MICROBIAL OCEANOGRAPHY

Ecology and Diversity of marine Microorganisms (ECODIM VII) of ASI

January 8 - 28, 2012

at the Estación Costera de Investigaciones Marinas (ECIM) of the Pontificia Universidad Católica de Chile (PUC) at Las Cruces, Chile,

Course Report

15 students, 10 full time and part time instructors and 6 symposium speakers from 7 countries (Chile, Argentina, Uruguay, Brazil, Colombia, Cuba, France, USA, Spain, The Netherlands and Switzerland) participated in the ECODIM course this year. The course is part of the International Graduate Course Series in Oceanography offered by the Austral Summer Institute of the University of Concepcion, Chile. Since the "home" of the earlier courses in Dichato was destroyed by the 2010 Tsunami, the 2012 ECODIM course took place at the Estación Costera de Investigaciones Marinas (ECIM) of the Pontificia Universidad Católica de Chile in Las Cruces, central Chile.

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ECIM of PUC at Las Cruces: A well-suited place for an intensive advanced course, well designed for research and learning in a beautiful environment high above the ocean.

Course Announcement & Organization

The advanced course on the ECOLOGY AND DIVERSITY OF MARINE MICROORGANISMS (ECODIM) was offered for the seventh time as part of the International Postgraduate Course Program in Oceanography, organized by the Universidad de Concepción. For the first time, the course took place at the Marine Station of the Pontificia Universidad Católica de Chile in Las Cruces (ECIM). The course was announced in May 2011 on a course poster that was sent to academic institutions world-wide and on the Internet under the addresses http://www.profc.udec.cl/ecodim/ and http://www.isme-microbes.org/events/meetings/past (Appendix 1). Relevant course information was also provided at the address: http://www.microeco.unizh.ch/chile/chile.html. The latter site will continue to be available for students with the intention of establishing lasting interactions among ECODIM alumni across geographical and institutional borders and to promote collaborations in the fields of microbial ecology in oceanography.

The course was organized by the 12th Austral Summer Institute of the Departamento de Oceanografía, Universidad de Concepción (UdeC). Since it will still take a few years to rebuild the Dichato station, which was destroyed by the 2010 Tsunami, we were offered to make the course at the Estación Costera de Investigaciones Marinas (ECIM) in Las Cruces, a well suited and beautifully located marine station of the Pontificia Universidad Católica de Chile. The Las Cruces marine station has excellent laboratory and lecturing facilities for student courses.

The course was directed by the team of instructors listed in Appendix 2. Organizational details are provided in Appendix 3. The course was held under the auspices of the Universidad de Concepción and supported through access to research facilities at ECIM by the Faculty of Biological Sciences of the Pontificial Catholic University of Chile. Funding for the course and the minisymposium was provided by the Agouron Institute, the Gordon and Betty Moore Foundation, the Graduate School of UdeC and the Faculty of Biological Sciences of PUC.

Making an excellent ZEISS microscope available free of charge and for the entire duration oft he course by the Reichmann company, and the use of a qPCR machine offered by ROCHE Chile Ltda. contributed significantly to the success of the course.

Salt evaporation ponds (i.e. salterns) in Cahuil were one of the study sites of this year's course

Course Participants and Staff

The 2012 course was attended by 15 students from 12 different academic departments in 7 Latin American and European countries (Argentina, Brazil, Chile, Colombia, Cuba, Uruguay and France). The course was open to graduate students and interested professionals. 15 students from a total of 48 applicants were selected on a competitive basis. In addition to the participants listed in Appendix 4 we received applications from from Peru, Croatia, Spain, El Salvador, Pakistan and the United States. The list with the full names of the selected participants, their home institution and their research interests are given in Appendices 4 and 5.

The course was taught by instructors from Chile, France, the USA and Switzerland. Speakers for the course symposium and for special lectures came from Chile, Spain, The Netherlands and France (Appendix 6). Most symposium speakers spent one extra day with the course and got involved in seminar-type activities with the students. The course instructors stayed with the course for 8 to 22 days.

Course Description and Structure

The main goal of ECODIM courses is building intellectual capacity and to transfer knowledge in areas of growing scientific interest in the marine biosciences. This is achieved by teaching concepts and providing an overview of the fields of microbial ecology, marine genomics and diversity of microorganisms. This year's course brought together various aspects of environmental, molecular and microbiological, as well as chemical, physical and bioinformatics methodologies needed to approach questions of biogeochemical, ecological, phylogenetic and genomic interests.

