

Molecular response to toxic diatom-derived aldehydes
in the sea urchin *Paracentrotus lividus*

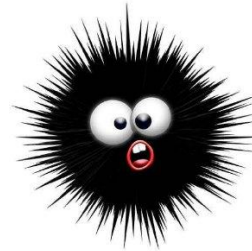


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Model organism: *Paracentrotus lividus*

- Relevant on structure of benthic marine community
- Mediterranean sea and Atlantic ocean
- Extraction and maintenance of gametes are easy
- Transparent embryos
- Embryos grow rapidly (pluteus stage at 48hpf)
- Long reproductive period



CLASSIFICATION

Domain: Eukaryota

Kingdom: Animalia

Phylum: Echinodermata

Class: Echinoidea

Order: Echinoida

Family: Echinidae

Species: *Paracentrotus lividus*

- Good model for ecotoxicological studies on response of marine invertebrate to environmental pollutants:

- ✓ physical and chemical xenobiotics
- ✓ low pH
- ✓ X-rays
- ✓ UVs

- ✓ antifoulings/pesticides
- ✓ heavy metals
- ✓ endocrine disrupters compounds
- ✓ oxylipins derived from diatoms

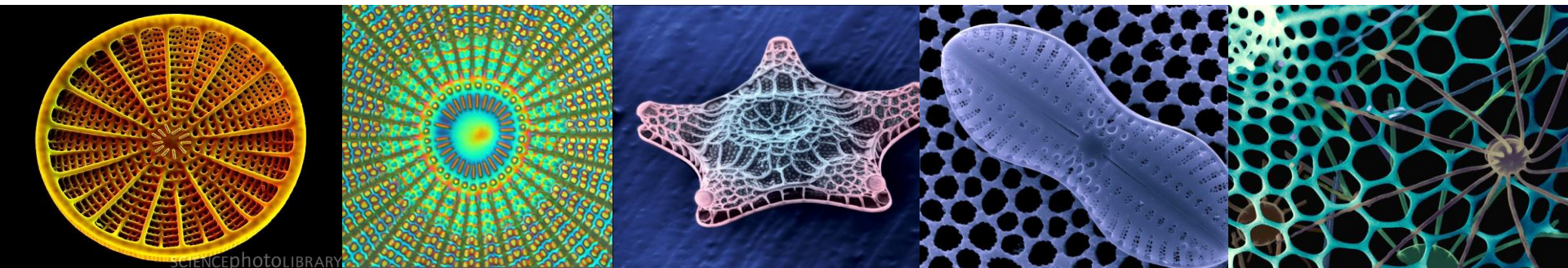


Diatoms

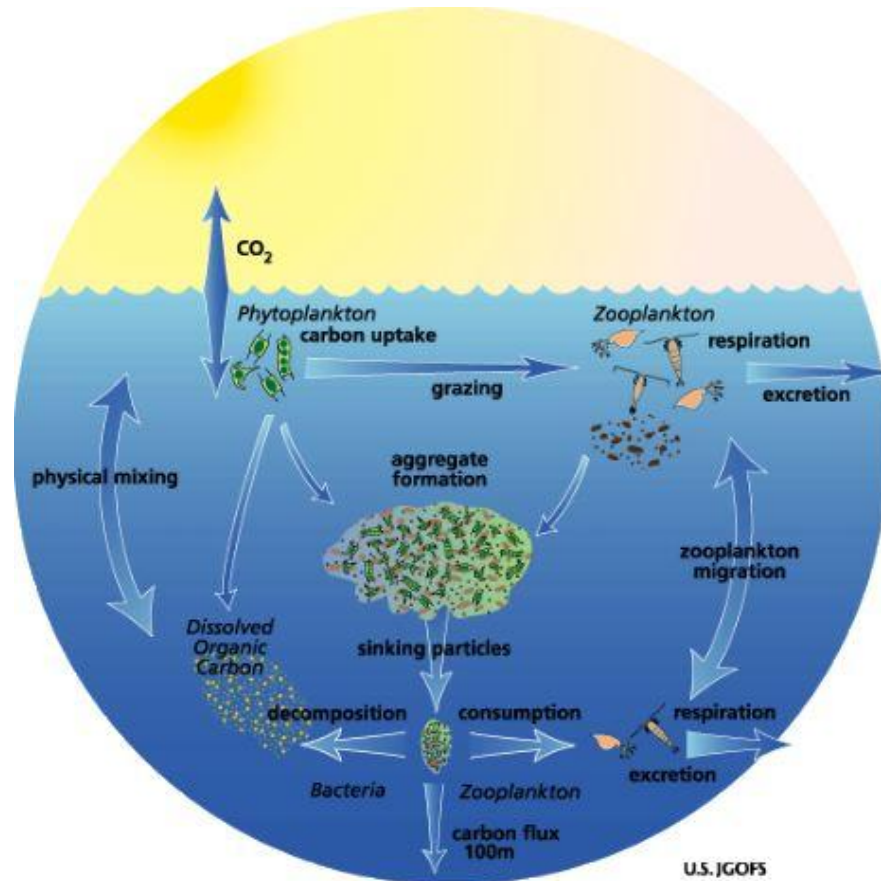
- There are more than 200 genera of living diatoms, and approximately 100,000 species.
- Diatoms live in the oceans and in freshwater.
- Most live in open water, although some live as surface films at the water-sediment interface (benthic), or even under damp atmospheric conditions.
- They are very important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



<http://deepbluehome.blogspot.it/search?q=diatom>



Marine food web



Beneficial role in supporting planktonic food web.
Diatoms are good food for the primary consumers of plankton.

