Report of the kick-off meeting of the RMP ROSEMEB (The ROle of SEcondary Metabolites in Ecosystem Biodiversity)

Ischia, Italy, 3 and 4 November 2005.

The meeting started in the morning of 3 November with the greetings of Dr. Cristina Gambi, who, on behalf of the Coordinator of the Benthos Ecology Laboratory of the Stazione Zoologica "A. Dohrn" (SZN), Dr. Cristina Buia, presented the Laboratory and its activities and welcomed the participants to the Laboratory. 18 people were present, including researchers from the SZN who were invited to listen to the presentations. Only representatives from LOV were absent. The first day was dedicated to scientific presentations, to give the audience the possibility to present and discuss the scientific research expertise present within the ROSEMEB consortium with the aim of integrating and building up potential collaborations. The following day was restricted to active ROSEMEB members and was dedicated to the organization and practical issues related to the deliverables of the RMP.

In the morning of November 3, **Adrianna Ianora** from the SZN, Italy, presented the aims and the organization of ROSEMEB, highlighting the main activities for the duration of the program.
She then gave a talk on the paradox of diatom-copepods interactions and on the studies being conducted at the Ecophysiology Laboratory of the SZN on this topic. This laboratory has pioneered studies on the effect of diatoms as food for copepods, together with Serge Poulet and co-workers of the Roscoff Marine Station in France, showing that diatoms negatively impact copepod reproduction, by affecting egg viability and reducing offsprimg fitness and survivorship to adulthood. In 1999, they published a paper (Miralto et al. 1999, Nature 402:173-176), where these effects were shown to occur during a *Skeletonema* bloom in the Northern Adriatic Sea; the molecules responsible for these effects were identifiedas the unsaturated aldehydes 2 trans, 4 trans, decadienal, 2 trans, 4 trans, 7 cis decatrienal and 2 trans, 4 cis, 7 cis decatrienal. A later paper (Ianora et al. 2004, Nature 429: 403-407) showed that the insidious effect of aldehydes does not deter the herbivore from feeding but impairs its recruitment and restrains the cohort size of the next generation.
Serge Poulet from Station Biologique of Roscoff, France, presented more recent results on the effects of polyunsaturated aldehydes on copepod reproduction. He highlighted the high variability in aldehyde production among diatom species and even strains of the same species. He pointed out the need for further investigations on the effects of natural diatom assemblages to assess the impact of aldehydes as well as other secondary metabolites on copepod reproduction in situ.
After the coffee break, **Raffaella Casotti** from the SZN presented recent data on the effect of polyunsaturated aldehydes on diatoms and on other phytoplankton. Aldehydes have been shown to affect growth and viability of diatoms and to trigger a putative cell death mechanism closely resembling apoptosis. The role of aldehydes may not be limited to toxicity, but there is preliminary evidence that these compounds act as infochemicals within the same diatom population, using NO and cytosolic calcium as signal

