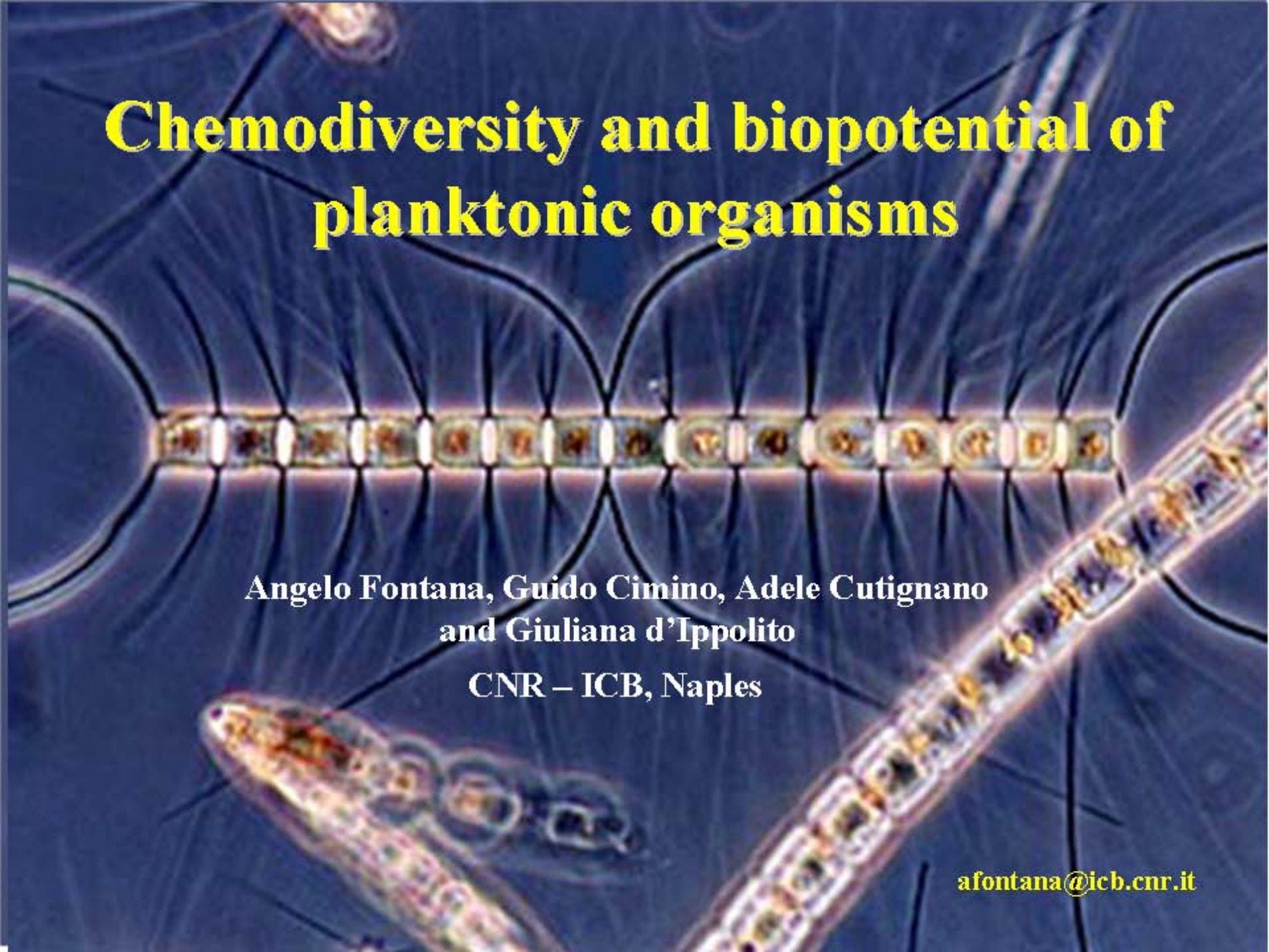
Chemodiversity and biopotential of planktonic organisms



Angelo Fontana, Guido Cimino, Adele Cutignano and Giuliana d'Ippolito

CNR – ICB, Naples

afontana@icb.cnr.it

Marine trophic chain

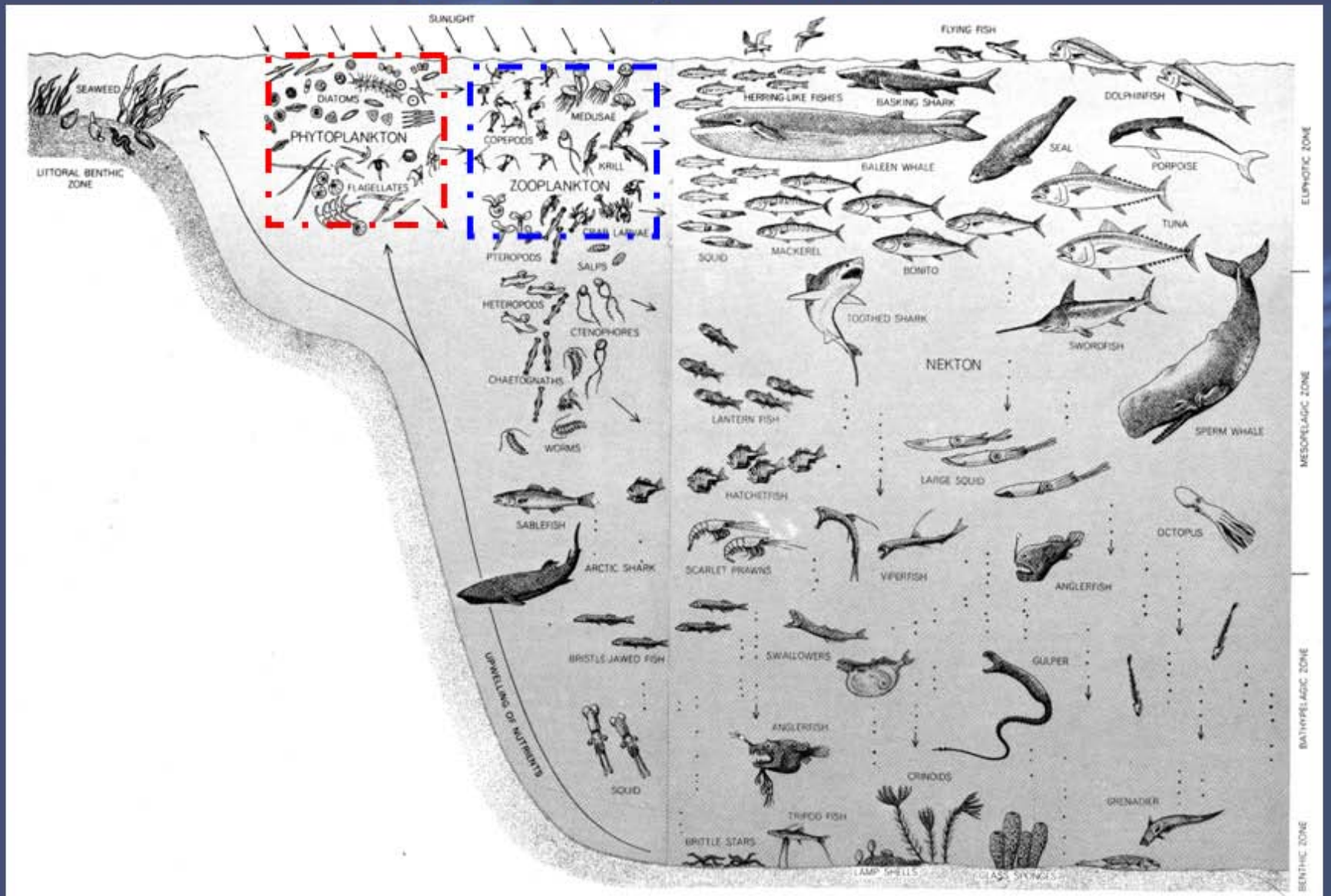
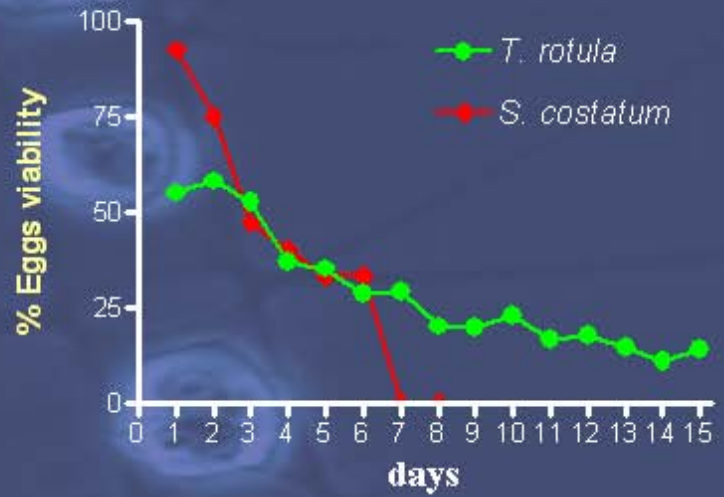
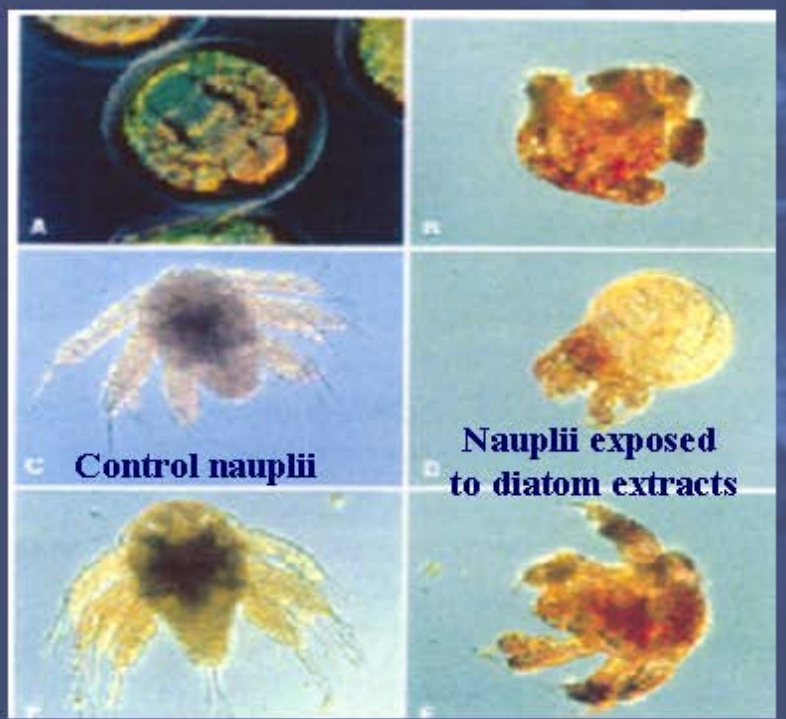
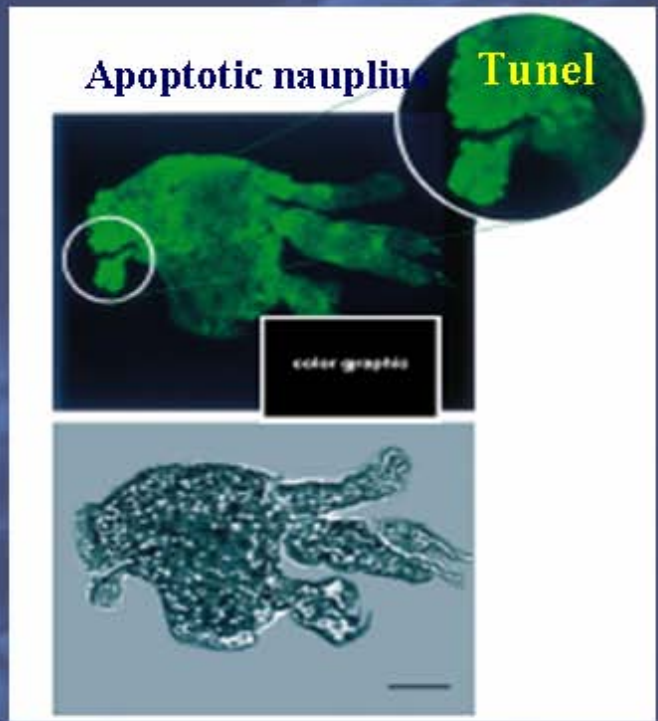


Figure 12–6. A sample of marine biota (organisms not drawn to scale) arranged to show the major food chain and depth relationships that link an entire ocean basin into a vast ecosystem. The diagram does not do justice to the microplankton and the "infauna" benthos, the importance of which is described 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.)

