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MarBEF

Marine Biodiversity and Ecosystem Functioning

EU Network of Excellence

Sustainable development, global change and ecosystems

D-7-CSP-5.2
MarBEF FISH (Flourescent in situ hybridization) TRAINING COURSE
Banyuls/mer, France, March 12-14, 2008

Due date of deliverable: March 2008
Actual submission date: May 13, 2008

Start date of project: 1 February 2004 Duration: 60 months

Organisation name of lead contractor for this deliverable

Framework Programme (2002-2006)

Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Report of the MarBEF FISH (Fluorescent In Situ Hybridization) TRAINING COURSE
Banyuls/mer, France
12-14/03/2008

Organisation :

Philippe Lebaron, Nyree West, Dominique Lamy (Laboratoire Arago, Banyuls/mer, France)
Nathalie Simon (Station Biologique de Roscoff, Roscoff, France)

1. Scientific report

Overall aims of the course :

The overall aims of the course were :
- to provide the basic principles and protocols of the FISH techniques for the monitoring of aquatic microbes (phytoplankton and bacteria)
- to discuss problems and biases associated to the FISH technique applied to marine samples.
- to discuss about the future applications and developments for the study of aquatic microbes with FISH.

Schedule and program of the course :

The course included theoretical presentations in the morning sessions and illustrations of theory and small practical experiments in the laboratory in the afternoon sessions. A copy of the program is attached to this report.

The course began with the arrival and registration of teachers and students, and greetings by Prof. Philippe Lebaron (director of the Laboratoire Arago).
The first day was dedicated to the principles of FISH and fluorescence techniques (lectures by Nyree West and Philippe Lebaron), and to a hands-on session in a practical room where the participants did work on natural microbial samples and probes designed to detect bacterial or phytoplankton phylogenetic groups. A round table allowed the particpants to discuss the problems associated to the preparation of samples for further FISH hybridization.

The second day was devoted to the analysis of hybridized samples (visualization under fluorescence microscopes and image analysis in the laboratory), and to a demonstration of the techniques used to design probes for microbial phylogenetic groups.

The third day was dedicated to the MICRO-FISH, a technique that allows the simultaneous detection/enumeration of aquatic microbes together with the monitoring of their metabolism. A lecture was given in the morning while the afternoon was dedicated to demonstrations.

Participants :

A total of 19 students (13 women and 6 men) from 9 different countries (Sweden, Italy, UK, Spain, France, Greece, Israël, Portugal, Slovenia) attended the course, most of which were PhD or post-doc students from MarBEF members or associate members institutions. The teaching staff consisted of 10 researchers mainly from the 2 institutions that participated to the organization (Station Biologique de Roscoff, and Laboratoir Arago), as well as from Spain.
The lists of participants and of persons that participated to the organization of the course are given below.

Products of the course :

1. Presentations, protocols and literature
The products from this course are available or will soon be available at the following addresses :

- web site of the plankton group in Roscoff : http://www.sb-roscoff.fr/Phyto/index.php?option=com_docman&task=cat_view&gid=77&Itemid=112
- web sites of the RMPs ROSEMEB and MARPLAN

They include mainly power-point presentations and protocols, as well as lists of publications that are of particular interest for the participants that will start using the FISH technique in their home laboratories, or for the participants that need to improve or adapt those techniques to their own samples.

2. Interaction and collaboration
The course allowed researchers from different fields and countries to interact. The round tables, coffee breaks and dinners were extremely important in this respect.

Feed-back from the course :

The course was an overall success, the students gave positive feedback and the teachers were all very satisfied with the level of attendance.
Copies of the feed-back that we obtained from the participants are available under request. A summary of the answers are given below.

Course Evaluation Questionnaire : Summary from 14 filled questionnaires

A. General Aspects of the Course

Course Content

1. What was your knowledge on the topics covered : Nothing **(3)**, basic **(8)**, advanced **(3)**
2. Did the course cover the topics you expected? Not at all **(0)**, Partially **(4)**, Totally **(10)**
3. What was the degree of difficulty? Too easy **(0)**, suitable **(14)**, Too difficult **(0)**

Course Organisation

4. Quality of the course outline (i.e. presentation detailing the course aims, content, assignment, etc.) : Very good **(10)**, good **(4)**, fair, poor **(0)**, very poor **(0)**
5. Organisations of courses activities (lectures, seminars, labs, etc.) : Very good **(10)**, good **(4)**, fair, poor **(0)**, very poor **(0)**

Teaching and Learning Support

6. Helpfulness of teaching staff: Very helpful **(12)**, helpful **(2)**, unhelpful, very unhelpful
7. Availability of course material: *(eg handouts, etc):* Very good **(8)**, good **(6)**, fair, poor **(0)**, very poor **(0)**
8. Clarity of presentations : Very good **(10)**, good **(3)**, fair **(1)**, poor, very poor

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B. Overall Evaluation

9. Overall, how would you rate the course content? Very Very good **(10)**, good **(4)**, fair, poor **(0)**, very poor **(0)**
10. Overall, how would you rate the organisation of the course? Very good **(9)**, good **(5)**, fair, poor **(0)**, very poor **(0)**
11. Overall, how would you rate the quality of the teaching? Very good **(10)**, good **(4)**, fair, poor **(0)**, very poor **(0)**
13. Good features of this course: **Practicals (4 students), equipment, lectures, discussions**
20. Poor features of this course: **too short (3 students), too focused on bacteria.**
21. How could this course be improved? **Add more time (6 students), invite more speakers, bring more material (hybridized slides)**

Schedule for the MARBEF FISH training course
Banyuls/mer, France, 12-14 March 2008