transduction effectors. She then presented preliminary results showing that aldehydes also affect other phytoplankton species, and that species differ in their sensitivity to these metabolites. In situ data during the North Adriatic *Skeletonema* bloom were also presented, where lysis rates were related to percentages of apoptotic cells in natural samples, as detected by the TUNEL assay. Higher lysis rates were estimated at the bloom peak phase, calling for a possible release of aldehydes (as well as other secondary metabolites) in the seawater following bloom decline. Hypotheses are advanced that such release may have an allelopathic effect on other phytoplankton present in the seawater.
Giuliana D'Ippolito from the SZN and IBC of Pozzuoli, Italy, presented the biosynthetic pathway of production of diatom-derived unsaturated aldehydes, highlighting the steps and putative precursor molecules involved. In addition, she presented evidence of new bioactive compounds resulting from enzymatic transformation of fatty acids after disruption of cell integrity and contact of the enzymes with their substrates.
Valerio Zupo from the SZN presented the aims and structure of a recently funded EC program, Pharmapox. This project aims at investigating the chemistry, pharmacology and bioactivity of novel apoptotic compounds as sex regulators in decapods. He presented the case study of Hippolyte, for which diet has a strong influence in sex determination and even sex reversal. He related these effects to apoptogenic molecules present in benthic diatoms. Aldehydes isolated from planktonic diatoms, however, had no effect on sex reversal, and was shown to have only a toxic effect on decapods.
After lunch in a nearby restaurant, the presentations resumed with **Maarten Boersma** from AWI, Helgoland, Germany, who presented the main structure of his institute, and the main lines of research that fit the aims of ROSEMEB. Within the Chemical Interactions department of AWI, different studies are conducted on a) the role and the effects of toxicants on marine mammals and their immunological responses, b) toxic substances in jellyfish, their chemical characterization and production, c) flatfish and their environment as indicators of stress and pollution, d) marine protists and their signaling substances. For the marine protists the research is aimed at identifying the producers, the compounds released, and their role (as deterrents, or in competition). Such substances are not only toxic, but can be used as well as infochemicals, or kairomones. He then presented some results on the effect of food quality in the link between algae and their predators, and discussed how variations in food quality may be due to environmental factors such as temperature. The perspective of this line of research would be, among others, to link food quality and aldehyde production in diatoms as food for zooplankton.
Gunilla Toth from TML in Tjarno, Sweden, presented the research activities conducted at her institute in the field of chemical ecology. Their research line is aimed at investigating chemical interactions in benthic macroalgae and macroinvertebrates. Gunilla showed the facilities available at TML, which will host the first ROSEMEB course on methods in chemical ecology to be held in September 2006. Tjarno is located along a coastal fjord, which is connected to the open sea, and it hosts the first marine national park in Sweden. Despite the proximity with the sea, the tidal range is limited. The laboratory has several boats of different dimensions for coastal sampling and it hosts a series of mesocosm facilities mainly used for ecosystem simulations in summer. The laboratory has basic chemistry equipment and the possibility of collecting and culturing several benthic and planktonic organisms. , Gunilla also presented the main lines of

research being developed at the institute. The first concerns herbivore defense mechanisms, which includes the study of fluorotannins as grazing deterrents, the role of invasive species and the biological reasons for their success in colonizing new environments. Antifouling compounds produced by sponges to avoid colonization by bacteria were also discussed, as well as the infochemistry of toxic dinoflagellates, and tritrophic interactions and their chemical mediators.

Helena Gaspar from CIMAR, Portugal, is a new addition to the ROSEMEB consortium, supported by IBC of Pozzuoli, Italy. The aim of her research is to study the chemical ecology of invertebrates, in a comparative perspective between Atlantic and Mediterranean species. Target species belong to Porifera and the chemical compounds released by these animals and their interaction with nudibranchs which are their natural predators. In particular, she showed evidence that compounds such as haminols, work as alarm pheromones and that these compounds are the same as those isolated from Mediterranean populations of nudibranchs.

Frederike Hoffman from MPIMM of Bremen, Germany, after a short overview of her institute and its structure, presented her research on sponge-microbe interactions. Sponges are a rich source of secondary metabolites which may also act as antifouling and antimicrobial agents. However, sponges and bacteria interact very strongly and there is a complex chemically-mediated communication among them so to optimize food utilization and production of useful substances. In fact, sponges produce waste molecules which are metabolized by bacteria, and bacteria may produce compounds necessary for the survival of the sponge. The cooperation among cells in the sponge is also regulated by chemical cues, mainly directed toward healing in the case of injury or defense against infections.

Ernesto Mollo from IBC of Pozzuoli, Italy, presented the results of his group in the chemical ecology of invasive species. His target species are mainly nudibranchs, which are very rich sources for toxic substances that are used as warning signals for potential predators. The aim of his research is to isolate the molecules involved in this defense mechanism, identify the origin of these metabolites and the mechanism of their bioaccumulation, biotransformation and biosynthesis. He then described the case of invasive species identified in a Greek bay, related to lessepsian migrations, highlighting how some compounds had a dietary origin, deriving from seagrasses upon which the animals were feeding at the time of collection.