Diatoms species produce secondary metabolites with cytotoxic activity

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[¶]

(Miralto et al. 1999)

Echinoderms



Crustaceans



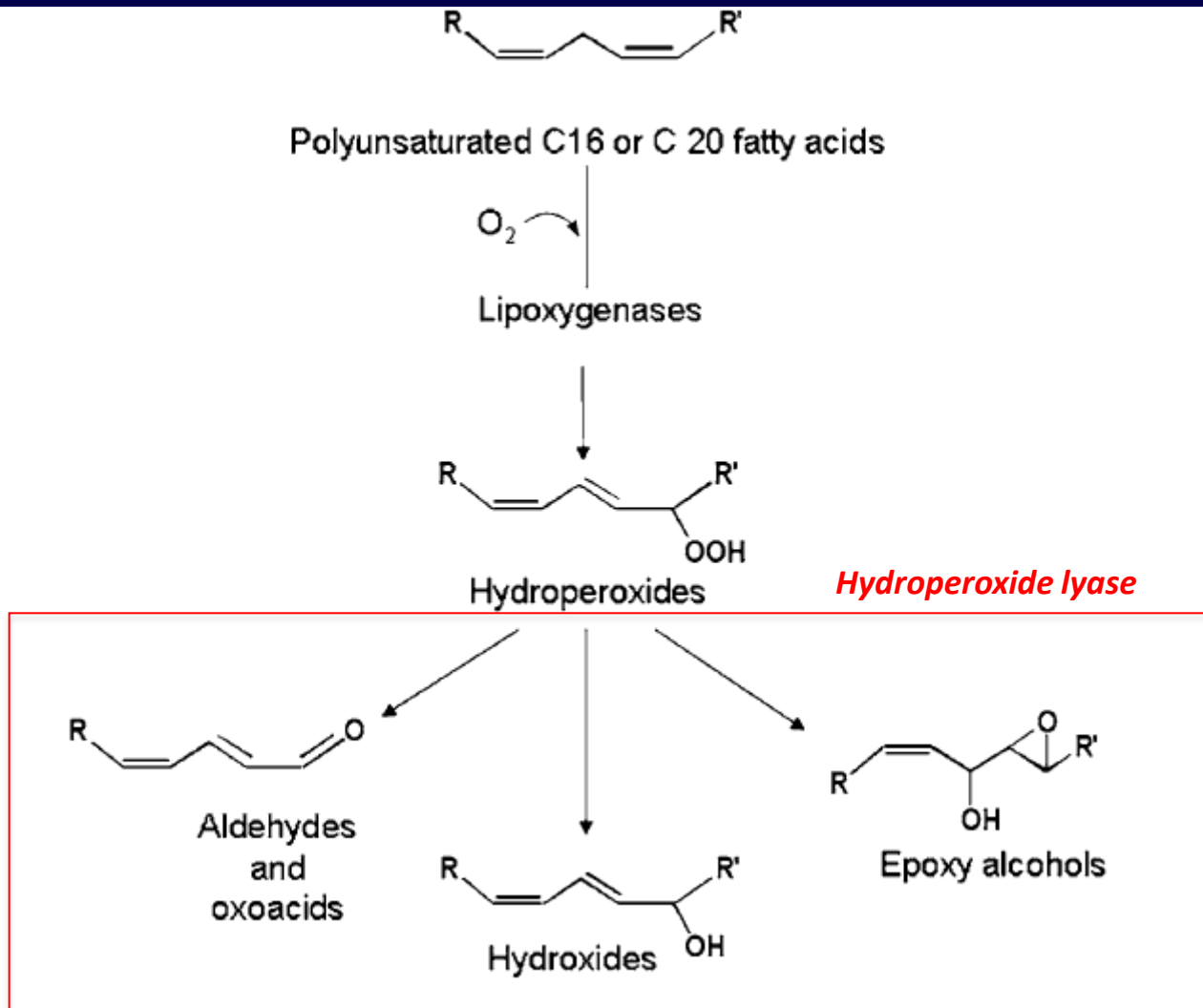
Polychaetes



(Ianora and Miralto 2010)

- Oocyte viability
- Sperm motility inhibition
- Fertilization success
- Cleavage inhibition
- Hatching reduction
- Larval toxicity

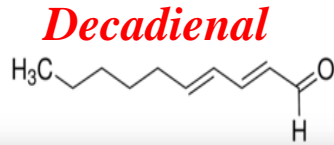
Oxidative metabolism of fatty acids in diatoms



OXYLIPINS

(Ianora and Miralto 2010)

The effects of decadienal on sea urchin embryos



The Journal of Experimental Biology 206, 3487-3494
 © 2003 The Company of Biologists Ltd
 doi:10.1242/jeb.00580

3487

A marine diatom-derived aldehyde induces apoptosis in copepod and sea urchin embryos

Giovanna Romano^{1,*}, Gian Luigi Russo², Isabella Buttino¹, Adrianna Ianora¹ and Antonio Miralto¹

¹Stazione Zoologica 'Anton Dohrn' Villa Comunale 1 – 80121 Napoli, Italy and ²Istituto Scienze dell'Alimentazione, Consiglio Nazionale delle Ricerche – 83100 Avellino, Italy

(Romano et al., 2003)

Mar. Drugs 2010, 8, 950-967; doi:10.3390/md8040950

OPEN ACCESS
Marine Drugs
 ISSN 1660-3397
 www.mdpi.com/journal/marinedrugs

Article

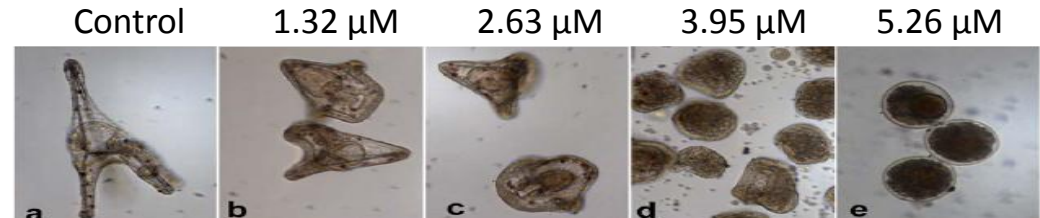
Teratogenic Effects of Diatom Metabolites on Sea Urchin *Paracentrotus lividus* Embryos