Diatom-induced toxicity on copepods





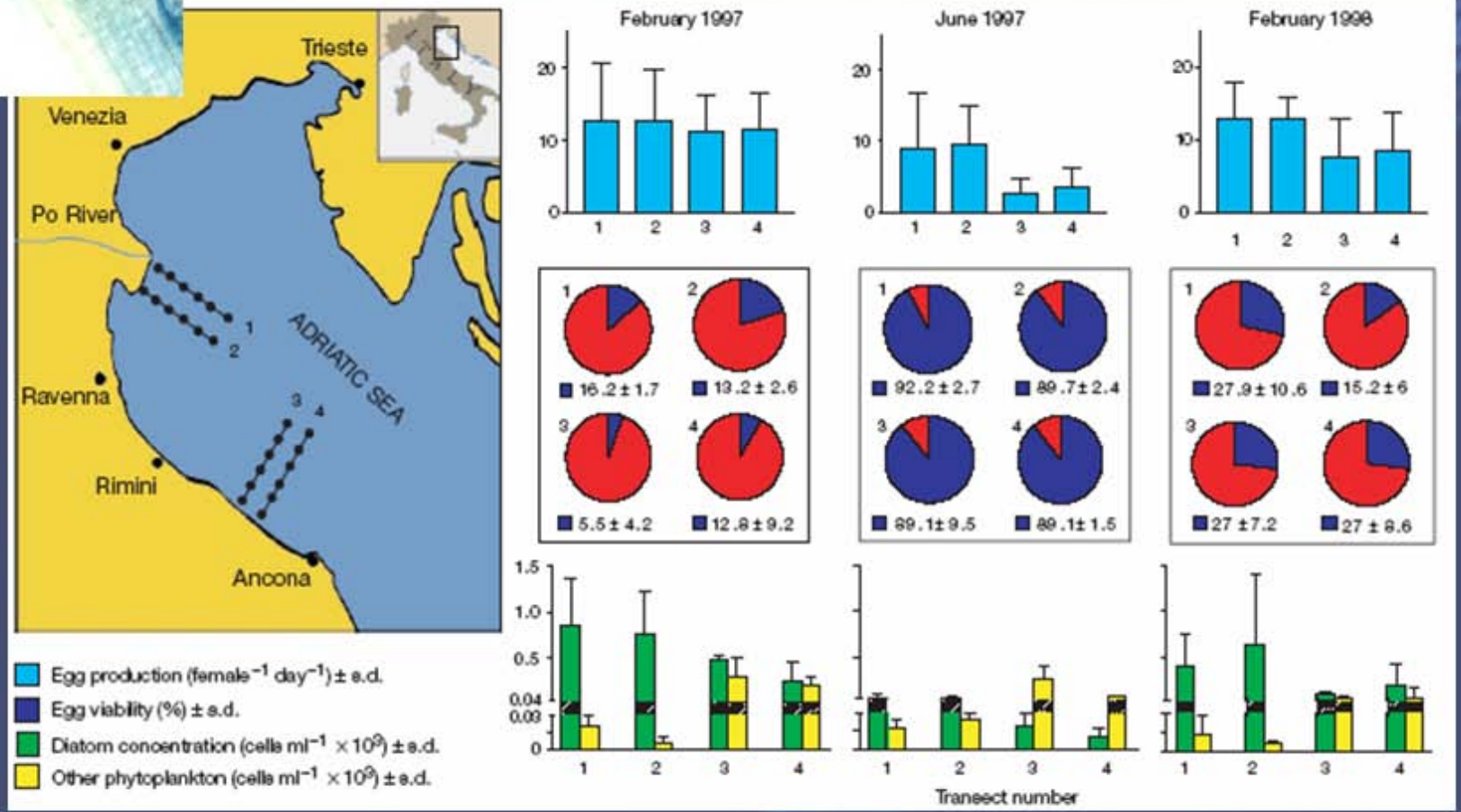


Poulet et al., 1994; Ianora et al., 2003

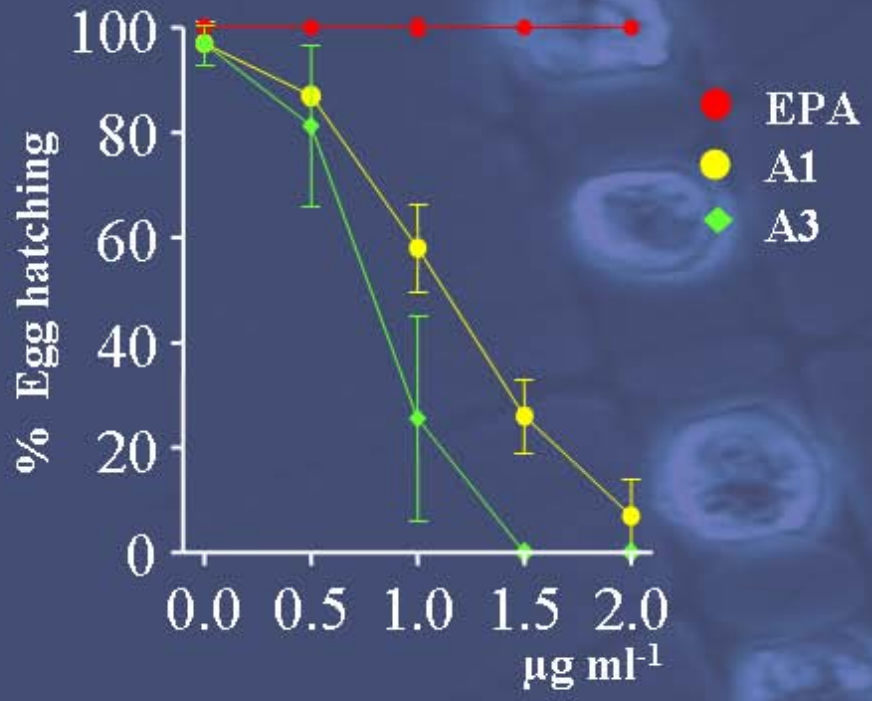
A1

A2

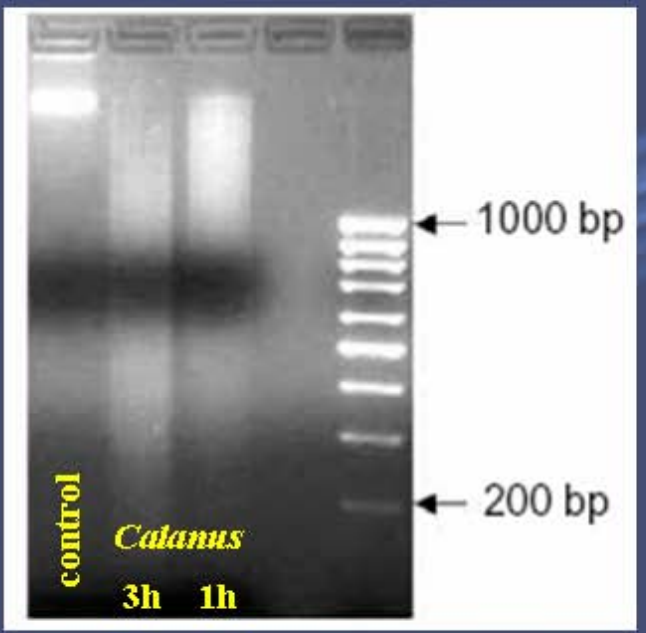
A3

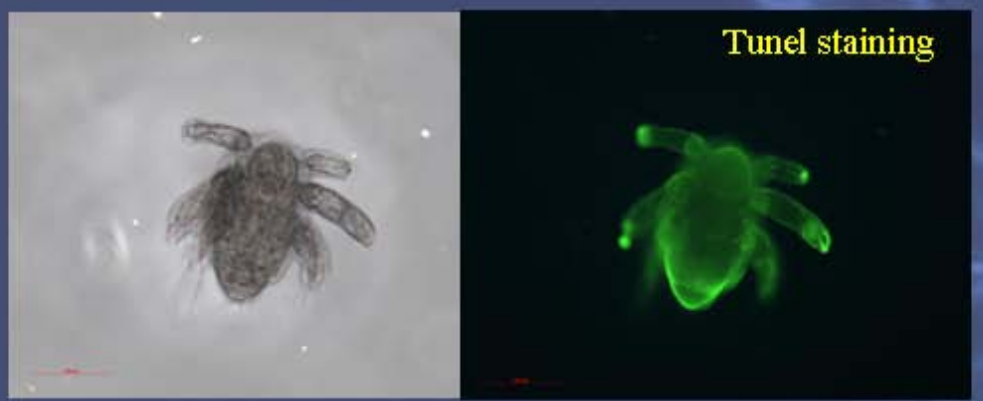


Miralto et al., 1999



DNA laddering of copepod egg exposed to A3 (decadienal)

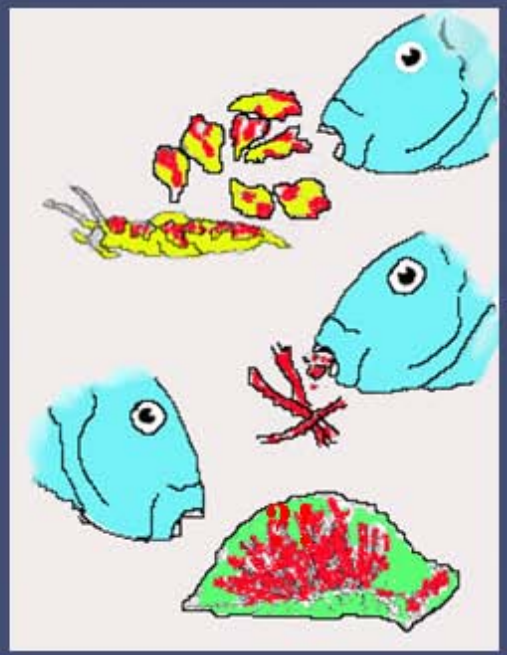




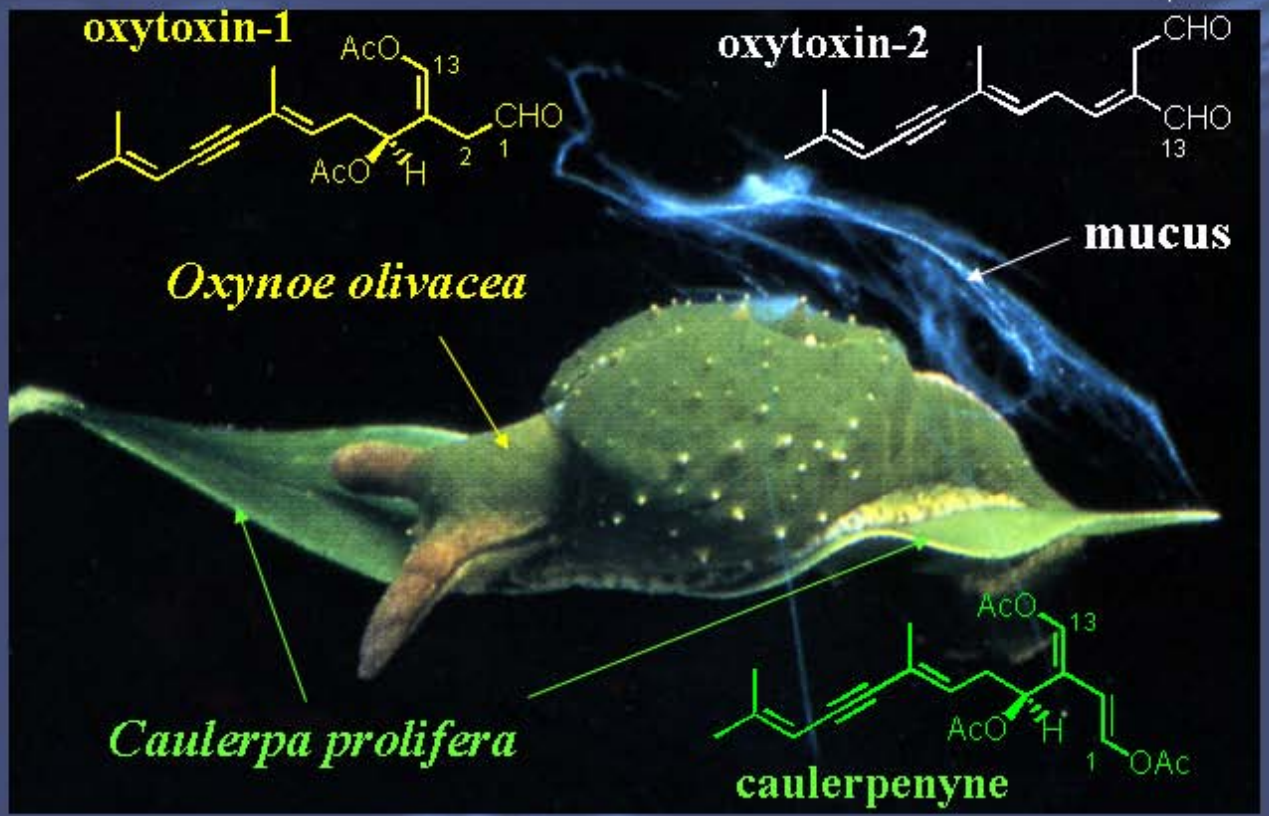
Newborn copepod nauplius exposed to A3 (decadienal)

Miralto et al., 1999; Romano et al., 2003

about chemical defense ….



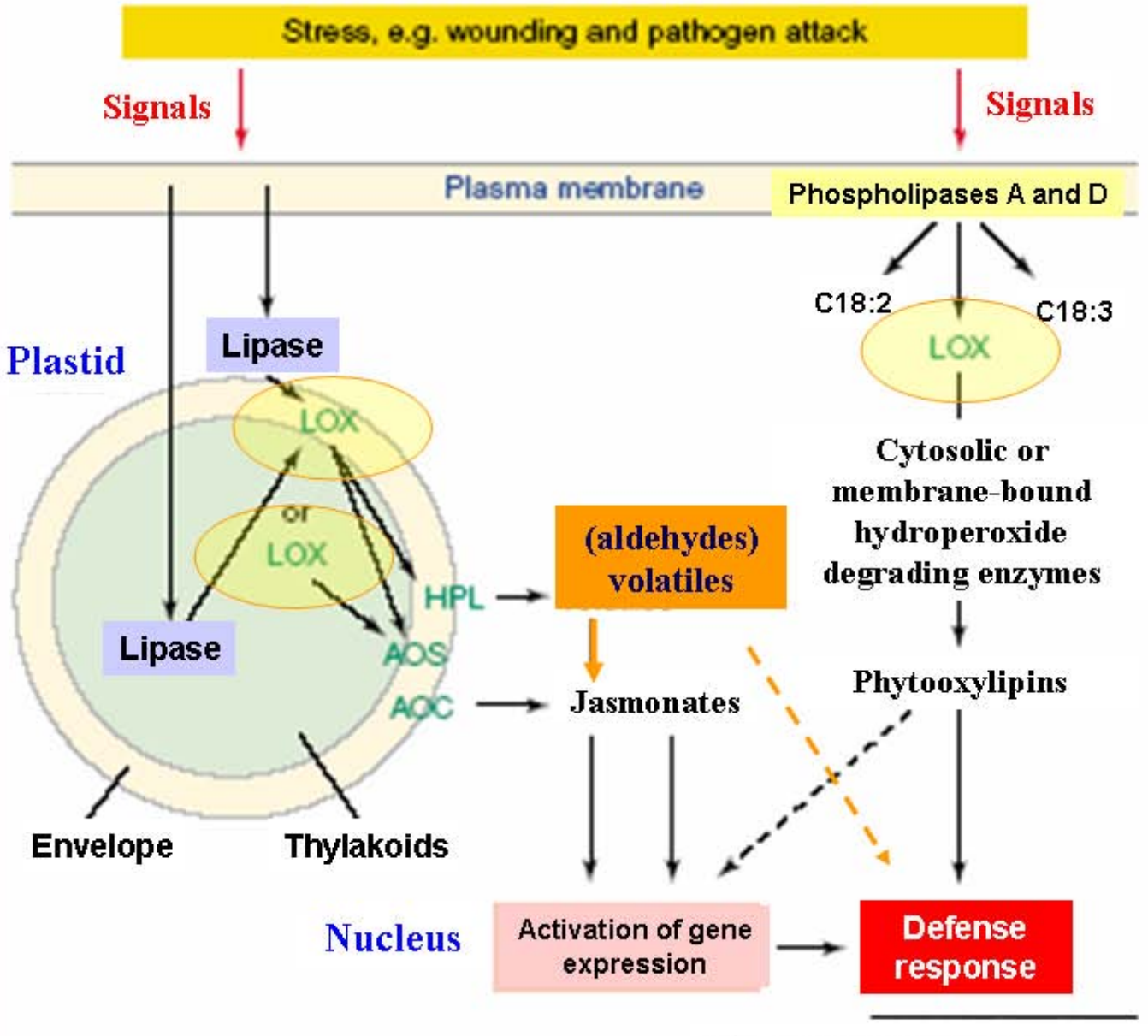
Deterrence and Unpalatability



Phycoaldehydes involved as mediators in diatom/copepod interactions

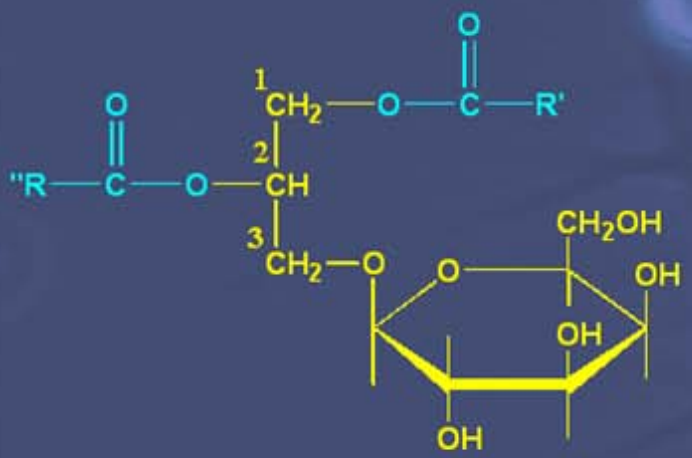
Tetrahedron Lett.,, 2002, 43, 6133 & 6137

