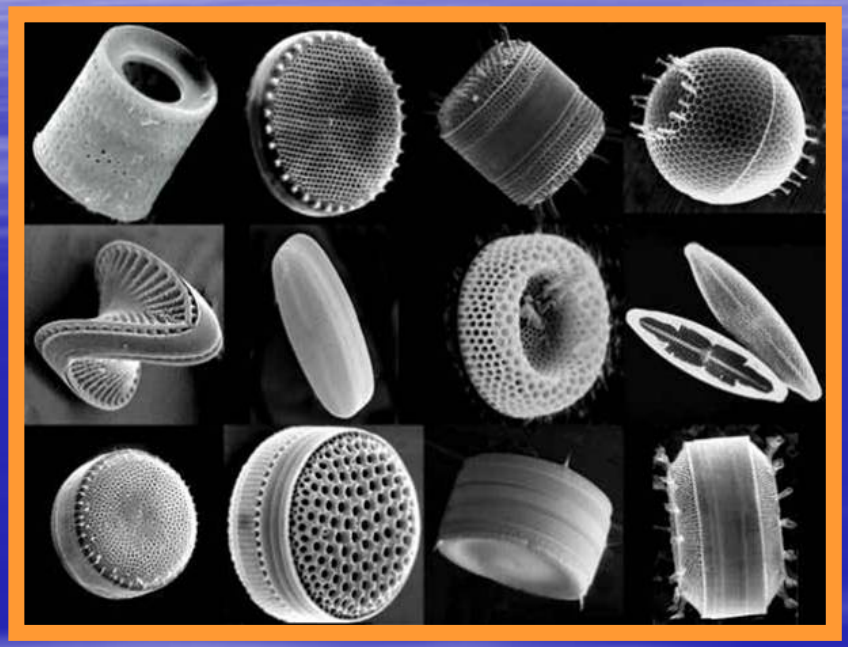
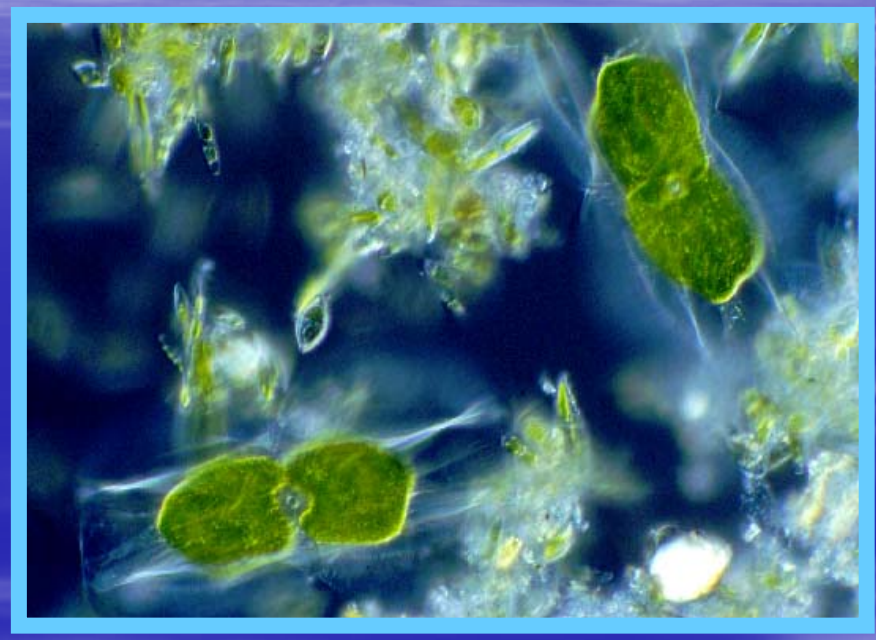
Ecotossicologia delle ossilipine da Diatomee.

Giovanna Romano
Stazione Zoologica Anton Dohrn

Diatoms





- There are more than 200 genera of living diatoms, and it is estimated that there are approximately 100,000 species
- Diatoms can be found in the oceans and in freshwater.
- Most live pelagically in open water, although some live as surface films at the water-sediment interface (benthic), or even under damp atmospheric conditions.
- They are especially important in oceans, where they are estimated to contribute up to 45% of the total oceanic primary production.

THE MARINE FOOD CHAIN

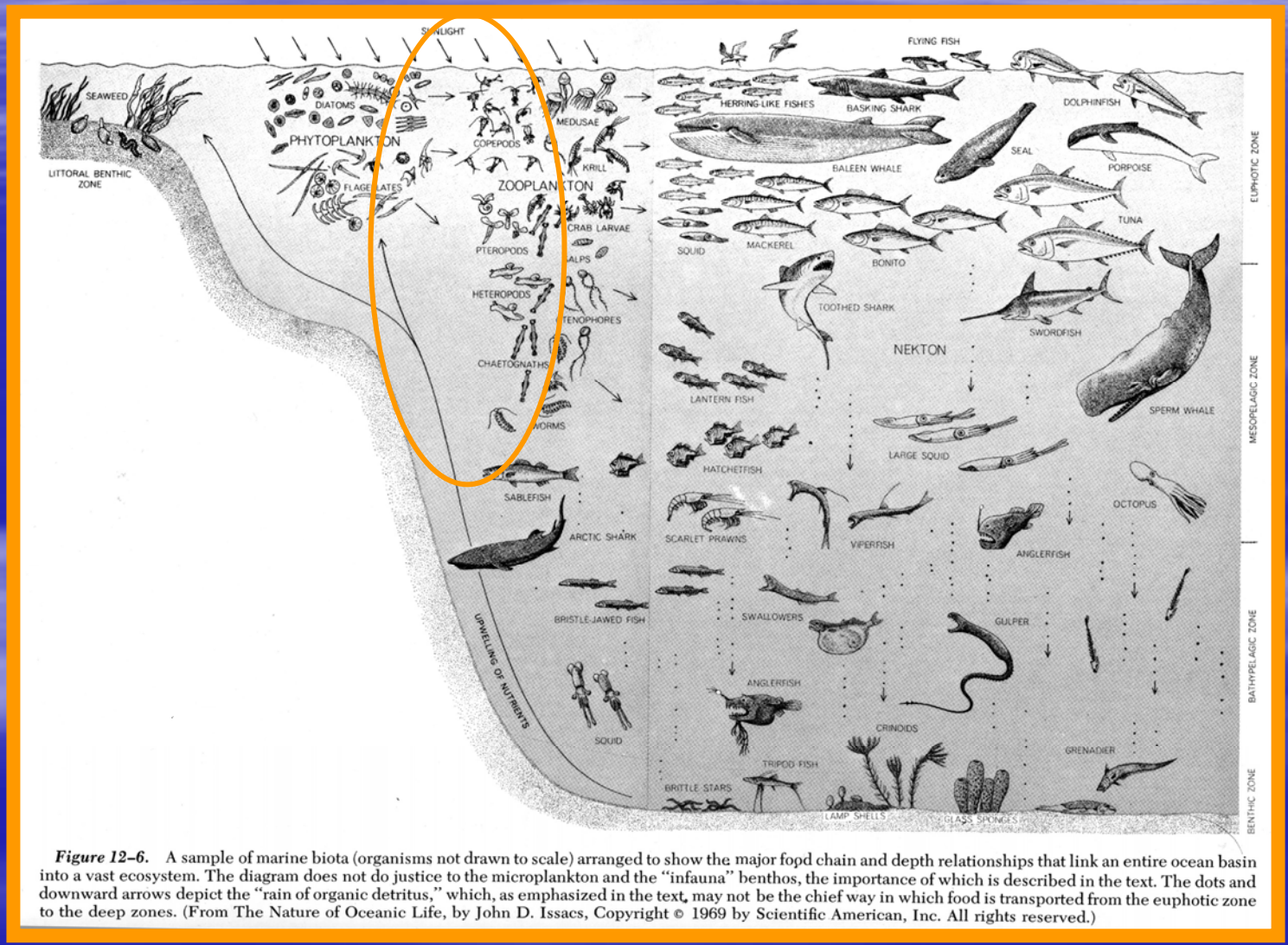


Figure 12–6. A sample of marine biota (organisms not drawn to scale) arranged to show the major food chain and depth relationships that link an entire ocean basin into a vast ecosystem. The diagram does not do justice to the microplankton and the "infauna" benthos, the importance of which is described in the text. The dots and downward arrows depict the "rain of organic detritus," which, as emphasized in the text, may not be the chief way in which food is transported from the euphotic zone to the deep zones. (From The Nature of Oceanic Life, by John D. Issacs, Copyright © 1969 by Scientific American, Inc. All rights reserved.)

The insidious effect of diatoms on copepod reproduction

A. Miralto*, G. Barone†, G. Romano*, S. A. Poulet‡, A. Ianora*, G. L. Russo§, I. Buttino*, G. Mazzarella§, M. Laabir*, M. Cabrini‖ & M. G. Giacobbe¶