Giovanna Romano *, Antonio Miralto and Adrianna Ianora

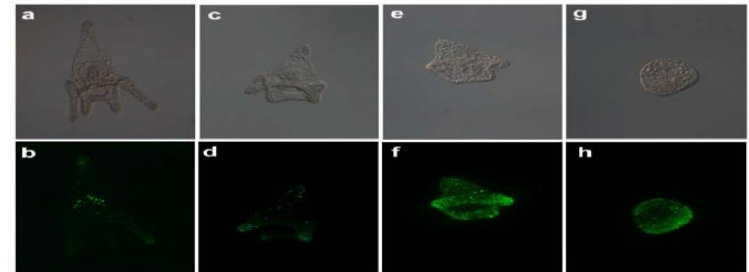
Stazione Zoologica Anton Dohrn, Villa Comunale 80121 Napoli, Italy;
 E-Mails: antonio.miralto@szn.it (A.M.); adrianna.ianora@szn.it (A.I.)

(Romano et al., 2010)

Decadienal induces teratogenesis and apoptosis



Control 1.32 μM 2.63 μM 3.95 μM



Increasing concentrations of decadienal →

First molecular studies

OPEN ACCESS Freely available online

PLoS one

Nitric Oxide Mediates the Stress Response Induced by Diatom Aldehydes in the Sea Urchin *Paracentrotus lividus*

Giovanna Romano¹, Maria Costantini², Isabella Buttino^{1*}, Adrianna Ianora¹, Anna Palumbo^{2*}

¹Laboratory of Functional and Evolutionary Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy, ²Laboratory of Cellular and Developmental Biology, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy

(Romano et al., 2011)

OPEN ACCESS Freely available online

PLoS one

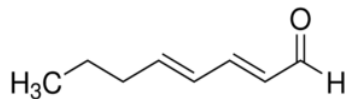
Defensome against Toxic Diatom Aldehydes in the Sea Urchin *Paracentrotus lividus*

Vincenzo Marrone¹, Marina Piscopo^{2*}, Giovanna Romano^{3*}, Adrianna Ianora³, Anna Palumbo¹, Maria Costantini^{1*}

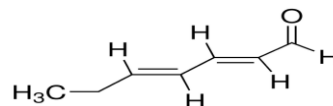
(Marrone et al., 2012)

..... The other PUAs?

Octadienal



Heptadienal



- Abundant compounds among PUAs
- No molecular evidences

Stress responses of sea urchin after exposure to heptadienal and octadienal



Morphologic

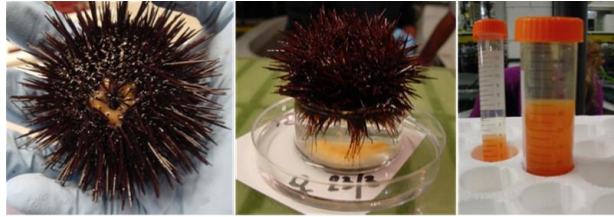
- ✓ Do PUAs affect the embryogenesis?
- ✓ When do PUAs act on embryo development?



Molecular

- ✓ Do PUAs have molecular targets?
- ✓ Could these targets be considered as possible biomarkers for environmental stress response?

Experimental procedures

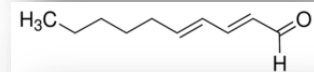


Sea urchin
eggs

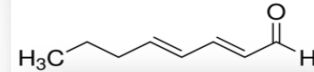
Incubation for
10 min with
aldehydes

Fertilization

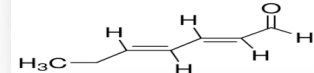
Morphological
observations
at 48 hpf



Decadienal



Octadienal



Heptadienal



<http://www.alnmag.com/news/2012/02/sperm-pathways-sea-urchins>



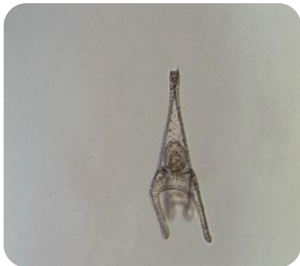
*Control embryos
at 48 hpf*

Do PUAs affect the embryogenesis?

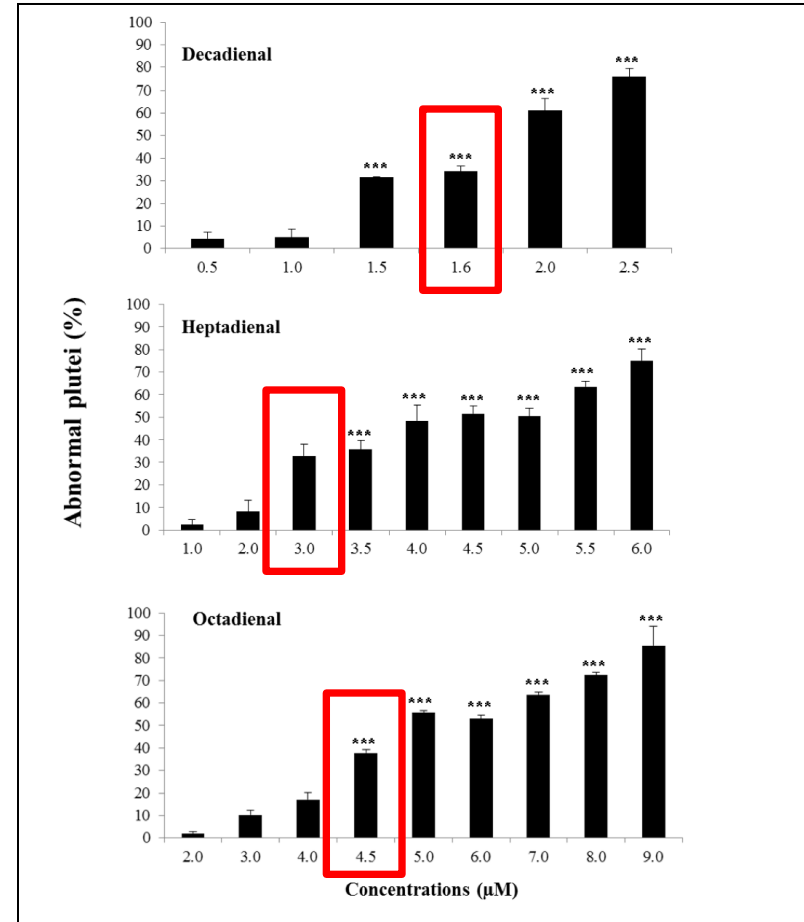
PUAs treatments induced **TERATOGENESIS**