Terrestrial plant-herbivores interaction

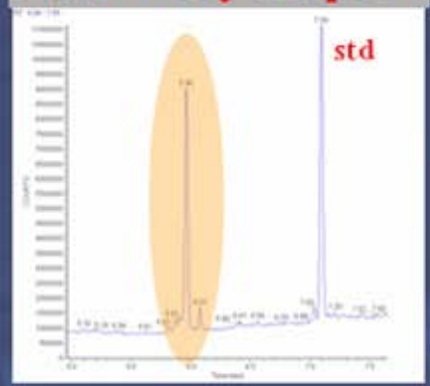


Blèe, 2002, Trends in Plant Science

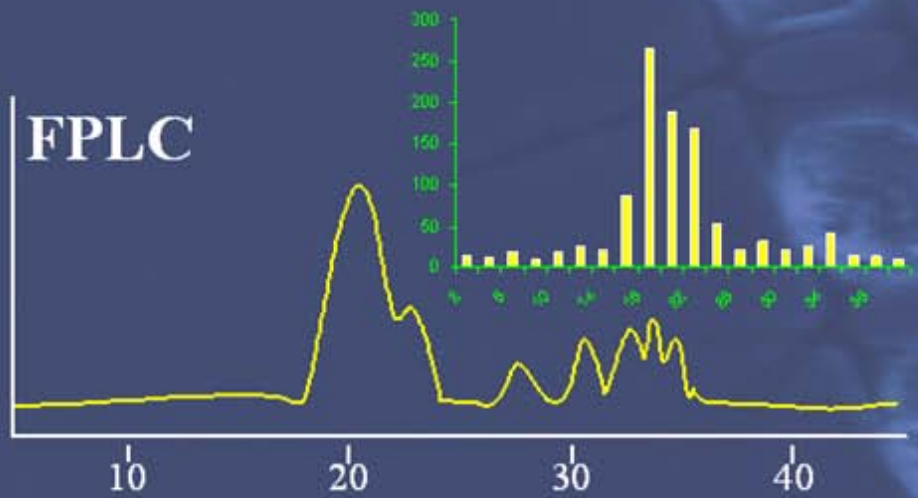
Synthesis of phycoaldehydes is triggered by galactolipase(s)

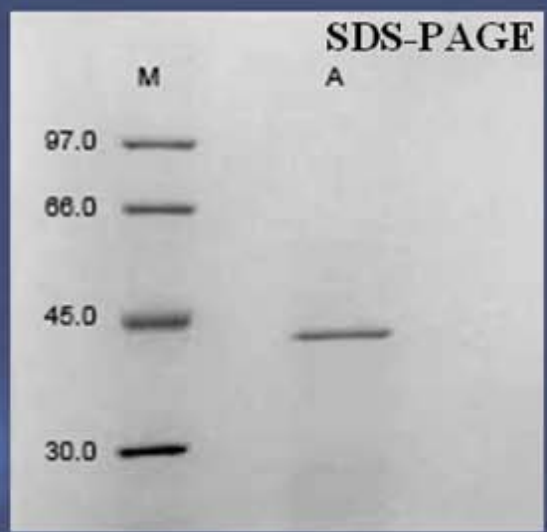


Enz + Glycolipid

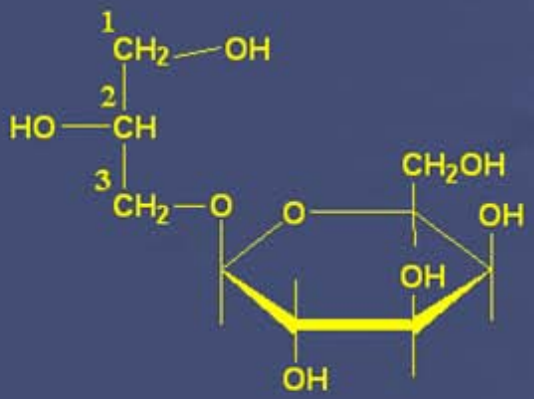


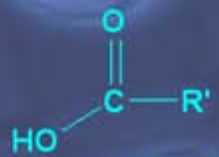
Enz+Phospholipid





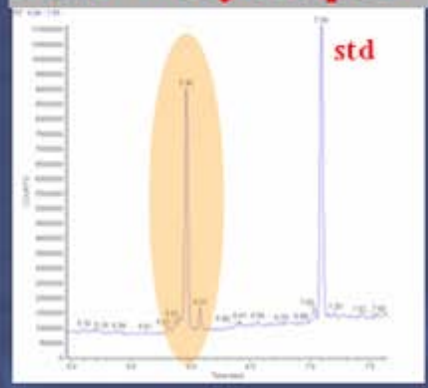
Synthesis of phycoaldehydes is triggered by galactolipase(s)



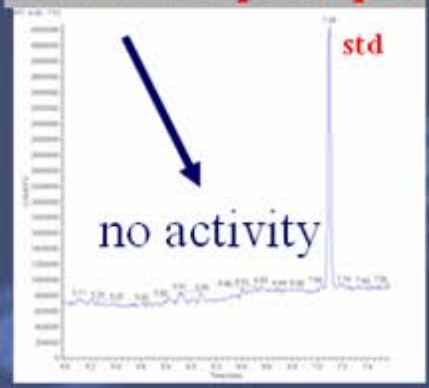


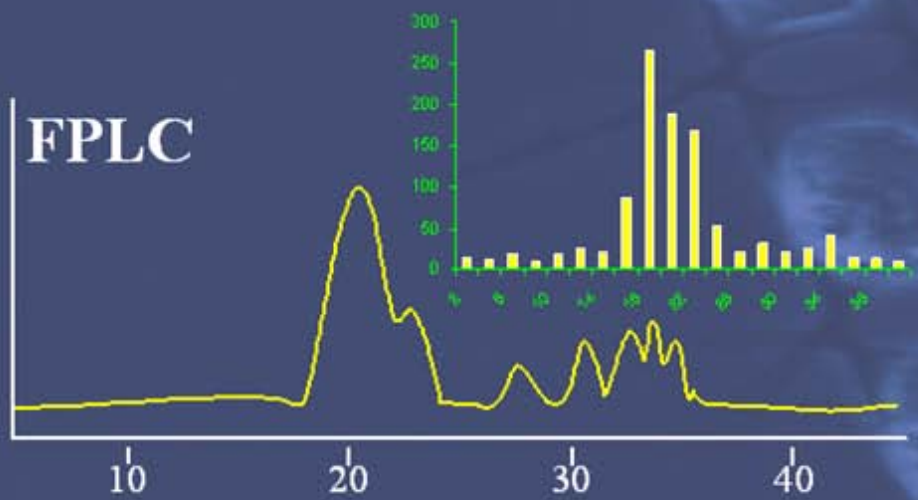
Free Fatty acids

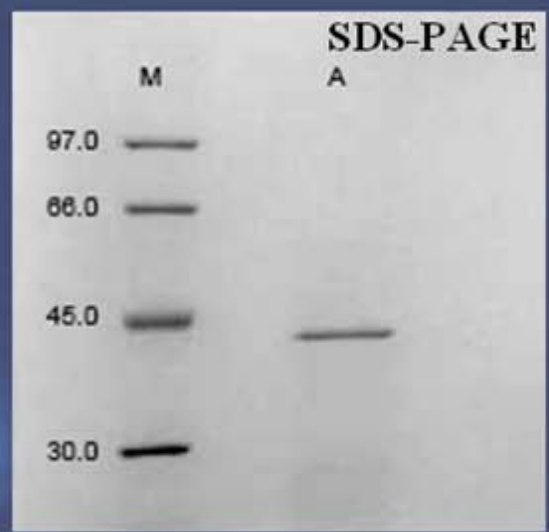
Enz + Glycolipid



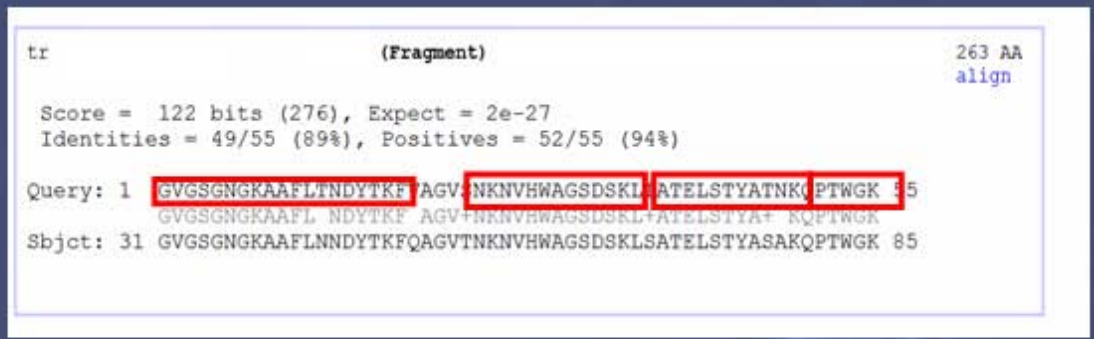
Enz+Phospholipid







The protein bearing most of the enzymatic activity in *T. rotula* is related to two proteins of unknown function from terrestrial plants.



tr (Fragment) 263 AA
align

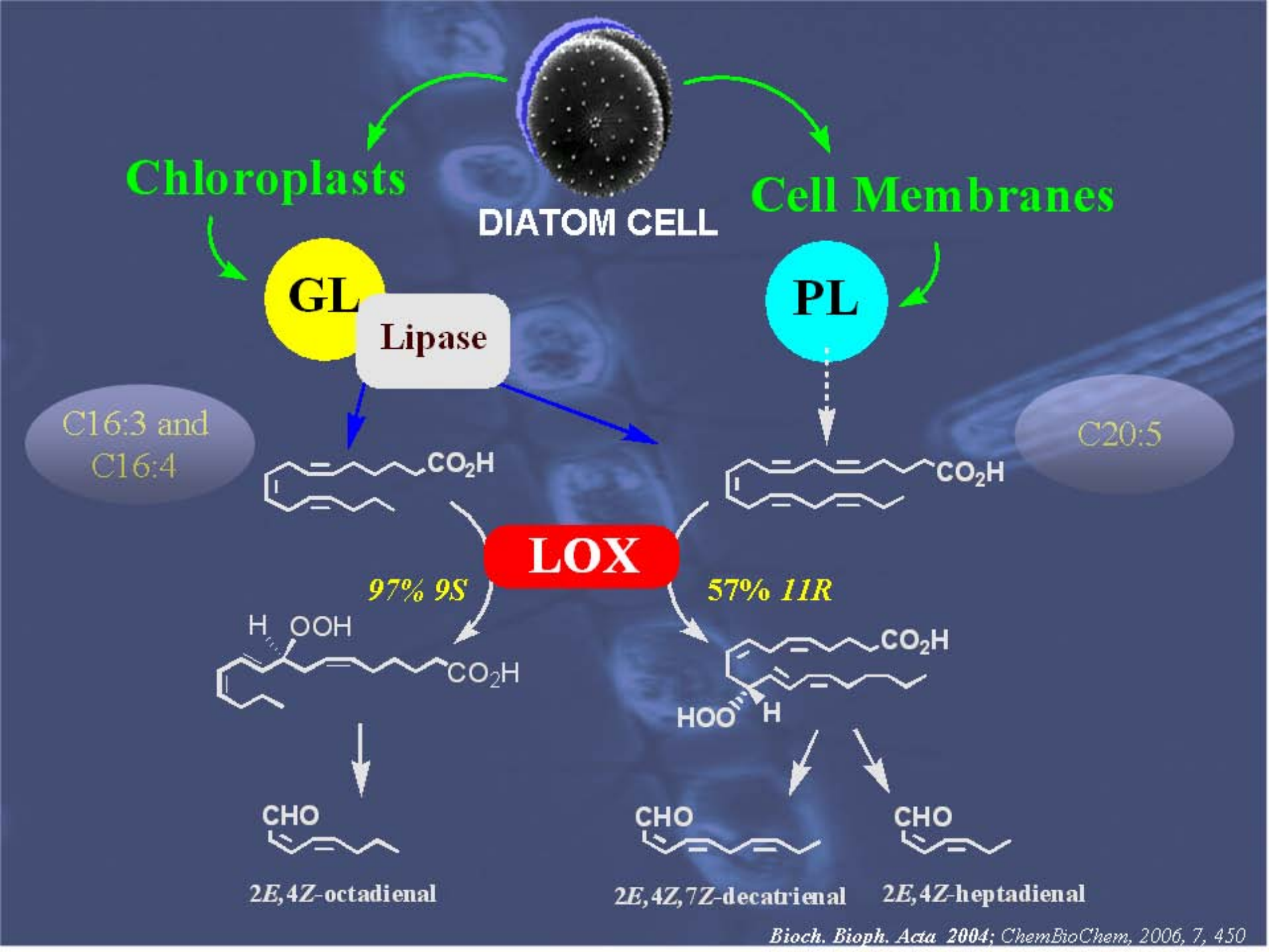
Score = 122 bits (276), Expect = 2e-27
Identities = 49/55 (89%), Positives = 52/55 (94%)

Query: 1 GVGSGNGKAAFLTNDYTKFTAGVTNKNVHWAGSDSKLSATELSTYATNKQPTWGK 55
GVGSGNGKAAFL NDYTKF AGV+NKNVHWAGSDSKL+ATELSTYA+ KQPTWGK
Sbjct: 31 GVGSGNGKAAFLNNDYTKFQAGVTNKNVHWAGSDSKLSATELSTYASAKQPTWGK 85

BLAST sequence alignment of protein 1.a (fragment 1-55) from *Arabdopsis thaliana* and protein 2.a (fragment 31-85) from *Hypericum perforatum*

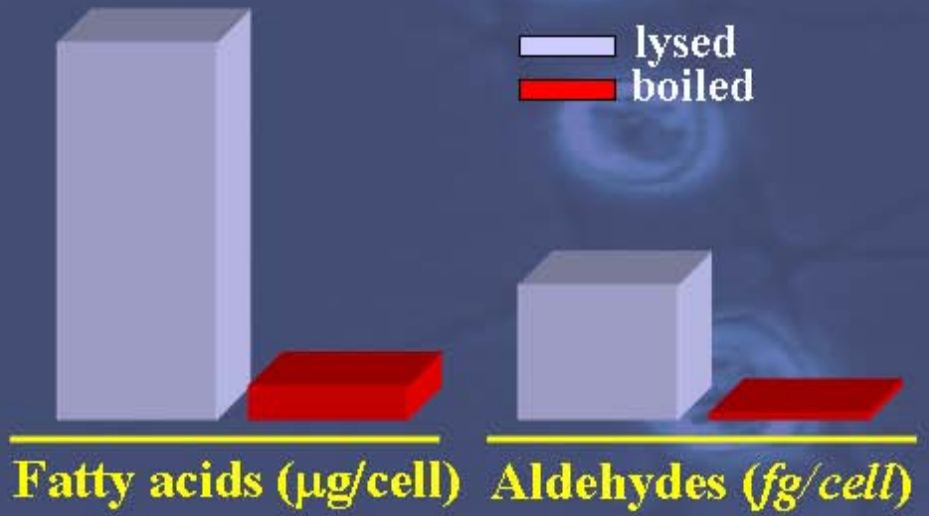
Peptide sequences of *T. rotula* palactolipase retrieved by ESI-Q-TOF MS/MS are highlighted.

