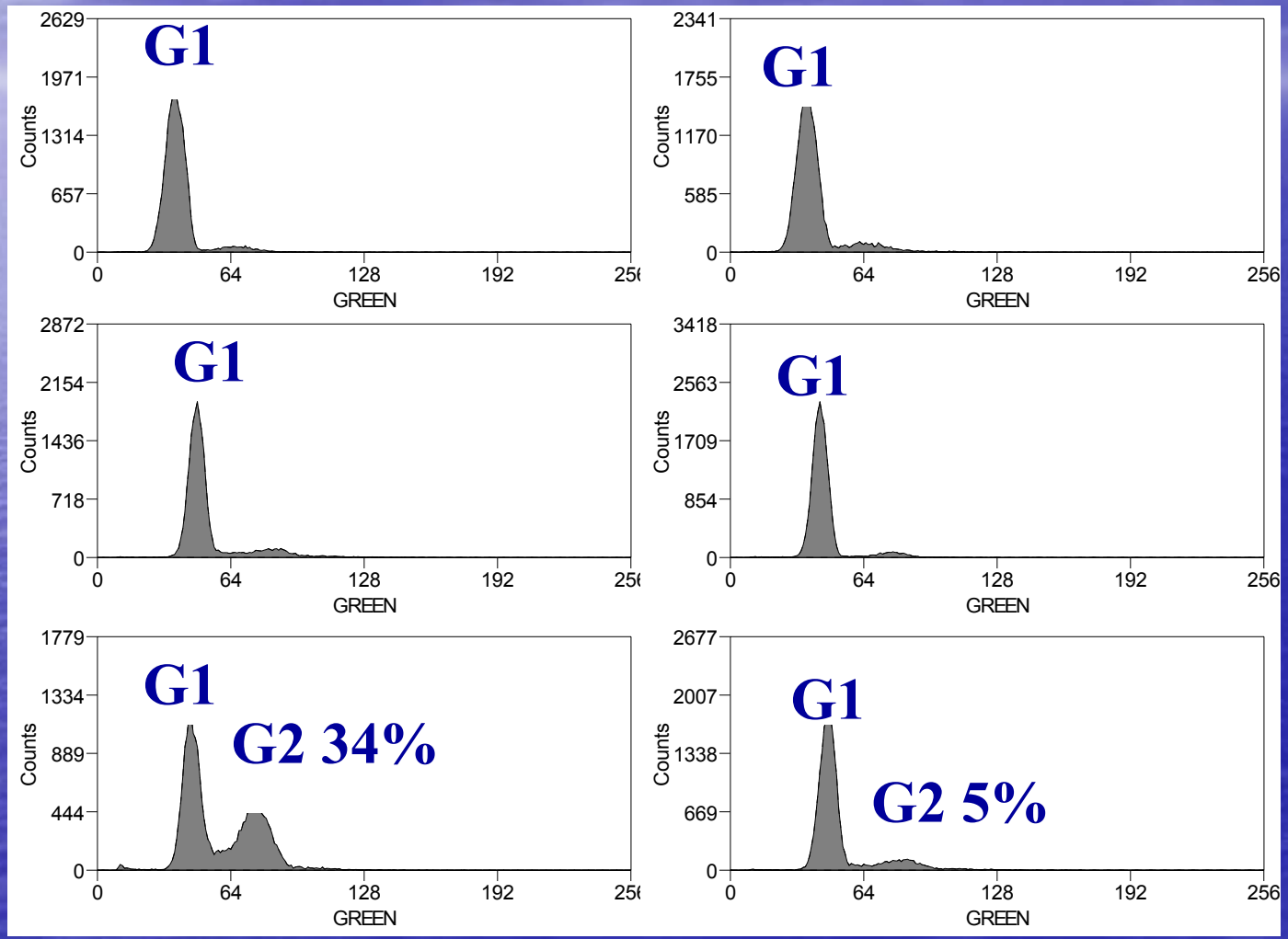




- **Photosynthesis is affected slower than esterase activity**

Cell cycle

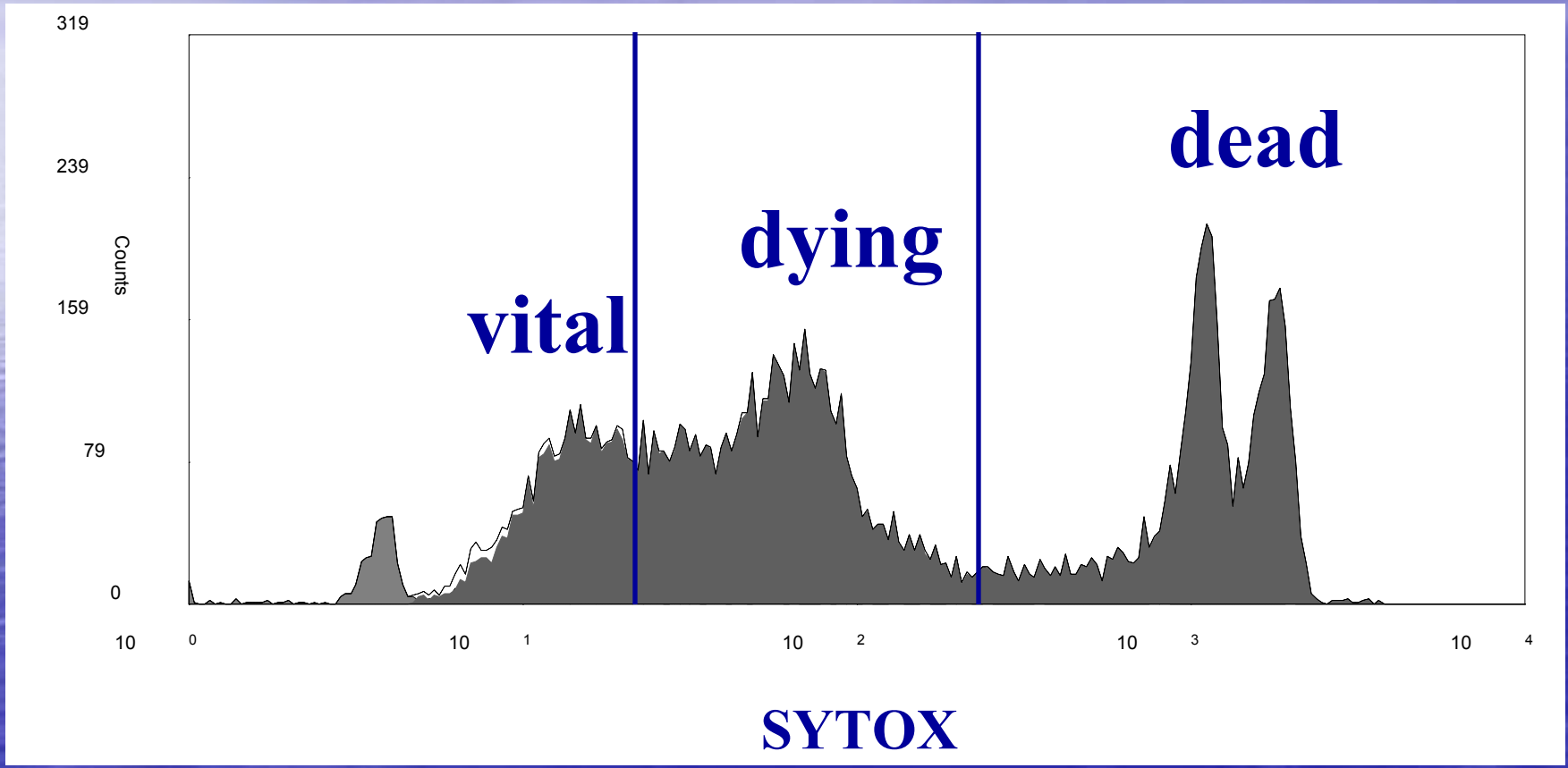
control *+ A3*



Casotti et al. 2005

DNA

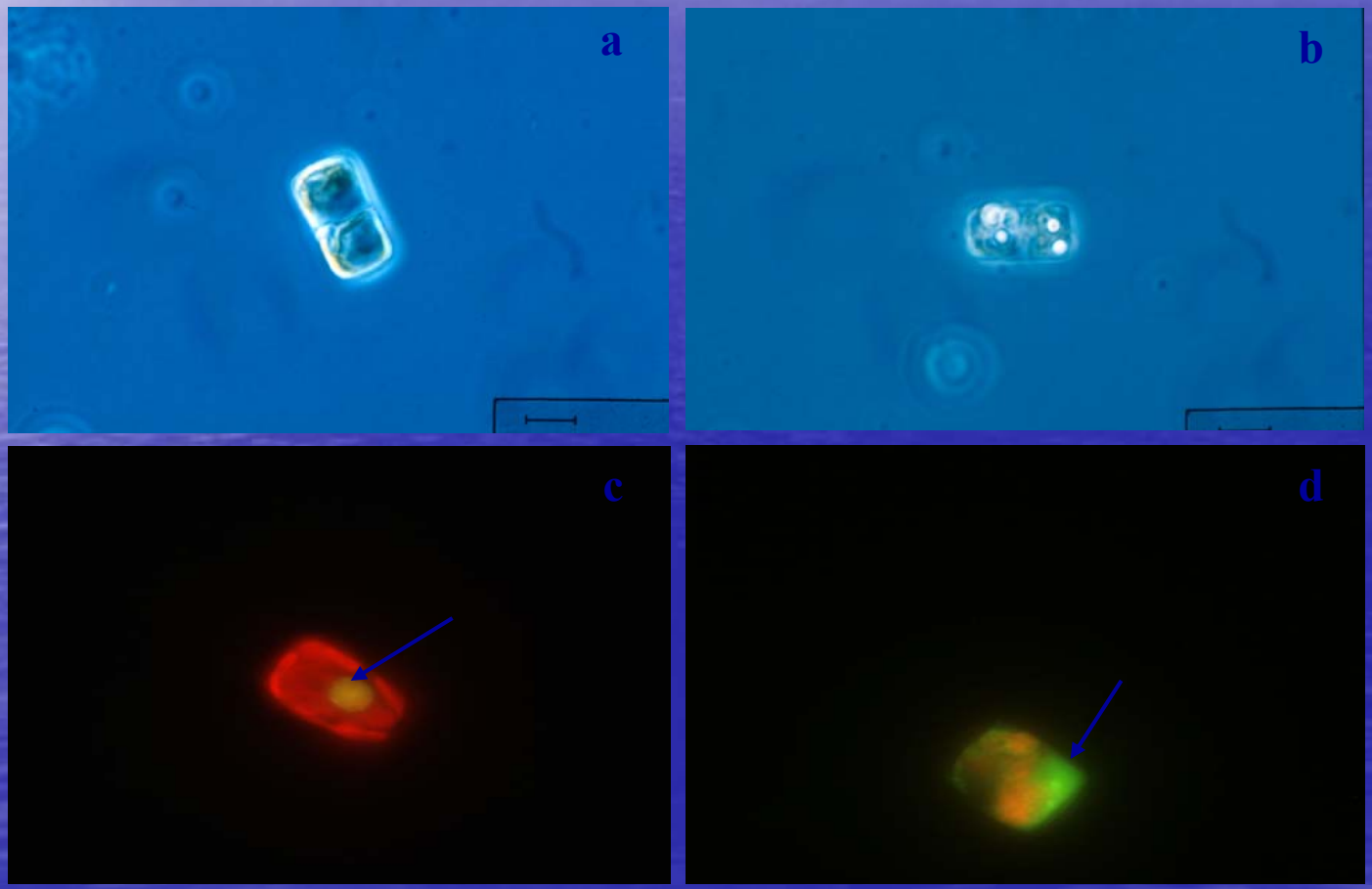
Subpopulations



Casotti et al. 2005

Active process of cell death

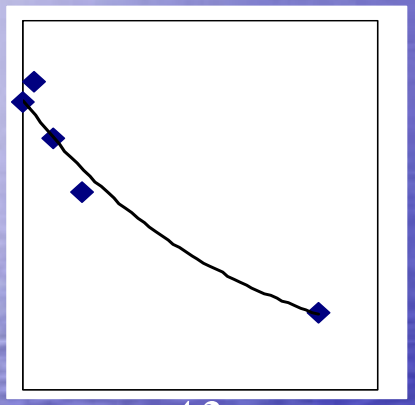
Morfologia



Casotti et al. 2005

TW

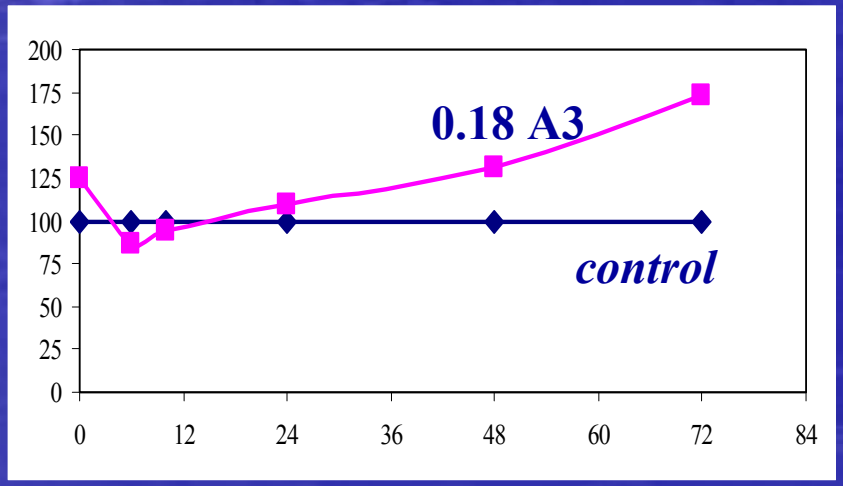