Control (embryos in sea water without aldehydes)



Dose-dependent increase of abnormal plutei



(Varrella et al., 2014)

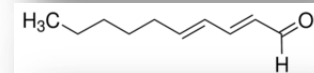
Experimental procedures

Sea urchin eggs

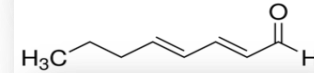
Incubation for
10 min with
aldehydes

Fertilization

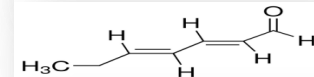
Morphological
observations at
72, 96 hpf
until one-week



Decadienal



Octadienal



Heptadienal



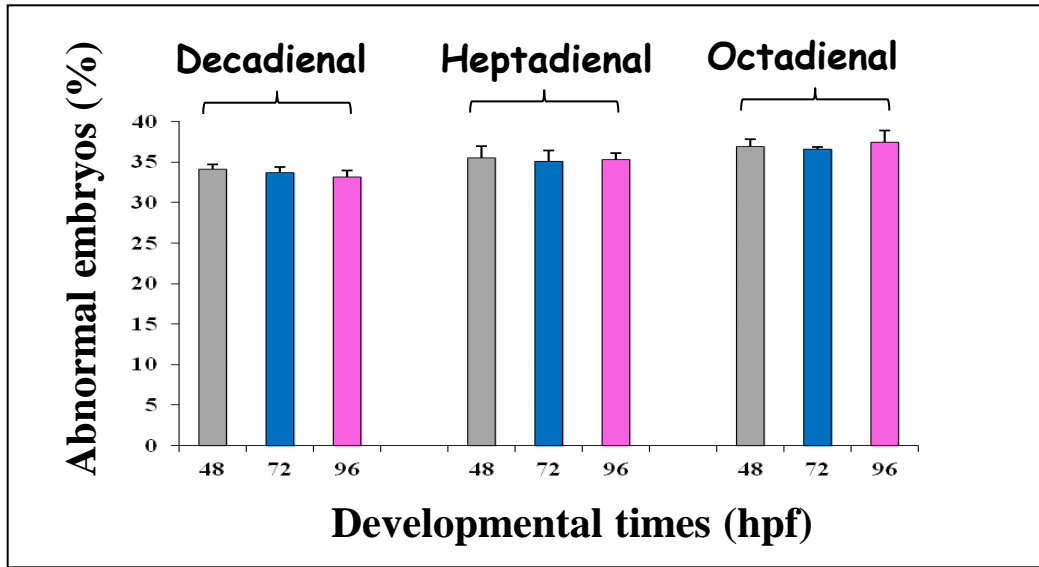
<http://www.alnmag.com/news/2012/02/sperm-pathways-sea-urchins>



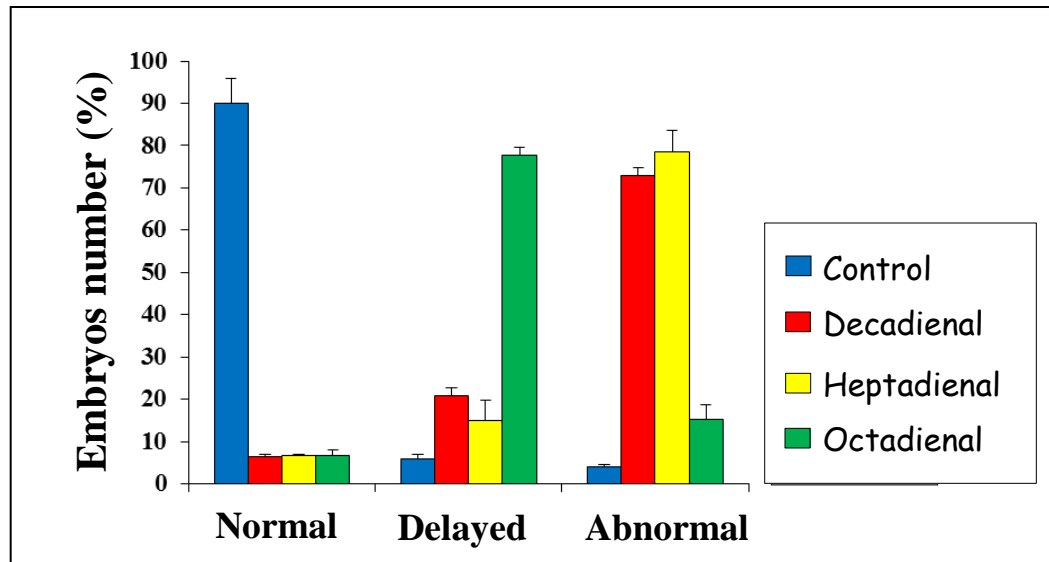
*Control embryos
at one week hpf*

Do PUAs affect the embryogenesis?