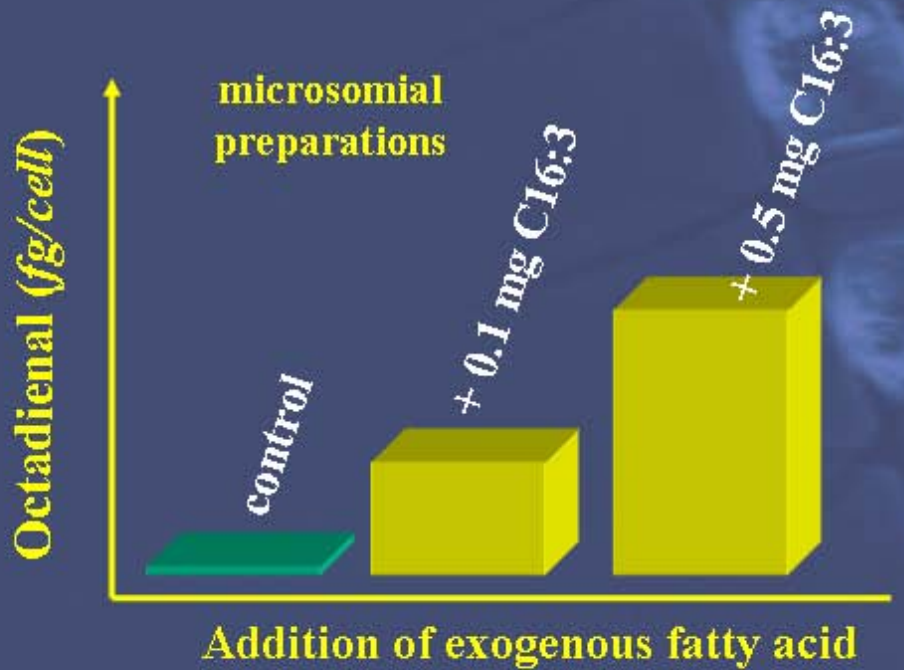
The protein, we called ThGAL1 contributes to lipid breakdown occurring during copepod grazing-induced defense and therefore provides fatty acid precursors for the synthesis of phycoaldehydes and other oxylipins. A detailed study of the expression profile of the activity is expected to reveal what triggers the synthesis of phycoaldehydes in marine diatoms. Furthermore, the use of lipases in industrial processes has steadily increasing as an alternative to chemical methods.

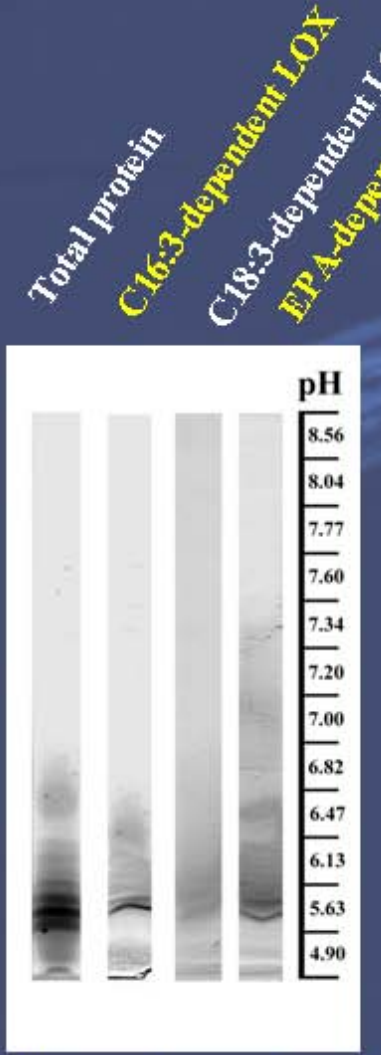


Bioch. Bioph. Acta **2004**; *ChemBioChem*, 2006, 7, 450

Characterization of the LOX-activity



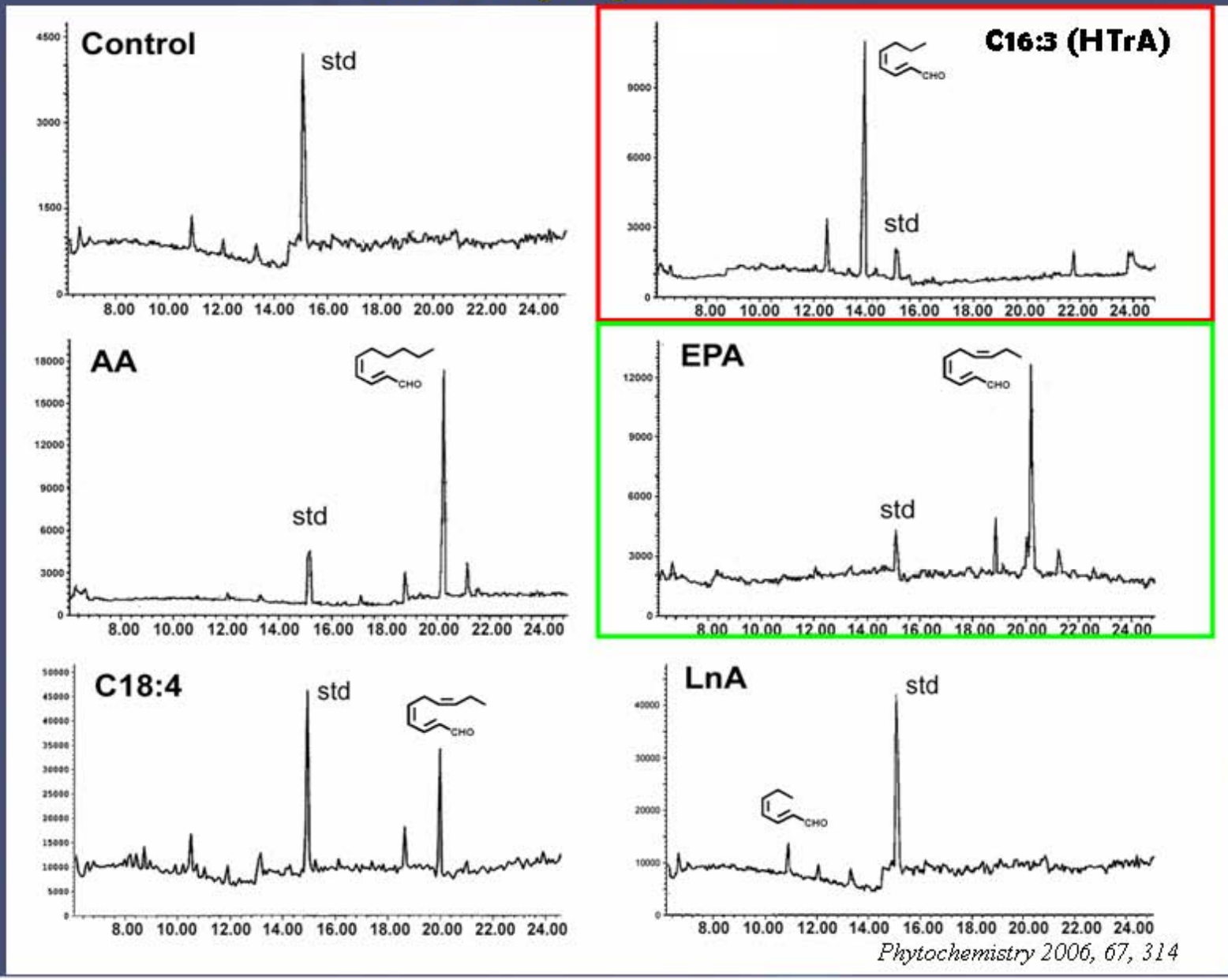




membrane-bound activity

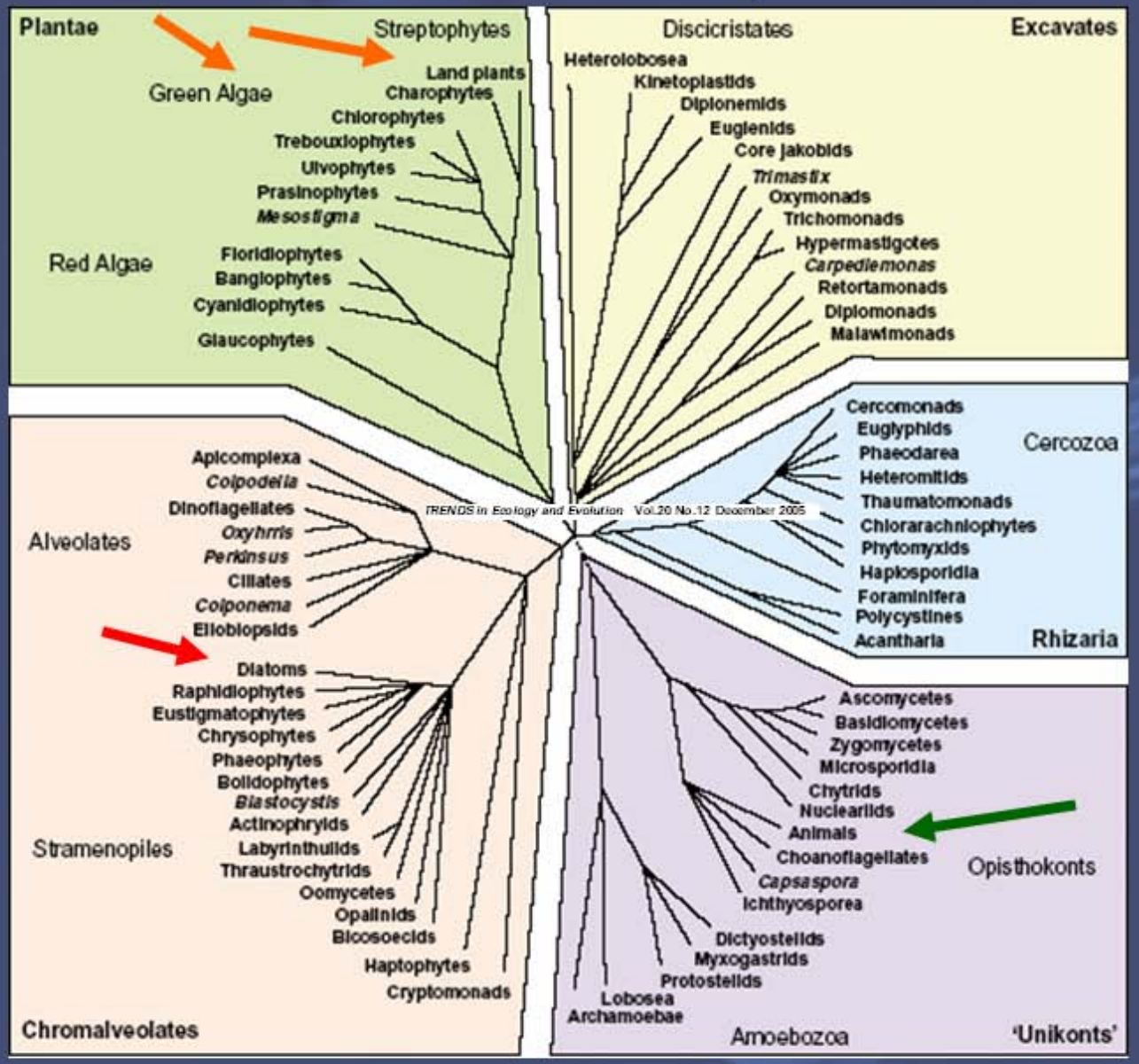
Org. Biomol. Chem., 2005, 3, 4065

Synthesis of phycoaldehydes by exogenous fatty acids and diatom preparations



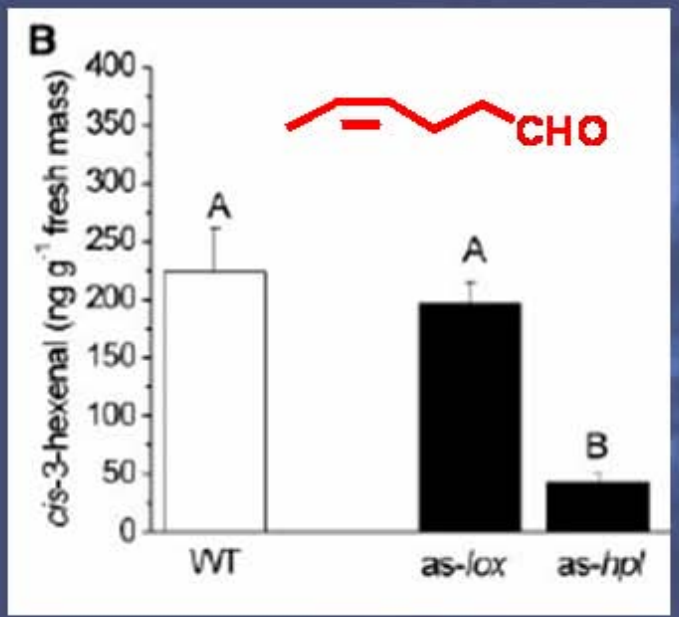
Phytochemistry 2006, 67, 314

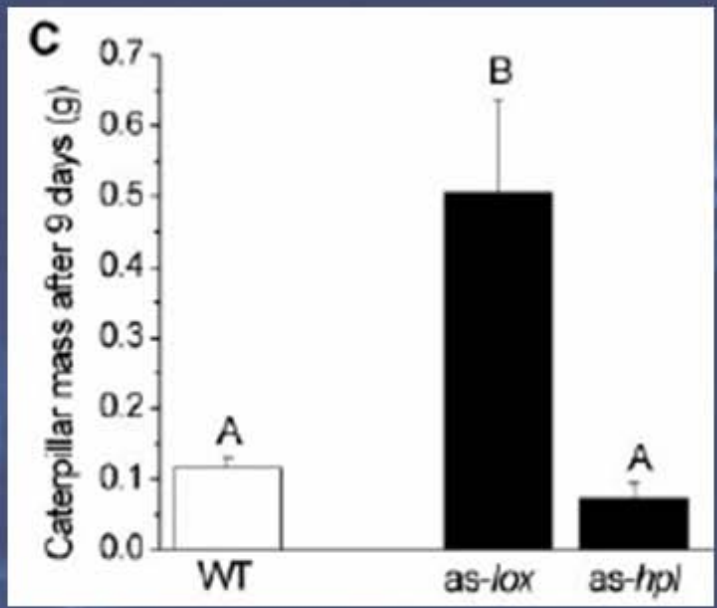
The tree of eukaryotes



Keeling et al., TRENDS in Ecology and Evolution 2005, 20, 670

Silencing lipoxygenase (lox) and hydroperoxide lyase (hpl) genes of native tobacco, *Nicotiana attenuata*, inhibits oxylipin signaling, leading to plants that are more vulnerable to adapted herbivores but also attracted novel herbivore species, which fed and reproduced successfully.