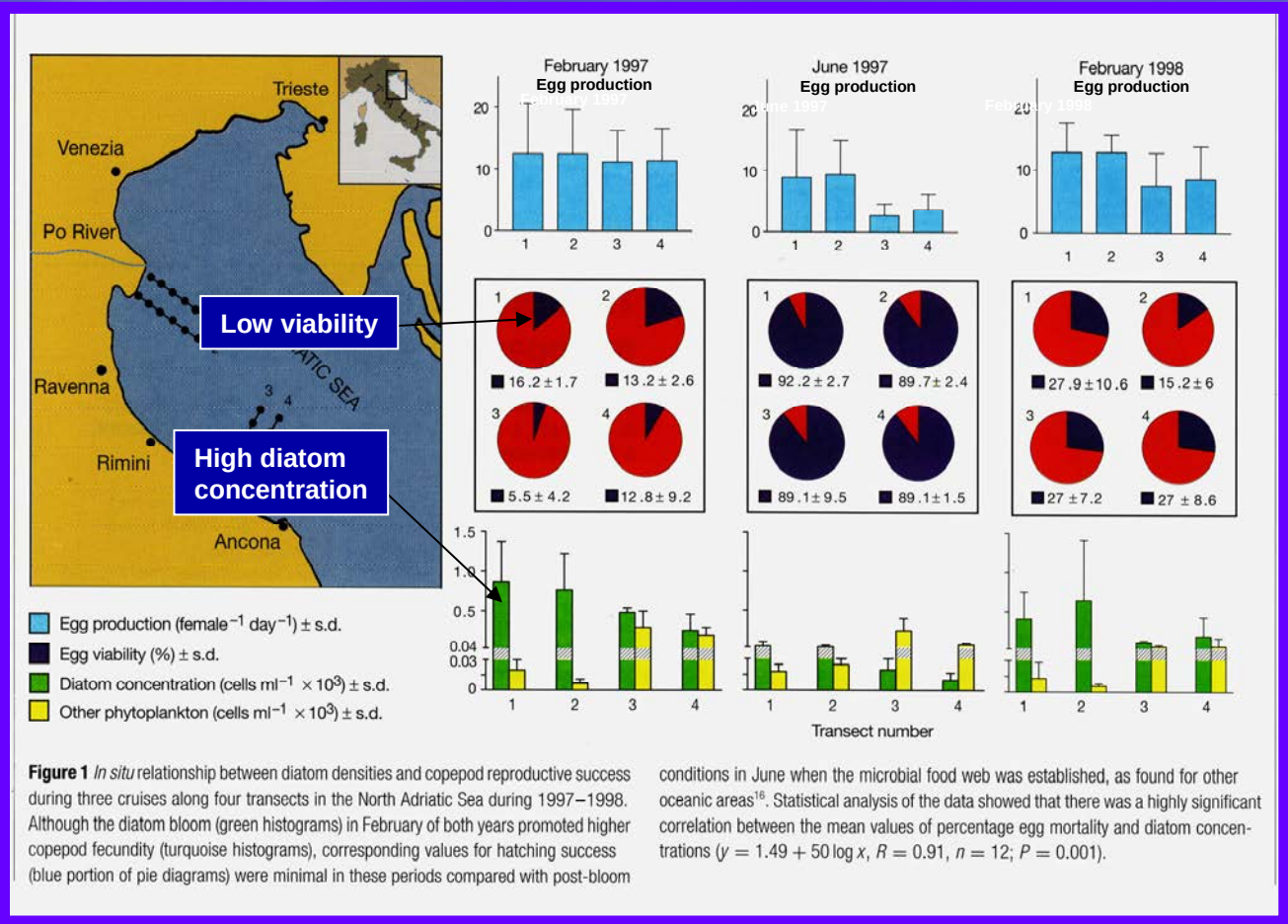
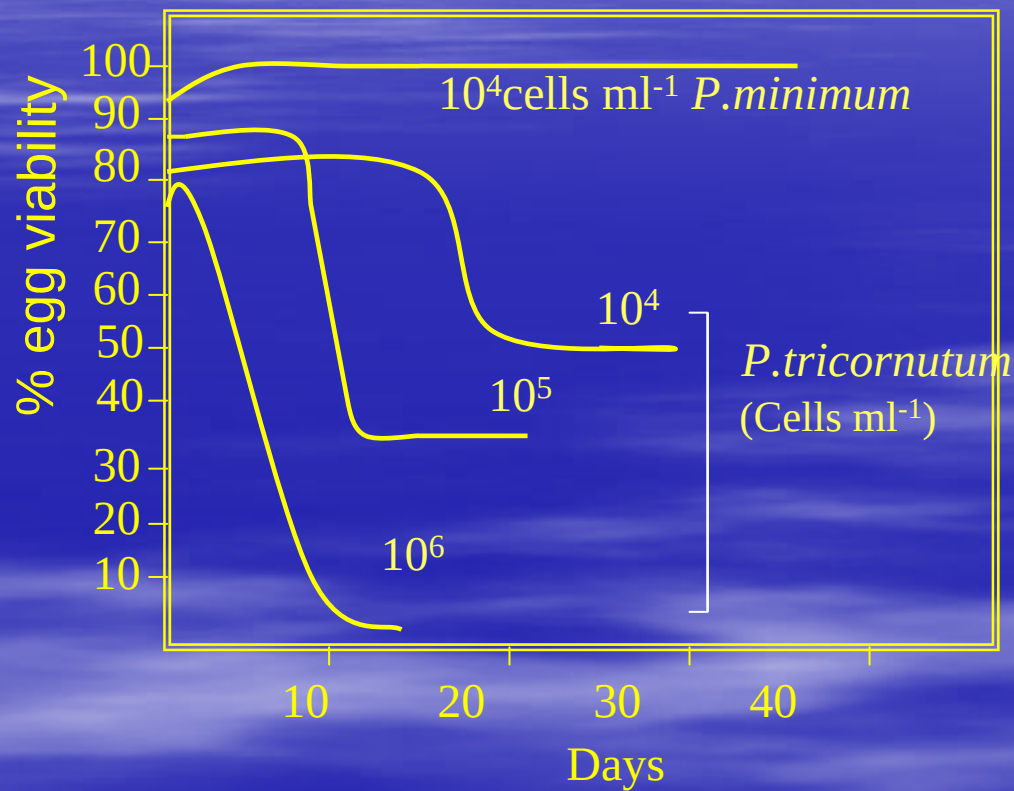


Figure 1 *In situ* relationship between diatom densities and copepod reproductive success during three cruises along four transects in the North Adriatic Sea during 1997–1998. Although the diatom bloom (green histograms) in February of both years promoted higher copepod fecundity (turquoise histograms), corresponding values for hatching success (blue portion of pie diagrams) were minimal in these periods compared with post-bloom conditions in June when the microbial food web was established, as found for other oceanic areas[16]. Statistical analysis of the data showed that there was a highly significant correlation between the mean values of percentage egg mortality and diatom concentrations ($y = 1.49 + 50 \log x$, $R = 0.91$, $n = 12$; $P = 0.001$).

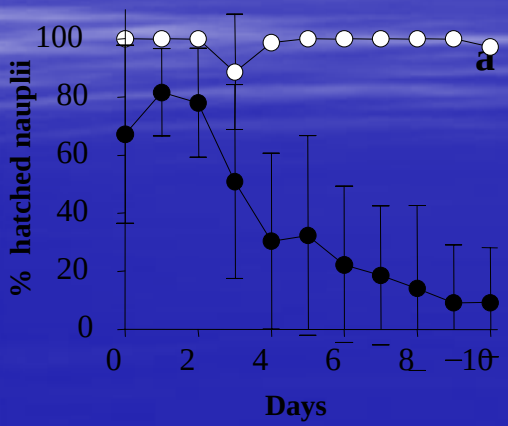
Miralto et al. Nature 1999

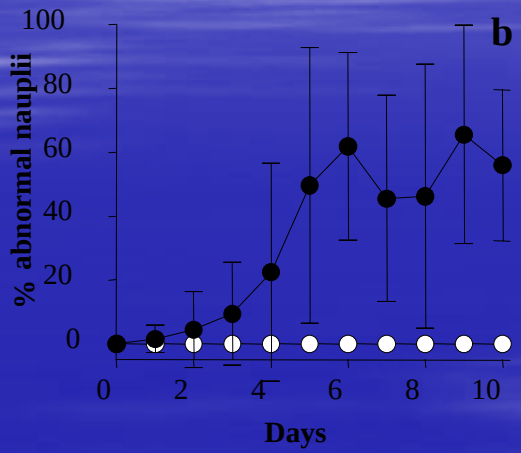
Diatom density-dependent effect on *Calanus* hatching success

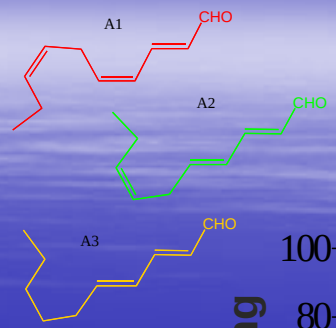


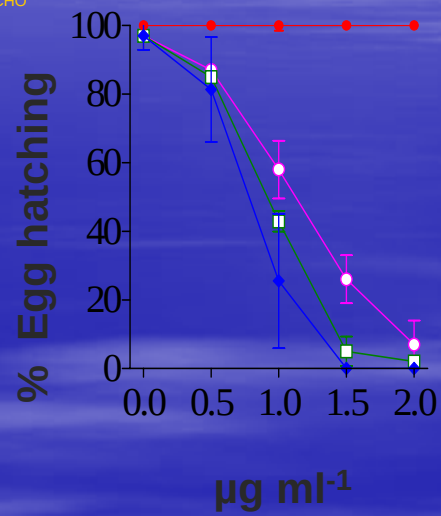
Chaudron et al.
Mar.Ecol.Prog.Ser. 1996

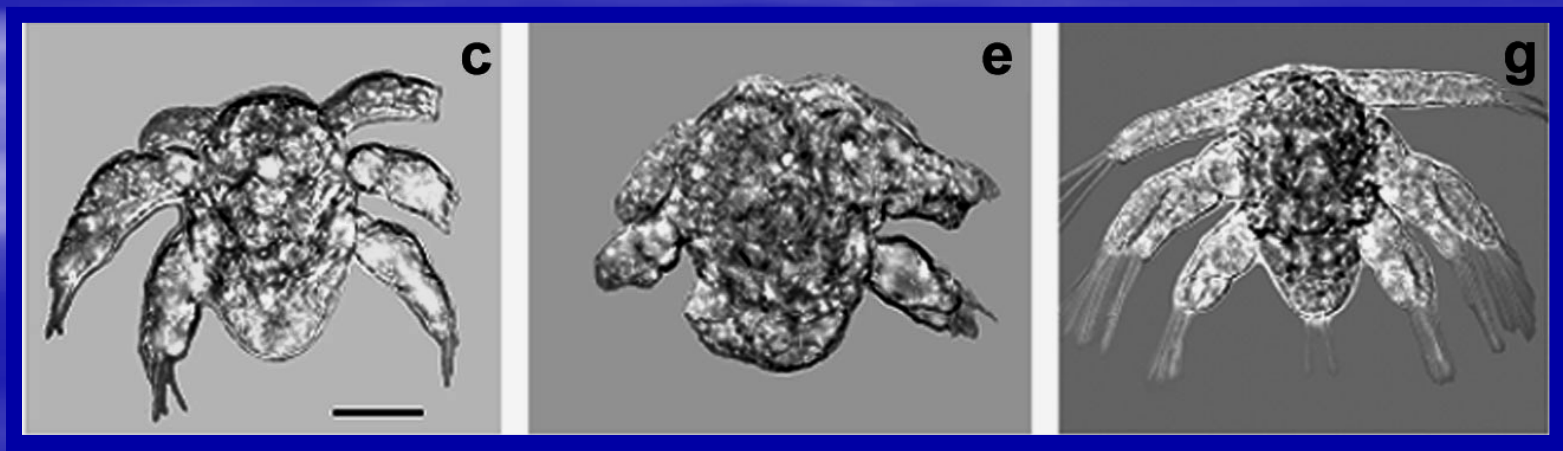
Effect of *Skeletonema marinoi* diet on *Calanus helgolandicus*