The most efficient training: Learning from each other in the field, in the laboratory and in the lecture hall

The course comprised field experiences, lectures and exercises in the morning, guided research work in the laboratory and tutored computer exercises in the afternoon, colloquia in the evening and two symposium days, one by invited speakers, the other organized by the students themselves. Preparatory discussions, exercises on particular course subjects, introduction to bioinformatics, computer aided thermodynamics in geochemical processes and metabolism were offered as exercises in small groups. With the didactic mix between lectures and talks by experts, workshops with turorials and the students' own efforts (paper presentations, experimental design, practical laboratory work), participants were introduced into topics from the research front in microbial oceanography. Students were also asked to suggest special topics and thus to define their particular needs.

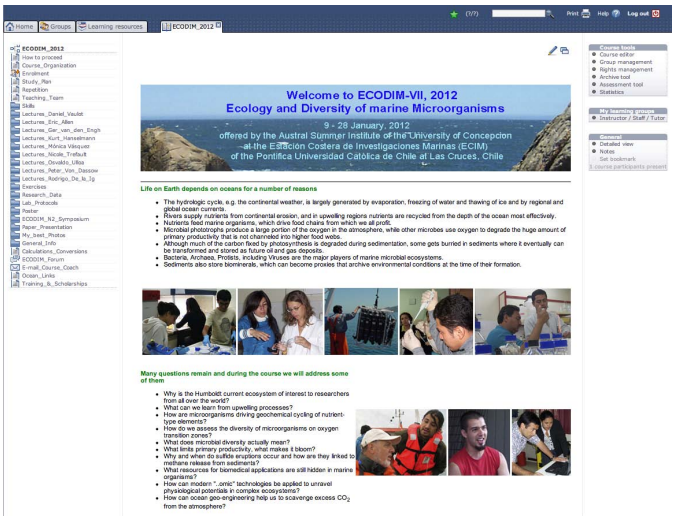
During the first week, the students introduced themselves with a short presentation about the research topics that are carried out at their "home institution" and their personal research interests. This activity provided to the participants and to the staff an overview of the background knowledge of each student.

In the field, we determined a number of environmental parameters of the sampling sites, collected fresh inocula for culturing of halophiles, anoxigenic phototrophs and cyanobacteria and for flow-cytometric analyses and biomass for DNA extraction. On a cruise to neaby coastal water sampling stations, some course participants gained first-hand experience of CTD measurement and water and sediment sampling and observation techniques of coastal and shelf environments. In addition, high salt environments were chosen this year as habitats to be investigated in detail. All students were exposed to these marine habitats and collected samples for the enrichment of microbes and the extraction and isolation of DNA. With the aid of molecular techniques, they investigated the presence of major genes and examined the morphological and functional diversity of microbial communities using DAPI staining and advanced epifluorescence microscopy and flow-cytometry. For the first time in the history of ECODIM, we made an attempt to define the genomic potential of a hypersaline habitat employing pyrosequencing of the entire DNA extracted from the planktonic microbial community.

An interactive website (right) for the exchange of course materials, lecture notes, papers, exercises etc. was created for this year's course on OLAT. It will remain active for a few months after the course has ended.

The laboratory part was designed to educate students in current techniques and to encourage independent research. Students carried out investigations in groups and independently with the aid of faculty and teaching assistants. The course culminated with the student project presentations whose results were summarized in posters available as pdf on the internet page of the ECODIM-VII course under http://www.microeco.unizh.ch/chile/chile.html and http://www.profc.udec.cl/ecodim/ .

With the paper presentations, students were trained to search the published literature via the internet, to screen and select the proper information, to quickly review the papers, to understand and extract key elements, present them to others in a didactically logical way and to be able to respond to questions relating to the topic.



