STAZIONE ZOOLOGICA ANTON DOHRN
NAPLES (ITALY), 3-24 OCTOBER 2015
REPORT
The 11th Advanced Phytoplankton Course (APC11) was held at the Stazione Zoologica Anton Dohrn in Naples (SZN) from 3 to 24 October 2015. This series of courses started in 1976 upon recommendation of the SCOR-WG on Phytoplankton Methods (WG 33), with the main goal of training and upgrading qualified students in the identification of phytoplankton species. The Course was organised jointly by the Stazione Zoologica, the University of Copenhagen and the IOC Science and Communication Centre on Harmful Algae. The organising committee of APC11 was formed by Marina Montresor, Diana Sarno, Adriana Zingone (SZN), Øjvind Moestrup (Univ. Copenhagen) and Henrik Enevoldsen (IOC) under the scientific direction by Adriana Zingone. The faculty included C.B. Lange and D. Sarno (diatoms), F. Cerino (cryptomonads), Ian Probert (haptophytes), J. Larsen, M. Montresor, K.A. Steidinger and Mona Hoppenrath (dinoflagellates), C. R. Tomas (raphidophytes and culturing), Øjvind Moestrup and Adriana Zingone (other flagellates) and W.H.C.F. Kooistra (molecular taxonomy and phylogeny).

Twenty-one participants from 16 countries were selected out of 57 applicants from 33 countries. The expertise in taxonomy and the role of participants in monitoring and research projects were taken into account in the selection process, along with the potential to train other people and contribute to the diffusion of the information acquired during the course.

The Course consisted of both theoretical and practical sessions. Lessons were given on the taxonomy, morphology and phylogeny of the different phytoplankton groups, followed by practical sessions. Materials consisted of a broad collection of unialgal cultures, permanent slides, as well as live or fixed natural samples. Relevant techniques were presented and shown to display taxonomic characters essential for identification in Light (LM) and Electron Microscopy (EM) and for culturing microalgal organisms. Special sessions included Transmission (TEM) and Scanning Electron Microscopy (SEM) observations. Serial dilution cultures set up at the beginning of the course from natural samples were examined by the participants, and the Most Probable Number (MPN) method to estimate species abundance was applied. Harmful or nuisance species received particular attention in the relevant sessions. Molecular methods for taxonomy and phylogeny were presented with theoretical lessons and dedicated seminars.

The manual on phytoplankton identification (Tomas C.R. ed., 1997) was used as the main textbook. A comprehensive collection of reprints and relevant literature was available to participants. At the end of the course all the material, including presentations in ppt format, tutorials and pictures taken in both LM and EM were shared with the student on Google drive©.

A final test was proposed to participants to evaluate the improvement in their skills. Participants also filled in a questionnaire with their evaluation of the course, which was also discussed during the closing session. Students declared their appreciation and acknowledged the faculty, the organisers and the secretary for their contribution to a successful Course, and provided useful indications to potentially improve future Courses.

Financial support for the Course was mainly provided by the SZN. Additional funds came from the Gordon and Betty Moore foundation, EuroMarine and the ISSHA (International Society for the Study of Harmful Algae). Carl Zeiss S.p.A. kindly lent 22 microscopes for the practical sessions. Cultures selected for display were provided free of charge by the Roscoff Culture Collection and the Scandinavian Culture Collection for Algae and Protozoa, while diatom permanent slides were kindly provided temporarily by the Grethe Hasle Collection.
1. BACKGROUND, ORGANIZATION AND GOALS