Raffaella Raniello from SZN, Italy, presented her recent results on the physiological plasticity of the invasive species *Caulerpa racemosa* and its allelopathic interactions with *Cymodocea nodosa* These chemical characteristics are under investigation and may explain the high success of this invasive species in the Mediterranean Sea.

Angelo Fontana from IBC, Pozzuoli, Italy concluded this series of presentations by presenting the research conducted at his institute on the chemistry of natural products from marine organisms. He presented several cases of chemical compounds extracted from marine organisms which present potentials for clinical applications as antitumour agents. First observations on fish behaviour suggested the presence of chemical cues as feeding deterrents in mollusks. Also the aposomatic coloration of some animals is related to warning signals to potential predators. In some cases these also originate from biotransformation of dietary metabolites. Some of these compounds hold similarities with the prostaglandins in humans. He also outlined the importance of investigating the

biosynthetic pathways involved and characterizing the enzymes in the synthesis of ecological mediators. He presented results on the chemical mechanisms of production of wound-induced defenses in marine diatoms, and suggested that mechanisms other than mechanical disruption of cell integrity may be responsible for the chemical triggering of aldehyde formation. In addition, he presented evidence that alternative compounds, other than aldehydes, may be produced by diatoms during blooms.

On the second day, November 4th, several practical aspects on the general activities of ROSEMEB were discussed.
Adrianna Ianora outlined the structure of the RMP and of MARBEF in general and gave clarifications on the budget allocation.
The discussion continued on the organization of the first ROSEMEB event, which is a Course on Chemical Ecology and Bioassay Methods, to be held in September 2006 in Tjarno, Sweden. The attendants agreed on the general structure of the course and on a list of potential speakers to be invited. This list will use the expertise present within MARBEF; but a certain number of external speakers will be invited, based upon the available budget and the costs of accommodation. Gunilla Toth from TML will provide precise figures about the costs at TML in the near future. The course will target about 20 graduate students and young researchers to be trained in chemical ecology concepts and practical issues. It will provide presentations on selected topics and will last 6 days. In the morning, speakers will be asked to give presentations on recent issues related to chemical ecology , while afternoon presentations will be dedicated to more practical aspects and to the description of protocols. When possible, simple experiments will be set up in the laboratory.

The participants then agreed to a series of additional activities, among which, another course on “Isolation and Characterization Methods in Marine Natural Product Chemistry”, that Angelo Fontana has agreed to organize in Pozzuoli, Italy, at the beginning of September 2007. This course will be open to 25 people approx. and will be open to MARBEF and non-MARBEF members.

The LOV of Villefranche, France, had agreed in the past to host the final workshop in 2008 in Villefranche on Advances in Chemical Ecology, among Rosemeb participants. However, since no representative of LOV was present, this will be discussed separately by the Coordinator and notified to the other participants.

Other activities include exchanges within institutes based on short-term collaborations, students’ stages. A potential list of exchanges include SZN and AWI (Casotti and Boersma), ICB-CIMAR (Fontana-Gaspar), ICB-MPIMM (Fontana-Hoffmann), MPIMM-TML (Hoffmann-Toth), SZN-SBR (Ianora-Poulet). The participants will report on these exchanges and their output(s) to the Coordinator.

Fontana, Gaspar and Ianora agreed to provide samples to other institutes belonging to the group.

Casotti will coordinate the redaction of a list of relevant publications in chemical ecology to be listed on the ROSEMEB website as reference material for interested readers. The participants agreed to provide this list of publications pertinent to their own area by January 2006, and the list should be made available on the ROSEMEB webpage in the first months of 2006.

The ROSEMEB consortium will also join Ianora's effort in writing an invited essay for the journal Estuary on "The role of organismal chemical interactions in estuarine and coastal processes". Participants agreed on the general structure of the paper and agreed to provide their own written section by the end of November. The essay should be published at the beginning of 2006.

Most of the participants formally agreed to provide the presentations of the previous day to be made available on the ROSEMEB webpage, within the MARBEF website.

The meeting ended at lunchtime of November 4th.