$$y = a\exp(bconc) + c$$

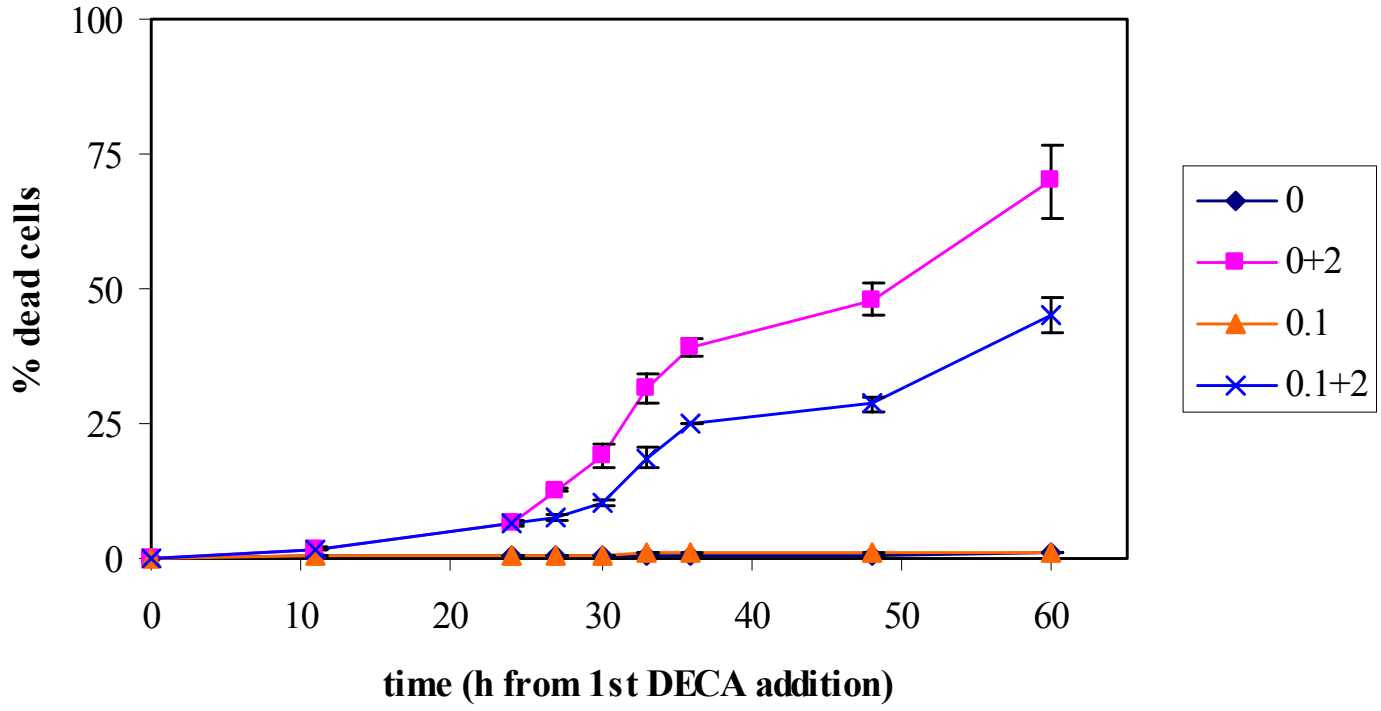


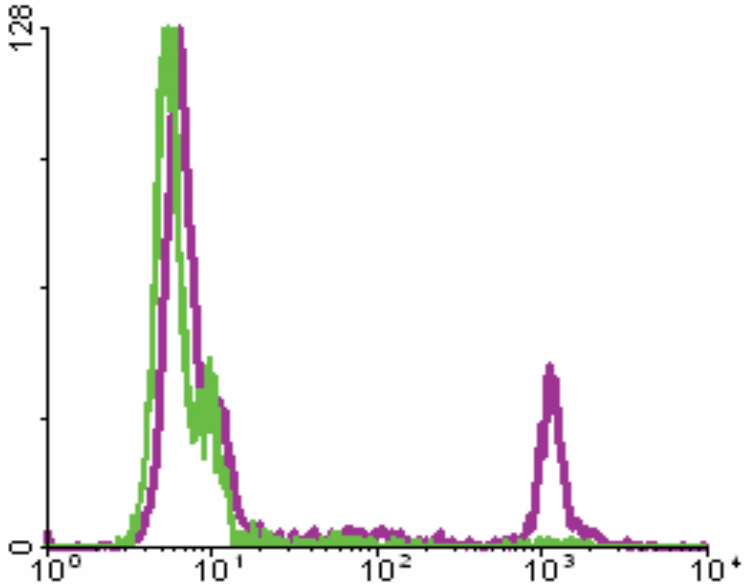
A3 conc

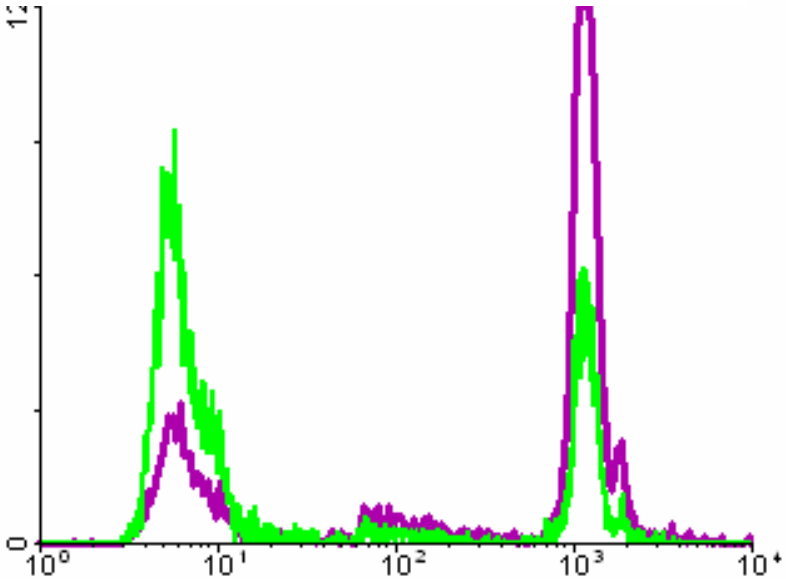
b values

FDA	2.97
cell ml^{-1}	1.37
red fluor.	0.88
SX+	0.48
Dt/(Dt+Dd)	0.42
Fv/Fm	0.27



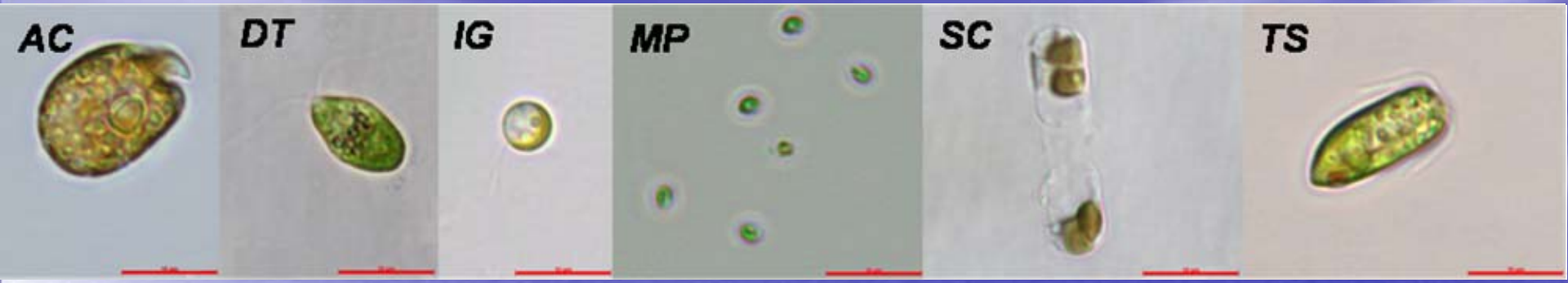

100
75
50
25
0
% dead cells
0
10
20
30
40
50
60
time (h from 1st DECA addition)
0
0+2
0.1
0.1+2


128
0
10^0
10^1
10^2
10^3
10^4


0
10^0
10^1
10^2
10^3
10^4

- Mediators of cell death (ROS or NO)
- Comparative analyses on other phytoplankton (F. Ribalet)

MODEL SPECIES



f/2 medium, under Light:Dark 12:12 at 150 µE.mol^{-1}.s^{-1} at 17°C in environmental chamber

SPECIES	CLASS	GROWTH RATE (d^{-1})
Amphidinium carterae (**AC**)	Dinoflagellate	0.43 ± 0.02
Dunaliella tertiolecta (**DT**)	Chlorophyceae	0.69 ± 0.09
Isochrysis galbana (**IG**)	Prymnesiophyceae	0.65 ± 0.03
Micromonas pusilla (**MP**)	Prasinophyceae	0.84 ± 0.05
Skeletonema costatum (**SC**)	Bacillariophyceae	0.85 ± 0.08
Tetraselmis suecica (**TS**)	Prasinophyceae	0.96 ± 0.07

AXENIC

50 mL of exponentially growing cultures (10 000 cells / mL) exposed to different concentrations of PUA (from 0.1 to 4 mg / L).

For each treatment, compilation of data from 2 series of experiments in triplicats