1.1. Background

Phytoplankton taxonomy is a very active field of research, which provides relevant information to other disciplines such as physiology, ecology, biogeography and molecular biology. Knowledge of phytoplankton species is also essential for practical issues, e.g. in biotechnology applications, monitoring and seafood control. It is therefore essential to keep a constant flux of information between taxonomists and taxonomy users in different fields. The series of Advanced Phytoplankton Courses (APC) was initiated in 1976 upon recommendation of the SCOR-Working Group of Phytoplankton Methods (WG 33), established in 1969 at the Marine Botany Section of the University of Oslo. After the first Course, two other advanced Courses were held at the Biological Station in Drøbak, in 1980 and 1983, with the teaching faculty basically including the same teachers (G.R. Hasle, J. Thronsden, K. Tangen, B. Heimdal and, from 1983, K.A. Steidinger). In the following years, seven APC have been organized at the SZN in 1985, 1990, 1995, 1998, 2005, 2008 and 2015, and one at the University of Copenhagen in 2012. Over the years, the faculty has included other teachers selected among the worldwide experts in the field of phytoplankton taxonomy (C.R. Tomas, M.J. Chrétiennot-Dinet, Ø. Moestrup, D. Sarno, A. Zingone, M. Montresor, C. Lange, J. Larsen, F. Cerino, M. Hoppenrath, W.H.C.F. Kooistra and I. Probert) who have flanked and later substituted the initial teachers.

A total number of 197 participants, representing 45 countries have attended the eleven APC so far organised. They constitute a special community that includes well-known scientists engaged, at times in leading positions, in the field of phytoplankton research all over the world.

1.2. Organization

The preparation for APC11 began about 18 months before its start with the invitation to the Faculty (Annex II). The Course was announced in July 2014 on the SZN Website (www.szn.it), the EuroMarine mailing list, the Bulletin of the Phycological Society of America and the Harmful Algal News Newsletter. The announcement included information on the venue, dates, aims, faculty, instructions and deadline for applications (November 15th, 2014) and was also disseminated through several mailing lists.

A total number of 57 applications were received from 33 different countries. Twenty-one applicants from 16 countries were selected (Annex III), with a waiting list of 10 more applicants. Criteria for selection included previous experience in phytoplankton, relevance of the Course to the present position of the applicant and to future involvement in monitoring and scientific projects, chances for participants to further spread information acquired during the course in their respective country. The country of origin was considered as well.

The Course was held in the seminar room and in the foyer of SZN. Students’ individual benches were equipped with a light microscope. The main desk had microscopes connected to a digital camera projecting images on a screen, computers, two video-projectors (for lectures in ppt format and images, respectively). A taxonomic library holding a wide book and reprint collection was arranged in the classroom. A large collection of pdf files of taxonomic publications was also available to the students together with an archived collection of literature references (Endnote).
Financial support for the Course was mainly provided by the SZN. Additional funds were obtained by the Gordon and Betty Moore foundation, EuroMarine, and the ISSHA (International Society for the Study of Harmful Algae). Carl Zeiss S.p.A. kindly lent 22 microscopes for the practical sessions. Cultures selected for display were provided free of charge by the Roscoff Culture Collection and Scandinavian Culture Collection for Algae and Protozoa, while diatom permanent slides were kindly provided temporarily by the Grethe Hasle Collection.

1.3. Goals

The Course was meant to upgrade and update the expertise of experienced researchers actively working in fields that require species identification such as phytoplankton ecology and monitoring, as well as taxonomy and systematics. The groups considered were diatoms, dinoflagellates, coccolithophores and other flagellate species. Special attention was given to species responsible for the formation of exceptional or harmful blooms. Information and training was provided on basic methods used in molecular taxonomy and phylogeny. In

2. CONTENT

The Course included the following activities, detailed in the program (Annex IV):

• Lessons on general taxonomic features of marine phytoplankton
• Direct observation at the LM and species identification
• Special LM techniques
• SEM and TEM techniques and observations
• Phytoplankton cultivation techniques
• Serial dilution technique and Most Probable Number (MPN) estimation
• Molecular phylogeny principles
• Invited seminars on specific topics/taxa

2.1. Opening and Introduction

On Saturday, October the 3rd 2015, participants registered at the Course desk at the SZN. APC11 was officially opened on Sunday October the 4th by Dr. Adriana Zingone who, on behalf of the Organizing Committee, welcomed participants and faculty, illustrated the scientific and social program of the next 3 weeks and presented all the sponsors and supporters of the Course.

A historical overview of APC was presented by the first of 5 invited speakers, Prof. Jahn Throndsen, who was part of the faculty of nine previous courses. The images showed many of the previous participants who are nowadays leaders in the field of phytoplankton research. Among the others, Barrie Dale, Trevor Platt, Gustaaf Hallegraeff, Victor Smetacek, Linda Medlin, Marta Estrada, Øjvind Moestrup and Malte Elbrachter. Almost all the members of the present faculty have been students at previous APCs. Jahn Throndsen presented
also a short overview of the life and scientific career of the late Prof. Grethe Hasle, who started the series of APCs and directed them until the 9th edition.

