

ROSEMEB

Role of Secondary Metabolites in Ecosystem Biodiversity

Adrianna Ianora and Raffaella Casotti



AIM: Integrate Activities on Chemical Ecology within the MarBEF NOE

Training Courses, Workshops, Sabbaticals

Other Activities (writing of position papers, exchange of information, data and samples)

List of Participants

- **Stazione Zoologica of Naples, Italy**
- **Roscoff Marine Laboratory, France**
- **Institute of Bio-Molecular Chemistry, Pozzuoli (Naples)**
- **Helgoland Marine Station (AWI), Germany**
- **Tjärnö Marine Laboratory, Sweden**
- **Centro Interdisciplinar de Investigação Marinha Ambiental(CIMAR)/
Instituto Nacional de Engenharia, Tecnologia e Inovação (INETI), Portugal**
- **Max-Planck-Institute for Marine Microbiology, Germany**
- **Villefranche Marine Station, France**

ROSEMEB Activities for 2005-2006

- Kick-off meeting in Ischia, Italy, 3- 4 November 2005
- Position paper “New Trends in Marine Chemical Ecology” by Ianora and 8 co-authors under revision for “Estuaries”
- List of relevant publications posted on MarBEF-ROSEMEB webpage
- Training course on “Bioassay Methods in Chemical Ecology”
9-14 September 2006 in Tjärnö, Sweden
- Exchange of samples
- Short sabbaticals



New reference list of relevant publications in chemical ecology

This is a list of published scientific papers, relevant to the general field of marine chemical ecology, meaning by this all fields of research dealing with secondary metabolites of marine organisms and their effects at the molecular, cellular, population, community, and ecosystem level. This list has several aims, from providing general reading to interested scientists, to offering a starting point for potential researchers in chemical ecology, to presenting supporting evidences from different models on the role of secondary metabolites in marine systems. It reflects the expertise of the participants in the MarBEF-ROSEMEB subproject, and is not intended to be exhaustive or final and will be updated as necessary.

MARBEF Advanced Course on
Chemical ecology and
bioassay methods

Marine Biological Laboratory
Tjärnö, Sweden, 9-14 September 2006

Course Coordinators

Adrianna Ianora
Henrik Pavia

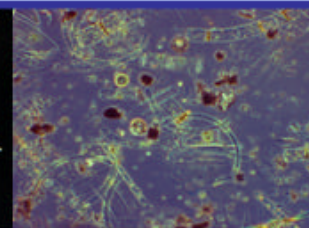
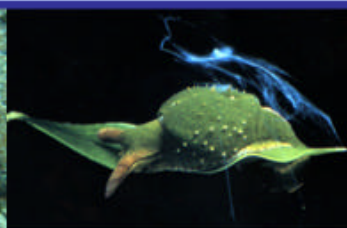
Scientific Board

Jens Harder
Raffaella Casotti
Adrianna Ianora
Gunilla Toth
Angelo Fontana

Faculty

Maarten Boersma (D)	Henrik Pavia (SE)
Raffaella Casotti (I)	Joe R. Pawlik (USA)
Angelo Fontana (I)	Philippe Potin (F)
Jens Harder (D)	Serge Poulet (F)
Mark E. Hay (USA)	Giovanna Romano (I)
Friederike Hoffmann (D)	Michael Steinke (UK)
Adrianna Ianora (I)	Gunilla Toth (SE)
Catherine Legrand (SE)	

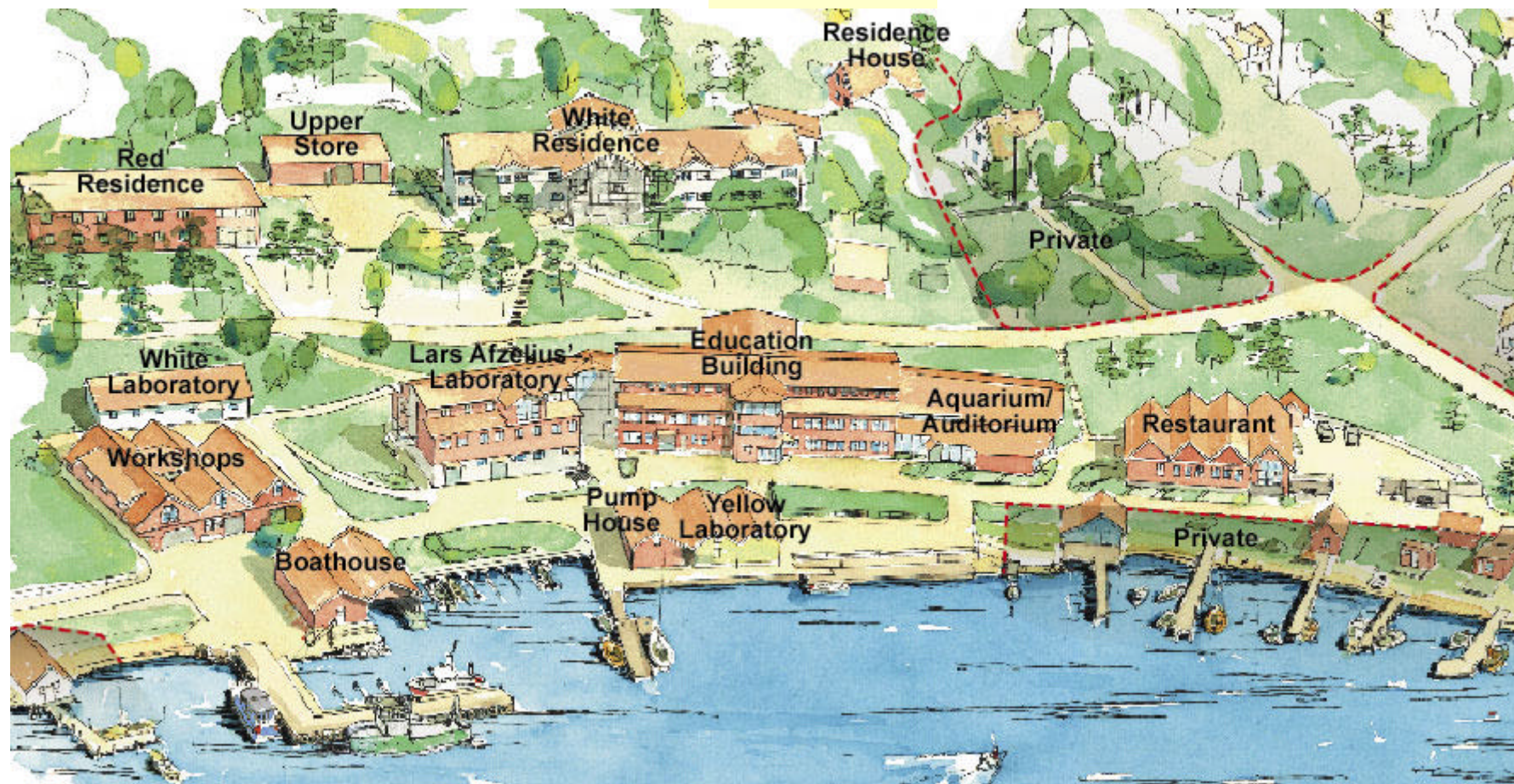
TOPICS: Chemical Ecology, Marine Natural Products, Ecotoxicology,
Allelochemicals, Bioassays of natural compounds, Chemical defense, Deterrence,
Trophic Interactions, Methods