Ribalet 2005

Statistical Analysis

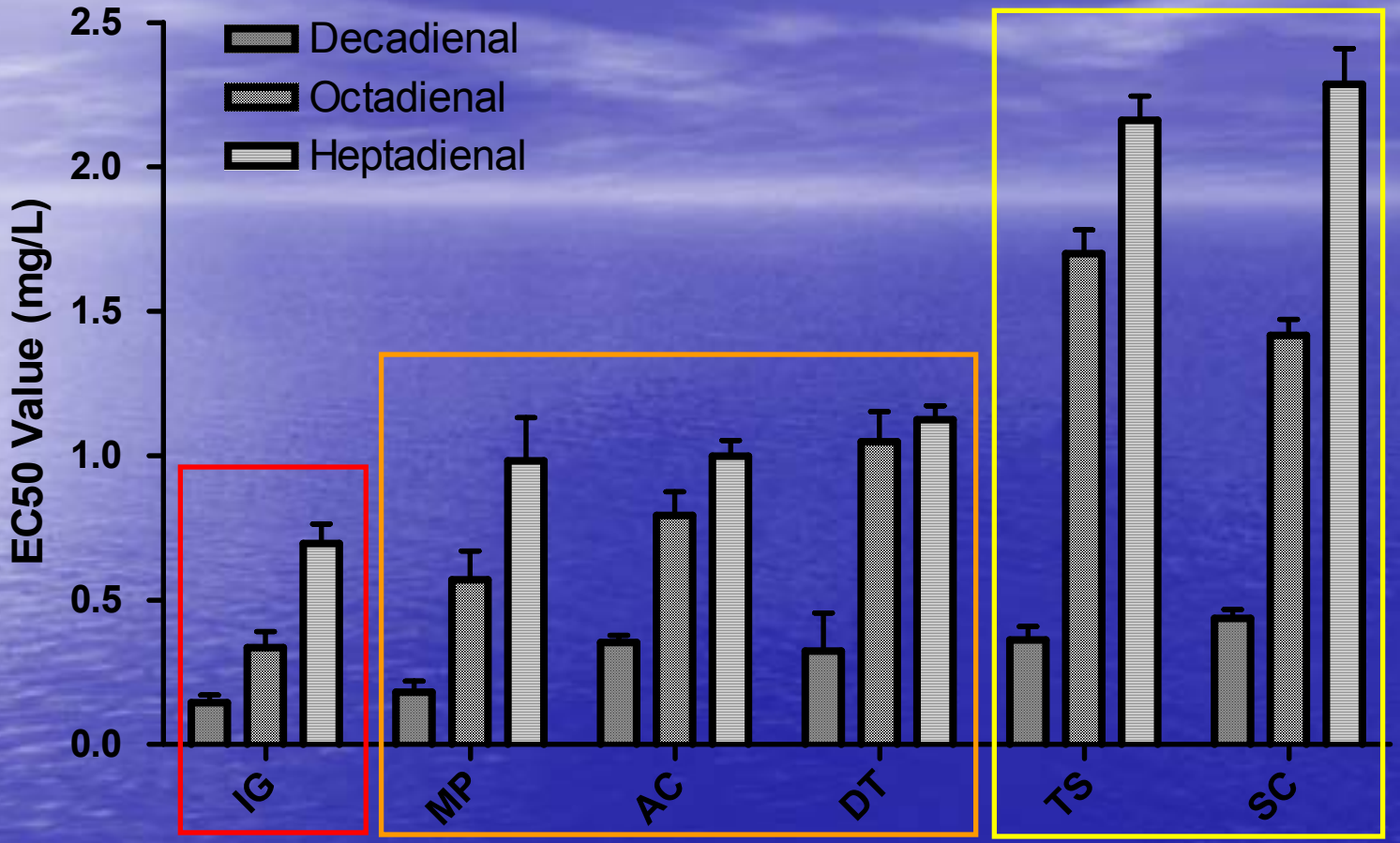


Figure 3: 24h-EC50 values for decadienal, octadienal and heptadienal for the different species. Data are means ± 95% IC (n=3).

Ribalet 2005

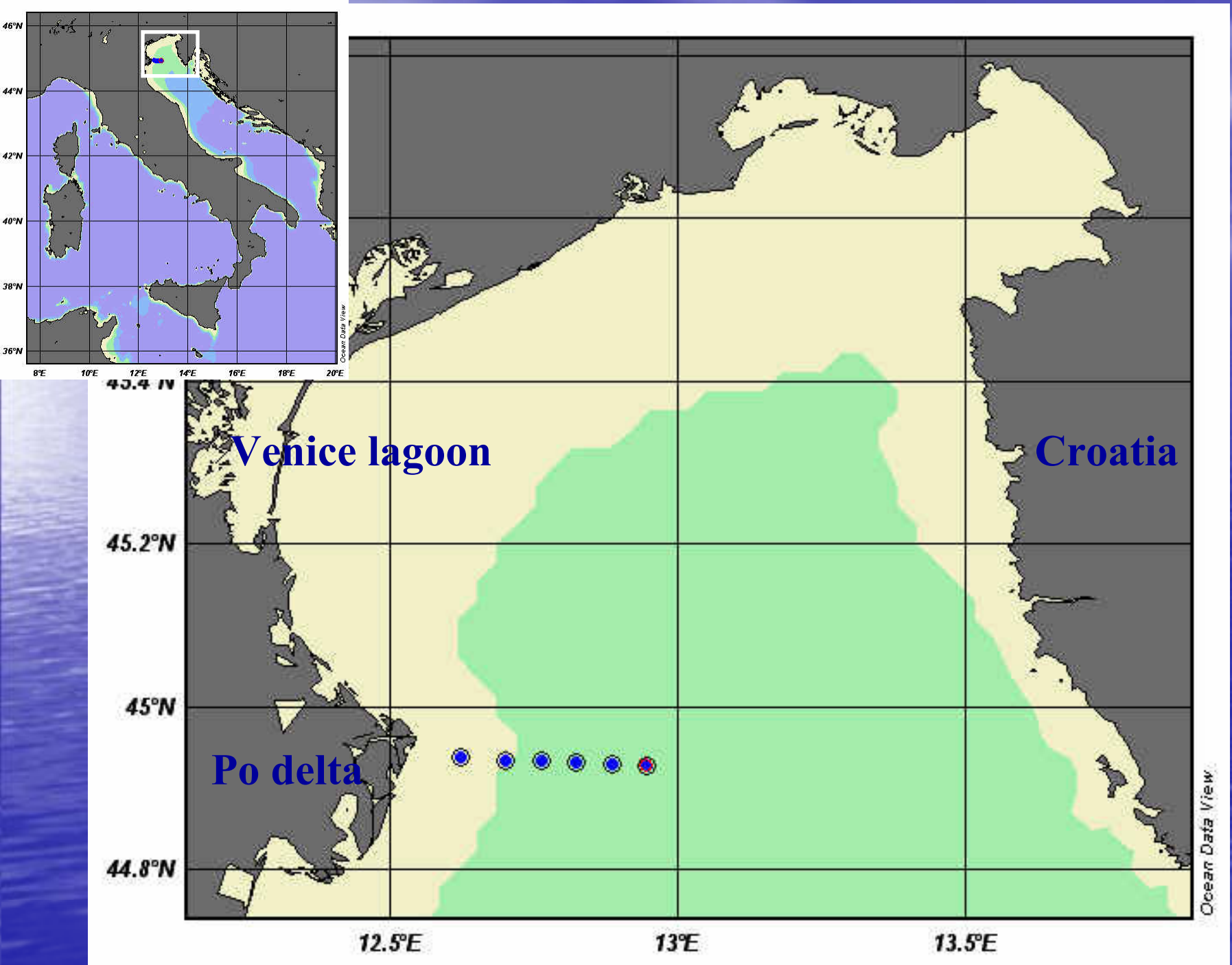
Producer of polyunsaturated aldehydes	Analysis of PUA					
	PUA	C7:2	C8:2	C8:3	C10:2	C10:3
	fmol/cell	% of total PUA				
Thalassiosira pacifica	9.81 ± 0.069	70	19	11	0	0[a]
Melosira nummuloides	8.68 ± 0.424	7	72	0	2	19
Thalassiosira rotula (CCMP 1647)	6.35 ± 0.289	5	32	11	2	50
Thalassiosira rotula (origin: Roscoff)	5.69 ± 0.472	7	16	18	0	58
Chaetoceros compressus	2.82 ± 0.635	31	12	0	8	49
Thalassiosira aestivalis	1.54 ± 0.266	78	16	6	0	0
Thalassiosira anguste-lineata	1.53 ± 0.079	69	17	14	0	0
Thalassiosira rotula (origin: Point Wells)	1.27 ± 0.108	24	8	41	0	27
Thalassiosira rotula (CCMP 1812)	1.04 ± 0.030	24	28	23	0	24
Skeletonema pseudocostatum	0.38 ± 0.041	50	49	1	0	0
Thalassiosira rotula (CCMP 1018)[c]	0.22 ± 0.048	31	45	24	0	0
Guinardia deliculata	0.18 ± 0.015	100	0	0	0	0
Skeletonema costatum (RCC 75)	0.13 ± 0.016	58	38	3	0	0
Fragilaria sp.	0.10 ± 0.010	65	32	0	3	0
Asterionellopsis glacialis	0.05 ± 0.005	68	28	4	0	0
Thalassiosira minima	0.05 ± 0.002	23	10	0	0	61
Skeletonema subsalsum	0.04 ± 0.014	0[a]	80	0	20	0