2.2. Materials

Materials for the Course consisted of fixed samples and cultures provided by the faculty as well as the students. More than 140 cultured strains were selected by the teachers and provided free of charge by the Roscoff Culture Collection (RCC) and Scandinavian Culture Collection of Algae and Protozoa (SCCAP), while permanent slides were lent by the Hasle Diatom Collection of Oslo.

The book ‘Identifying Marine Diatoms and dinoflagellates’, edited by C.R. Tomas (1997), was used as a general textbook. A collection of about 2000 taxonomic papers and several books were available to the students. For the methods and for selected groups of species, handouts and ppt files on the techniques illustrated were distributed.

The presentations, pictures taken during the Course and other material were shared with the participants and the faculty by uploading them on Google drive©.

2.3. Lessons

The course included a total of 120 hours of lessons, direct observations at the LM, exercises and seminars (see Annex IV). General features of diatoms, dinoflagellates, coccolithophores and other flagellates were illustrated during the different sessions. Morphological characters of a wide number of species as seen in light and electron microscopy were presented and distinctive features were highlighted. A lesson on molecular approaches in phytoplankton taxonomy was given. Participants were informed on recent taxonomic changes. Updated literature references were illustrated and discussed.

2.4. Practical activities

2.4.1 Species observation

Species observations were made on selected material, including permanent slides, live unialgal cultures and fixed samples.
The material was distributed to each participant for observation in the LM and shown on a screen through a digital video camera connected to a microscope. Additional information and/or iconographic material were shown on a second screen and the relevant literature was mentioned. Ultrastructural features were displayed by pictures projected on the screen during species observation. Toxic and/or harmful species were given special attention and shown in comparison to related non-harmful species. Pictures were taken for most of the material shown during the practical activities.

2.4.2. Techniques

The Course included a number of methodological lessons, spanning from special techniques for observations in LM and EM to single cell isolation, serial dilution techniques, culturing and molecular methods. The following methods were presented:
- Serial dilution cultures established from natural samples for flagellate identification and abundance assessment using MPN.
- Single cell isolation by micropipetting and culturing techniques.
- Squashing and thecal plate staining for thecate dinoflagellates.
- Preparation of TEM grids and SEM stubs using both culture material and mixed samples.

2.4.3. Exercises

Exercises during the lessons included identification of species from SEM/TEM pictures and carving of dinoflagellate species with their plate pattern.

The last days of APC11 were devoted to the observation of mixed natural samples from different geographic areas provided by the faculty or by participants. Participants worked together to compile species lists for each sample. Exercises with the simultaneous observation of similar species were also organized to highlight the distinctive features and improve identification capabilities.

The dilution cultures set up during the first day were observed and live flagellates as well as other microalgae were identified. The report logs were unified and the concentration of the single species in the original sample was estimated as MPN.
2.5. Scanning (SEM) and Transmission Electron Microscope (TEM)

The EM session aimed at showing the potential of the techniques rather than training students on EM species identification, which would require a separate course. Students were divided into subgroups which alternated at the SEM and TEM to observe material under the guidance of the teachers. Diagnostic ultrastructural details were shown for selected diatom, coccolithophore, cryptophycean, prasinophycean and naked dinoflagellate species.

2.6. Special sessions

Seminars on specific topics were given by invited speakers. Lectures included historical aspects of taxonomy (Throndsen), new molecular HTS approaches to the study of natural communities (Massana and Vaulot), challenging issues in diatom taxonomy (Mann) and technological development of in situ imaging for phytoplankton (Campbell). Seminars were followed by open discussions with the participants and, in the case of imaging, by an exercise on image identification. Seminars were also given on the history of the Stazione Zoologica (Maja), and on issues concerning quality control of phytoplankton data (Zingone).

Posters were prepared by the participants illustrating their scientific activity in relation with phytoplankton taxonomy and displayed in the classroom. An evening session was dedicated to the discussion of the posters.