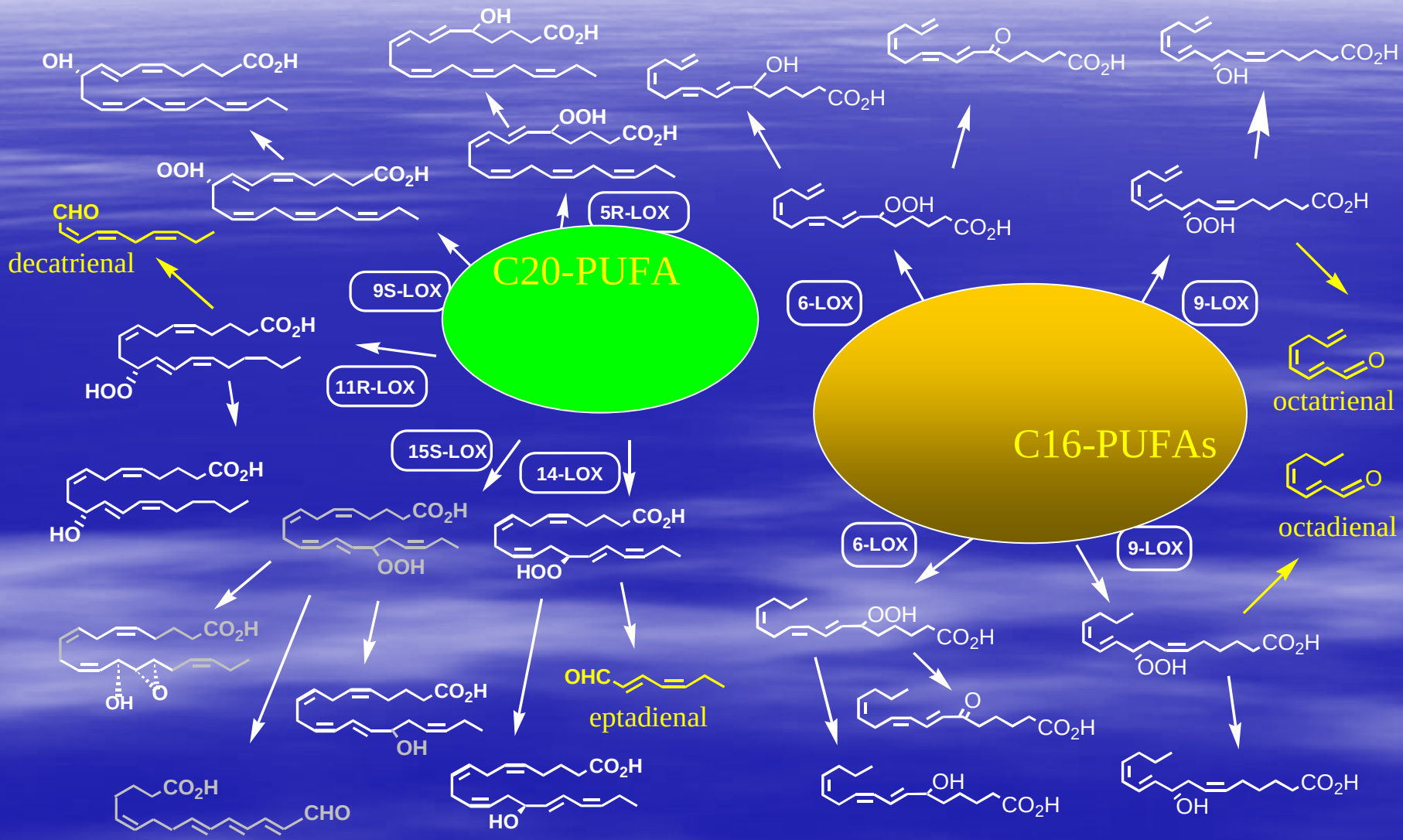






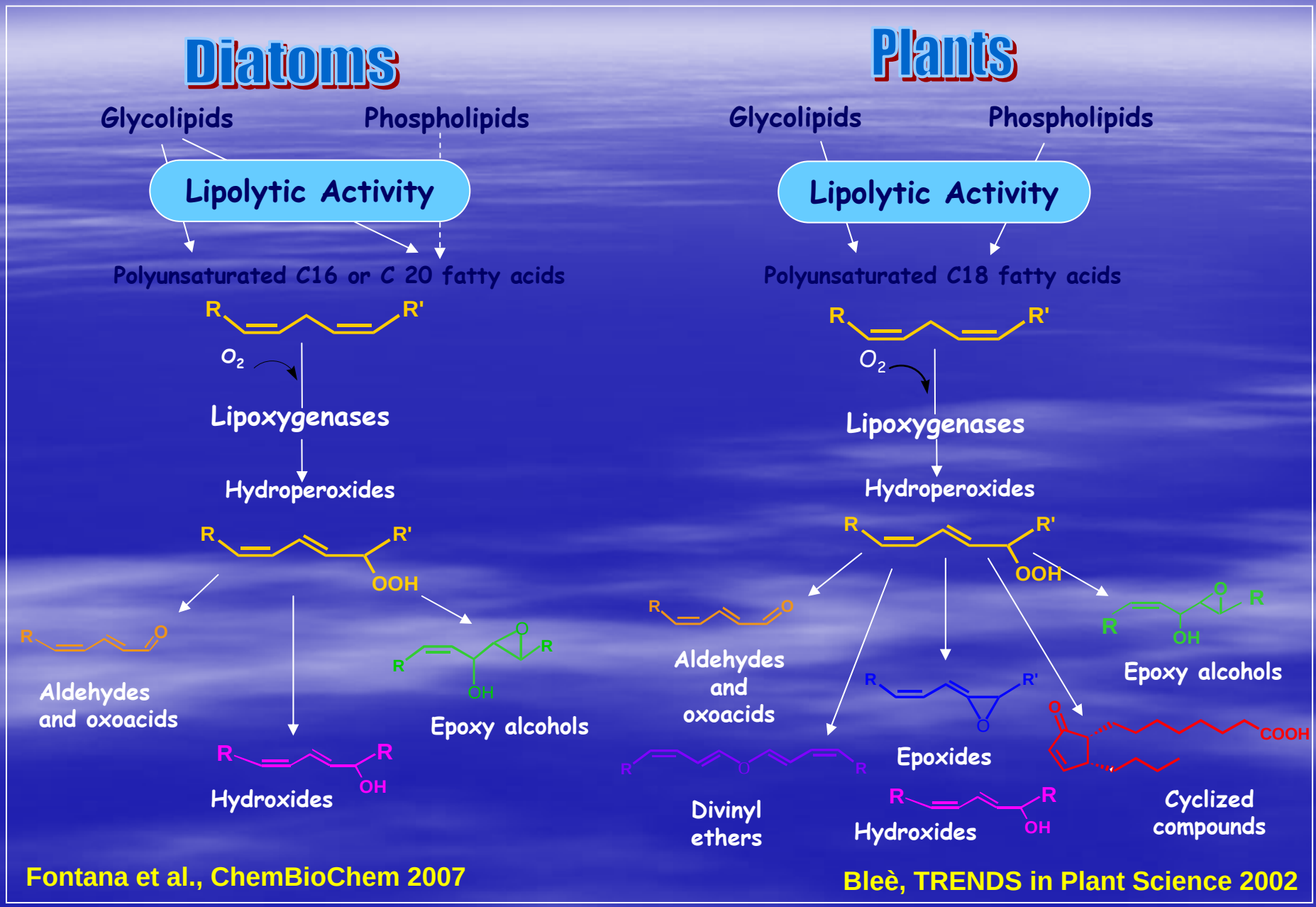
Ianora et al. Nature 2004

Oxylipins in diatoms



Fontana et al 2007

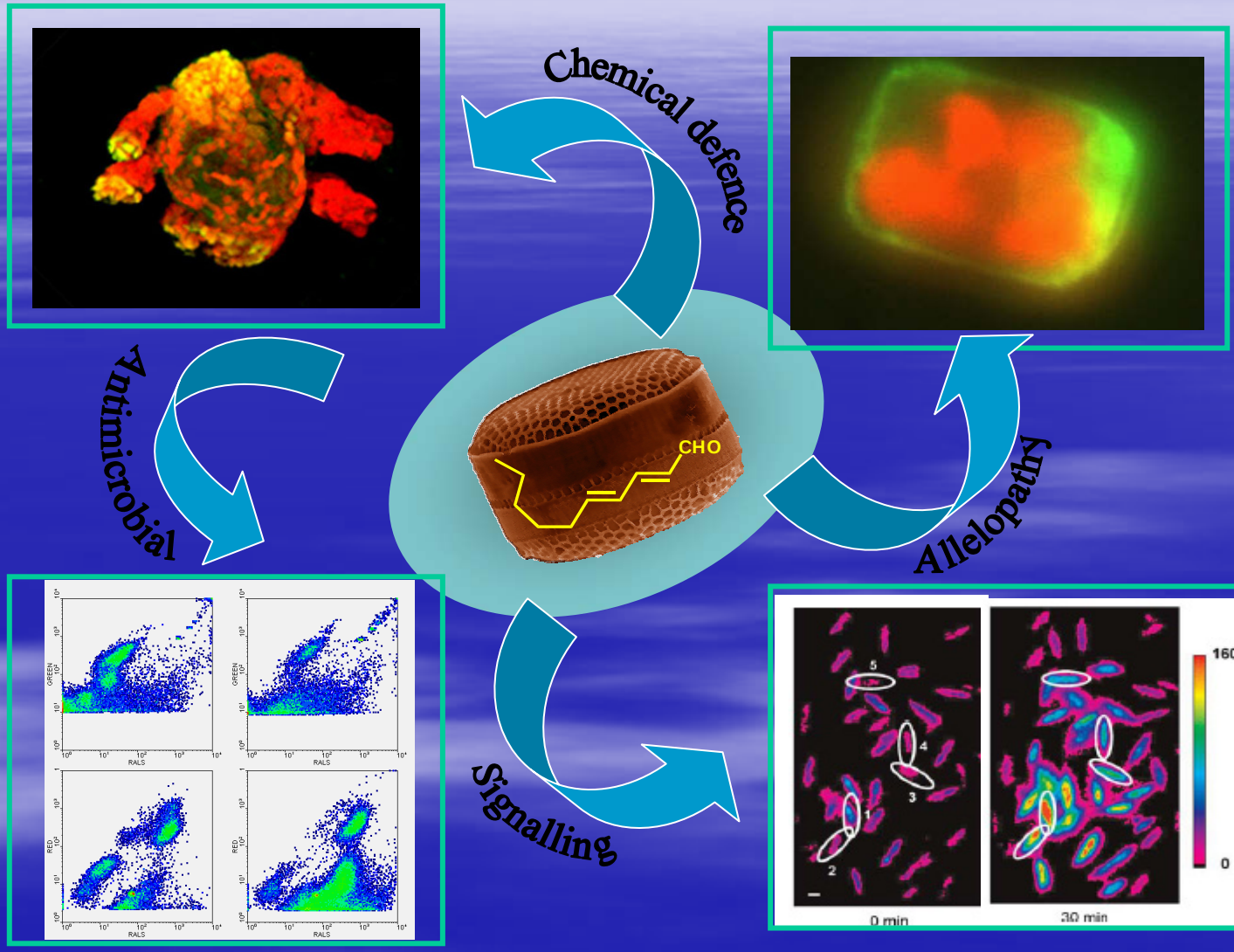
Oxylipin metabolism



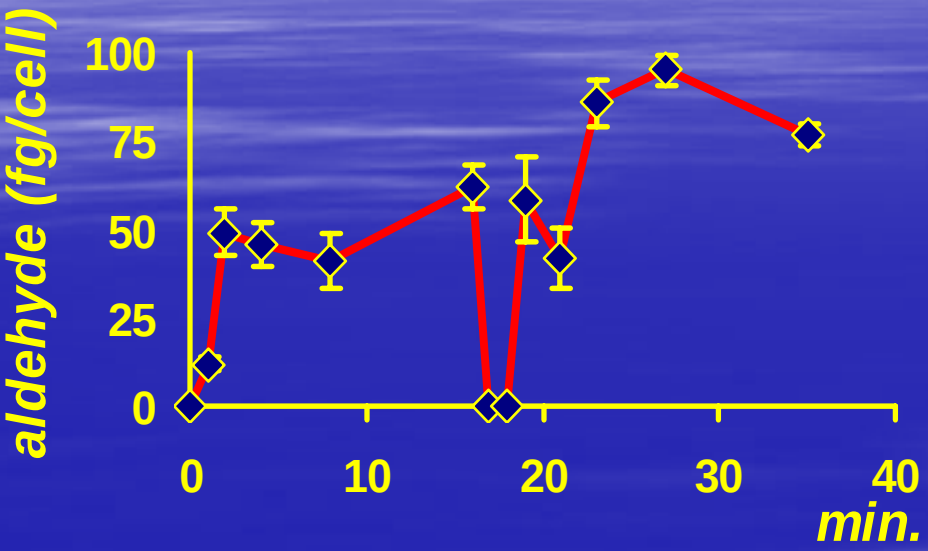
Fontana et al., ChemBioChem 2007

Bleè, TRENDS in Plant Science 2002

Wound-activated defence

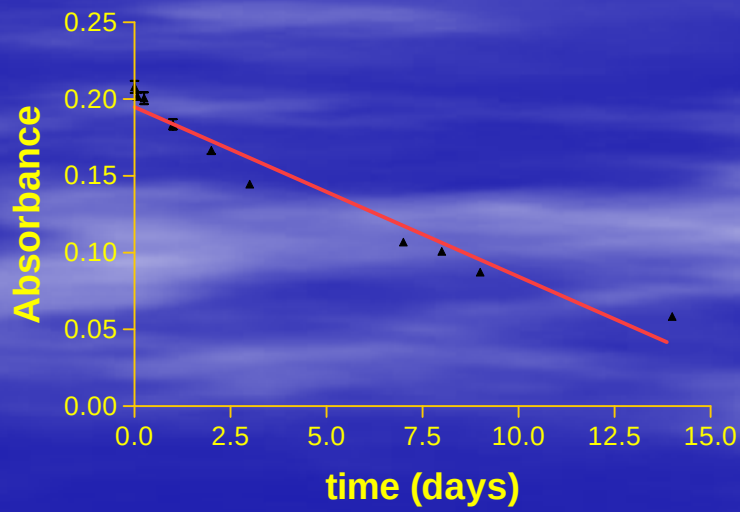


Aldehyde synthesis in *S. marinoi*



Fontana et al., 2007

Aldehyde detection in sea water



Romano et al. 2010

Effect of diatom aldehydes on marine organisms

- Cleavage inhibition
- Hatching reduction
- Larval toxicity
- Inhibition of sperm motility

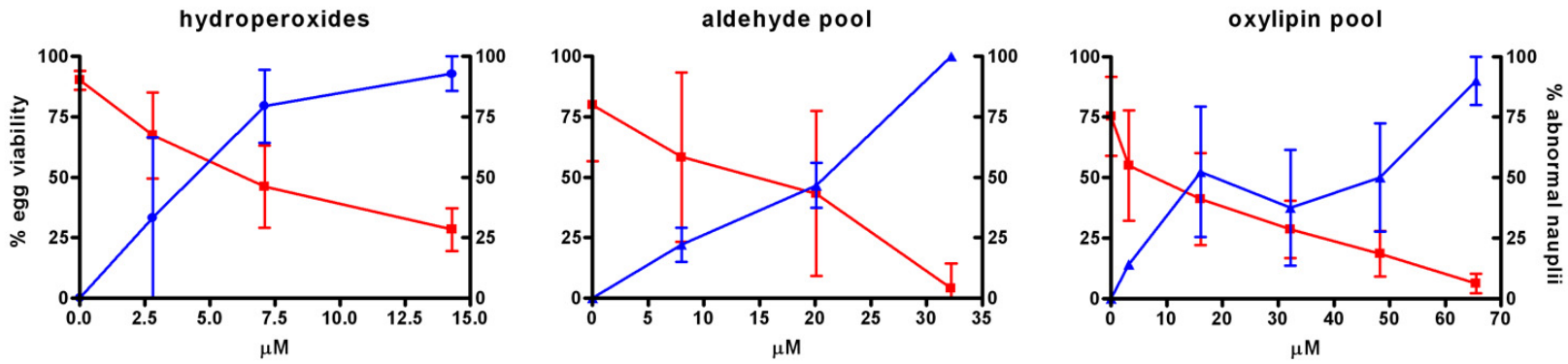
Polychaeta (Anellida)
- *Arenicola marina*
- *Nereis virens*

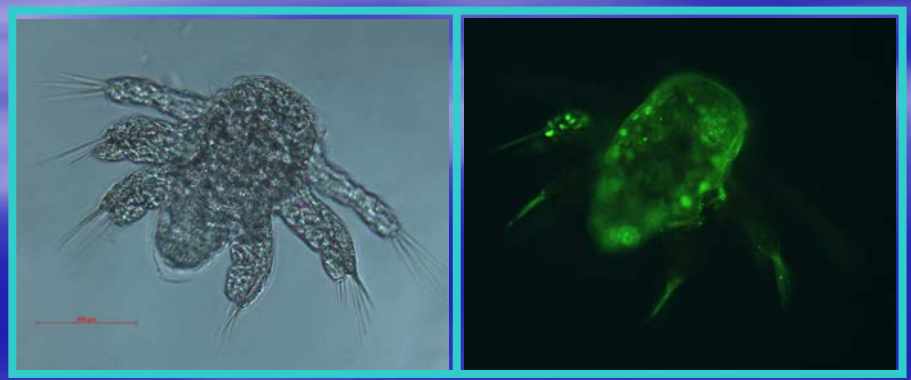
Echinodermata
- *Asterias rubens*