Course Contents

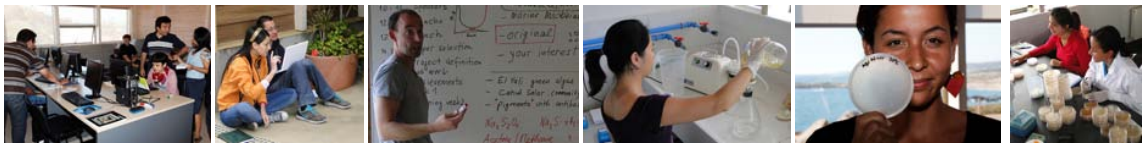
ECODIM-VII focused on 4 main topics with the following contents:

Microbial Ecology: Field observations and collection of environmental determinants (metadata); nutrient and energetic constraints in ocean habitats; molecular evolution of biogeochemical processes (N-, S-cycling); microscopic and cytofluorometric analyses of population changes; techniques for growing and isolating microorganisms.

Diversity: Molecular and microscopic methods for the identification of OTUs and microorganisms from all three domains of life and viruses, pigment analyses; collection of DNA and its sequence analyses; detection and description of genotypic diversity; studying phylogenetic relationships and constructing trees based on statistical clustering techniques.

Population genetics: Structure, size and dynamics of microbial populations; gene flow.

Genomics: High throughput sequencing approaches; bioinformatics, data analyses and annotating genomic information; database searches for full genome and metagenome comparison.



Only hard work leads to success

Short introductions to the particular conditions in hypersaline salars and in oxygen minimum zone (OMZ) along the continental shelf of South America and in parts of the eastern South Pacific during the first few days were followed by a one day cruise to collect samples from the water column and the sediments. Another two field days were devoted to sampling at the Cahuil salterns and the El Yali salt ponds.

Introductory lectures and practical exercises emphasized the chemical, phylogenetic, metagenomic and energetic basics of marine microbial ecology, photosynthesis and ocean biogeochemical cycling, as well as the molecular and culturing methodologies, microscopy and flow cytometry for the study of the molecular ecology of microorganisms in ocean waters and sediments. The 2012 course addressed close to 30 topics in lectures, workshops and minisymposia (for details see Appendices 7-9).

Computer labs were designed to familiarize students with the most common data bases available for phylogenetic and metagenomic analyses. The tutorials focused on the design and validation of nucleic acid probes and the application of bio-thermodynamic models to examine metabolic processes in geochemical cycles.

The lectures were delivered by the course instructors (Appendix 5). The goal of all lectures was to illustrate how basic concepts in microbial ecology and chemistry can be applied to a number of questions relating to microbial oceanography. Additional speakers were invited to deliver special lectures during the mini-symposium in Santiago on January 13 (Appendices 7 & 8). All lectures and the details of the course activities are outlined in the weekly schedules (Appendix 7). Most speakers made their slides available for personal use by the course participants.

Places visited for Field Work

This year, the main course topics dealt with the richness of microbes and their metabolic activities in the near shore waters of the South Pacific Ocean (33°23'S; 71°45'W) and in high salt environments of the Cahuil salars (34°28'41"S 72°1'6"W) and the El Yali salt ponds (33° 44'S; 71°39'W). This allowed us to study the regulation effects of salt concentrations on community selection.

Solar salterns consist of a series of shallow, interlinked ponds through which seawater flows, becoming gradually concentrated in salt until NaCl is precipitated. Gypsum and carbonates precipitate during the first stages and, finally, when seawater has evaporated to about 1/10th of the original volume, NaCl crystallizes and can be harvested.

Important ecological changes occur through this gradient: decreasing biodiversity with increasing salinity, and high density of prokaryotic cells and short food chains. Each pond contains characteristic microorganisms that are well adapted to live in increasingly higher salt concentrations. The color of hypersaline ponds are caused by different types of red, orange or purple pigments present in halophilic microorganisms.