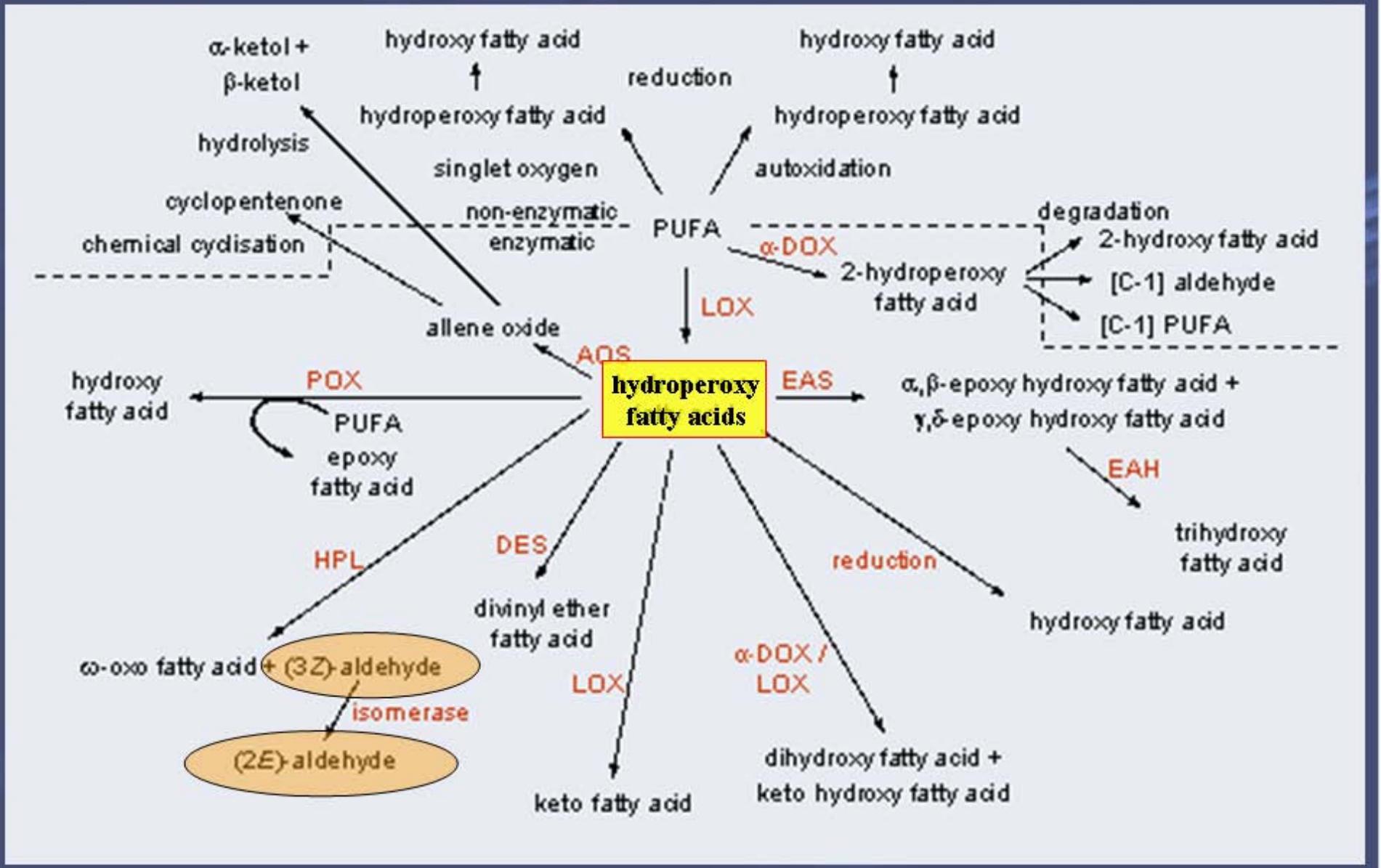




…… in nature, these results show that lipoxygenase dependent signaling determines host selection for opportunistic herbivores and that induced defenses influence herbivore community composition.

Kessler et al. 2004

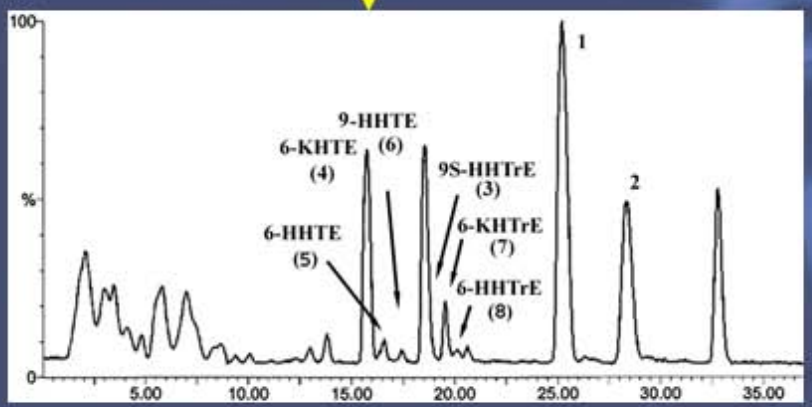
LOX-pathways in plants

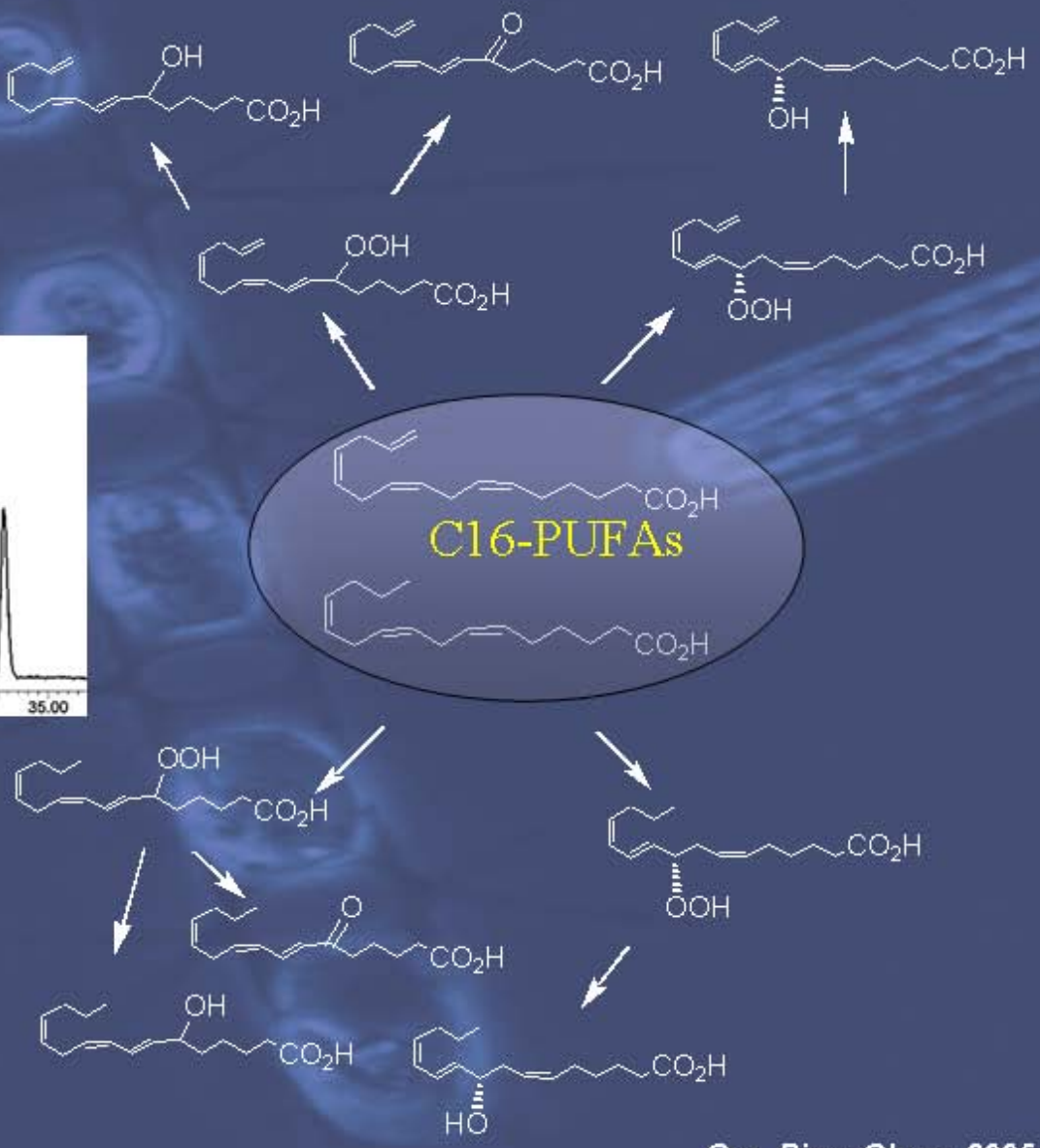


Other Oxylipins in Thalassiosira rotula (lab culture)

