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

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

**CLASSIFICATION**
- Domain: Eukaryota
- Kingdom: Animalia
- Phylum: Echinodermata
- Class: Echinoidea
- Order: Echinoida
- Family: Echinidae
- Species: Paracentrotus lividus
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
Beneficial role in supporting planctonic 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

(Miralto et al. 1999)

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

Echinoderms

Polychaetes

Crustaceans

(Ianora and Miralto 2010)
Oxidative metabolism of fatty acids in diatoms

Polyunsaturated C16 or C 20 fatty acids

O₂

Lipoxygenases

R

R

Hydroperoxides

R

R

Hydroperoxide lyase

Hydroperoxides

Aldehydes and oxoacids

R

R

Epoxy alcohols

Hydroperoxide lyase

OXYLIPINS

(Ianora and Miralto 2010)
The effects of decadienal on sea urchin embryos

Decadienal

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

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

Decadienal induces teratogenesis and apoptosis

First molecular studies

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

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(Mar. Drugs 2011, 9, 1035-1045; doi: 10.3390/md9111035)

Defensins against Toxic Diatom Aldehydes in the Sea Urchin Paracentrotus lividus

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(Mar. Drugs 2012, 10, 976-988; doi: 10.3390/md10090976)

Increasing concentrations of decadienal
….. The other PUAs?

- 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

1. **Sea urchin eggs**
2. **Incubation for 10 min with aldehydes**
3. **Fertilization**
4. **Morphological observations at 48 hpf**

Additional information:
- Decadienal
- Octadienal
- Heptadienal
- Control embryos at 48 hpf

[Source](http://www.alnmag.com/news/2012/02/sperm-pathways-sea-urchins)
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)
Sea urchin eggs

Incubation for 10 min with aldehydes

Fertilization

Morphological observations at 72, 96 hpf until one-week

Experimental procedures

Decadienal
Octadienal
Heptadienal

Control embryos at one week hpf

Do PUAs affect the embryogenesis?

72, 96 hpf

One week
Experimental procedures

Sea urchin eggs

Incubation for 10 min with aldehydes

Fertilization

10 min pf
2 blastomeres
40 min pf
8 blastomeres
2 hpf
32 blastomeres
3 hpf
Early blastula
5 hpf
Late blastula
8 hpf

PUAs additions

Control embryos at 48 hpf

Morphological observations at 48 hpf

When do PUAs act on embryo development?

Aldehydes additions at different developmental times

(Varrella et al., 2014)
PUAs action time

10 minutes before

fertilization

10 minutes after

PUAs
Experimental procedures

Sea urchin eggs

Incubation for 10 min with aldehydes

Fertilization

Determination of teratogenic and dose-dependent concentrations of aldehydes

Analysis of 31 by Real Time q-PCR

Decadienal
Octadienal
Heptadienal

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
- **Swimming blastula**
  - 9 hpf
  - Pluteus
- 24 hpf
- 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

(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.

✓ When do PUAs act on embryo development?

The aldehydes could compromise the normal embryonic development affecting embryos before and/or soon after the fertilization.
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.
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
- Oxyditave stress effects induced by Oxylipins