- *Psammechinus miliaris*
- *Sphaerechinus granularis*
- *Paracentrotus lividus*

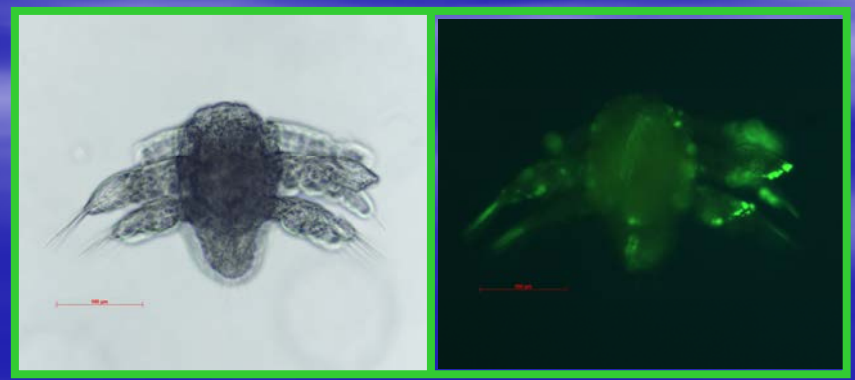
Crustacea
- *Tisbe holothuriae* *(copepod)*
- *Artemia salina*
- various species of Copepods

Caldwell 2009; Ianora and Miralto 2009

Effect of hydroperoxydes, aldehydes and oxylipins on egg viability and naupliar abnormalities



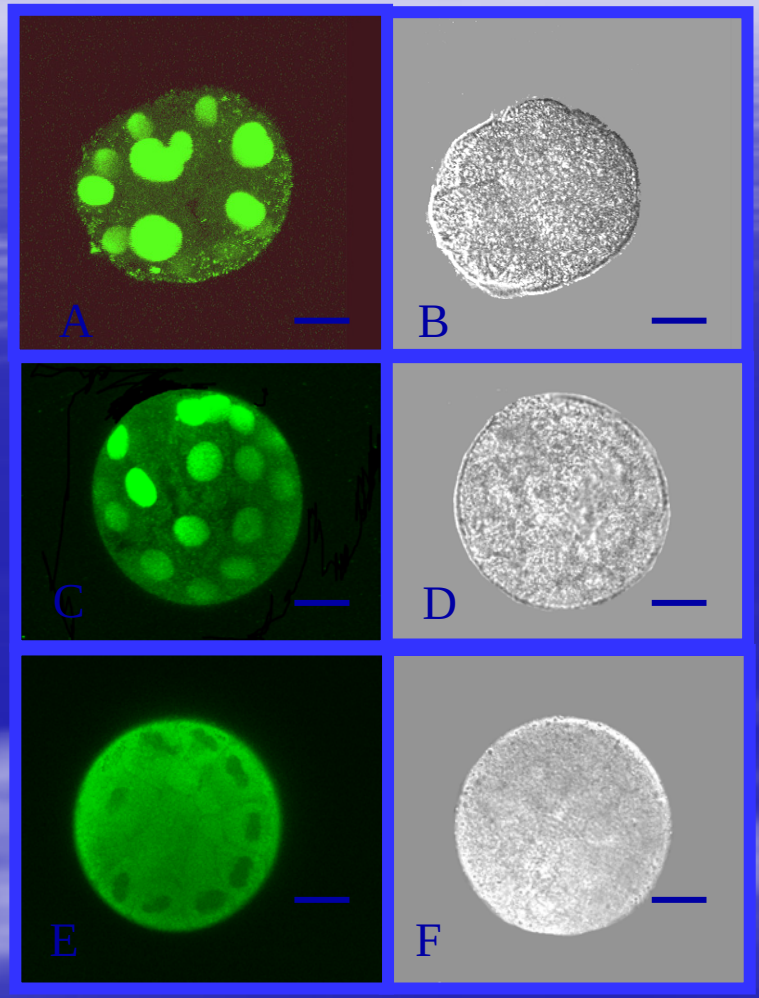




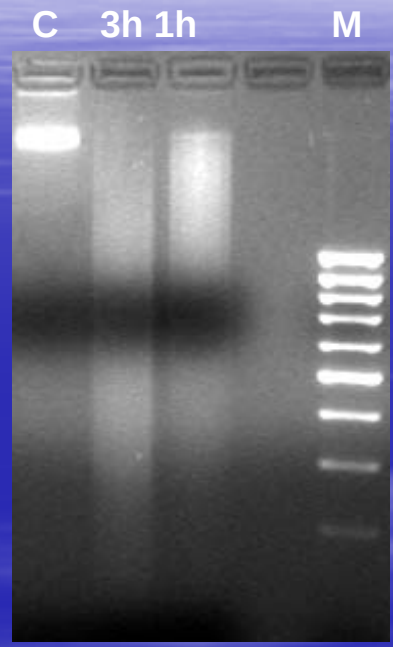
Fontana et al. 2007

Apoptosis Induction

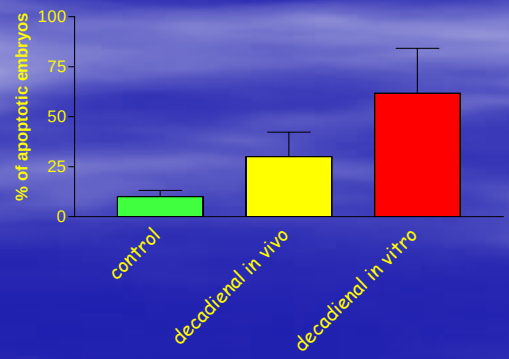
Copepods



Calanus helgolandicus embryos observed by confocal laser scanning microscopy in fluorescent (A, C, E) and in trasmitted (B, D, F) light. In (A) and (C) nuclei are positively stained (green) by TUNEL. Bar, 40µm. (E) Three-dimensional image of control embryo. Nuclei are not stained in green and appear as black shadows.