Successful research demands making proper decisions in the field

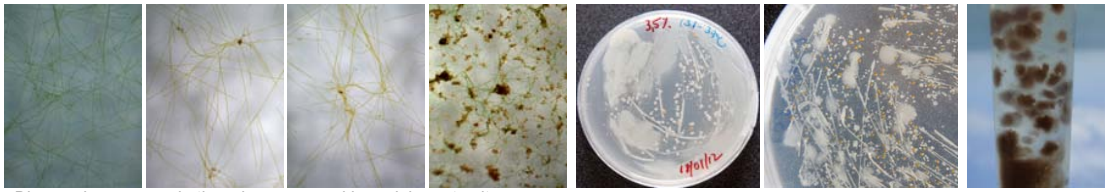
Course Research

The research part in the lab focused on growing phototrophic and heterotrophic Bacteria, Archaea and Viruses from the above mentioned ecosystems, isolating DNA for molecular analyses and metagenome pyrosequencing, microscopy, flow-cytometry, characterizing pigments by UV-Vis spectroscopy and elucidating the regulation of growth in microcosm experiments. It was the goal to study the trophic and organismic interactions, pigment change and filament length variation of cyanobacteria and the role microbes play in the geochemical cyling of matter in the particular marine environments. The techniques were instructed to small groups in rotations. Molecular, flow-cytometric and culturing techniques were applied in particular for the study of the diversity and abundances of marine prokaryotic and eukaryotic microbes applying 16S DGGE analysis for bacteria, cyanobacteria, archaea and eukarya. The techniques were applied to small research projects, and their range of applicability and the limitations were studied by four student research groups. Course participants gained experience in the collection and preservation of samples in the field, they were trained in designing experiments, including the use of controls and replication, in maintaining cultures of microbes and in the analysis and interpretation of results using phylogenetic methods as well as biosystem modelling with the aid of databases and in bio-thermodynamics applied to the understanding of microbially mediated processes in biogeochemical cycles.

A first group explored the changes in bacterial and archaeal diversity and abundances in different ponds expressing increasing salt concentrations up to the point of halite precipitation in the Cahuil solar salterns. They employed DNA extraction, 16S rRNA DGGE analysis,

Microscopy, Pigment extraction in Acetone / Methanol, Culturing and Flow Cytometry. Although microbial diversity and chlorophyll containing microorganisms decrease along the salt gradient of the saltern ponds, the same Archaea populations seem to exist in ponds with salinities above about 10% (w/w).

With another student group, we investigated the distribution of oxygenic and anoxygenic phototrophic microorganisms in layered hypersaline microbial mats and the metabolic, behavioral and ecological strategies they employ. For this project we used selective enrichment and exposure to salt stress, epifluorescence microscopy, pigment extraction combined with UV-VIS spectroscopy, cytofluorometry and 16S rRNA DGGE analysis. We found that stress-related conditions may generate physiological changes aimed at optimizing photosynthesis. The pigment patterns from mat organisms point to different adaptation strategies for photosynthesis under high salt concentrations, e.g. cyanobacteria tend to loose the ability to synthesize active chlorophylls and grow in shorter filaments. Some populations (presented as OTUs) of the mat community can exchange between different zones and some are common under very different salt concentrations.



Discoveries as seen in the micoscope and in enrichment cultures

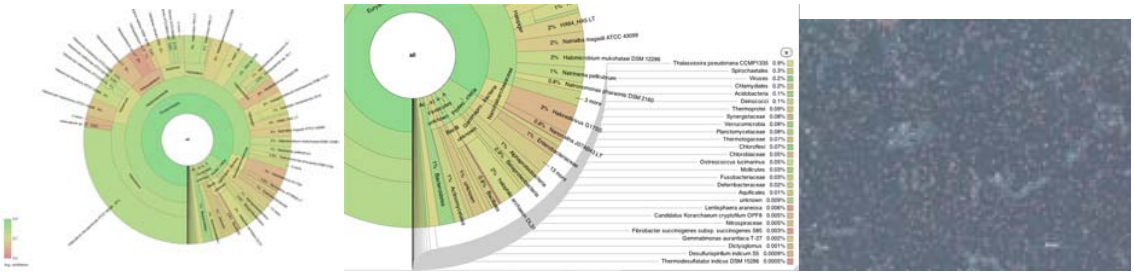
Although we were originally skeptical that the group setting out to find viruses in hypersaline environments would succeeded, they did. They tried to prove the hypothesis that there must be viruses in hypersaline environments that can infect and lyse Bacteria/Archaea isolated form the same location. The importance of viruses in ecosystems lies in the regulation of microbial community composition through highly specific host-virus interactions. Although most of the abundant extremely halophilic Archaea and Bacteria can be cultivated, according to the literature, no viruses were isolated so far that could infect them. The group obtained a morphologically diverse collection of Bacteria/Archaea from low NaCl-containing solid media. Isolation of more extreme halophiles would require several weeks of incubation, which was outside of the scope of the course. Viral lytic infections (plaque development) in a bacterial and possibly in an archaeal strain were observed. Since they detected different plaque morphologies (clear vs. turbid-bordered plaques) they could infer that more than one viral strain lysed the hosts.