Diatom lysate

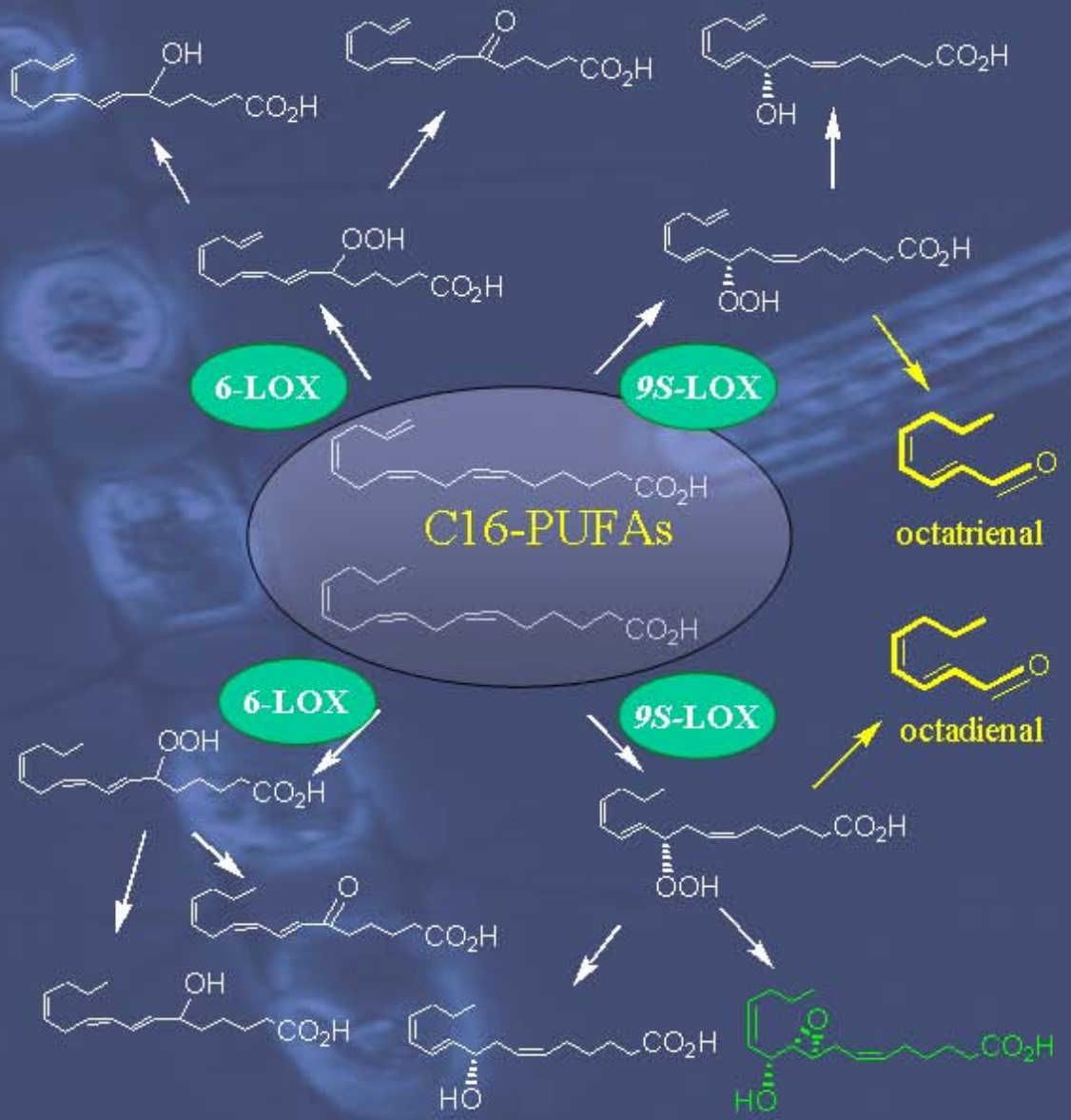
LC/MS-MS



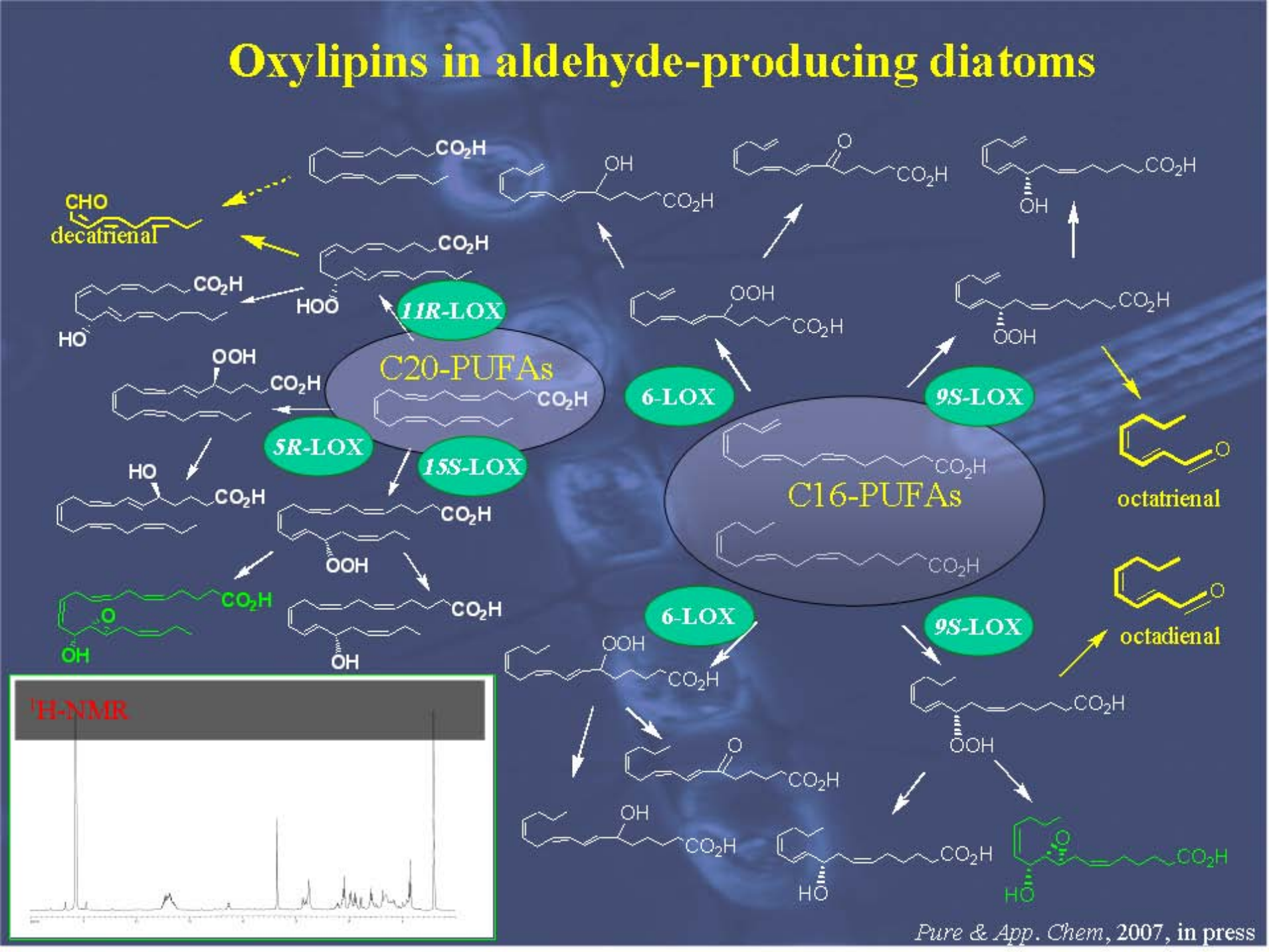


Org. Biom.Chem, 2005

Oxylipins in aldehyde-producing diatoms

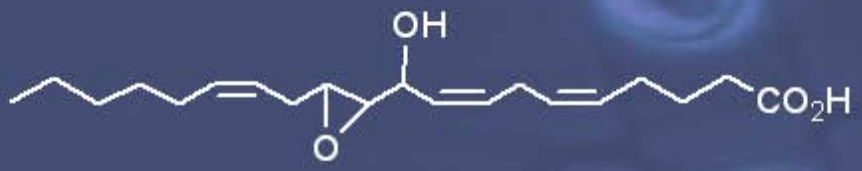


Oxylipins in aldehyde-producing diatoms



Pure & App. Chem, 2007, in press

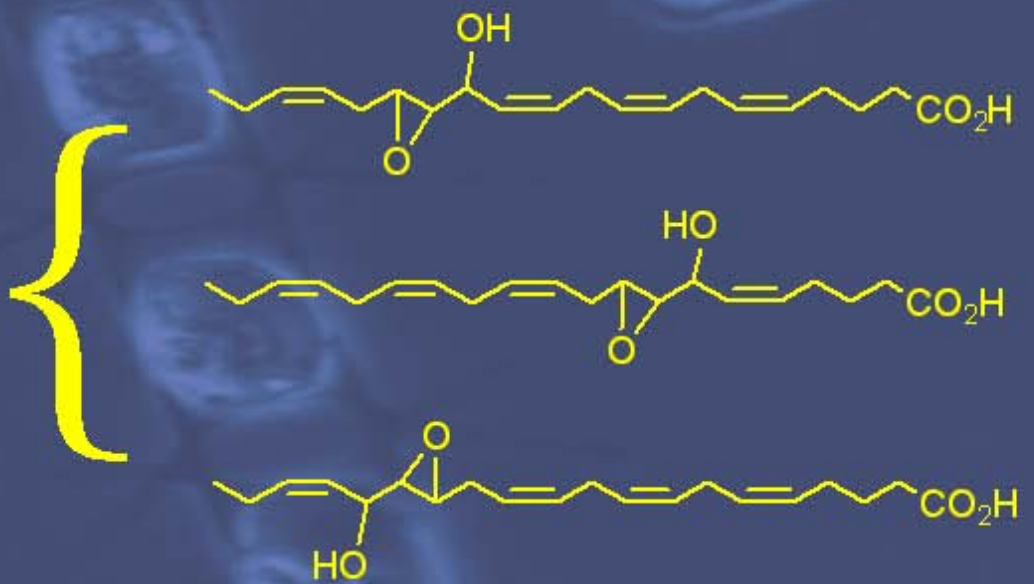
Hepoxilin B-series



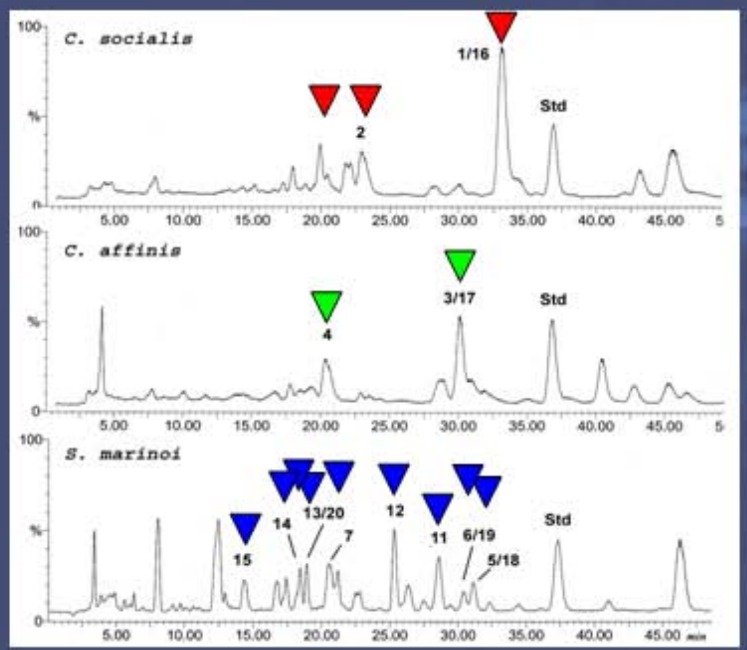
mammalians

calcium release from intracellular stores by a G-protein coupled receptor-mediated action in mammalian cells

diatoms



manuscript submitted


C. socialis
1/16
Std
2
C. affinis
3/17
Std
4
S. marinoi
14
13/20
12
11
6/19
Std
15
7
5/18
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00

LAH
stimulus
LAH
PL
MGDG
free fatty acid
LOX
OOH
fatty acid hydroperoxide
RED
HPL
EAS
OH
hydroxy-fatty acid
aldehyde & ω
oxo-acids
O
OH
epoxy-alcohol

Many other species show a similar complexity of metabolites and metabolisms:

- Studies of 11 other species or strains of diatoms have been completed;
- Evidence of 8 different oxygenase pathways not always leading to aldehydes
- Large array of oxylipins, suggesting enzymatic activities other than those of *S. costatum* and *T. rotula.*
- Species-specific distribution
- C20:5 and C16:3 fatty acids largely predominant as source substrate
- Pivotal role of chloroplastic glycolipids in all studied species
- Processes detected also in phytoplankton (North Adriatic - Mediterranean Sea and Terra Nova Bay - Antarctica)

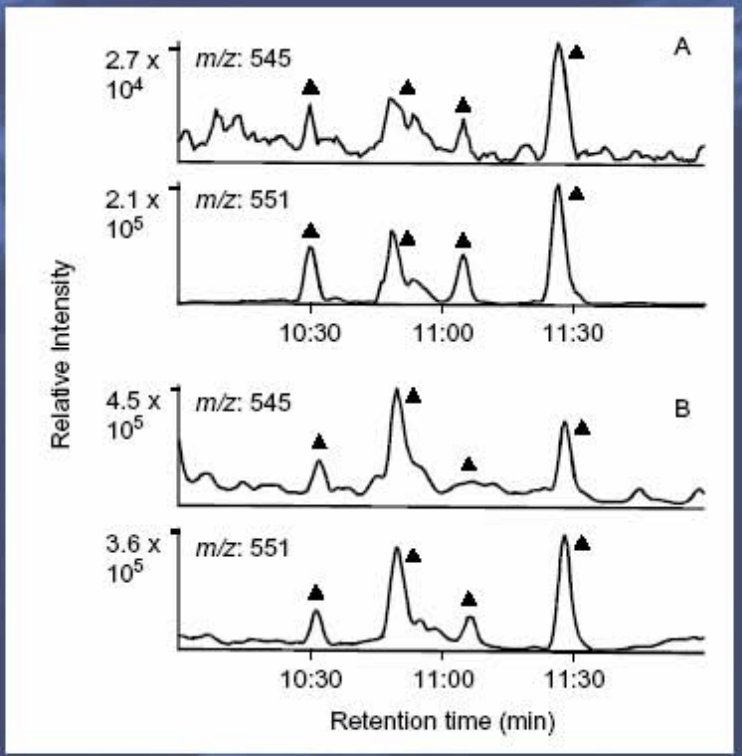
Are these products in somehow related to diatom-induced failure of copepod reproduction?

Diet-derived oxylipins in mammalians

Evidence is emerging that certain biologically active oxidized fatty acids, called phytoprostanes (PPs) may modulate the function of mammalian immune cells, displaying potent antiinflammatory and apoptosis inducing activities similar to some prostaglandins (Mediterranean Diet).

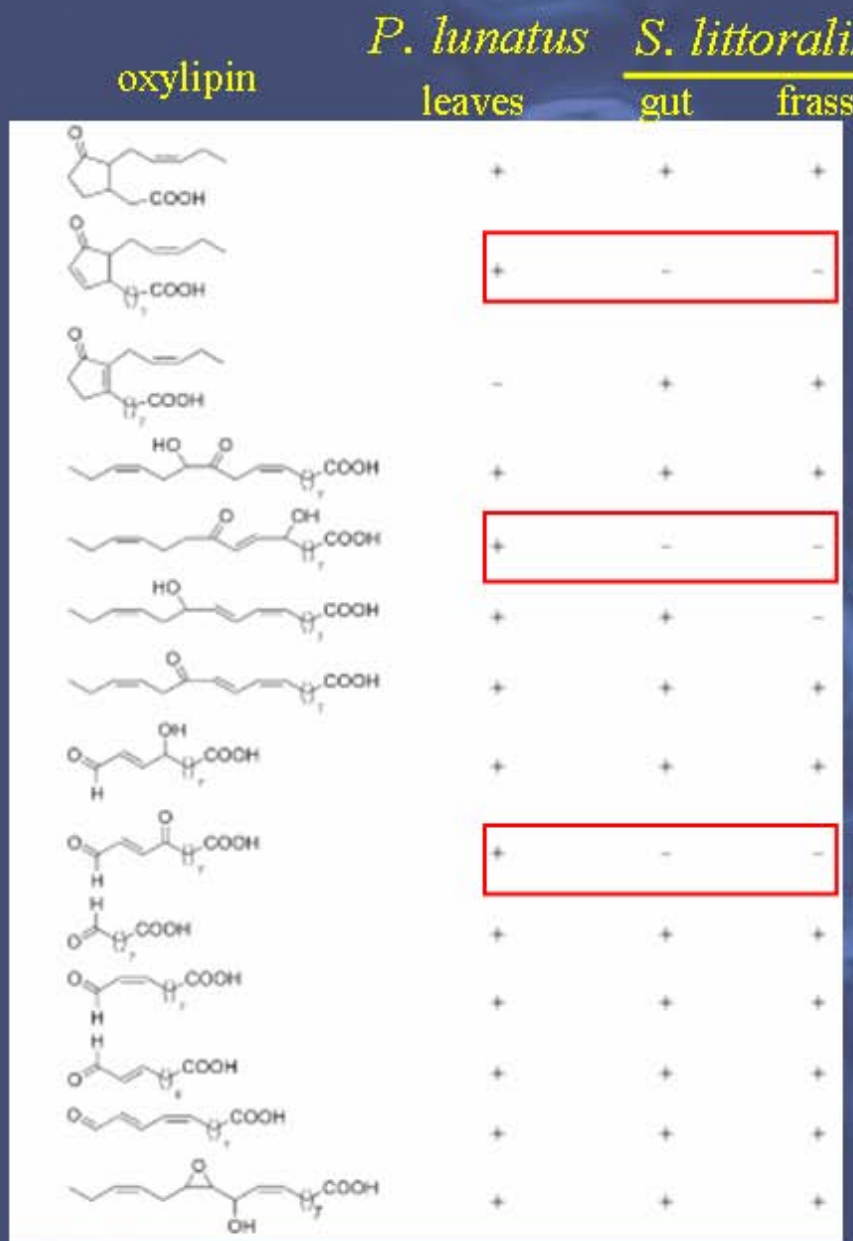
After oral consumption of vegetables rich in polyunsaturated fatty acids, PPs were absorbed, found to circulate in plasma (A) in conjugated form and excreted in free form into urine (B).

PPs detection by LC-MS in plasma (A) and urine (B)