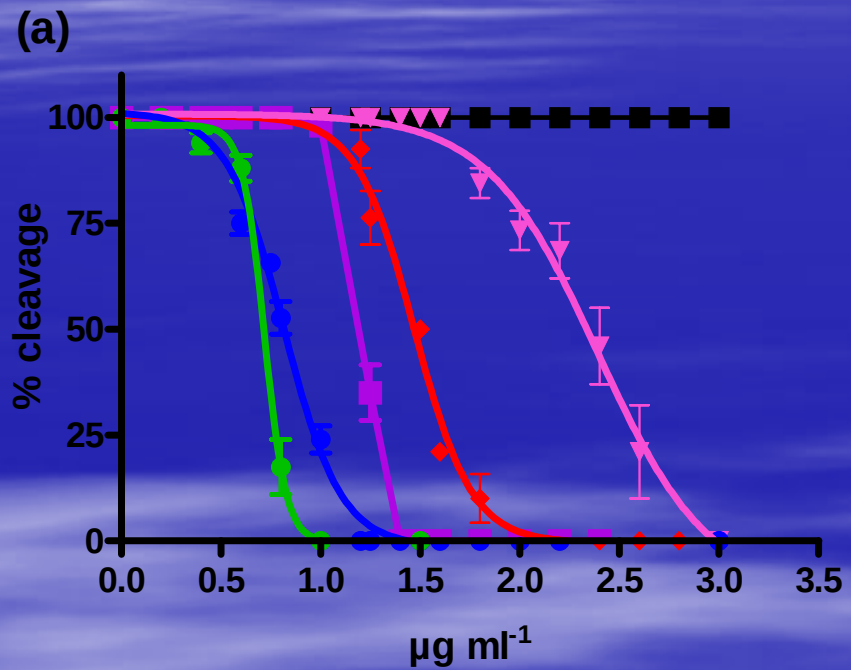


Agarose gel analysis of PCR amplified DNA isolated from copepod embryos.
(C: control; 3h and 1h: time of incubation in 5 µg/ml DD; M: markers)

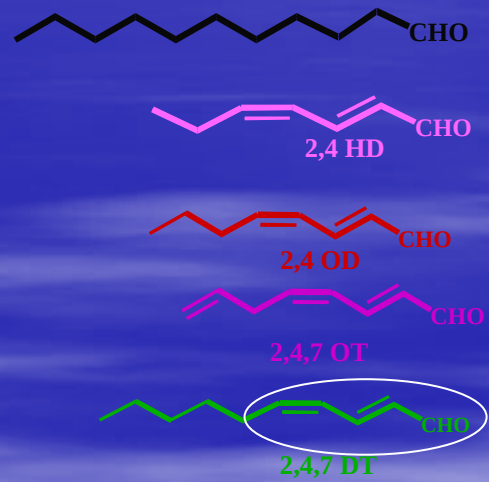


Romano et al, J Exp Biol 2003

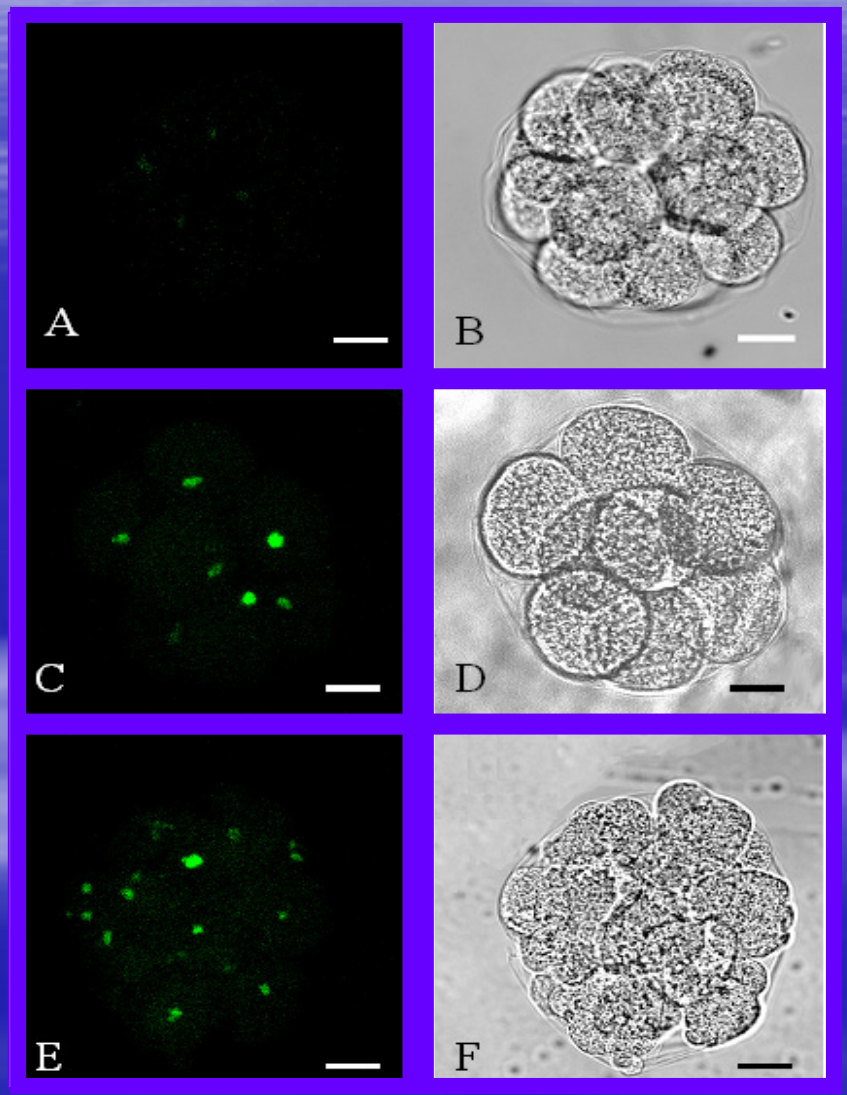
Effect of aldehydes on sea urchin embryos

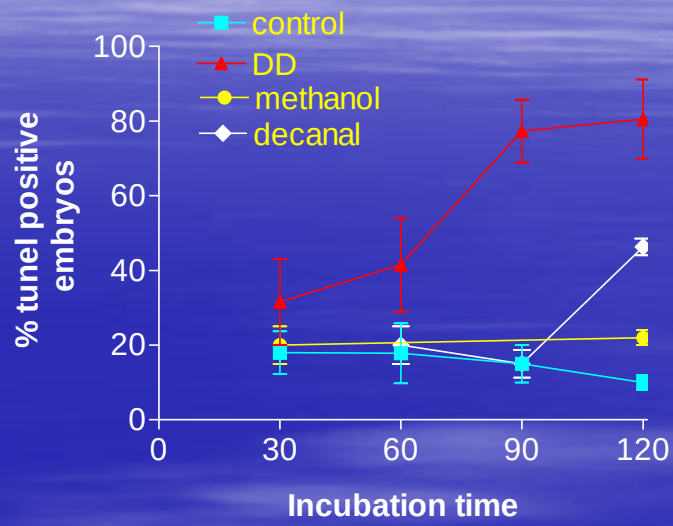


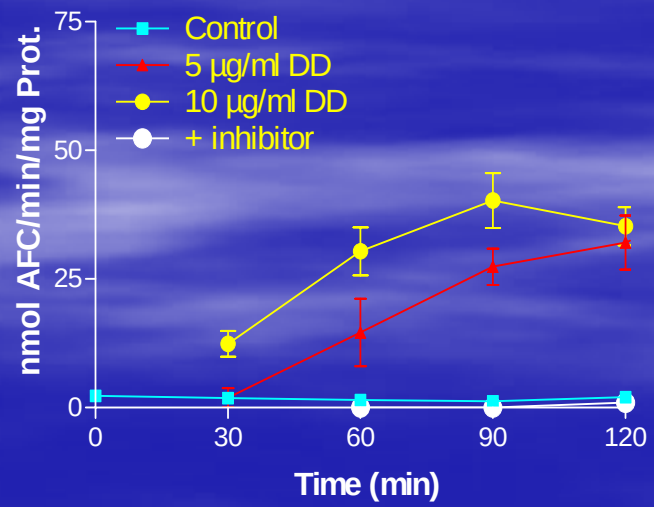
- tridecanal
- eptadienale
- octadienal
- mix HD+OD+DD
- octatrienal
- decadienal