The forth group investigated the role of the light regime on the picoplankton community in El Quisco Bay. They defined the *in situ* conditions by measuring temperature, salinity and fluorescence as a function of depth and counted the number of picoeukaryotes, *Synechococcus sp.* and total bacteria. These data were used to set up four microcosm tanks, which were kept at a constant temperature inside a water tank and shaded to reduce the natural light intensities from 100% to almost complete darkness. They then followed population changes by flow cytometry and epifluorescence microscopy and found that the picophytoeukaryotes seem to be most sensitive to radiation changes, and that communities kept in a low light regime showed higher fluorescence, probably due to an increase in pigment formation as a response to the limiting radiation.

The results of the student projects are summarized in posters, which are available as appendices 13, 14, 15 and 16.

- Detection of Viruses in a hypersaline Environment (A13)
- Photosynthetic Strategies in a hypersaline Microbial Mat (A14)
- Microbial Diversity and Pigment Succession along the Cahuil Saltern Ponds (A15)
- The Effect of Light Regimes on Picoplankton Communities from El Quisco Bay (A16)

In addition, we investigated the microbiota of a hypersaline, NaCl-saturated crystallizer pond at the same site using pyrosequencing. Analyses of the metagenome (nearly 117 Mb) revealed the dominance of *Halorubrum spp.* and other *Haloarchaea*, but also a number of probably novel Euryarchaeota groups and, with lower abundances, a great diversity of halotolerant or halophilic bacteria. Since the analysis of the sequence data is still in progress, the results were not yet included in the posters. We are searching for key metabolic genes and markers for haloviruses.



Our first metagenome from the Cahuil salar

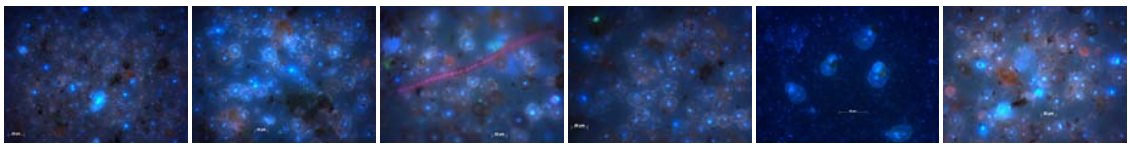
Juan Ugalde, an ECODIM alumnus, and now a PhD student in marine biology / bioinformatics at UCSD is helping us to analyze the pyrosequencing data of the Cahuil Salar. So far, we have found that the Euryarchaeota are the dominant Phylum; 80% of all Euryarchaeota belong to the family Halobacteriaceae. *Halorubrum spp.* dominate with 29% of all Halobacteriaceae, *Halorubrum lacusprofundi* alone accounts for 27%, different *Haloquadratum spp.*, which we never found in the microscope, still make up 7% of all, but only 9% of the Halobacteriaceae. *Halorubrum lacusprofundi*, an orgnoheterotroph, originally isolated from a deep lake in Antarctica, grows at temperatures as low as 0° and up to about 42°C It is a good candidate to look for in Antarctica with ECODIM-VIII. This organism was well studied, which allows us to have access to the genome map from the *Halorubrum lacusprofundi* genome database http://halo4.umbi.umd.edu/cgi-bin/haloweb/hla.pl?operation=hla located at the University of Maryland http://halo4.umbi.umd.edu/ . From http://genome.ornl.gov/microbial/hlac/ one finds that it has almost a complete set of the enzymes for the reductive TCA as a CO_2-fixing metabolism. The metagenomic data will be deposited in Camera (http://camera.calit2.net/) within the next few months.

Questions relating to bacteriorhodopsin synthesis genes in the Cahuil salar, the origin of the orange-pink pigment and many other questions can now be approached with the metagenome in hand.

Mini-Symposia

A a one-day symposium entitled “NITROGEN FIXATION IN AQUATIC ENVIRONMENTS: STRATEGIES AND ECOLOGY“ took place as a „public“ event offered by the course on Friday January 13 at the main campus of the Pontificia Universidad Católica de Chile in Santiago. It was organized by Beatriz Díez and Mónica Vásquez, both from the P. Universidad Católica de Chile and took place in the beautiful auditorium of the Facultad de Ciencias Biologicas. The detailed program is enclosed as Appendix 8. The speakers are listed in Appendix 6. Established investigators representing different research institutions from Chile and from abroad presented their research, stimulated discussion on newest approaches, initiated new research ideas among the symposium participants and strengthened interactions between different research groups and centers. The course students were in charge of hosting some of the guest speakers for after-symposium discussion groups. The symposium was sponsored by the Agouron Institute, the Gordon and Betty Moore Foundation, the Austral Summer Institute and the P. Universidad Católica de Chile.