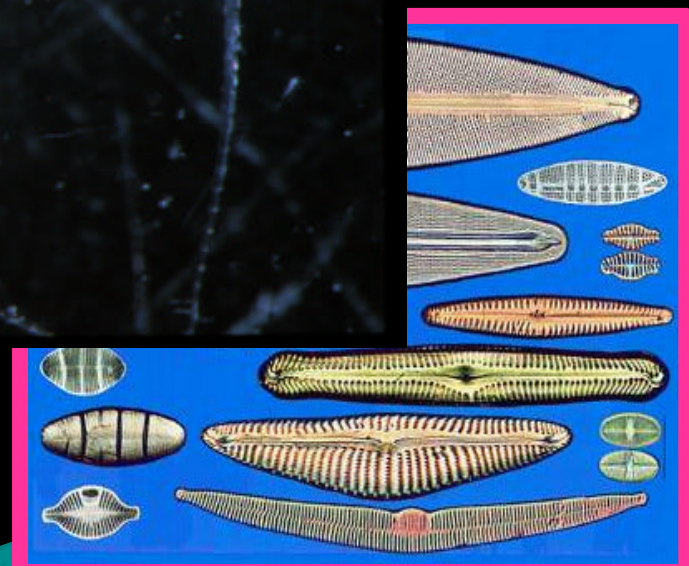
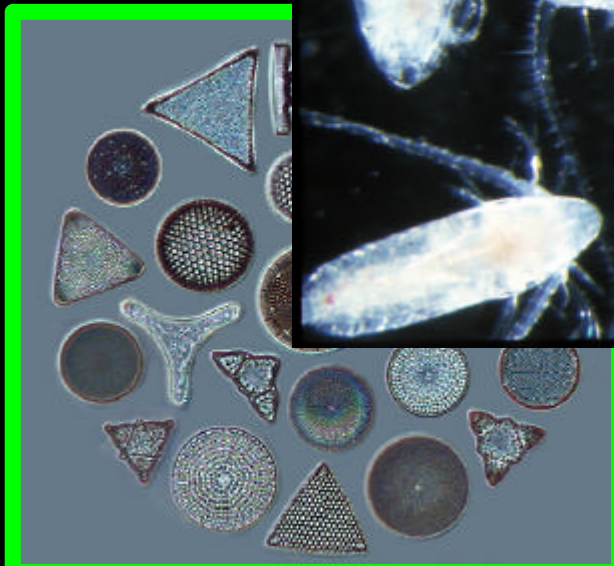
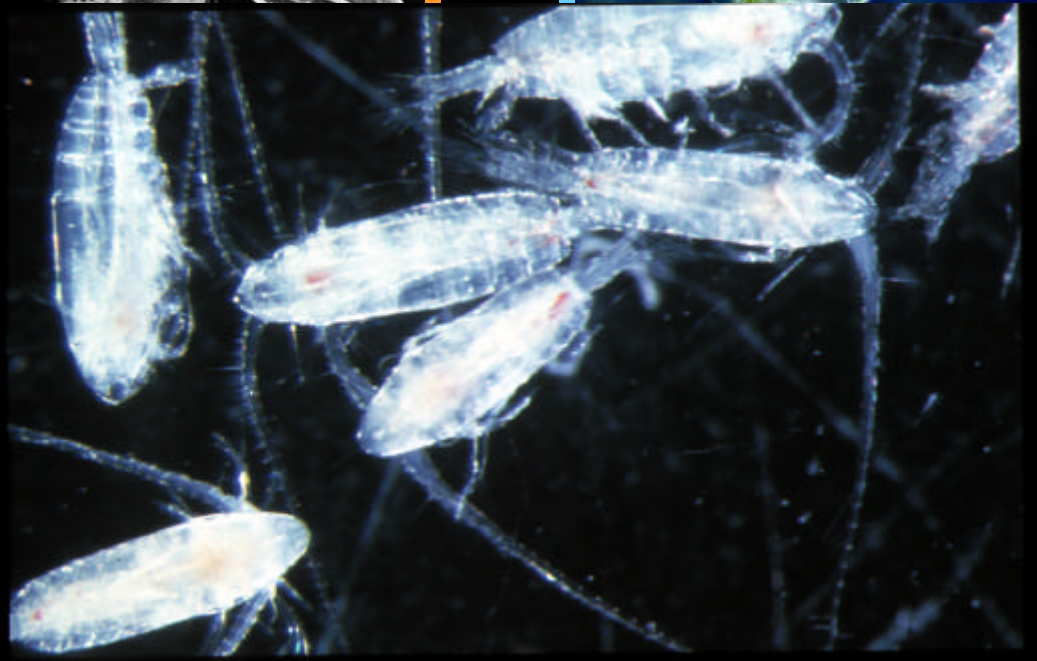
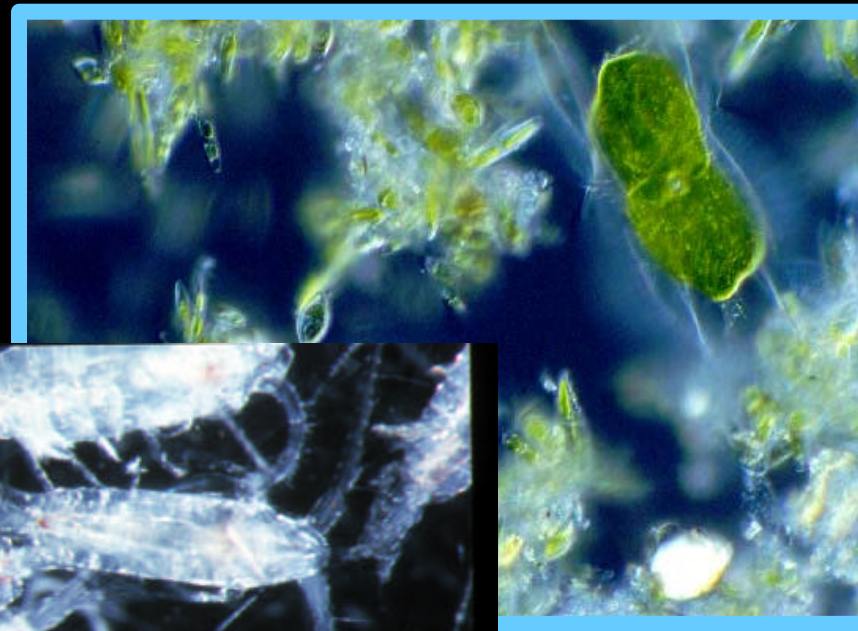
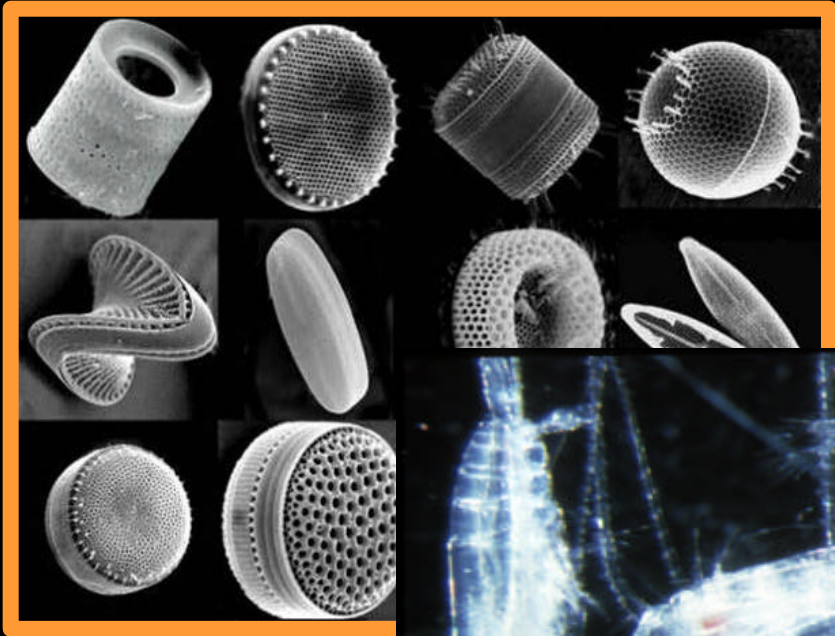
More info and application forms: www.marbef.org