3. SOCIAL EVENTS

Social events were organised to allow students and faculty to relax during the intensive work programme and enhance the cohesion of the group. Social activities included the welcome party, dinners in restaurants or pizza places, guided tours to Pompei and the Catacombs of San Gennaro and a social dinner the night before the APC closure.
4. EVALUATIONS

4.1. Final test

A test was proposed to the students with detailed taxonomic questions on several species and groups illustrated during the Course. The test was discussed with the students clarifying doubtful points emerged from their answers.

4.2. Questionnaire

At the end of the Course participants were distributed an evaluation questionnaire on both scientific and logistic aspects of the course. Participants expressed a very positive evaluation of APC11, in terms of general organization, quality of the lessons, samples and cultures, material provided (lessons, literature), logistic and social activities. The seminars given by the invited speakers were highly appreciated. Participants admitted that the course was very intense and even so there were genera that were left out: a shorter course would not be a good option and a longer course would be too exhausting. Several good suggestions emerged that in part reflected the specific needs of the single participants. Despite the different expertise, interests and duties of participants, apparently the APC11 format was a good compromise that offered everybody the possibility to gain more detailed knowledge in phytoplankton taxonomy.

5. CLOSING SESSION

During the closing ceremony, each student was distributed a participation certificate. APC 11 was closed on Saturday, October 24th.
ANNEXES

I  Organizing Committee and technical support
II  Faculty
III  List of participants
IV  Budget
V  Programme
Annex I

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- G. Lanzotti: video and audio services
- F. Iamunno and R. Graziano: electron microscopy
- R. Trimarco, R. Sepe, V. Monfrecola and A. Pedone: technical support and logistic
- S. Orfano, S. Addezio and S. Scotti: administration support
- M. Cannavacciuolo, V. Stefanino and G. Zazo: sampling
- C. Minucci: cultures and assistance in the classroom
- F. Tramontano: sampling and assistance in the classroom
- I. Percopo: teaching assistance
- M. Maja: History and art of the Stazione Zoologica
- M. Groeben: web site assistance

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ANNEX III

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## ANNEX IV

### BUDGET

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<th>Expenditures (€)</th>
<th>Notes</th>
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<td>Faculty (travel, hotel, meals)</td>
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<td>Guest speakers (travel, hotel, meals)</td>
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<td>Materials and consumables</td>
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<td>Contract for scientific/secretarial support</td>
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<th>Incomes (€)</th>
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<tr>
<td>SZN contribution</td>
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<td>Fee (600*19 participants)</td>
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# ANNEX V
## Program

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<th>Day</th>
<th>Registration (from 6:00 pm)</th>
<th>Welcome Party</th>
<th>Morning 1 (8:30-10:30)</th>
<th>Morning 2 (11:00-12:30)</th>
<th>Afternoon 1 (14:00-16:00)</th>
<th>Afternoon 2 (16:30-18:00)</th>
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<tr>
<td>03.10 Sat</td>
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<td>Day Morning 1 (8:30-10:30)</td>
<td>Morning 2 (11:00-12:30)</td>
<td>Afternoon 1 (14:00-16:00)</td>
<td>Afternoon 2 (16:30-18:00)</td>
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<td>04.10 Sun</td>
<td></td>
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<td>Opening of the Course, welcome and introduction A. Zingone</td>
<td>Principle of molecular methods in taxonomy; molecular identification, phylogenetic trees, metabarcoding W. Kooistra</td>
<td>Dinoflagellates, unarmoured species</td>
<td>Dinoflagellates, unarmoured species</td>
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<td></td>
<td></td>
<td></td>
<td>A. Zingone</td>
<td>W. Kooistra</td>
<td>Jahn Throndsen, University of Oslo. ‘History of APC and taxonomy’</td>
<td>Jahn Throndsen, University of Oslo. ‘Grethe Hasle: in Memoriam’</td>
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<td>05.10 Mon</td>
<td>Dinoflagellates, unarmoured species</td>
<td>Karlodinium, Takayama J. Larsen Karenia, Brachidinium K. Steidinger</td>
<td>Serial Dilution cultures from natural samples Theory and demonstration A. Zingone</td>
<td>Dinoflagellates, unarmoured species Pelagodinium, Symbiodinium, Polarella, M. Montresor</td>
<td>Dinoflagellates, unarmoured species</td>
<td>Dinoflagellates, unarmoured species</td>
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<td>07.10 Wed</td>
<td>Dinoflagellates, Peridiniales Introduction, Prorocentrum, M. Hoppenrath</td>
<td>Thoracosphaeraceae, i.e. Scrippsiella, Pentapharsodinium, Pfiesteria and related genera M. Montresor</td>
<td>Dinoflagellates, Peridiniales Azadinium, Amphidoma M. Hoppenrath Armoured species</td>
<td>Dinoflagellates, Peridiniales Heterocapsa, Amphidoma, M. Hoppenrath</td>
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<td>Dinoflagellates, Peridiniales Introduction, Protoperidinium, Diplopsalis group, Podolampas, Blepharocysta K. Steidinger</td>
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<td>Dinoflagellates, Dinophysiales Introduction, Dinophysis, Phalacroma, J. Larsen Amphisolenia, Ornithocercus, Histioneis, Oxyphysis, K. Steidinger</td>
<td>Dinoflagellates</td>
<td>Participants’ mixed samples</td>
<td></td>
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</tbody>
</table>