Karg et al., 2006

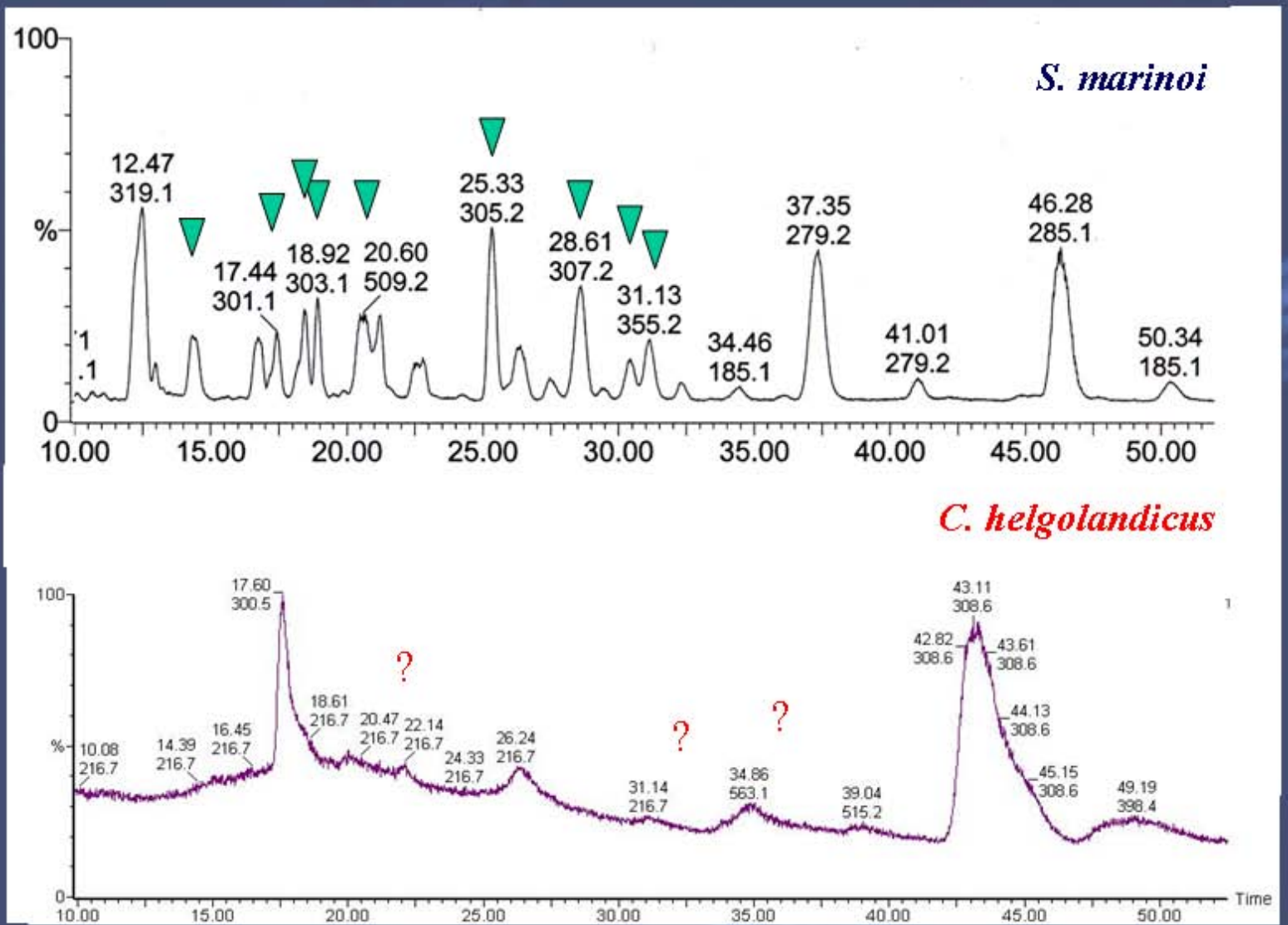
Diet-derived oxylipins in insect larvae

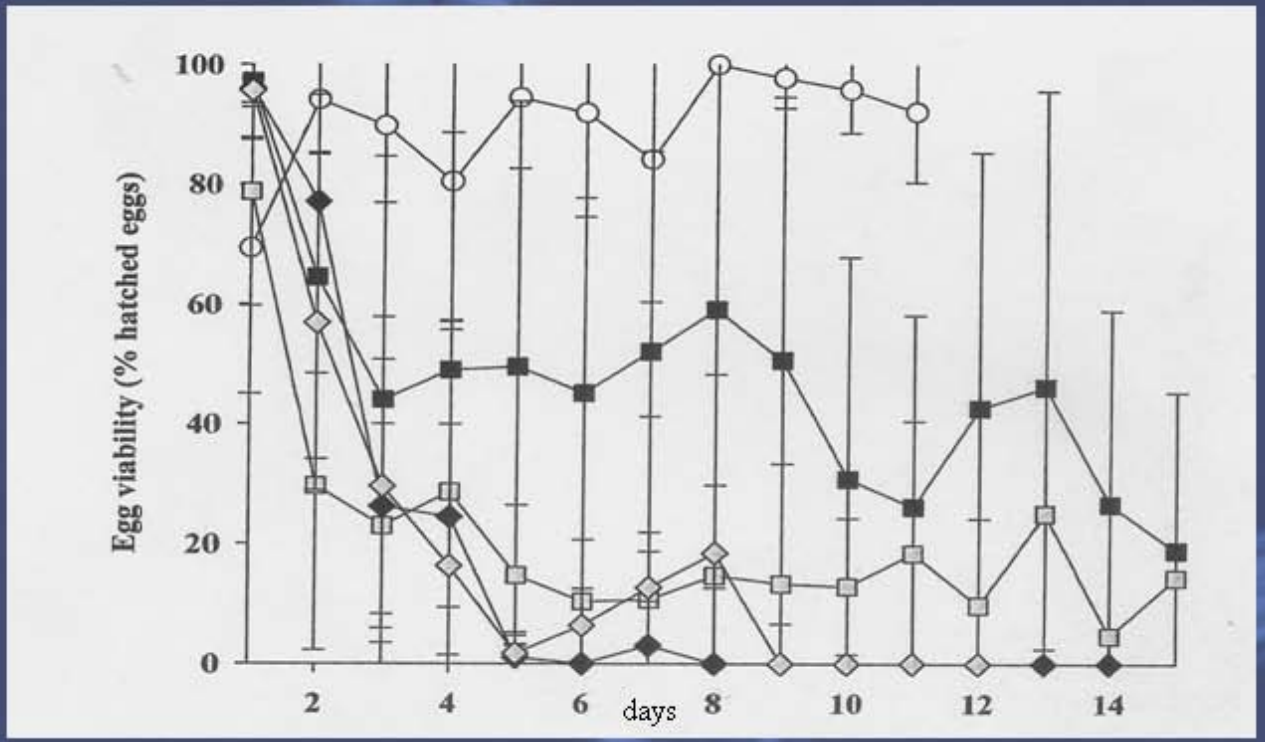


In response to feeding larvae of the Mediterranean climbing cutworm (*Spodoptera littoralis*), leaves of the lima bean (*Phaseolus lunatus*) produce oxylipins ..... Accordingly, the feeding insect experiences high local concentrations of oxylipins, which are taken up into the alimentary canal and are finally excreted with the feces.

Boland et al., 2007

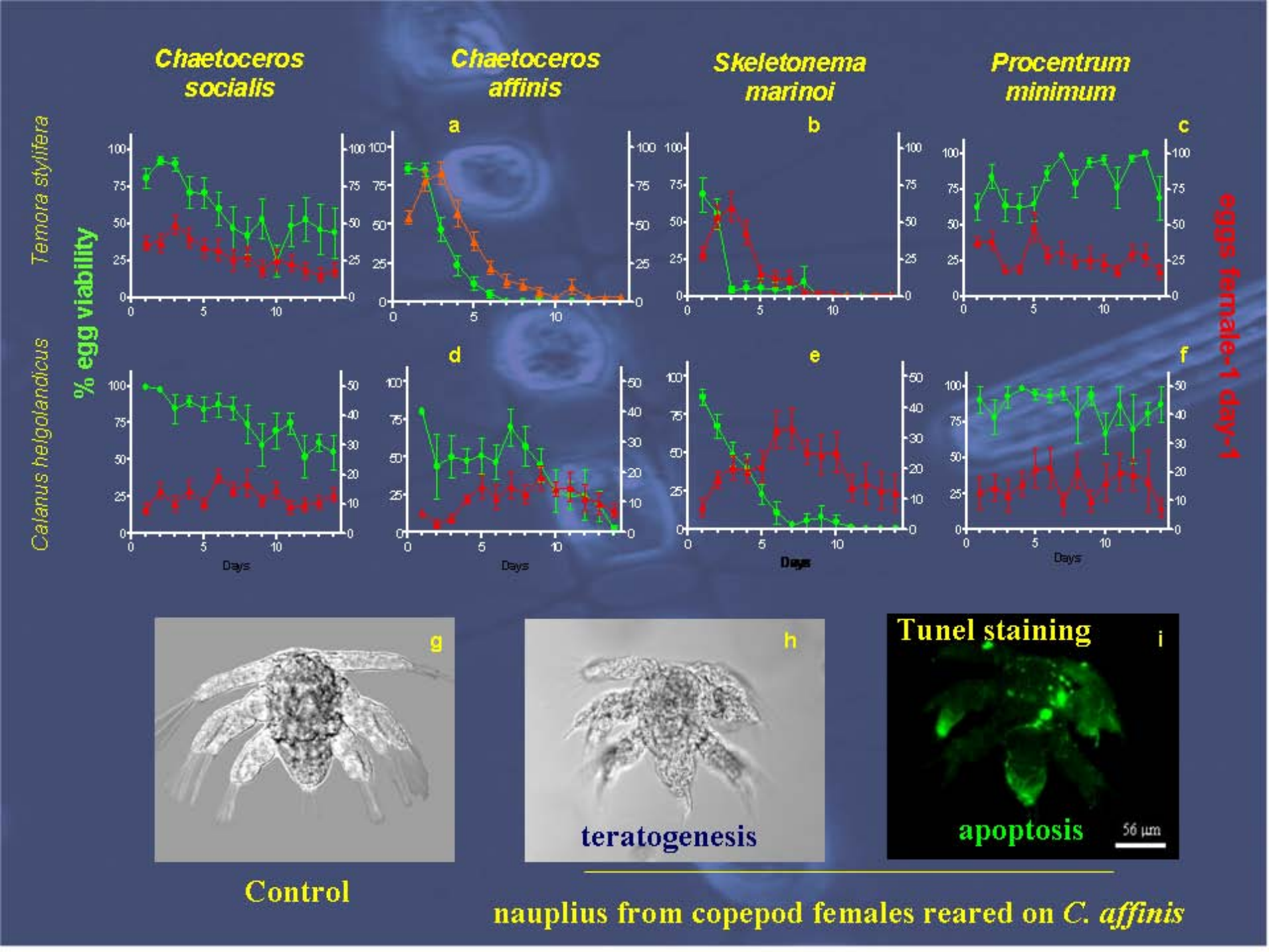
Diet-derived oxylipins in copepods

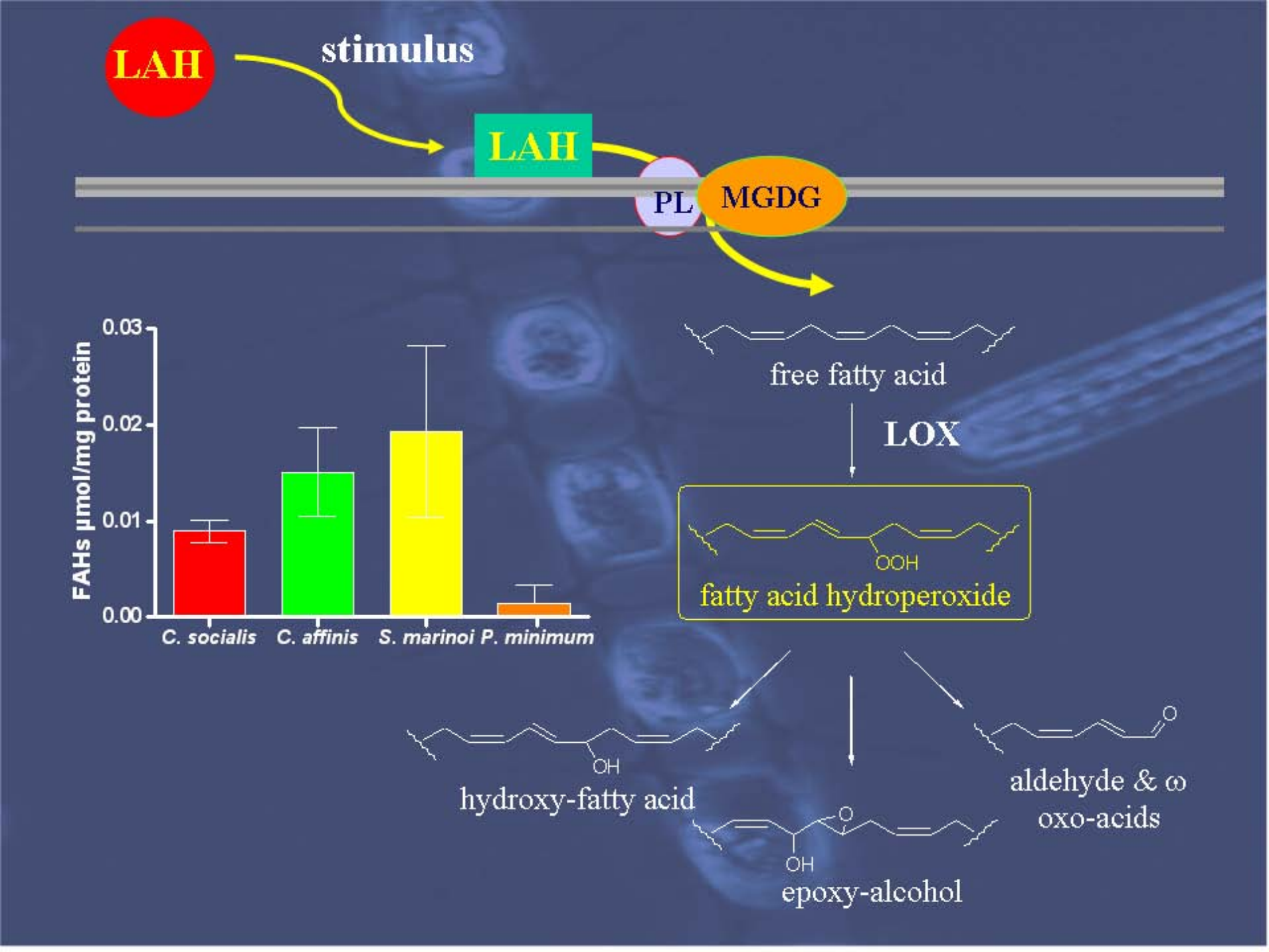


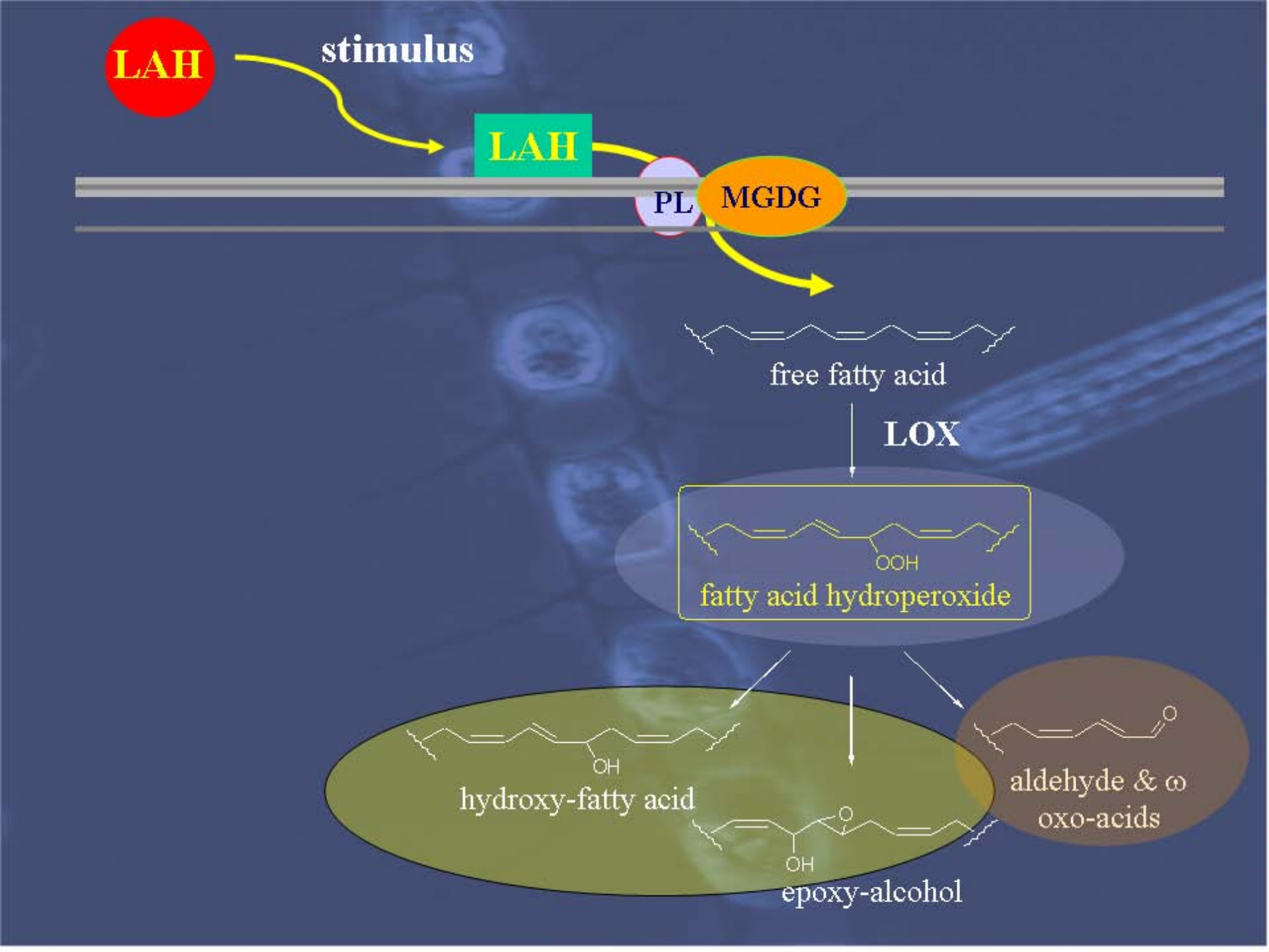


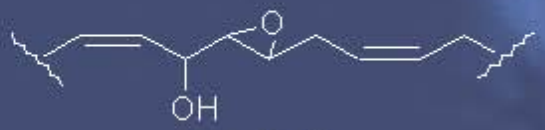
Species	phycoaldehydes	phycooxylipins
○ *P. minimum*	-	-
■ *T. rotula st#1*	+++	++
□ *T. rotula st#2*	+/-	++
◆ *S. costatum*	++	+++
◇ *S. pseudocostatum*	-	+++