Contact person: gunilla.toth@tmbl.gu.se

Deadline for application: June 30th, 2006



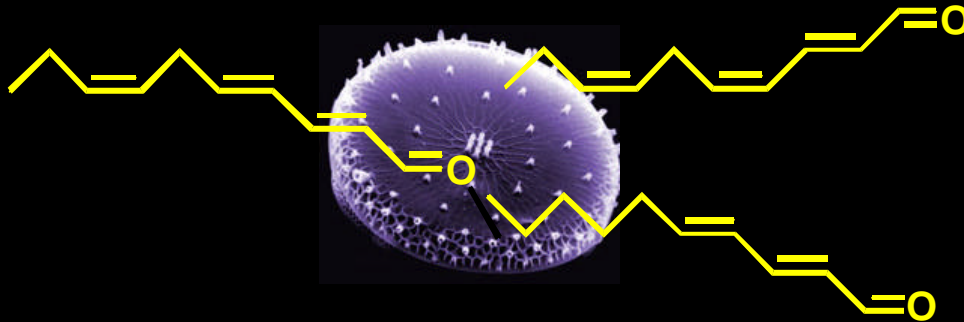
Diatom-Copepod Interactions



Cytotoxic PUAs are produced by *specific diatoms*

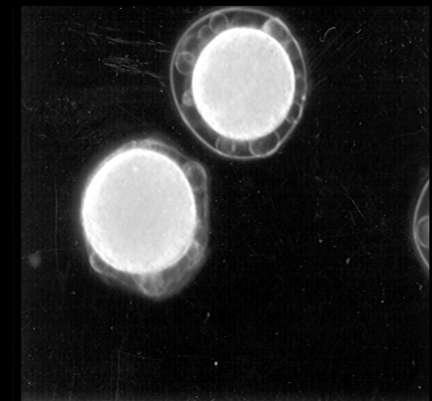
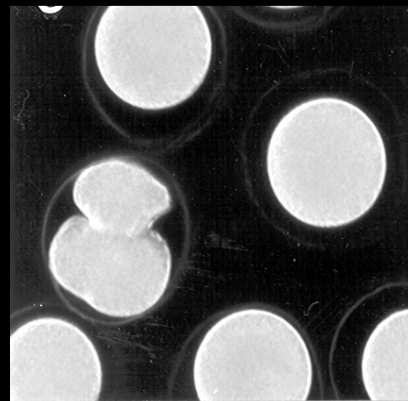
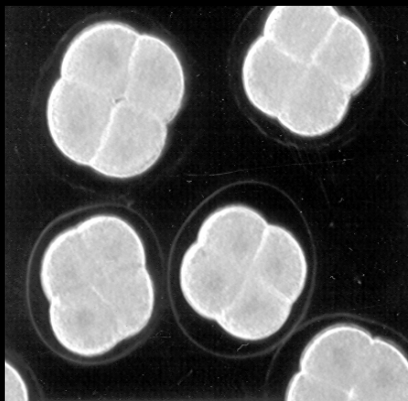
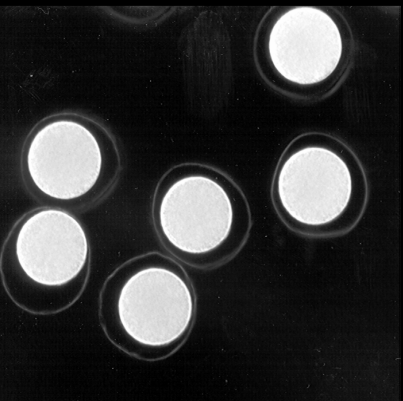
Aldehydes from *T. rotula*, and *S. costatum* have been shown to inhibit cell division in copepods and other invertebrates.

Miralto *et al.* Nature 1999
Ivanora *et al.* Nature 2004



Presence of PUAs in diatoms does not reduce the feeding activity of herbivorous copepods.

Bioassays with sea urchin eggs



Control t = 10 min

Control t = 4h

After addition
of decadienal
(1mg / l)