Pizza
<table>
<thead>
<tr>
<th>Day</th>
<th>Morning 1 (8:30-10:30)</th>
<th>Morning 2 (11:00-12:30)</th>
<th>Afternoon 1 (14:00-16:00)</th>
<th>Afternoon 2 (16:30-18:00)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.10 Fri</td>
<td>Flagellates Morphology, terminology, general systematics techniques (examples) Ø. Moestrup</td>
<td>Heterokontophyta Morphology, taxonomy and diversity. Dictyochophyceae Pseudochlathomma, Dictyocha, Florenciella, Apedinella Ø. Moestrup</td>
<td>Guest lecture Daniel Vaulot, Station Biologique de Roscoff ‘Diversity and Oceanic distribution of two picoiplankton groups: prasinophytes clade VII and Parmales/Bolidophytes 1’</td>
<td>Heterokontophyta Chrysophyceae, Ochromonas, Dinobryon, Pelagophyceae, Aureococcus, Pelagomonas Bolidophyceae, Bolidomomas, Pinguiophyceae, Phaeomonas Ø. Moestrup, A. Zingone</td>
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<tr>
<td>10.10 Sat</td>
<td>FREE</td>
<td></td>
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<td>Wine &amp; Cheese Pub</td>
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<tr>
<td>12.10 Mon</td>
<td>Haptophyta, Coccolithophores Diversity, morphology, taxonomy, life cycles I. Probert</td>
<td>Haptophyta, Coccolithophores Isochrysidales, Coccolithales Observations of cultures I. Probert, A. Zingone</td>
<td>Haptophyta, Coccolithophores Syracosphaerales, Zygodiscales, others. Observations of cultures I. Probert, A. Zingone</td>
<td>Haptophyta, Coccolithophores Observations of natural samples I. Probert, A. Zingone</td>
</tr>
<tr>
<td>13.10 Tue</td>
<td>Cryptophyta Morphology, Taxonomy, Identification F. Cerino Observations: Rhodomonas, Storeatula F. Cerino, A. Zingone</td>
<td>Cryptophyta Observations: Hemiselmis, Teleaulax, Proteomonas F. Cerino, A. Zingone</td>
<td>Culture methods (group 1) C. Tomas</td>
<td>Culture methods (group2) C. Tomas</td>
</tr>
<tr>
<td>15.10 Thu</td>
<td>Flagellates Serial Dilution Cultures (group1) SEM/TEM SESSION (group2) Ø. Moestrup, A. Zingone, F. Cerino</td>
<td>Flagellates Serial Dilution Cultures (group 2) SEM/TEM SESSION (group 1) Ø. Moestrup, A. Zingone, F. Cerino</td>
<td>Diatoms Introduction to diatom morphology, terminology, general systematics C. Lange, W. Kooistra</td>
<td>Diatoms Leptocylindraceae, Corethraceae Theoretical lessons, examination of samples D. Sarno</td>
</tr>
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*Poster Session
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</tr>
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</table>
| 16.10 Fri | Diatoms\textsuperscript{1}\textsuperscript{2}\textsuperscript{3} Rhizosoleniaceae  \textsuperscript{(1st part)}  
Theoretical lessons, examination of samples, C. Lange | Diatoms\textsuperscript{1}\textsuperscript{2}\textsuperscript{3} Rhizosoleniaceae \textsuperscript{(2nd part)}  
Theoretical lessons, examination of samples, C. Lange | Diatoms\textsuperscript{1}\textsuperscript{2}\textsuperscript{3} Melosiraceae, Coscinodiscaceae  
Theoretical lessons, examination of samples, C. Lange | Diatoms, Coscinodiscaceae, 
Stellarimaceae  
Theoretical lessons, examination of samples, C. Lange |