Becerro et al., manuscript in preparation

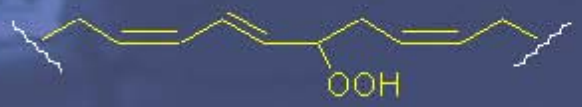

Chaetoceros socialis
Chaetoceros affinis
Skeletonema marinoi
Procentrum minimum
Temora stylifera
Calanus helgolandicus
% egg viability
eggs female-1 day-1
a
b
c
d
e
f
Days
g
h
teratogenesis
Tunel staining
i
apoptosis
56 µm
Control
nauplius from copepod females reared on C. affinis


LAH
stimulus
LAH
PL
MGDG
FAHs µmol/mg protein
0.03
0.02
0.01
0.00
C. socialis
C. affinis
S. marinoi
P. minimum
free fatty acid
LOX
OOH
fatty acid hydroperoxide
OH
hydroxy-fatty acid
aldehyde & ω
oxo-acids
O
OH
epoxy-alcohol


LAH
stimulus
LAH
PL
MGDG
free fatty acid
LOX
OOH
fatty acid hydroperoxide
OH
hydroxy-fatty acid
O
OH
epoxy-alcohol
O
aldehyde & ω
oxo-acids

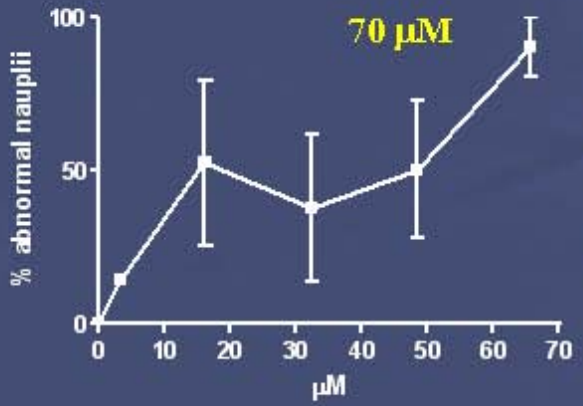


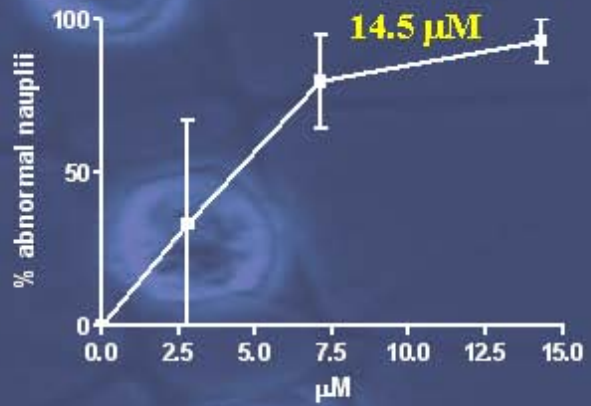
oxylipins

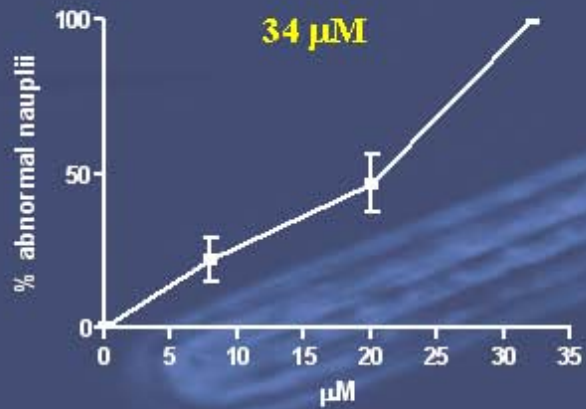


fatty acid hydroperoxides

aldehydes

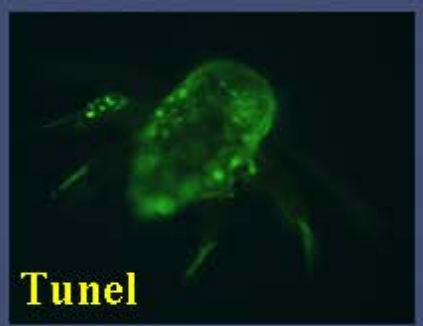


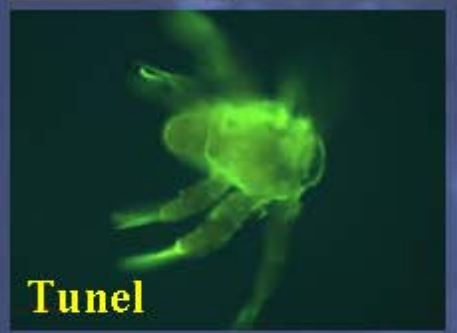




Teratogenesis

Apoptosis



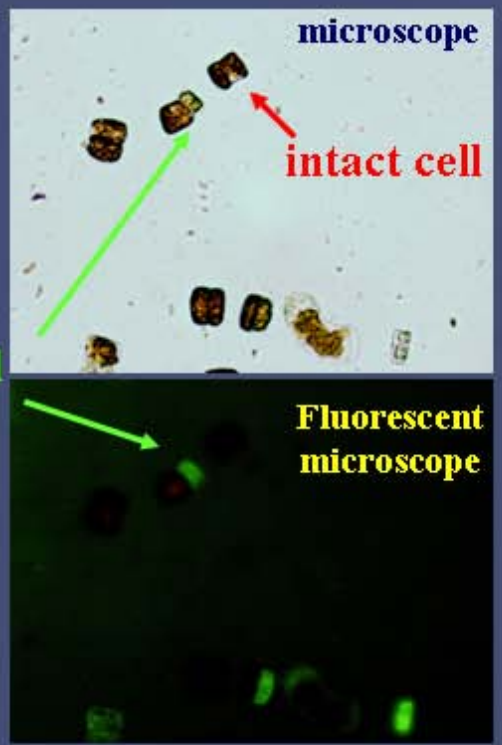


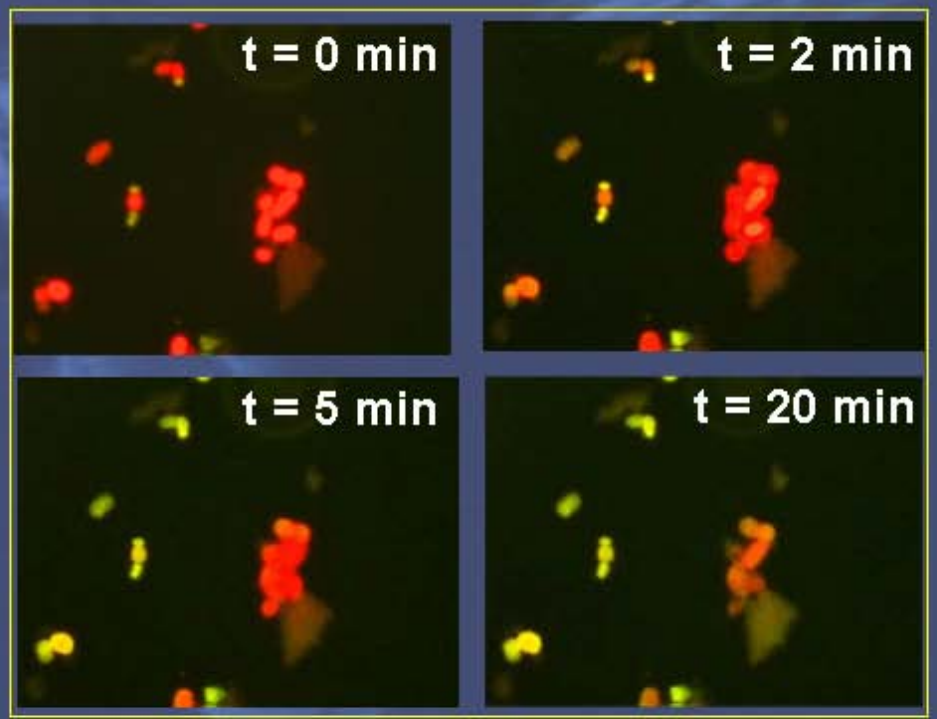
manuscript submitted

OXIDATIVE STRESS INDUCED BY LOX ACTIVITY

Green fluorescence

hROS (highly reactive oxygen species) induce apoptosis, DNA and protein damage

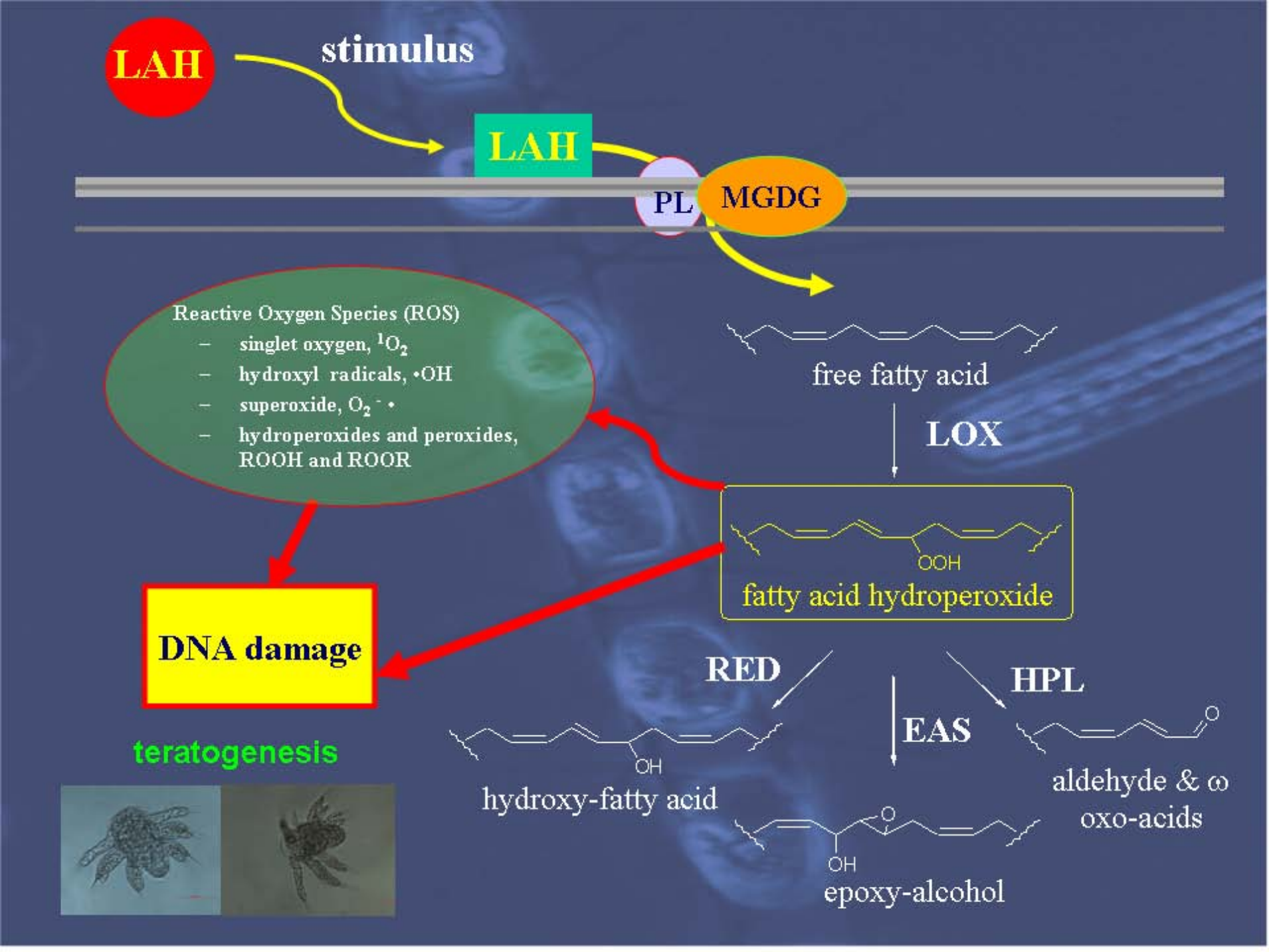




red = chlorophyll green = ROS

Conclusions

- Synthesis of phycoaldehydes is essentially due to decompartmentation and mixing of substrate (mostly, glycolipids) and enzymes;
- The process is triggered by a galactolipase activity (in addition to a phospholipase?) and requires lipoxygenase enzyme(s) showing high substrate affinity for chloroplast-derived C16- and C20-fatty acids;
- The process is dynamic and (in lab) lasts for several minutes - until glycolipids fuel the downstream oxygenase pathway;
- Presence of phycoaldehydes seems to be restricted to genera *Thalassiosira* and *Skeletonema;*
- Synthesis of aldehydes is however associated/complemented by production of other fatty acid derivatives (oxylipins), including the unstable fatty acid hydroperoxides (FAHs);
- Even in absence of aldehydes, some of these metabolites (e.g., FAHs) can mimic the toxic effect (apoptosis and teratogenesis) induced on copepods by diet rich in diatoms;
- LOX-dependent lipid peroxidation boosts oxidative burst, leading to synthesis of proapoptotic and teratatogenic chemical radicals.


LAH
stimulus
LAH
PL
MGDG
Reactive Oxygen Species (ROS)
– singlet oxygen, $^1O_2$
– hydroxyl radicals, •OH
– superoxide, O_2^{-} •
– hydroperoxides and peroxides, ROOH and ROOR
free fatty acid
LOX
OOH
fatty acid hydroperoxide
DNA damage
RED
HPL
EAS
OH
hydroxy-fatty acid
O
aldehyde & ω oxo-acids
O
OH
epoxy-alcohol
teratogenesis

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