Toxic effects

Adolph *et al.*, 2004; Poulet 2005

Aldehyde suppression of copepod larval growth

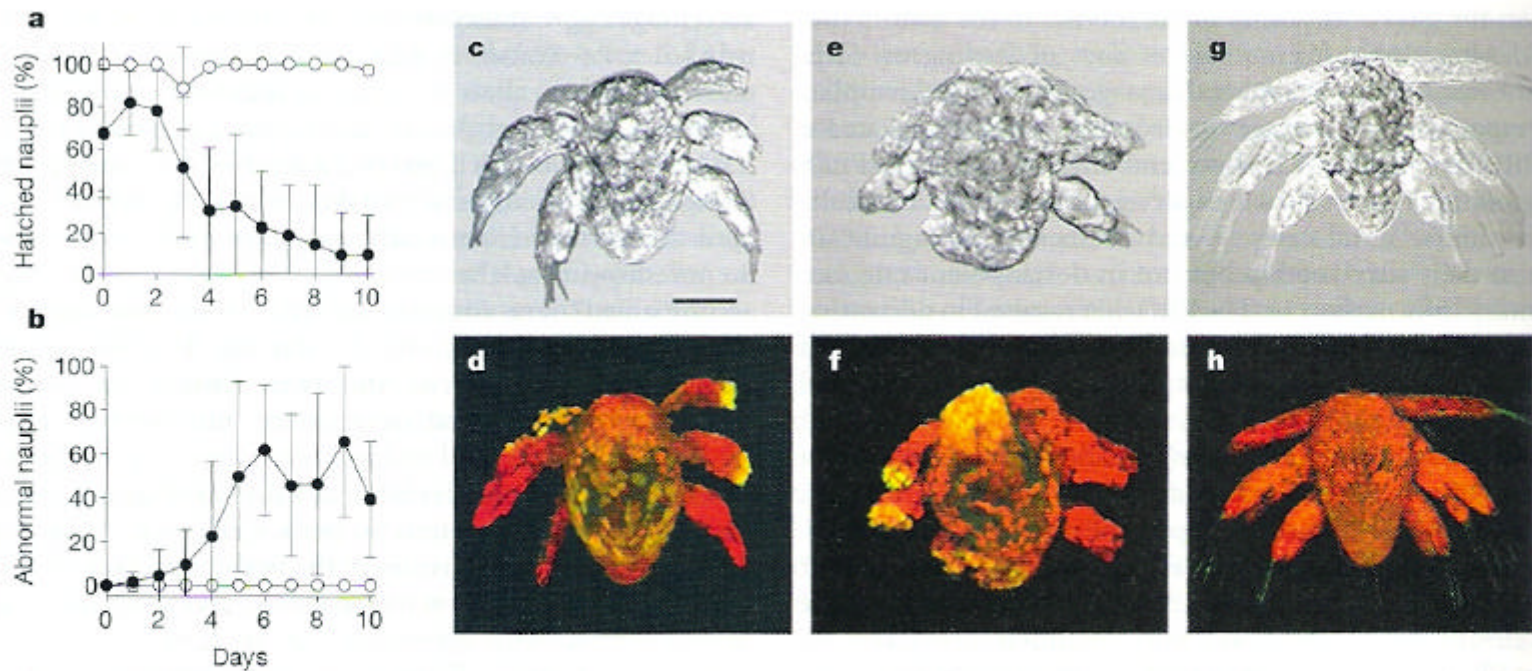