A second mini-symposium (DISCOVERIES IN MICROBIAL ECOLOGY) was organized in Las Cruces as a course activity on January 21, with 16 presentations offered by the course participants themselves and by Mónica Vásquez. Each student selected a published research paper which represented a recent discovery in microbial oceanography and which was linked to the student’s research interest. The one-day symposium was open to the course participants (students and faculty) as well as to interested researchers and students from ECIM in Las Cruces. The selected paper titles and the symposium program are available as Appendix 9.



The epifluorescence microscope reveals unexpected froms and abundances of microbes from natural samples and from enrichments

Student Evaluation

All participants were highly-motivated, actively-participating, hard-working students who expressed great interest in every aspect of the subjects which were taught. We expect ECODIM students, who successfully completed the course to become leaders in their fields in their countries and to contribute significantly to marine microbiology in the coming years. Since the course was taken by several students for credit, an evaluation was made for all students. Full credit required:

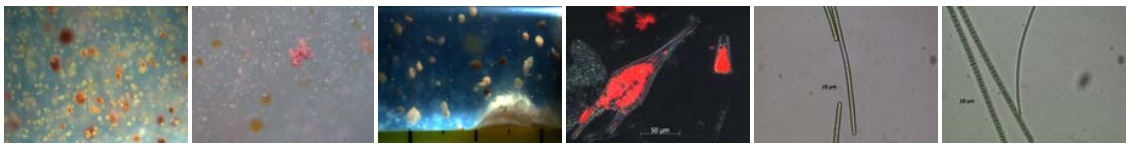
a) giving a 15 minute presentation on the work the student is presently involved in at his/her home institution (at the beginning of the course).
b) presenting the essence of a published scientific paper, selected by the student, in 25 minutes (incl. discussion) in English and being able to respond to questions related to it. The paper should be relevant to the course topics (microbiology, ecology, microbial diversity in ocean habitats) and the particular scientific interest of the student (counted as individual exam towards the middle of the course). The presentations are listed in Appendix 9.
c) the presentation of the course research results (counted as an individual and group effort)
d) the contribution to the final poster design (group effort)

All students passed the exams and were given credits for a grade A or B (Appendix 10), according to the scale recommended by UdeC

Grade scale:

Points	Grade	Scale	Points	Grade	Scale
100 - 88	A	excellent	< 50	D	insufficient
87 - 68	B	good	0	F	failed
67 - 50	C	sufficient		**I**	incomplete
				X	withdrawn

Students, assistants and instructors received a certificate for successful passing and participating to the course, respectively (A11a-A11c)



Ingenious "tricks" for enrichment and combinations of microscopic techniques

Thanks

It is one of the most important components of an intensive course like ECODIM that the group spirit remains high in spite of the late hours and sometimes the shortage of sleep. A special thank goes to all those who supported the course in one way or another. Uncomplicated interactions, friendship and a great hospitality made the course work enjoyable, easy, productive and memorable for the students as well as for the instructors. Investigators from PUC contributed to the course by sacrifycing research time, equipment, and funds for course experiments. Everybody was very helpful in getting together the necessary infrastructure for the lab, the computer exercises, and the field trips in a timely manner.

The course profited immensely from the hard work and dedication of:

- ALL instructors and speakers of the course; they are listed in Appendix 6.
- NICOLE TREFAULT, this year's young investigator teaching trainee,
- CARLA GIMPEL and JUAN FRANCISCO SANTIBAÑEZ, the on-campus teaching assistants,
- MONICA SORONDO, the course coordinator,
- ESTEBAN ALARCÓN who guaranteed safe transportation,
- JUAN ARELLANO and FERNANDO CARMONA from W. Reichmann Y Cía. Ltda. for the use of the Zeiss microscope,
- MANUEL ARAYA from ROCHE Chile Ltda., for the use of their real time PCR machine,
- GER VAN DEN ENGH from the BD Advanced Cytometry group, Seattle, USA for making the transport of the Cytometry container from Concepción to Las Cruces possible,
- The Center of Genomics and Bioinformatics at the Biotechnology Institute, Universidad Mayor, for adjusting the price of the metagenome 454 sequencing to accommodate the strictures of the course budget,
- SERGIO NAVARRETE, the station director, for hosting the course at ECIM,
- RICARDO CALDERON and RANDY FINKE, the divers and boat captains,
- The staff of the Marine Station at Las Cruces, in particular GLENDA LLANOS and all those who loaned us equipment and who tolerated our late night working hours,
- PAULA NUÑEZ and her team for serving us great meals,
- The staff of the CABAÑAS who read wishes from our eyes.