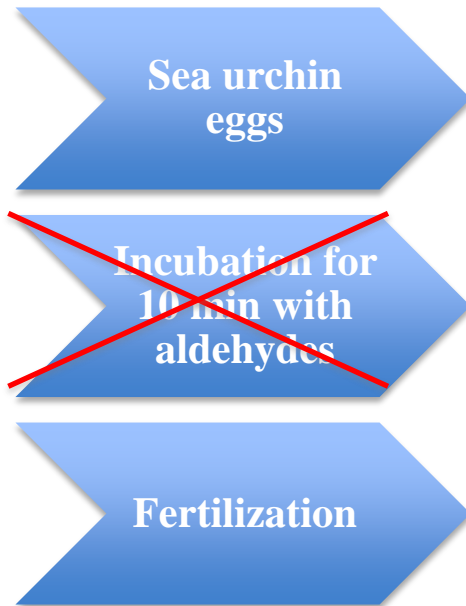
72, 96 hpf



One week



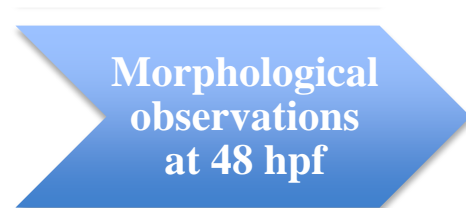
Experimental procedures



<http://www.alnmag.com/news/2012/02/sperm-pathways-sea-urchins>



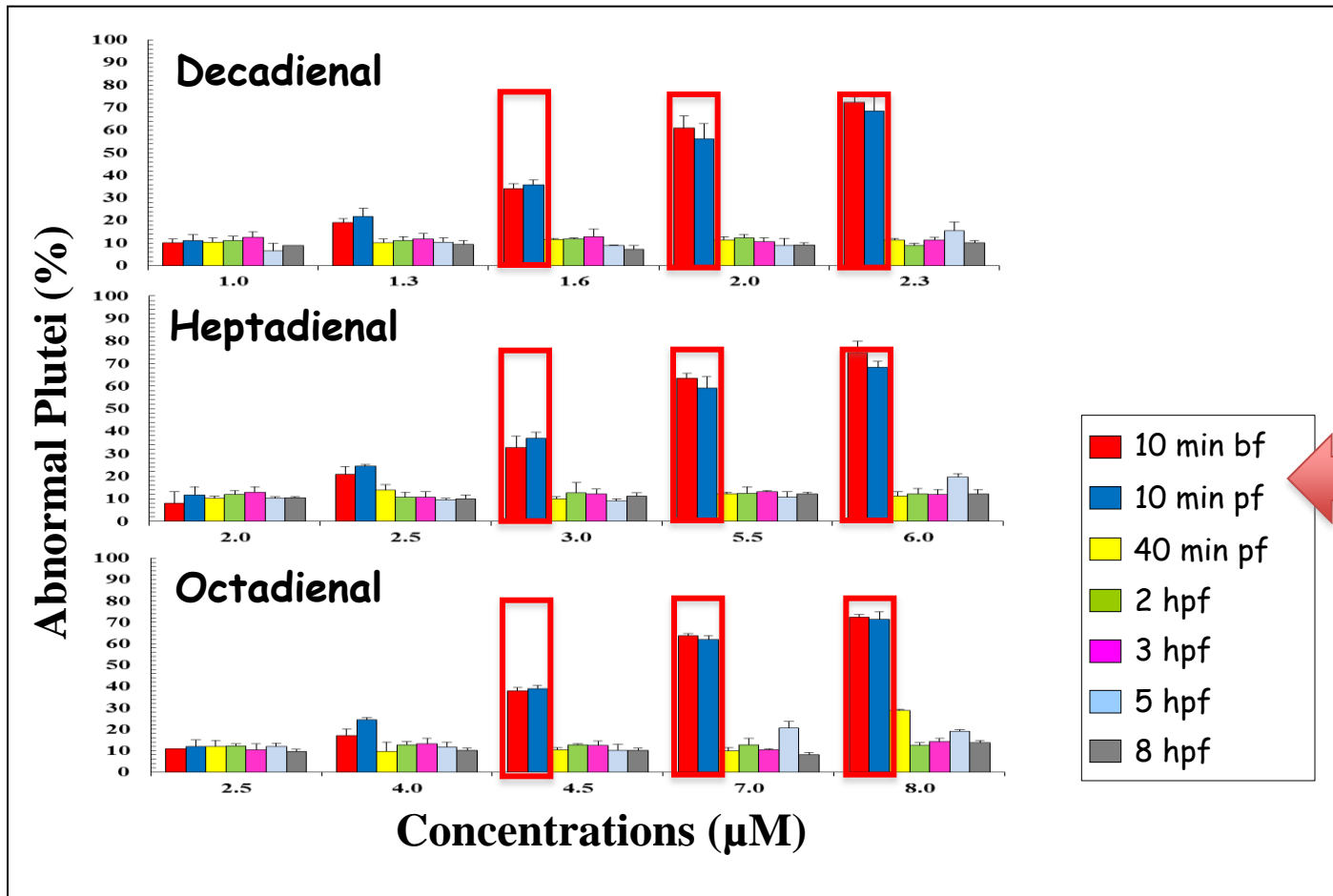
PUAs additions



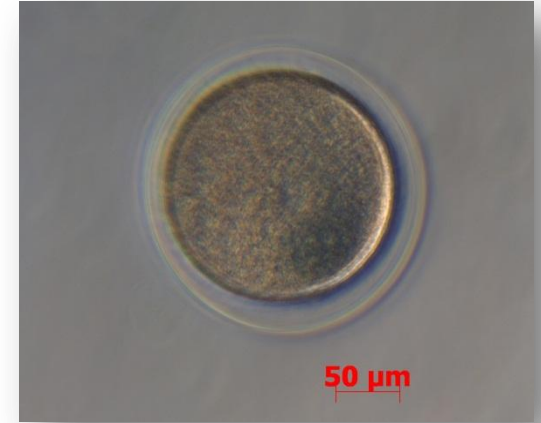
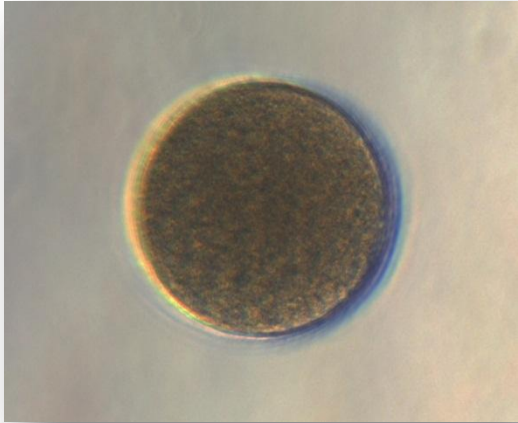
Control embryos at 48 hpf

When do PUAs act on embryo development?