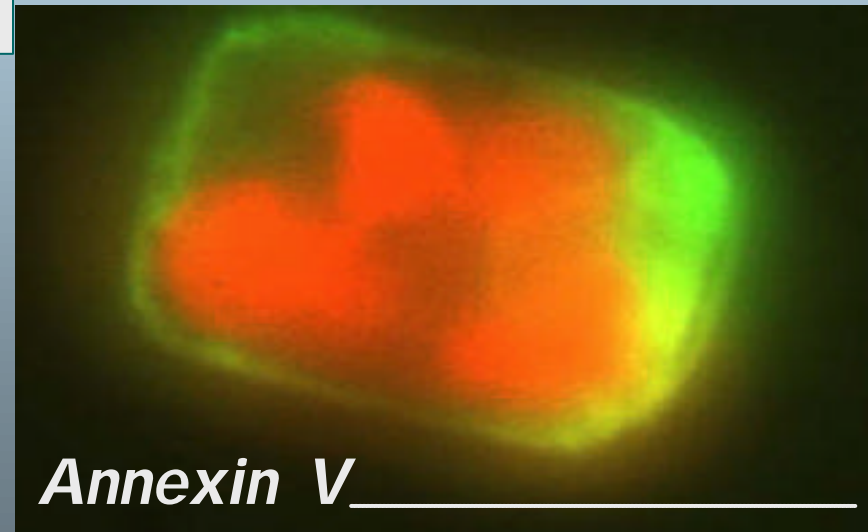
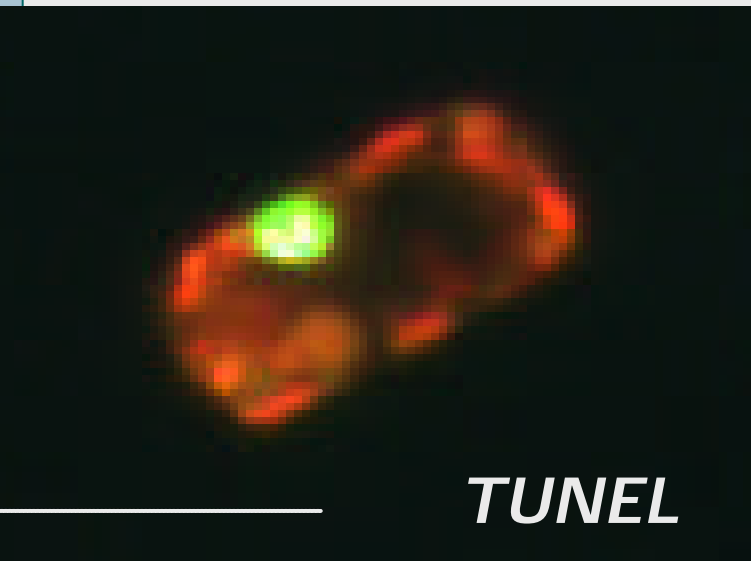
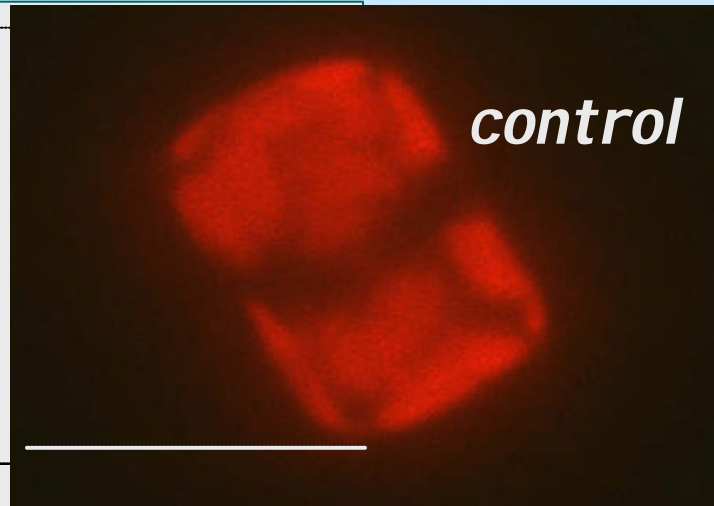
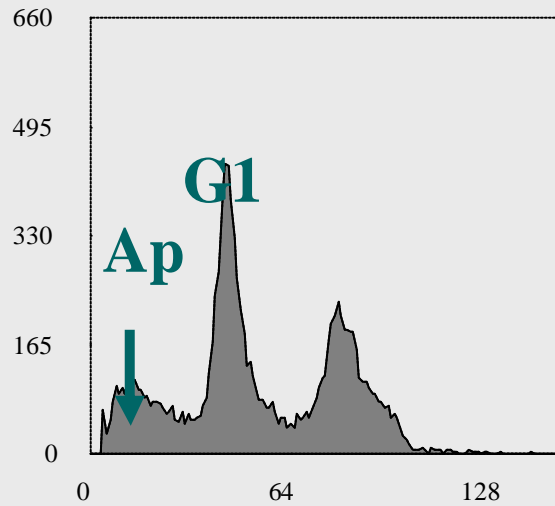