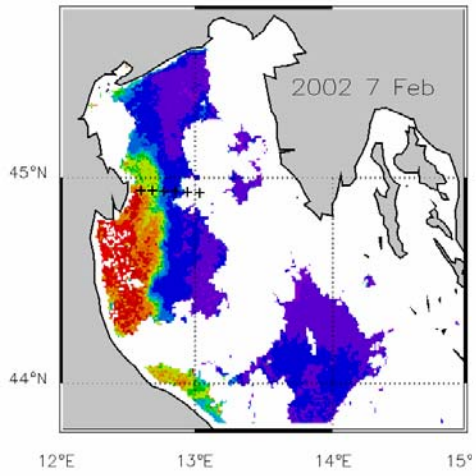
Ribalet 2005

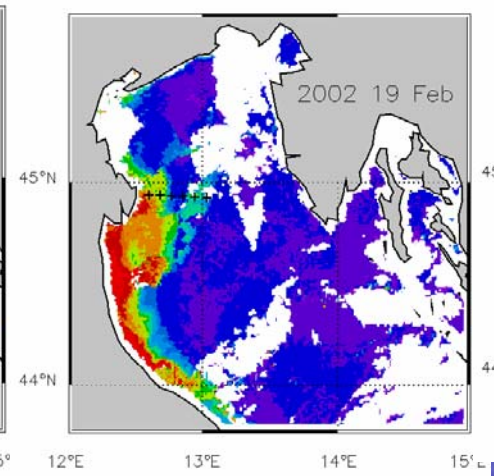
(Wichard et al., 2005)

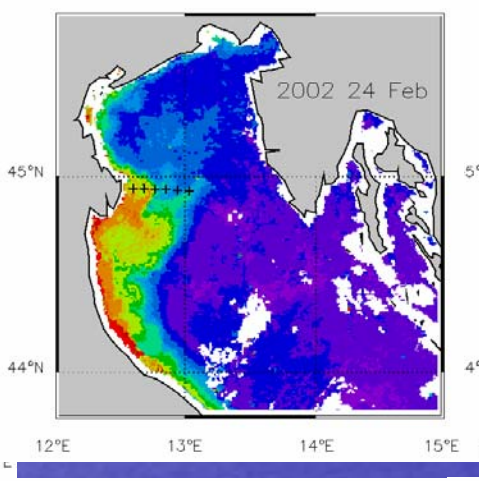
In situ work (more questions than answers)

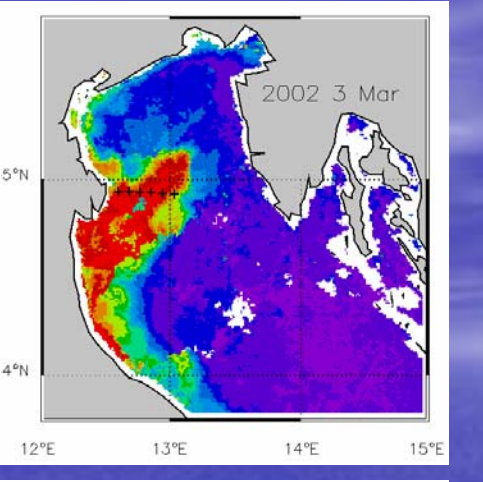
- Study of a diatom bloom: *Skeletonema costatum* in the North Adriatic Sea

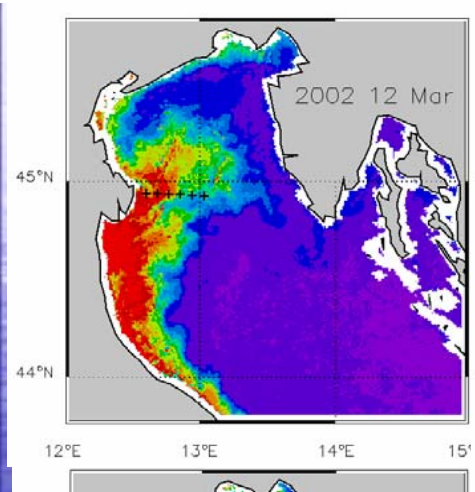

46°N
44°N
42°N
40°N
38°N
36°N
8°E
10°E
12°E
14°E
16°E
18°E
20°E
Ocean Data View
Venice lagoon
Croatia
45.2°N
45°N
Po delta
44.8°N
12.5°E
13°E
13.5°E
Ocean Data View