Wednesday 12.03.08	**Activity**	**Place**
09:00 – 09:30	Welcome, general organisation	Building B – 1st floor Amphitheatre
09:30 – 10:30	*Seminar* : Principles of the FISH methods applied to aquatic microbes (NW)	Building B – 1st floor Amphitheatre
10:30-10:45	*Coffee Break*	*Building B – 3rd Floor Coffee room*
10:45 – 11:45	*Seminar* : Fluorescence, principles and precautions (PL)	Building B – 1st floor Amphitheatre
12:30 – 13:30	*Lunch*	*Grand Hotel*
13:30 – 18:00	Presentation f the practical (NS)*Practical* **(Part I)** : TSA(CARD)-FISH applied to detect, identify and enumerate prokaryotes and eukaryotes	Building A – 1st floor TP room 3
16:00-16:15	*Coffee Break (during lysozyme treatment step)*	*Building B – 3rd Floor Coffee room*
18:00 – 19:00	*Round table* : Precautions to take when preparing samples for FISH (fixatives, long term storage, etc.)	Building A – 1st floor TP room 3
Thursday 13.03.08		
08:30 – 13:00	*Practicals* **(Part II)** : TSA(CARD)-FISH applied to detect, identify and enumerate prokaryotes and eukaryotes	Building A – 1st floor TP room 3
09:30 – 10:45	*Lecture/practical* **(during hybridisation step)**: Designing and in silico testing of probes for FISH, optimizing hybridisation conditions (NW)	Building A – 1st floor TP room 3
10:45-11:00	*Coffee Break (during hybridization step)*	*Building B – 3rd Floor Coffee room*
13:00 – 14:00	*Lunch*	*Grand Hotel*
14:00 – 18:00	*Demonstration/Practical* : Counting labelled cells in FISH samples. Image analysis. automatic counting (PC)	Building A – 1st floor TP room 3 Building B – 2nd floor microscope room
16:00-16:15	*Coffee Break*	*Building B – 3rd Floor Coffee room*

Friday 14.03.08		
09:00 – 10:00	*Lecture*: MICRO-FISH, detection, identification and enumeration of microbes together with the monitoring of their metabolism (PG)	Building B – 1st floor Amphitheatre
10:00-11:15	*Coffee Break*	*Building B – 3rd Floor Coffee room*
10:15 – 13:00	*Demonstration/Practical* : Demonstration of the MICRO-FISH technique (PC) Demonstration of counting labelled cells in MICRO-FISH samples on image analysis	Building B – 2nd floor dark room Building B – 2nd floor microscope room TP3 room
13:00 – 14:00	*Lunch*	*Grand Hotel*
14:00 – 15:00	*Round table* : Future applications and development for the study of aquatic microbes with FISH	Building B – 1st floor Amphitheatre

* Students may bring their own hybridized samples.

Part I CARD-FISH = Short presentation of the different steps, cut of membranes, treatment with agarose and lysozyme; storage of the membranes at -20°C until the Part II (hybridisation step)
Part II CARD-FISH = Hybridisation, Wash step, Equilibriation in TNT buffer, TSA reaction, Wash step

Speakers and demonstrators :

PL : Philippe Lebaron, Observatoire Océanologique, Banyuls/mer, France
NW : Nyree West, Observatoire Océanologique, Banyuls/mer, France
PG : Josep Gasol, Institut de Ciències del Mar, Barcelona, Catalunya, Spain
NS : Nathalie Simon Station Biologique, Roscoff, France

PC : Philippe Catala, Observatoire Océanologique, Banyuls/mer, France
CM : Carmen Manes, Observatoire Océanologique, Banyuls/mer, France
ML : Melissa Laghdass, Observatoire Océanologique, Banyuls/mer, France
NB : Nicole Batailler, Observatoire Océanologique, Banyuls/mer, France
DL : Dominique Lamy, Observatoire Océanologique, Banyuls/mer, France

EF : Elodie Foulon, Station Biologique, Roscoff, France
AC : Aurélie Chambouvet, Station Biologique, Roscoff, France
MF : Miguel Frada, Station Biologique, Roscoff, France
SM : Sylvie Masquelier, Station Biologique, Roscoff, France

List of participants

Last name	First name	Institution	Town	Country
Pedrotti	Maria-Luisa	Laboratoire d'océanographie de Villefrance	Villefranche/mer	France
Thor	Peter	Göteborg University, Dept. of Marine Ecology	Kristineberg	Sweden
Tinta	Tinkara	National Institut of Biology, Marine Biological station Piran	Piran	Slovenija
Lino Carreira de Carvalo	Silvia	Department of Oceanography and Fisheries University of the Azores	Horta, Azores	Portugal
Domingos	Catia	Instituto de Investigationes Marinas	Vigo	Spain
Saramento	Hugo	Institut de ciencies del Mar - CMIMA CSIC	Barcelona	Spain
Jimenez	Eroteida	Institut de ciencies del Mar - CMIMA CSIC	Barcelona	Spain
Pachiadaki	Maria	Hellenic center for marine research	Anavyssos	Greeece
Blihoghe	Daniela	Tel Aviv University + ICB-CNR Napoli	Tel Aviv	Israel
Celussi	Mauro	National Institute of Oceanography and Experimental Geophysics (OGS)	Trieste	Italie
Siano	Raffaele	Stazione Zoologica di Napoli	Naples	Italy
Balestra	Cecilia	Statione zoologica di Napoli	Naples	Italy
Cerino	Federica	Department of Marine Sciences - Polytechnic University of Marche	Ancona	Italy
Tait	Karen	Plymouth Marine Laboratory	Plymouth	United Kingdom
Lønborg	Christian	The Scottish Association for Marine Science, Dunstaffnage Marine Laboratory	Oban	United Kingdom