Figure 3 Effects of diet on *C. helgolandicus* offspring fitness. **a**, After ten days of feeding, the viability of eggs spawned by *C. helgolandicus* females fed the diatom *S. costatum* SKE (filled circles) dropped to <20% compared with >95% with the control dinoflagellate *P. minimum* PRO (open circles). **b**, After five days of feeding on SKE, 45–65% of the hatched nauplii were abnormal. **c**, **d**, Such nauplii had deformed limbs that were positive

for TUNEL staining (yellow, **d**) specific for apoptosis. **e**, **f**, After nine days of feeding on SKE, the degree of teratogenesis increased and nauplii were strongly deformed. **g**, **h**, Nauplii generated from females fed the control PRO diet appeared normal and stained negatively with TUNEL (**h**), indicating that nuclei were not apoptotic. Scale bar: 90 μ m.

Effects of unsaturated aldehydes on diatoms

DNA





Effect of three algal aldehydes on several strains of marine bacteria



Francois Ribalet¹, Laurent Intertaglia², Raffaella Casotti¹, and Philippe Lebaron²

¹ Station Zoologica A. Cochin & Napoli, Italy, ² Observatoire Océanologique de Banyuls-sur-mer, France

*ribalet@ozn.it

Introduction

Several species of diatoms are able to produce different unsaturated aldehydes, such as heptadecenal, octadecenal and docosadecenal (Richard et al. 2005). The antifouling properties of these unsaturated aldehydes are reported for many different biological models, including sponges (Janora et al., 2003), ascidians (Dahl et al. 2003), tunicates (Dietz et al. 1998), echinoderms (Romano et al. 2003), polychaete embryos (Cachet et al. 2003) and sponges (Cachet et al. 2003). It has been recently shown that these unsaturated aldehydes are also toxic to phytoplankton of different taxonomical groups including diatoms (Casotti et al. 2005, Ribalet et al. in preparation). Cytotoxicity of diatom-derived aldehydes has also been demonstrated for non-marine bacteria (Adolph et al. 2004). In order to test if diatom-derived aldehydes are toxic on marine bacteria as well, we have used cultures of 13 bacterial species, well represented in marine areas, and followed their growth after exposure to different concentrations of heptadecenal, octadecenal and docosadecenal.

The experiments have been performed in the framework of the Research Vessel MARPLAN, as a collaborative action between the Station Zoologica A. Cochin & Napoli and the Observatoire Océanologique de Banyuls-sur-mer. This work has also been in the objectives of two other MARBLE Research Vessel Projects, ROSEMBE (Role of secondary metabolites in driving ecosystem functionality and maintaining ecosystem biodiversity) and "Biodiversity diversity and ecosystem functions: concepts, open questions and recommendations for integration of models into general ecological frameworks".

Materials and Methods

12 marine and one non-marine bacterial cultures, belonging to six different phyla, were grown in marine broth medium at room temperature in darkness. Exponentially growing cultures (10⁷-10⁸ cells mL⁻¹) were exposed to different concentrations of aldehydes (from 13 to 145 µM). The cell density was followed every 3 hours by measuring optical density using a micro reader (Fig 1). Working solutions of three aldehydes (docosadecenal, octadecenal and heptadecenal) were prepared by diluting the stock in absolute methanol.

Results

Species	code	Docosadecenal µM	Octadecenal µM	Heptadecenal µM
<i>Flavobacter divinis</i>	FLA00106	13	18	18
<i>Flavobacter putrescentis</i>	FLA00107	13	18	18
<i>Roseobacter maris</i>	ROSE00108	13	18	18
<i>Alphaproteobacteria maris</i>	ALP00109	26	36	36
<i>Cytophaster citreus</i>	CYT00110	26	36	36
<i>Bacillus aquimaris</i>	BAC00111	53	129	145
<i>Enterococcus faecalis*</i>	ENT00112	3	4	4
<i>Alphaproteobacteria maris</i>	ALP00113	26	36	36
<i>Sulfobacter putrescentis</i>	SUL00114	No response	No response	No response
<i>Alphaproteobacteria maris</i>	ALP00115	No response	No response	No response
<i>Alphaproteobacteria maris</i>	ALP00116	No response	No response	No response
<i>Oligo maris</i>	OLI00117	No response	No response	No response
<i>Flavobacter sp.</i>	FLA00118	No response	No response	No response

Table 1. List of the bacterial strains used in the study and the aldehyde concentrations inducing a response on cell density (compared to the control that induces a reduction in cell growth, upon an increase in the cell growth and grey indicates no effect relative to the control). Species names of the same color belong to the same phylum. * non marine bacteria

Conclusions

Exposure to the tested aldehydes induced different responses according to the species. Reduced growth rates were observed for the three Alpha-proteobacteria (*Roseobacter* and *Cytophaster citreus*), for *Alphaproteobacteria maris*, and for the Gamma-proteobacteria, *Bacillus aquimaris*. Also the non marine bacteria *Enterococcus faecalis* showed sensitivity to the aldehydes and resulted to be the most sensitive of all strains used.