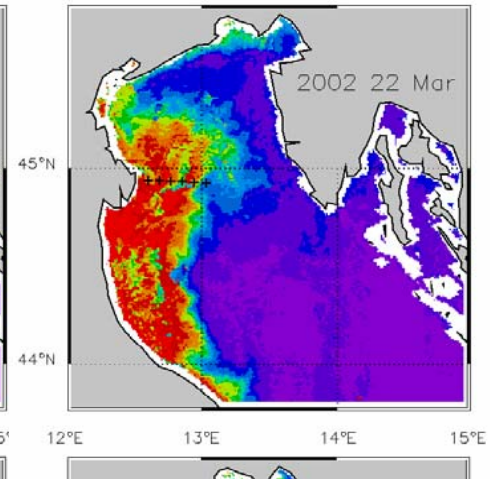

2002 7 Feb
45°N
44°N
12°E
13°E
14°E

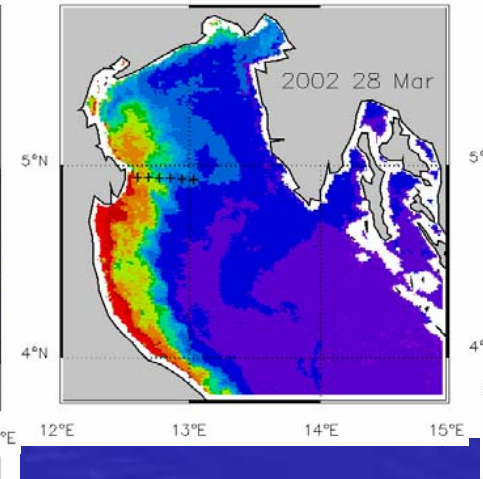

2002 19 Feb
45°N
44°N
12°E
13°E
14°E

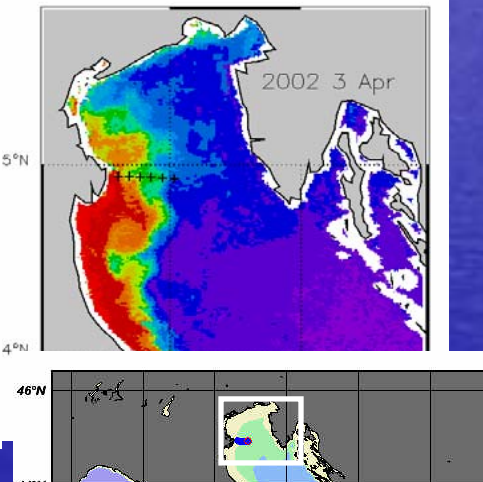

2002 24 Feb
45°N
44°N
12°E
13°E
14°E
15°E

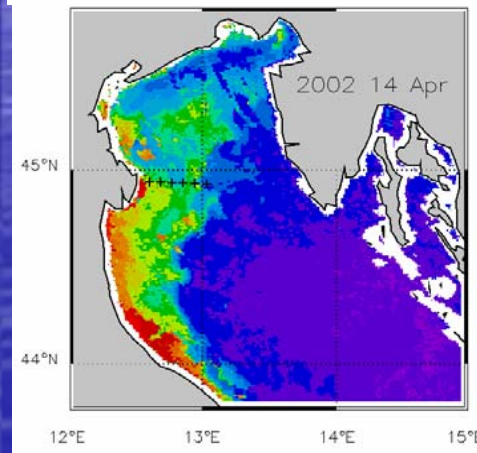

2002 3 Mar
5°N
4°N
12°E
13°E
14°E
15°E

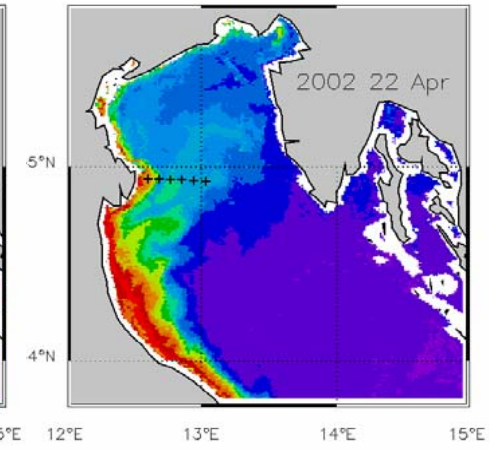

2002 12 Mar
45°N
44°N
12°E
13°E
14°E

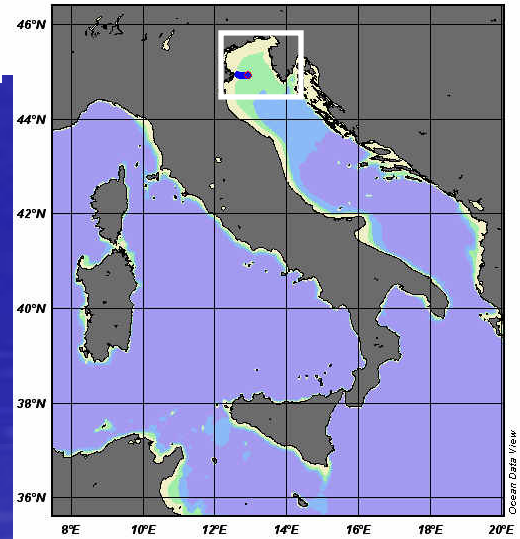

2002 22 Mar
45°N
44°N
12°E
13°E
14°E
15°E


2002 28 Mar
5°N
4°N
12°E
13°E
14°E
15°E


2002 3 Apr
5°N
12°E
13°E
14°E


2002 14 Apr
45°N
44°N
12°E
13°E
14°E
15°E


2002 22 Apr
5°N
4°N
12°E
13°E
14°E
15°E


46°N
44°N
42°N
40°N
38°N
36°N
8°E
10°E
12°E
14°E
16°E
18°E
20°E
Ocean Data View

Aldehydes can be effectors of a bloom at sea?