Excellent working conditions at the ECIM labs and successful differentioation of organismic pigments

Outlook, Outreach and Recommendations

January is a good time for an intensive microbial oceanography course like ECODIM, because the students have finished their academic commitments for the year and could fully dedicate their time to the course. The new ECIM facilities in Las Cruces are well suited to host an activity like the Microbial Ecology and Diversity Course, because there is ready access to a variety of marine ecosystems; the laboratories can temporarily be equipped quickly with the necessary instruments, computer facilities are suitable for the needs of the course and students and instructors are able to stay in houses near the station, which stimulates active exchanges and full dedication to the course objectives.

Most of the demands, which emerged from previous courses at Dichato could also be fulfilled in las Cruces in 2012. For future courses of this kind it is recommended:

1. that the basic structure of the course and the timing be maintained and that its duration be extended by a few days,
2. that the mini-symposia be maintained as a means of introducing the students to frontier research, with the participation of additional speakers from abroad,
3. that enough time be reserved for the course research project and other laboratory work, since these are the activities during which the students get practical experience and where questions emerge,
4. that enough independent study time be integrated into the program for reading and discussing key papers,
5. that a facility allowing for larger scale growth experiments, the culturing of anaerobic organisms, and the sorting of cells into growth media, be made available,
6. that relations with equipment and supply companies be used further for course purposes. Lending modern equipment, computers etc. to an international course and having students work with good reagents and molecular products is attractive for the companies involved as well as to provide state-of-the-art materials for the course.
7. The need for fast Internet connection for journal and library access, as well as for bioinformatics and modelling exercises which need software that is only available online remains a necessity for future courses.
8. Thanks to generous grants from the Agouron Institute and the Gordon and Betty Moore Foundation it was possible to purchase laboratory equipment and consumables to allow 15 students to work efficiently.

Given the favourable circumstances at the Las Cruces station, the strong financial support by a number of institutions (see Appendix 1), and the dedication of people from various university campuses and ECIM, the course instructors were able to achieve the course objectives with almost everything they had intended to do. The organizers were glad ASI decided to offer the course, in spite of the fact that we were not able to do it in Dichato as we did in earlier years. Since it will probably still take a few years to rebuild the Dichato station, we were thus pleased when we heard that ASI accepted the offer by the Estación Costera de Investigaciones Marinas (ECIM) to make the 2012 course at Las Cruces. ECIM is a well suited and beautifully located marine station of the Pontificia Universidad Católica de Chile. It has excellent laboratory and lecturing facilities for student courses. We have learned this year, that we can make ECODIM at other locations and are looking for an attractive place along the Chilean coast or on Antarctic Islands.

Since its start in 2000, ECODIM has established itself as a widely recognized training opportunity for marine microbiology in South America. A number of people, who were tained by ECODIM are now active in the field in Chile and in numerous other South American countries, in the US and in Europe. This not only strengthens the field, it also assures the successful continuation of ECODIM. The course has also gained

attention outside of the field of marine sciences and microbial ecology through coverage in public media (Universia: http://servicios.universia.cl/universidades/noticias/161303/cientificos-dictan-importante-curso-internacional-en-chile , Radio UDEC: http://www.radioudec.cl/index.php/noticias-udec/1733-cientificos-dictaron-en-chile-importante-curso-internacional-sobre-microorganismos-marinos.html , an article that was published in "El Sur", the main newspaper in Concepción and a number of reports about ECODIM in University News media. They are compiled in A17.

Students' responses to the course content, the teaching approaches and the facilities were positive and encouraging. The contacts which have been established among the students and between the students and the instructors will last into the future and may lead to study periods abroad or to joint research activities with people who met during ECODIM – VII.

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Appendices (pdf files)

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Las Cruces, January 29, 2012