Apoptosis Induction







Romano et al, J Exp Biol 2003

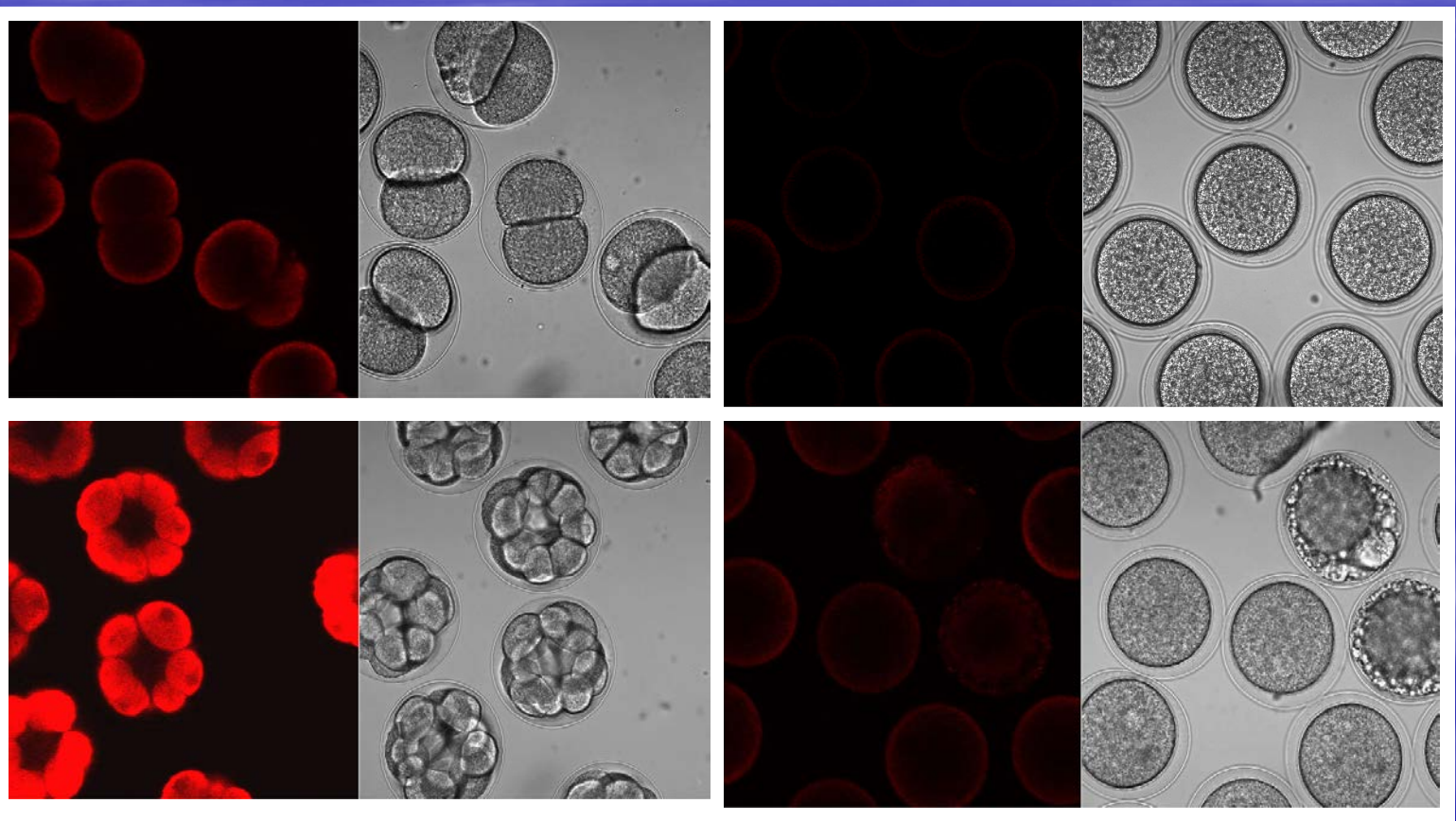
Apoptosis Induction

Mitochondrial integrity assessment

Control | Decadienal

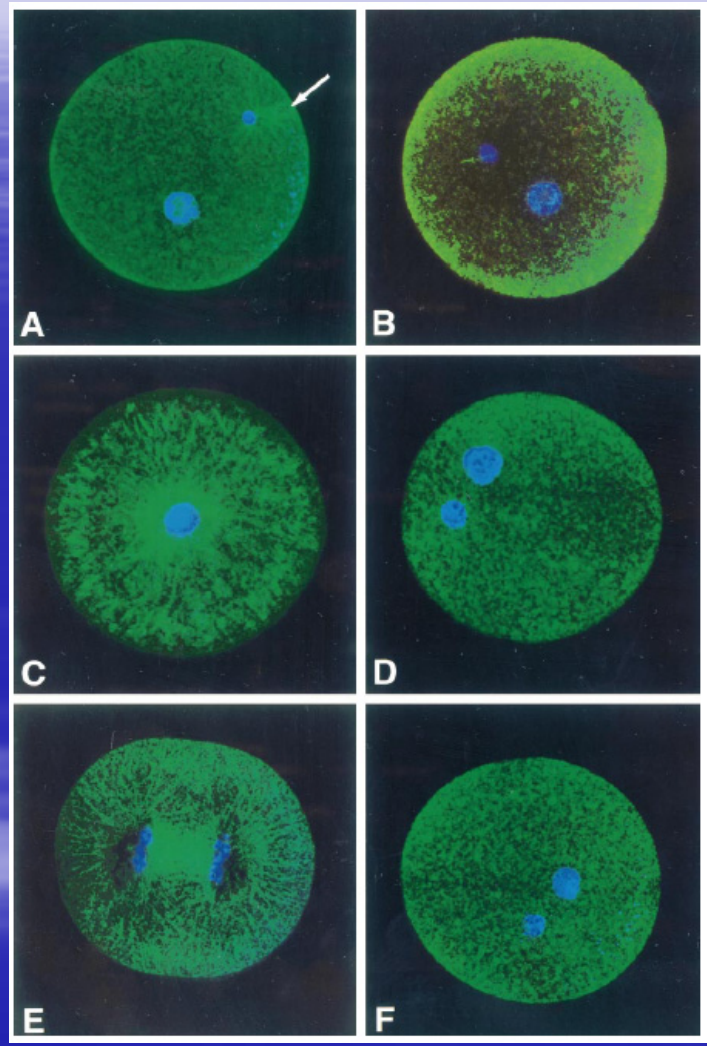
30 min

130 min



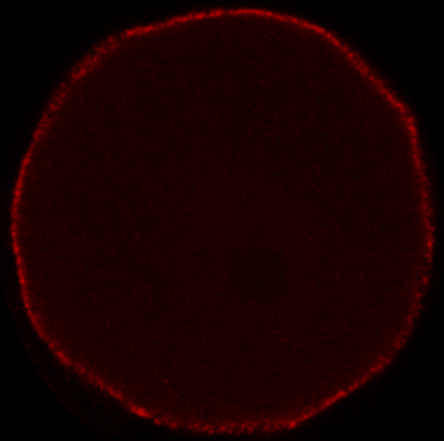
Romano, unpublished

Disruption of Tubulin polymerization in sea urchins

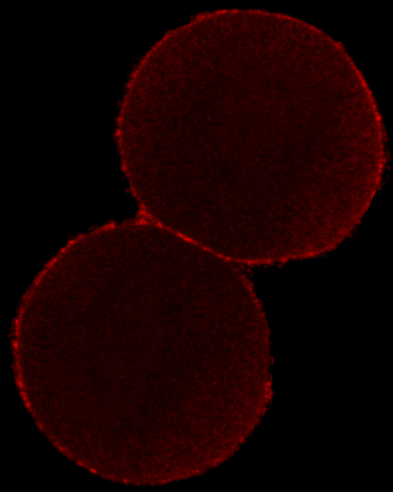


Buttino et al. 1999

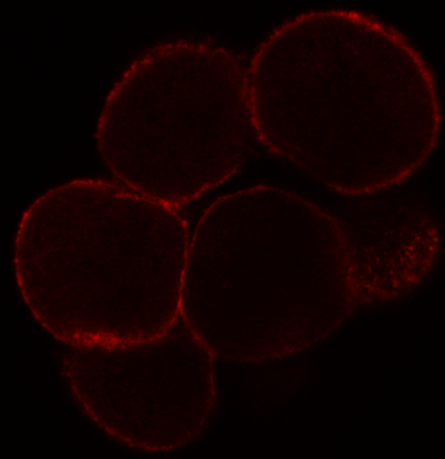
Interference on Actin microfilament reorganization