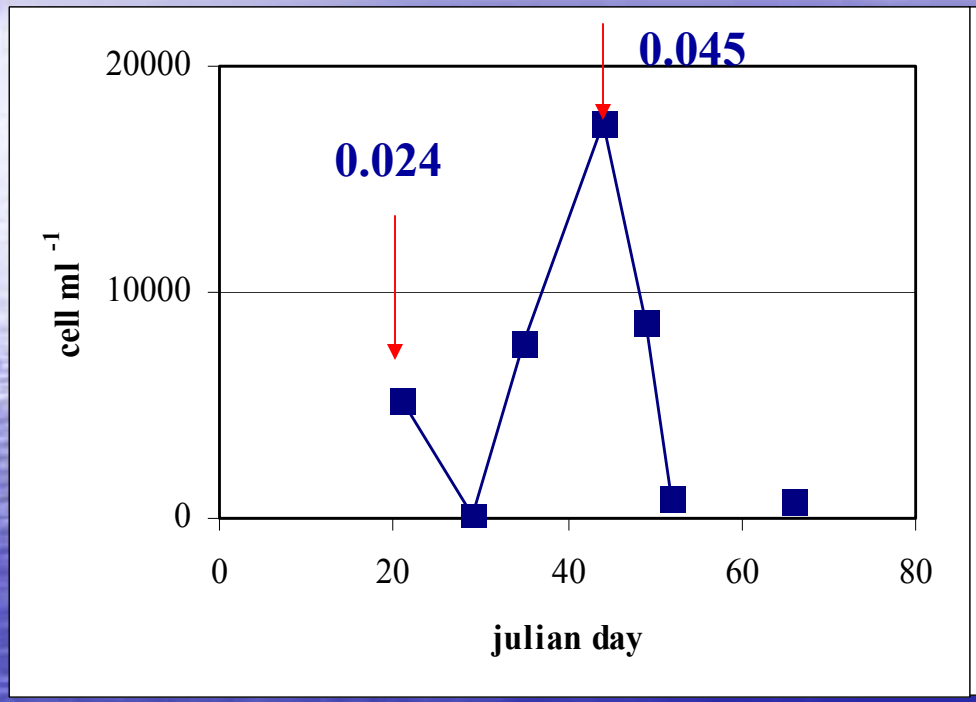
- Mastication by grazers
- Autolysis

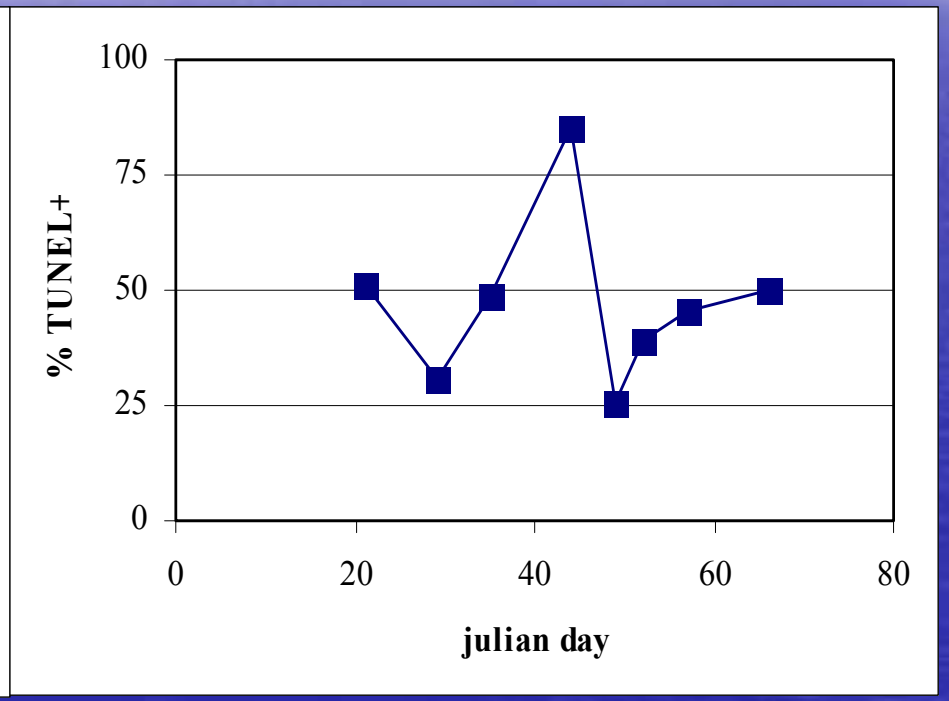
- Endogenous (ciclo cellulare)
- Exogenous (virus, bacteria, UV, mixing, nutrients)

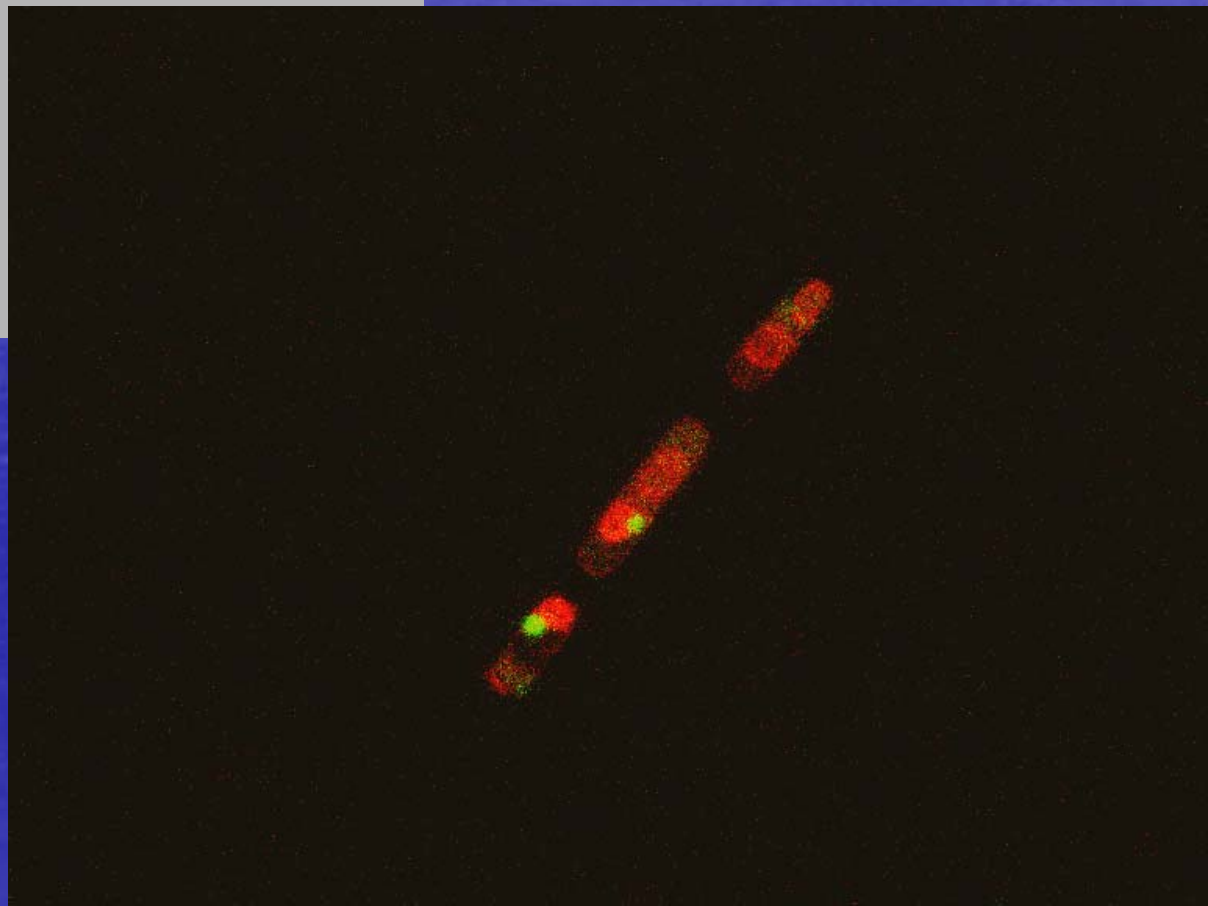
Tentative approach for *in situ* studies

- Characterize the *Skeletonema* bloom dynamics (growth rates, interactions with other phytoplankton)
- Estimate lysis rates (FDA method)
- Estimate apoptotic cells

- Relate to effects on copepods
- Presence of aldehydes dissolved?
- Enhancement due to environmental factors?


0.045
0.024
20000
10000
0
cell ml -1
0
20
40
60
80
julian day


100
75
50
25
0
% TUNEL+
0
20
40
60
80
julian day



TUNEL