Aldehydes additions at different developmental times



PUAs action time



10 minutes before

fertilization

10 minutes after

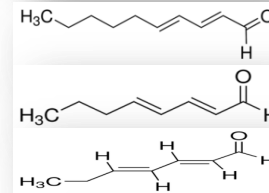
PUAs

Experimental procedures

Sea urchin eggs



Incubation for 10 min
with aldehydes



Decadienal

Octadienal

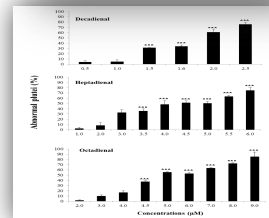
Heptadienal

Fertilization



<http://www.alnmag.com/news/2012/02/sperm-pathways-sea-urchins>

Determination of **teratogenic**
and **dose-dependent**
concentrations of aldehydes



Analysis of 31 by Real
Time q-PCR



Do PUAs have molecular targets?

Genes analyzed by Real-Time qPCR

Stress

Hsp70
Hsp60
Hsp56
MT
MTase
GS
cytb
p38 MAPK
14-3-3ε

Skeletogenesis

SM30
BMP5-7
SM50
Nec
uni
p16
p19

Development and differentiation

hat
sox9
BP10
Blimp
Alix
Wnt5
Wnt6
Wnt8

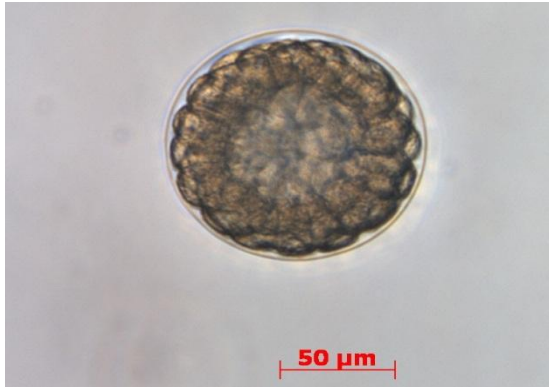
Detoxification

MT
MT4
MT5
MT6
MT7
MT8
MDR1
CAT

(Marrone et al., 2012; Varrella et al., 2014)

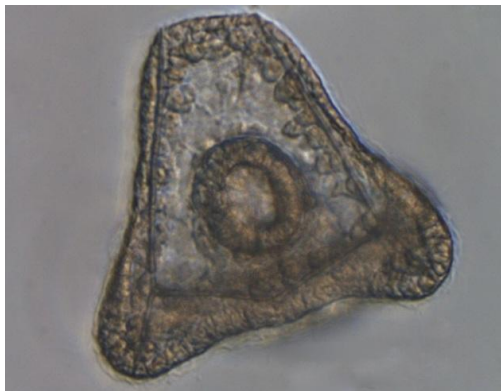
The key stages of *P. lividus* embryogenesis

Early blastula



5 hpf

Prism



24 hpf

Swimming blastula



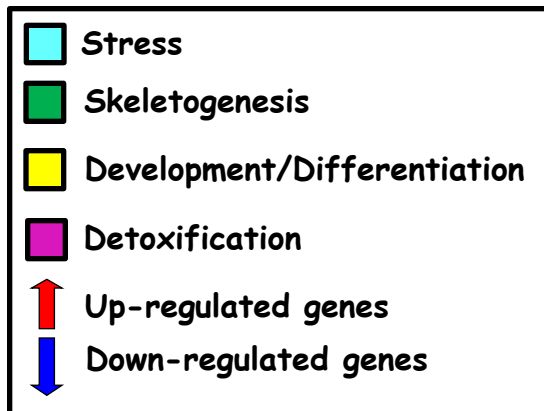
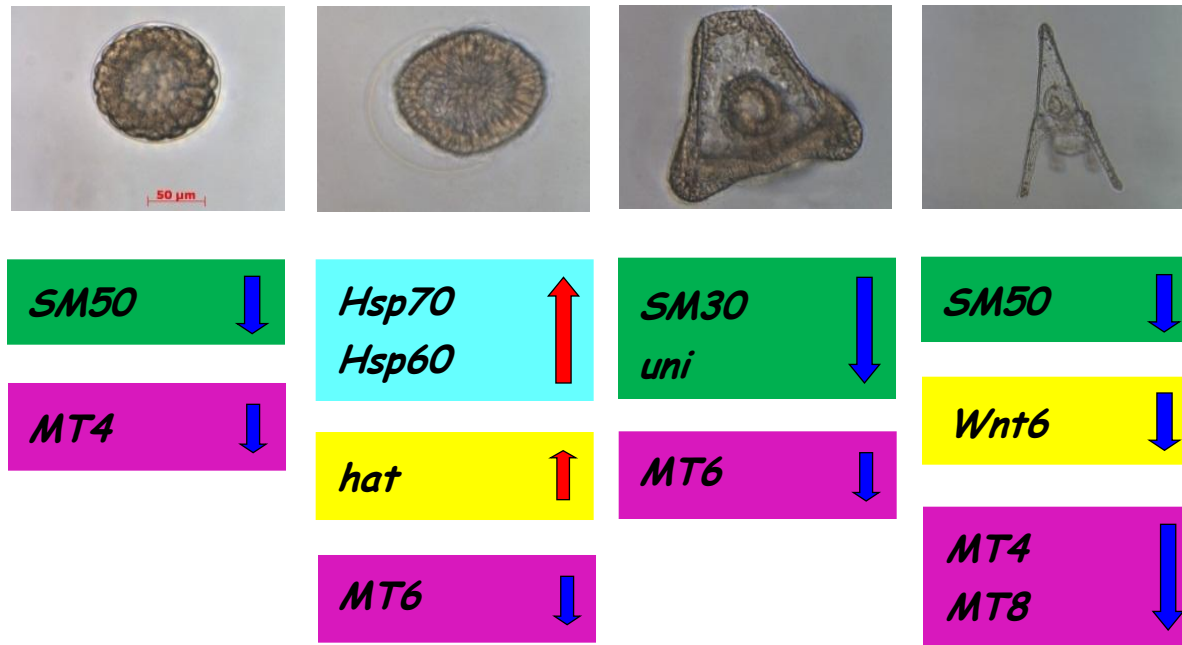
9 hpf