| 17.10 Sat | **FREE – EXCURSION TO POMPEI**                                                         |                                                                                        |                                                                                        |                                                                                        |
| 18.10 Sun | Diatoms, Hemidiscaceae, Asteroalumpraceae  
Theoretical lessons, examination of samples, C. Lange | Diatoms, Thalassiosiraceae \textsuperscript{(1st part)}  
Theoretical lessons, examination of samples, C. Lange, D. Sarno | Diatoms\textsuperscript{1}\textsuperscript{2}\textsuperscript{3} Thalassiosiraceae \textsuperscript{(2nd part)}  
Theoretical lessons, examination of samples, C. Lange, D. Sarno | Diatoms, Lithodesmiaceae, 
Eupodiscaceae  
Theoretical lessons, examination of samples, D. Sarno |
| 19.10 Mon | Diatoms\textsuperscript{1}\textsuperscript{2}\textsuperscript{3} Hemiaulaceae  
Theoretical lessons, examination of samples  
D. Sarno | Diatoms, Chaetocerotaceae  
Theoretical lessons, examination of samples  
D. Sarno, N. Lundholm | Diatoms, Chaetocerotaceae  
Theoretical lessons, examination of samples  
D. Sarno, N. Lundholm | Diatoms, Introduction to Pennate 
Diatoms, C. Lange 
Fragilariae  
Morphology, terminology  
Examination of samples, N. Lundholm |
| 20.10 Tue | Diatoms, Thalassionemataceae  
Theoretical lessons, examination of samples  
C. Lange | Diatoms, Bacillariaceae  
Theoretical lessons, examination of samples  
N. Lundholm | Guest lecture: \textit{David Mann}  
Royal Botanical Garden, Edinburgh  
Title: Diatom taxonomy is terrible, terrifying, terrific | Diatoms, Bacillariaceae  
Theoretical lessons, examination of samples  
N. Lundholm | Visit to 
Catacombe di San Gennaro 
and dinner |
| 21.10 Wed | Diatoms, SEM/TEM (group1)  
C. Lange, D. Sarno | Diatoms, SEM/TEM (group2)  
C. Lange, D. Sarno | **FREE AFTERNOON** | **FREE AFTERNOON** |
|           | Serial Dilution Cultures (g2)  
A. Zingone, F. Cerino | Serial Dilution Cultures (g1)  
A. Zingone, F. Cerino |                                                                                        |                                                                                        |
| 22.10 Thu | Mixed samples from different geographical regions (participants’ samples) LM  
SEM/TEM session on mixed samples | Mixed samples from different geographical regions (participants’ samples) LM  
SEM/TEM session on mixed samples | Guest lecture: \textit{Lisa Campbell}  
Texas A&M University, ImagingFlowCytobot: What we can learn from a high temporal resolution phytoplankton timeseries | Demo: In situ imaging for phytoplankton monitoring  
Lisa Campbell |
| 23.10 Fri | Final student evaluation | Mixed samples from different geographical (participants’ samples) LM  
SEM/TEM session on mixed samples | Mixed samples from different geographical regions (participants’ samples) | General discussion on taxonomic issues | Social Dinner |
| 24.10 Sat | **Summary:** Course evaluation and general conclusion | **Summary:** Course evaluation and general conclusion |                                                                                        |                                                                                        |