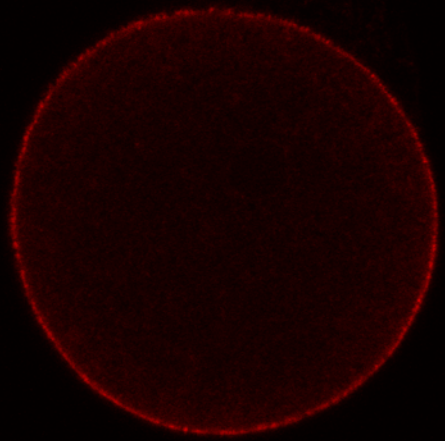
Control 10 min



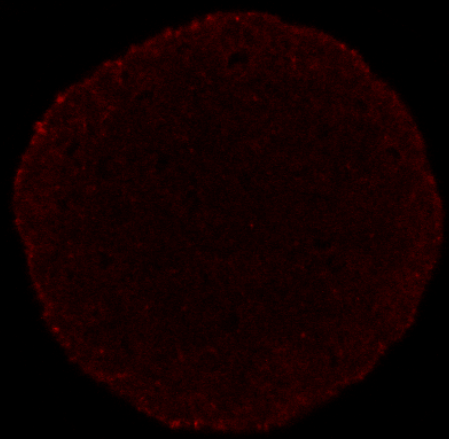
Control 90min



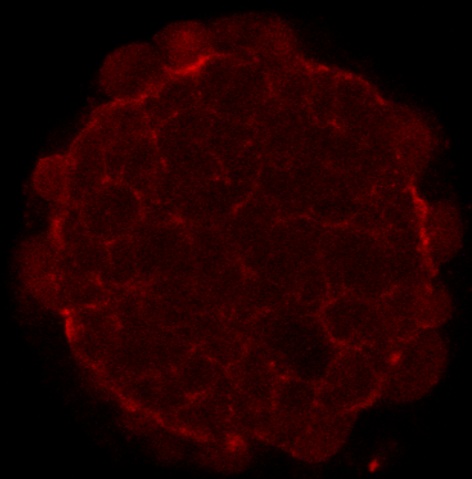
Control 120min



DD5ug 10min



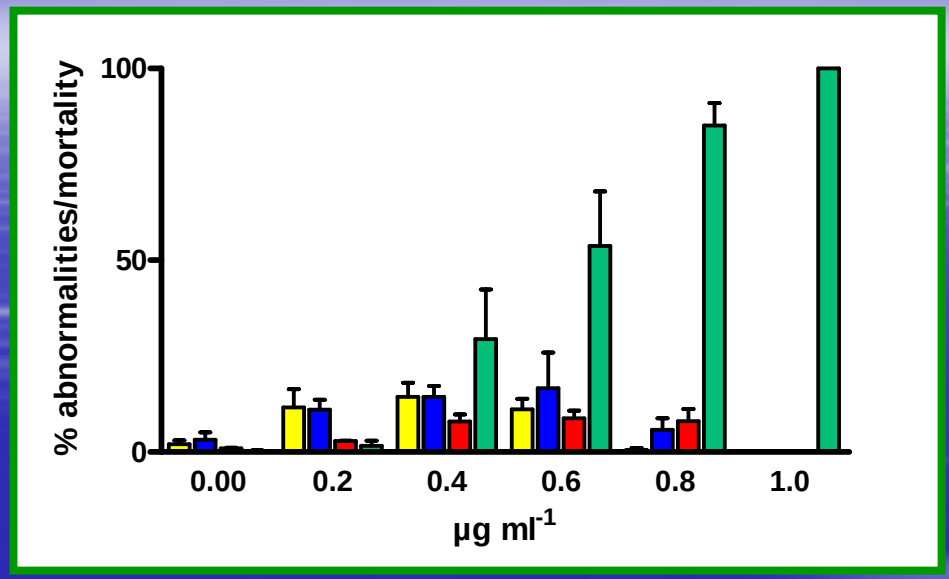
DD5ug 90min



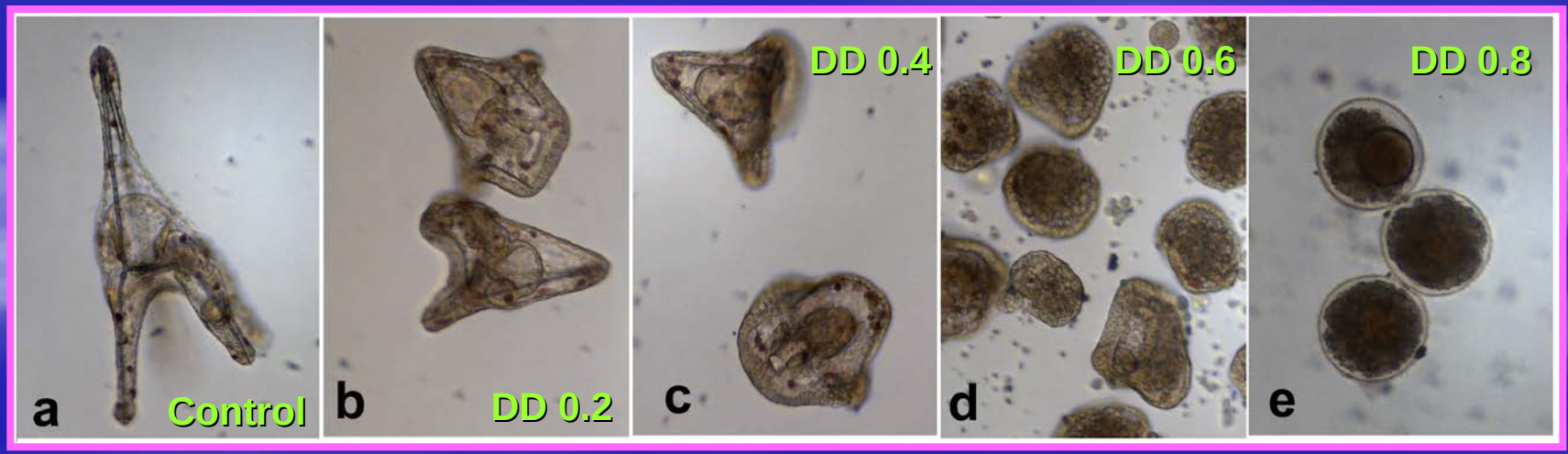
DD5ug 120min

Romano unpublished

Long term exposure to DD sub-lethal concentrations

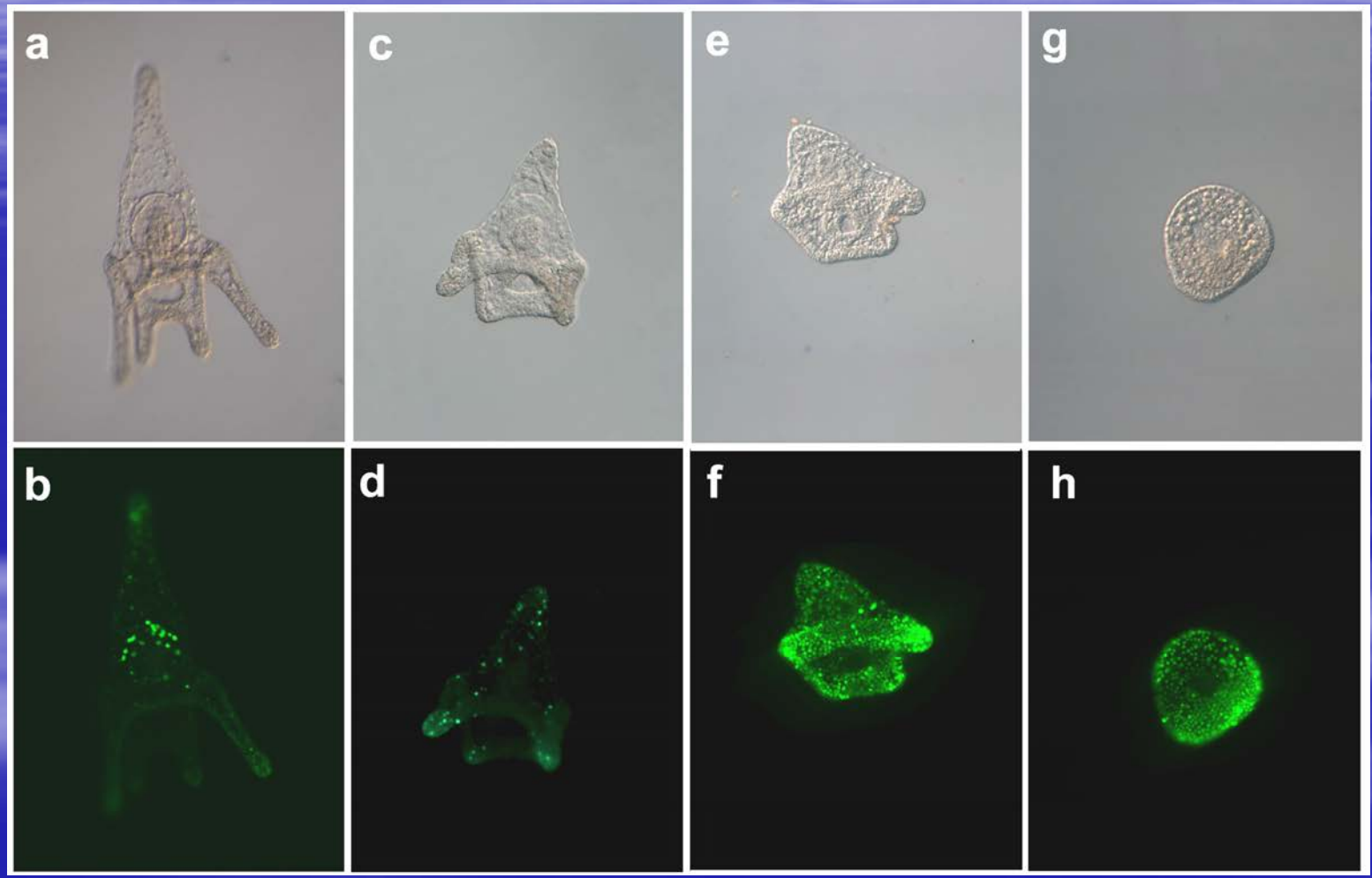


Impairment of larval fitness



Romano et al. 2010

Induction of apoptosis at larval stage



Romano et al. 2010

Cell Stress & Chaperones (2007) 12 (1), 44–50
© Cell Stress Society International 2007
Article no. csac. 2007.CSC-229R

Cadmium induces an apoptotic response in sea urchin embryos

Maria Agnello,[1] Simone Filosto,[1] Rosaria Scudiero,[2] Anna M. Rinaldi,[1] and Maria C. Roccheri[1]

[1]Dipartimento di Biologia Cellulare e dello Sviluppo "A. Monroy" Università degli Studi di Palermo, Viale delle Scienze ed.16, 90128 Palermo, Italy
[2]Dipartimento Delle Scienze Biologiche–Sezione di Biologia evolutiva e Comparata, Università Federico II di Napoli, Via Mezzocannone 8-80134 Napoli, Italy

Abstract Cadmium is a heavy metal toxic for living organisms even at low concentrations. It does not have any biological role, and since it is a permanent metal ion, it is accumulated by many organisms. In the present paper we have studied the apoptotic effects of continuous exposure to subacute/sublethal cadmium concentrations on a model system: *Paracentrotus lividus* embryos. We demonstrated, by atomic absorption spectrometry, that the intracellular amount of metal increased during exposure time. We found, using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay, that long treatments with cadmium triggered a severe DNA fragmentation. We demonstrated, by immunocytochemistry on whole-mount embryos, that treatment with cadmium causes activation of caspase-3 and cleavage of death substrates α-fodrin and lamin A. Incubating the embryos since fertilization with Z-DEVD FMK, a caspase-3 inhibitor, we found, by immunocytochemistry, that cleavage by caspase-3 and cleavage of death substrates were inactivated.

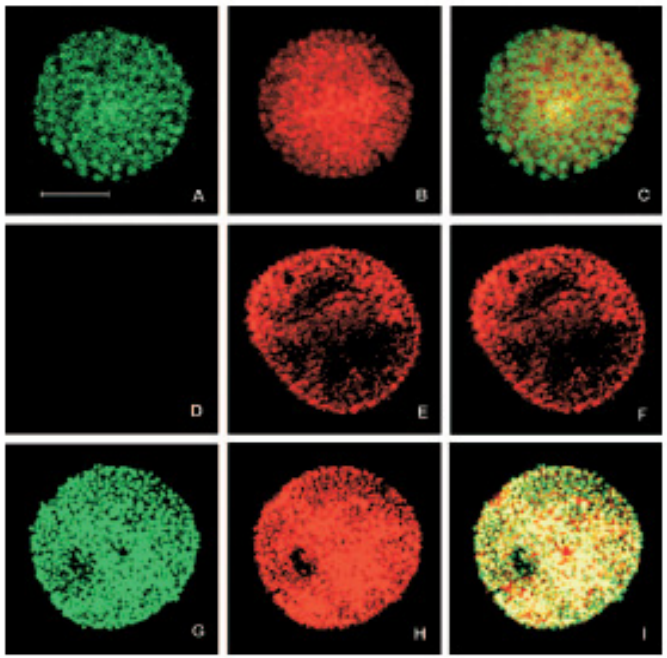


Fig 2. Evaluation of DNA fragmentation by TUNEL assay. The images show equatorial sections observed under confocal laser microscopy. In green DNA fragmentation (A, D, G); in red (propidium iodide) totality of nuclei (B, E, H); merging of green and red (C, F, I). Embryos treated for 24 hours with 1 mM $CdCl_2$ (A–C); control embryos (D–F); control embryos incubated for 10 minutes with DNA-ase I (G–I). Bar = 40 μM.