Pluteus



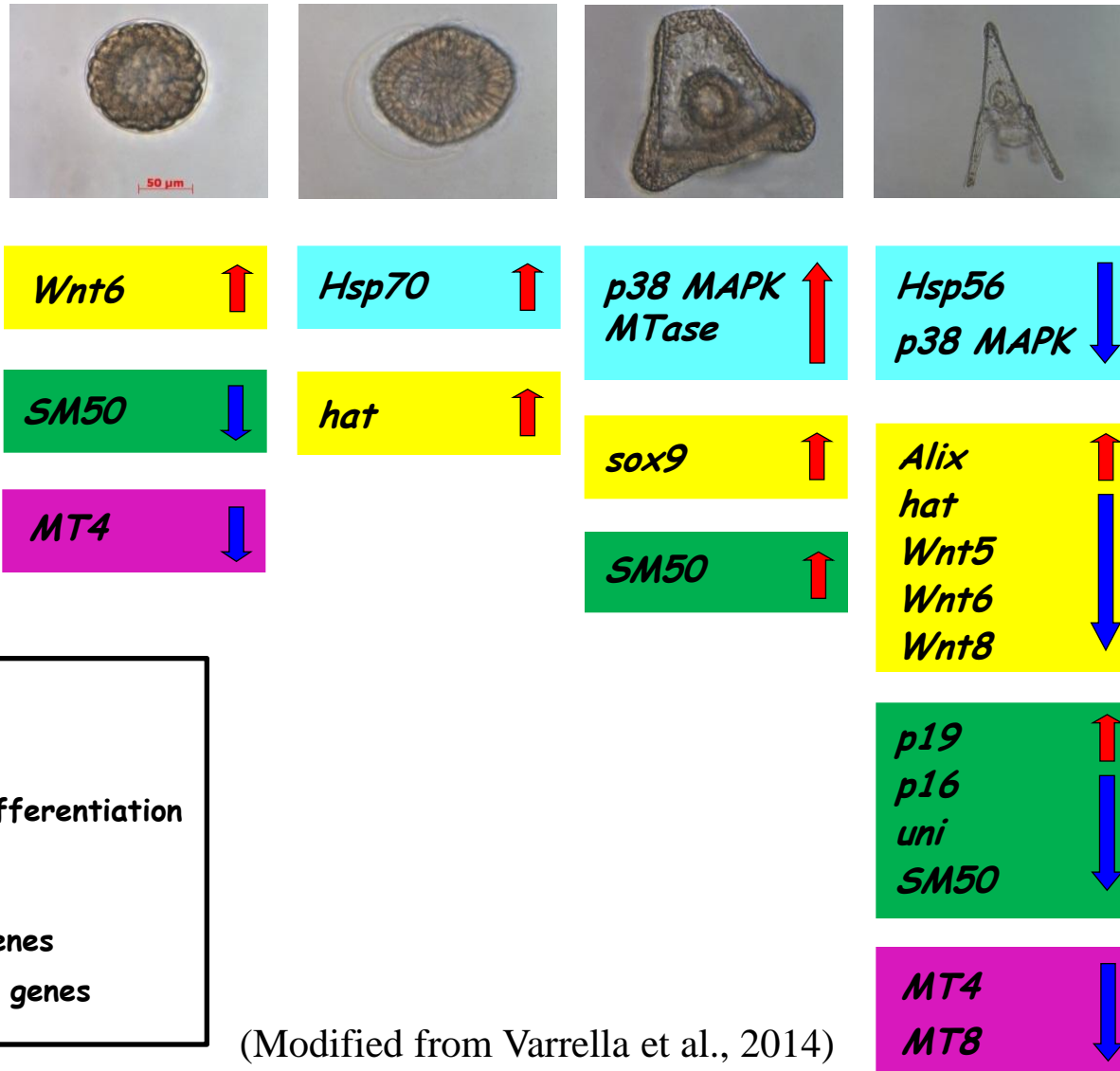
48 hpf

Effects of decadienal on gene expressions at different developmental stages



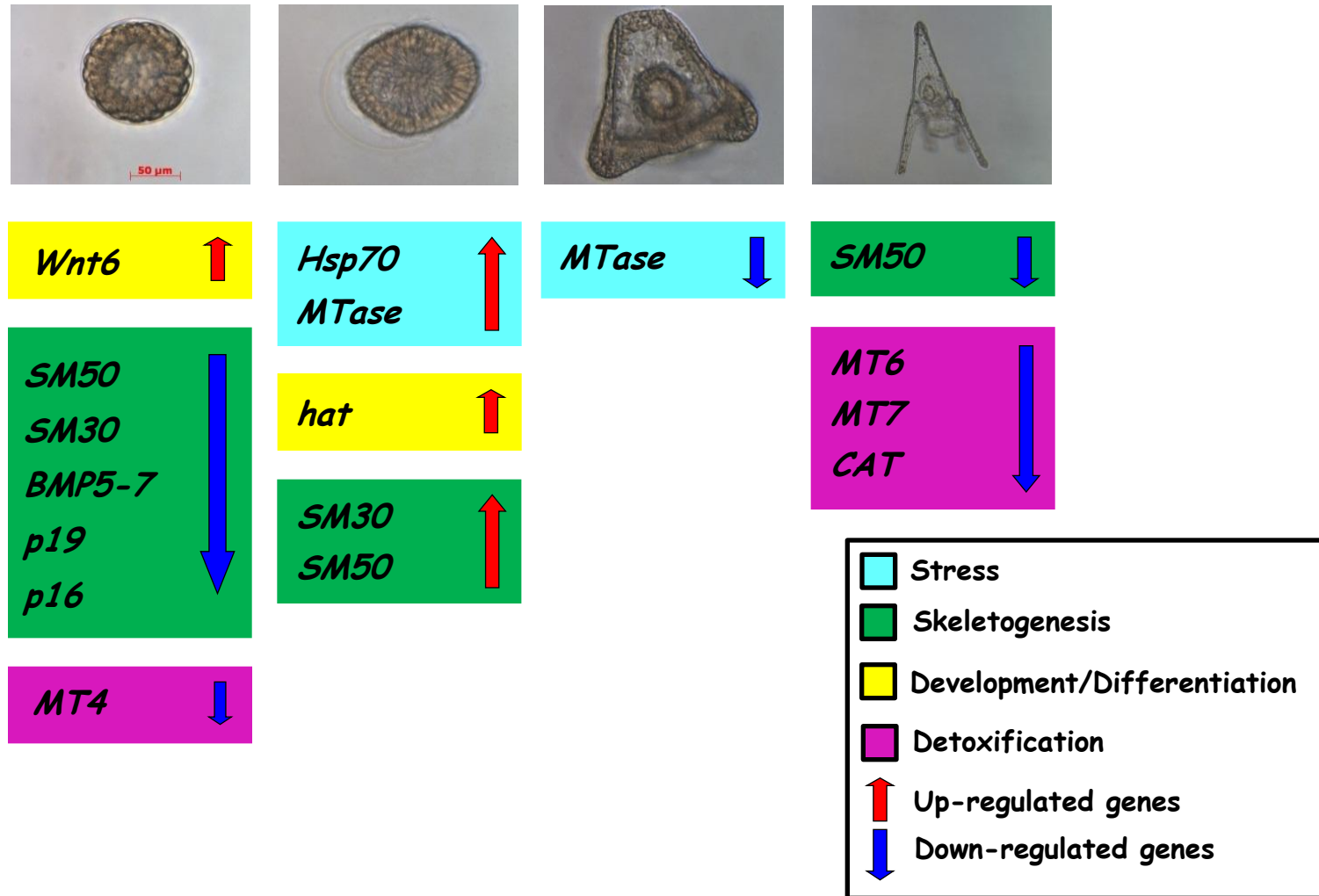
(Modified from Varrella et al., 2014)

Effects of heptadienal on gene expressions at different developmental stages



(Modified from Varrella et al., 2014)

Effects of octadienal on gene expressions at different developmental stages



(Modified from Varrella et al., 2014)

Summary

Decadienal

Hsp70 ↑
Hsp60 ↑

SM50 ↓
SM30 ↓
uni ↓

hat ↑
Wnt6 ↓

MT4 ↓
MT6 ↓
MT8 ↓

Heptadienal

Hsp70 ↑
MTase ↑
hsp56 ↓
p38 MAPK ↓

SM50 ↑↓
uni ↓
p19 ↓

Wnt5 ↑
Wnt6 ↑
Wnt8 ↑
hat ↓
sox9 ↓
Alix ↓

MT4 ↓
MT6 ↓
MT8 ↓

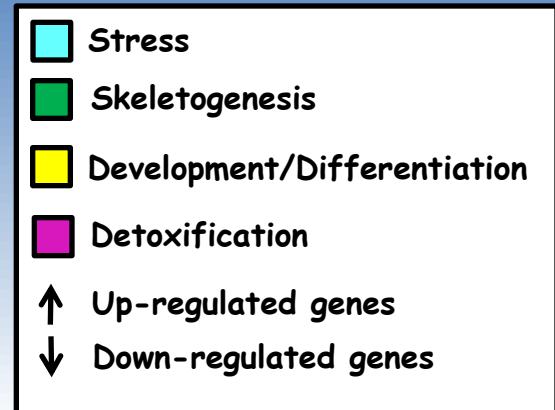
Octadienal

Hsp70 ↑
MTase ↑

SM50 ↑↓
SM30 ↑↓
BMP5-7 ↓
p16 ↓
p19 ↓

hat ↑
Wnt6 ↓

CAT ↓
MT4 ↓
MT6 ↓
MT8 ↓



(Modified from Varrella et al., 2014)

Morphological conclusions

✓ Do PUAs affect the sea urchin embryogenesis?

The aldehydes induced teratogenesis on sea urchin embryos in a dose-dependent manner.

The teratogenic effect after a week of PUAs exposure appeared to be stronger for decadienal and heptadienal.

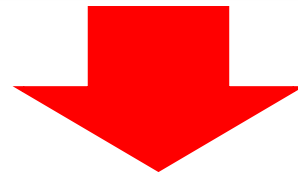
✓ When do PUAs act on embryo development?

The aldehydes could compromise the normal embryonic development affecting embryos before and/or soon after the fertilization

Molecular conclusions

- ✓ Do PUAs have molecular targets? And could these targets be considered as possible biomarkers for environmental stress response?

The aldehydes have different molecular targets, affecting the expression levels of different genes at different times of embryonic development.



**Stress response
Biomarkers**

....more in general

Ecological relevance considering the importance of diatoms blooms in nutrient-rich aquatic environments.....

....molecular evidence for the toxic effects of the diatom-derived PUAs

....novel tools for understanding the cellular mechanisms of the response to aldehydes exposure to the benthic organisms.

Future perspectives

- Effects of many other Oxylipins (HEPEs)
- Functional networks of the PUAs target genes
- Oxidative stress effects induced by Oxylipins