No increase of the maximum cell density in presence of the aldehydes was observed in *Alphaproteobacteria maris*, suggesting that this strain might use aldehydes as a carbon source.

A remarkable resistance to aldehydes was shown by the two Cytophaga-Flavobacterium-Bacteroides *Oligo maris* and *Flavobacter* sp. and the two *Alphaproteobacteria* genera (*Gamma-proteobacteria* phylum), *A. stellipolius* and *A. maris* with no effect with concentrations up to 145 µM.

The response to aldehydes is variable within each phylum, indicating that sensitivity to aldehydes is not linked to taxonomy. Indeed, it appears to be linked to other parameters, probably physiological, morphological and ecological that need to be further investigated.

These results indicate that secondary metabolites of diatoms such as polyunsaturated aldehydes may play a role in shaping bacterial community composition and functioning, for example during blooms, representing selection effectors and/or ruling competition and resource utilization among species.

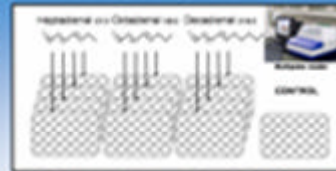


Figure 1. Experimental design used in the study. Each 24 well plate contained 12 marine bacterial strains in duplicate. 4 different concentrations for each aldehyde have been tested.

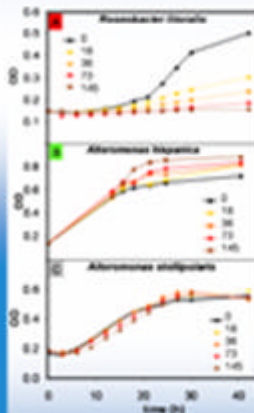
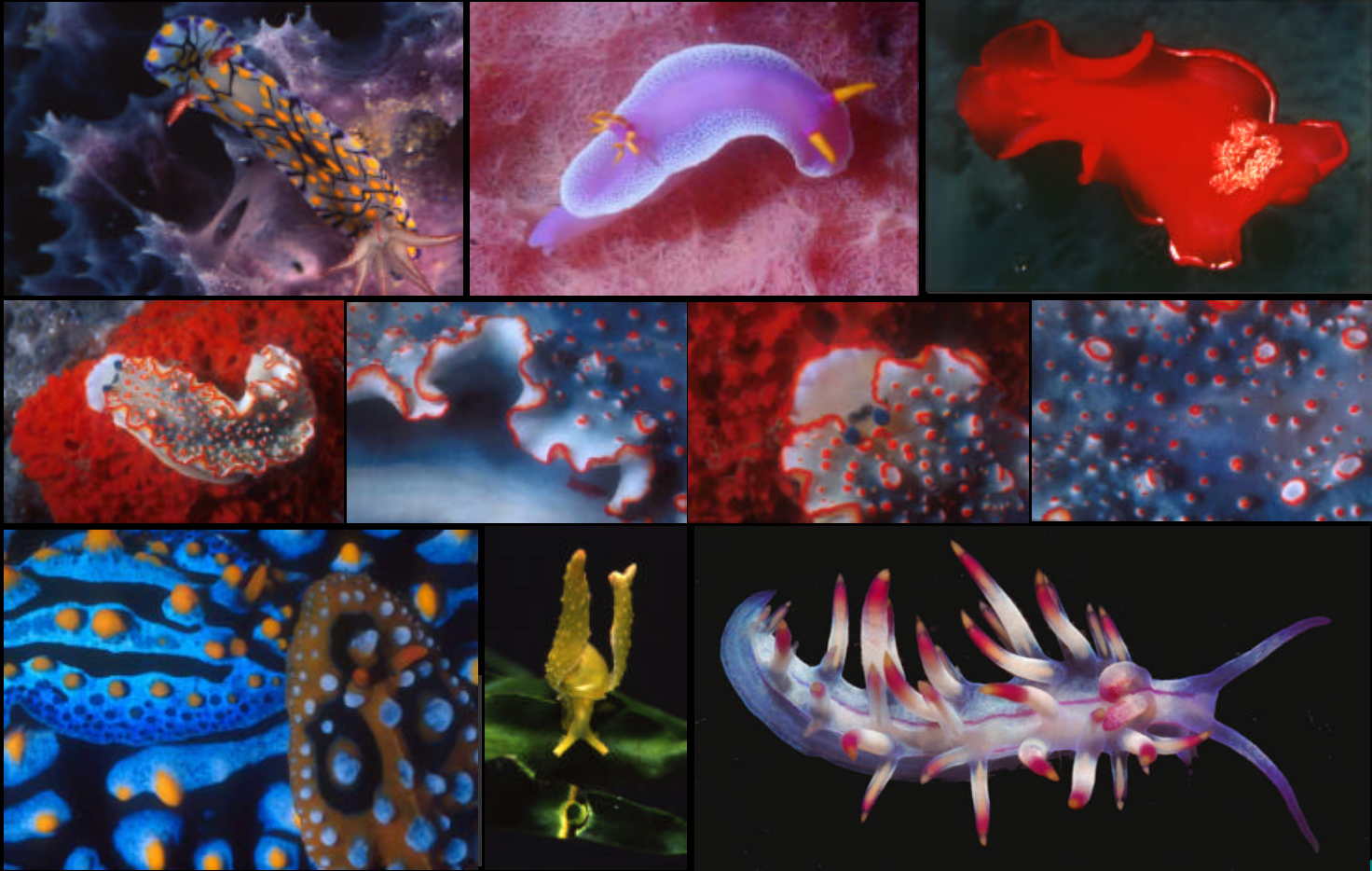