Apoptosis
DOI 10.1007/s10495-009-0420-0

UNUSUAL MODEL SYSTEMS FOR CELL DEATH RESEARCH

Apoptosis: focus on sea urchin development

Maria Agnello · Maria Carmela Roccheri

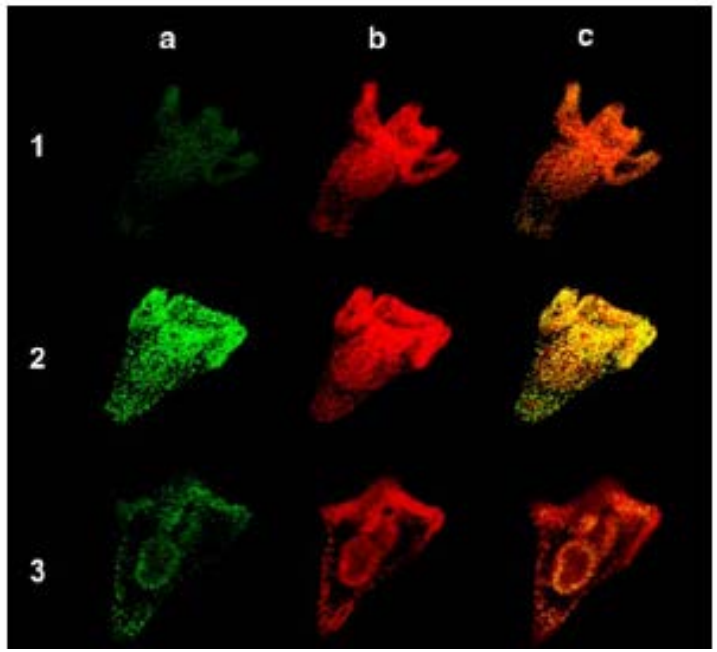
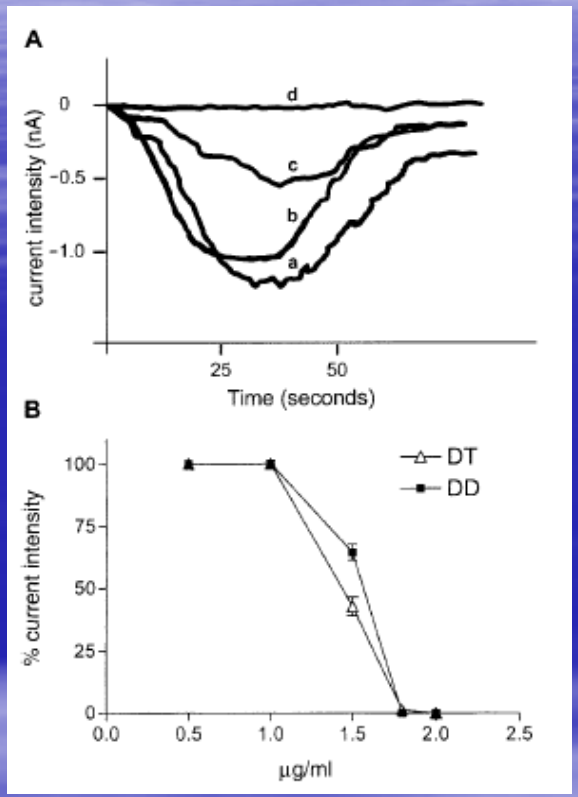
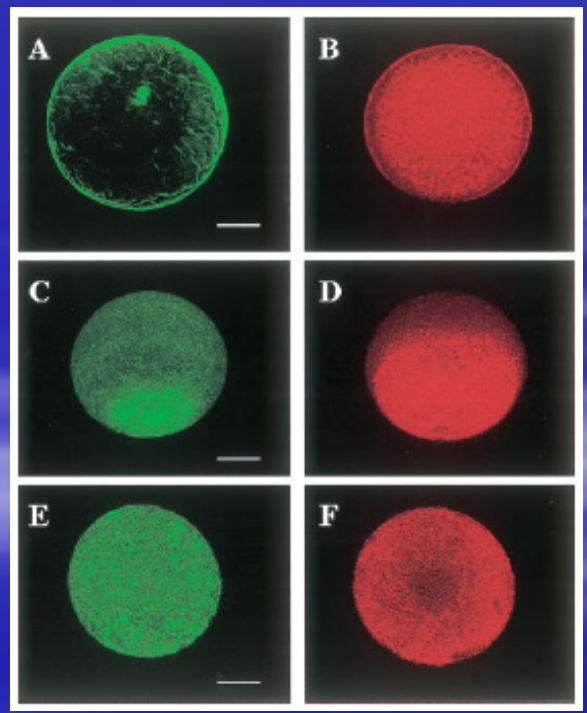
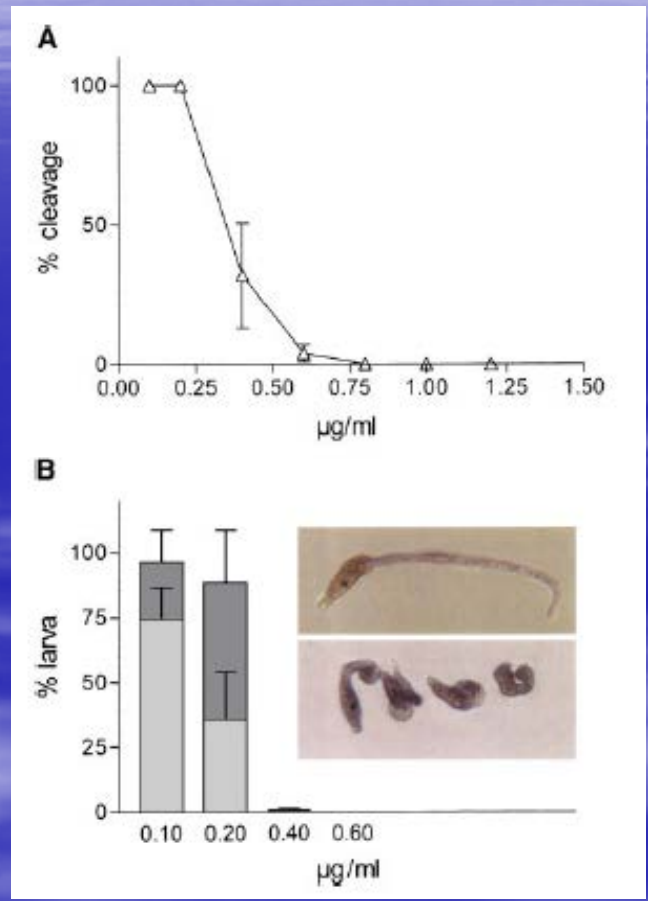


Fig. 1 TUNEL assay showing DNA fragmentation in P. lividus embryos, at pluteus stage. Equatorial optical sections of embryos observed under Confocal Laser Scanning Microscopy. DNA fragmentation (green channel) (a); totality of nuclei by propidium iodide (red channel) (b); merging of a and b (c). Control, 36 h of development (1); TPA ? 31C treated (2); CdCl2 1 mM treated (3

Effect of aldehydes on sea urchin embryos

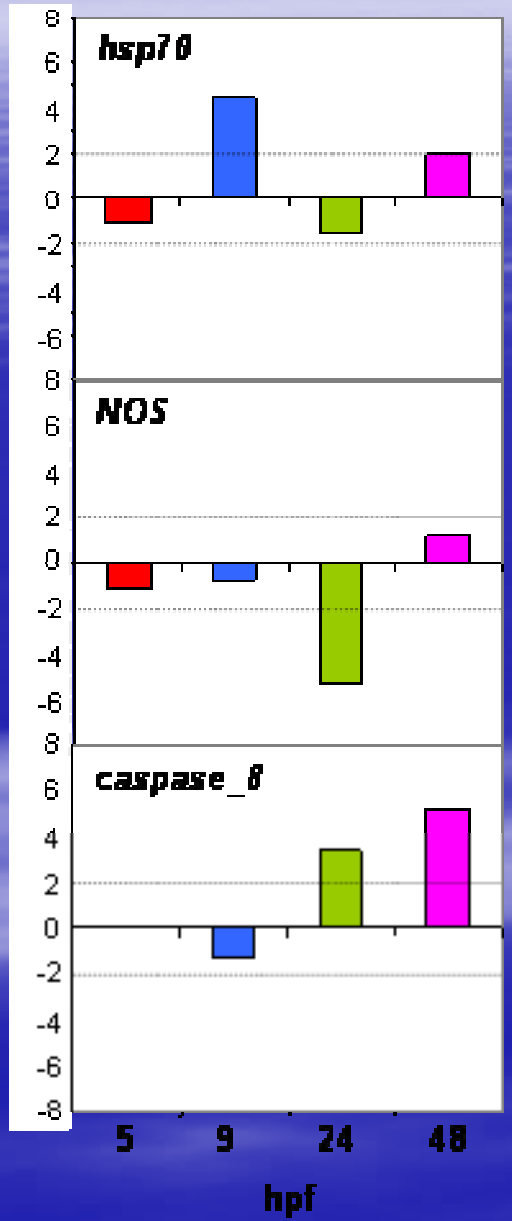


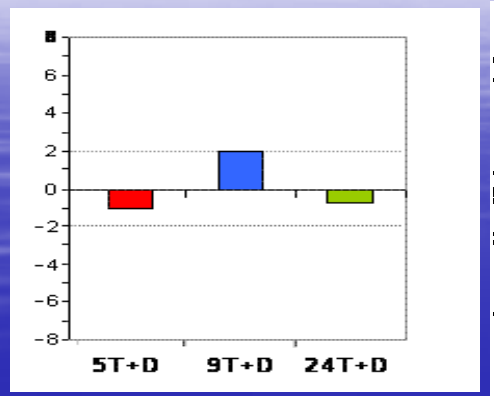


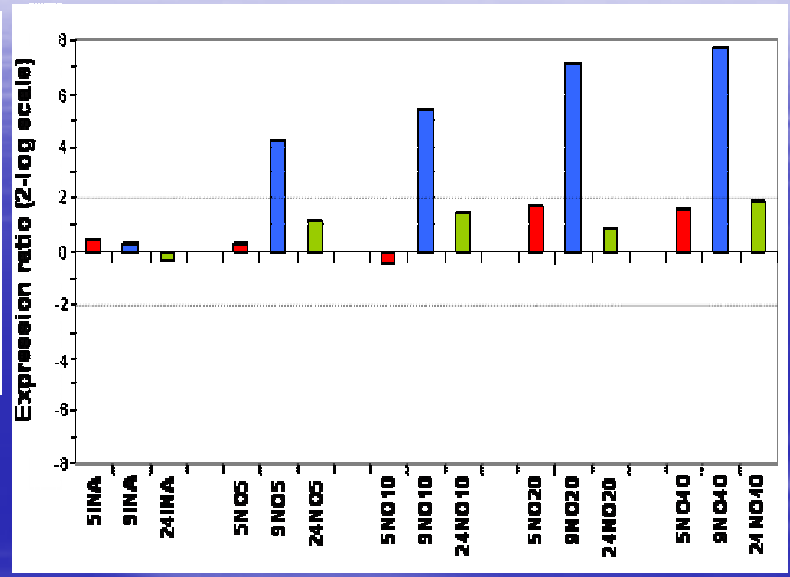


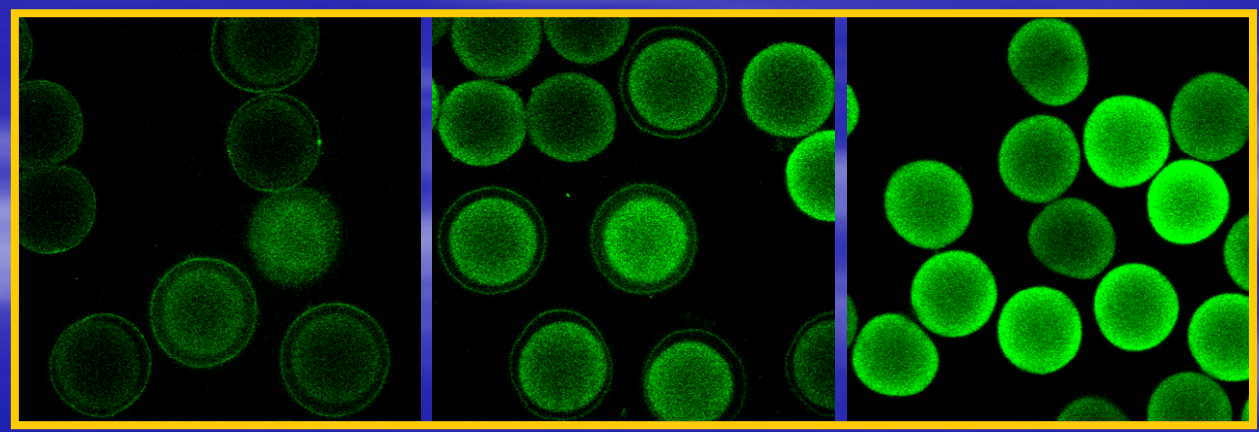
Tosti et al. 2003

Genes expression in response to sub-lethal aldehyde concentration









Nitric oxyde increase after treatment with 1, 2.5 and 5 µg/ml decadienal, revealed with the fluorescent specific dye DAF-FM

Romano et al, in preparation

Expression of stress-related genes in C. helgolandicus

Approaches:

- Induce stress (feeding) and quantify gene expression with Quantitative Real-Time PCR (qPCR)
- cDNA libraries of *C. helgolandicus* after diatom feeding "Suppression Subtractive Hybridization"

C. helgolandicus

Quantitative expression of stress-related genes:

Genes of interest	Function
Aldehyde dehydrogenases (ALDH)	Aldehyde detoxification
Cytochrome P450 (CYP)	Biotransformation
Glutathione S-transferase (GST)	Biotransformation
Heat-shock proteins (HSP)	General stress response
Superoxide dismutase (SOD)	Radical detoxification
Catalase (CAT)	Radical detoxification

Mechanism of action of diatom aldehydes

Effects: cleavage inhibition
teratogenesis
developmental delay

- **Induction of Apoptosis**
- **Disruption of Tubulin polymerization**
- **Interference on Actin microfilament reorganization**
- **Influence on stress-related gene expression**

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Giovanna Romano
Stazione Zoologica Anton Dohrn