Figure 2

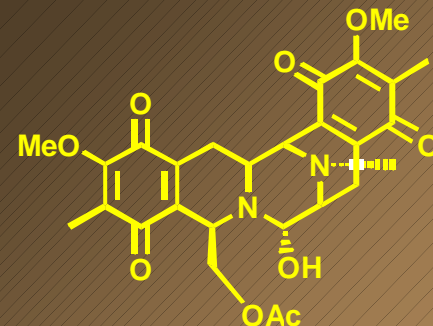
Overview of the effect of aldehyde concentration on bacterial cell density. (A) heptadecenal induced a negative effect on the cell growth of *Roseobacter divinis*, including cell growth at concentrations as low as 13 µM. (B) heptadecenal induced the growth of *Alphaproteobacteria maris* at concentrations as low as 36 µM. (C) heptadecenal had no effect on growth of *Alphaproteobacteria maris*, with respect to the control.

Repulsive for potential predators



Attractive for marine chemists

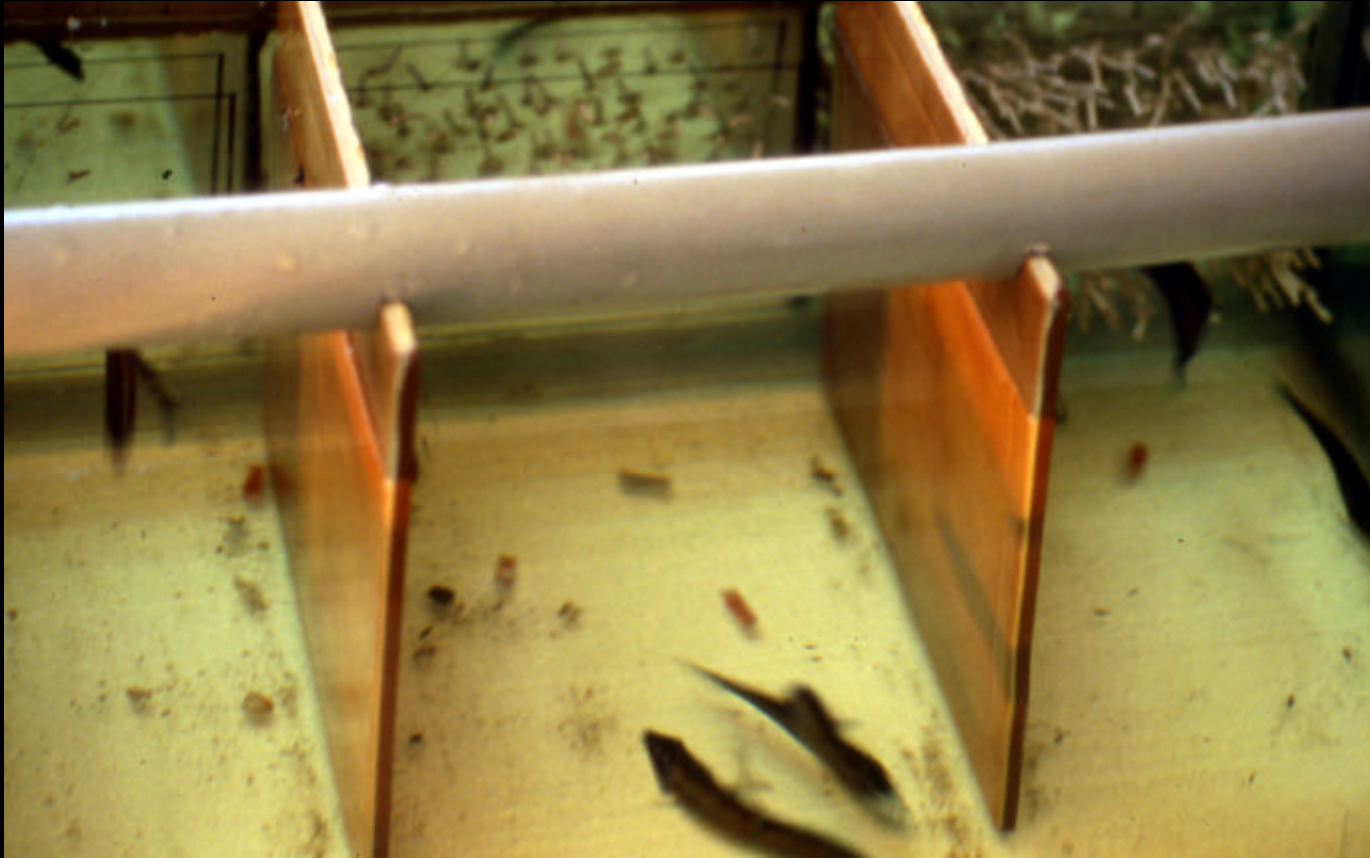
Zalypsis®



jorumycin

- Zalypsis® (PM00104/50) is PharmaMar's first marine derived compound that has begun Phase I clinical trials for the treatment of solid tumours.
- Zalypsis® is a novel chemical entity related to the marine natural compounds Jorumycin and the family of Renieramycins, obtained from molluscs and sponges, respectively.

Ecological activity: feeding deterrency of marine opisthobranchs



Fontana et al. 2005

Summary:

to compare the biological activity of secondary metabolites and distinguish those that act as allelochemicals and growth inhibitors, or induce antifouling, feeding deterrence, toxicity and death

to differentiate between the effects induced due to nutritional deficiency and from those induced by toxins and growth inhibitors

to study the biochemical pathways and the biochemical control that underlie synthesis of secondary metabolites

to exchange samples for chemical and biological screening among network members

to intercalibrate methods