# EU-LIFEHAB: Expanding the Discussion on the Life Cycles of Harmful Algae

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## Participants

Donald M. Anderson, Stephen S. Bates, Susan Blackburn, Chris J. S. Bolch, Barrie Dale, Malte Elbrächter, Paul E. Hargraves, Ichiro Imai, Anke Kremp, Jane M. Lewis, Marina Montresor, Louis Peperzak, Christopher A. Scholin, and Carmelo R. Tomas

## Background

The workshop "LIFEHAB: Life history of microalgal species causing harmful blooms," funded by the Fifth Framework Programme (Energy, Environment and Sustainable Development) of the Commission of the European Communities, was held in Calvià (Mallorca, Spain), 24–27 October 2001. Complex and heteromorphic life cycles are part of the adaptive strategies of organisms causing harmful algal blooms (HAB). They can influence the intrinsic potential for growth, persistence and dispersal, allowing the species to occupy different ecological niches. Information about life cycle strategies are very important for understanding bloom dynamics and population structure of HAB species.

The workshop report (Garcés *et al.,* 2002) contains extended abstracts, reports of the discussion groups, tables with summarized information on diatom, dinoflagellate, haptophyte and raphidophyte life cycles and a very comprehensive bibliography. The report is currently available at: http://www.icm.csic.es/bio/projects/lifehab/.

The objectives of LIFEHAB were to

- review current knowledge on the life cycles of phytoplankton organisms, focusing on harmful species;
- identify the role of heteromorphic life cycles in population dynamics;
- define future HAB research directions to fill existing gaps in knowledge;
- debate the most appropriate approaches and methods;
- promote the development of cooperative scientific initiatives.

The aim of the roundtable held during the Xth International Conference on Harmful Algae was to expand the discussion so as to include non-EU scientists who were not involved on the LIFEHAB Workshop.

**S. Bates** noted that although considerable knowledge has been gained on *Pseudo-nitzschia* species, little progress has been made on other diatom genera. Advances in species-specific molecular probe design that allow the detection of different sexual stages in the field and the differentiation of male from female gametangia would be most useful, in order to determine the proportion of these cells in a population. Mating compatibility studies could clarify questions concerning species definitions. Other approaches, such as the use of image analysis or the identification of condensed chloroplasts, could prove effective in tracking changes in size spectra and resting stages. The identification, localization and physiology of possible overwintering stages are other key issues for understanding the population dynamics of *Pseudo-nitzschia* species.

**P. Hargraves** focused on triggering mechanisms for diatom auxospore (size, environment, mating types, pheromones) and resting cell (light, nutrients, temperature) formation. Ecological and physiological studies, as well as monitoring programs, are as good as taxonomic quality allows them to be. The definition of the species/taxonomic units is thus another crucial issue, and the correct approach can be seen as a "three-legged stool" coupling morphology, life cycles, and molecular systematics. Four fields of priority research can be identified in this context: i) relation between life cycle events and interspecific competition at the biochemical level; ii) the role of parasites and pathogens in the control of blooms; iii) the enhancement of toxicity through symbiosis with bacteria; and iv) phylogenetic distribution of toxins, including those affecting organisms other than humans.

**J. Lewis** identified as priority activities: i) investigation of mutation rates in cultures; ii) organization of workshops on dinoflagellate-culturing techniques; iii) development of markers for gametes and viable cysts; iv) investigation of the role of temporary cysts in life cycles; v) search for overwintering stages of species where cysts are not known; and vii) improved methodologies for detailed water-column monitoring and for estimates of *in situ* germination rates.

**S. Blackburn** illustrated examples of crossing matrixes for sexual mating, showing that dinoflagellates have rather complex mating systems, including multiple mating types. She outlined the importance of sexual compatibility among strains, which will ultimately affect cyst production rates and end up in the genetic structure of the population.

**M. Elbrächter** pointed out that little is known about different cell division modalities among dinoflagellates and recalled that in some cases, non-motile stages are involved in asexual reproduction. He also mentioned aspects of dinoflagellate morphology and life cycle traits that have been misinterpreted, as was the case for *Pyrocystis* and *Dissodinium,* the latter with lunate-shaped secondary cysts in which up to 8 asexual planospores are formed, or the asex-

ual pellicle cysts in *Lingulodinium polyedrum.* In his view, more attention should be paid to understanding internal clocks, circannual clocks, social behaviour, communication, and chemical signalling mechanisms.

**L. Peperzak** explained that prymnesiophytes are quite complex because they include motile/non-motile and haplontic/diplontic stages. Molecular probes and flow cytometry are needed to identify species and ploidy levels. The need to develop stage-specific probes and to identify factors influencing life cycle transitions was also mentioned. He speculated on the *quorum sensing* (QS) abilities of microalgae as a way to detect the abundance of the same species by secreting species- and strain-specific competence activators. In bacteria, QS is involved in genetic transformation and sporulation (see Dummy and Williams, 1999). Hypothetically, QS could be involved in syngamy/meiosis and cyst/colony formation in prymnesiophytes. Preliminary tests with *Phaeocystis* indicated that high cell densities induced colony formation. Proving the existence of QS, including among others competence activators, would provide a new perspective in the study of HAB dynamics.

**C. Tomas** and **I. Imai** noted that raphidophytes are naked pleomorphic species not easy to study because they are difficult to preserve without deforming or bursting the cells. Here, the combination of morphology, pigment composition and molecular probes on live and preserved specimens becomes truly a strong argument. While some life cycle information occurs in reports of blooms or cultures, there has been no concerted effort to define the life cycle stages of the different raphidophytes. Given their increasing importance as HAB species, there is a need to re-examine the life cycle phases using a number of techniques now available (gene sequencing, nuclear staining, etc.) as well as traditional ones using clonal cultures. Little is known about processes undertaken in dark and cold bottom waters. Signalling between cells could be through high density or through infochemicals. This kind of communication has not been proven for any HAB species, yet it could be a means for timing life cycle changes in populations capable of forming dense blooms.

**M. Montresor** summarized research priorities for understanding the importance of life cycle events in HAB ecology: a) role of life history stages in bloom dynamics, *e.g.,* when are resting stages produced? how many? how many are viable in the sediments? is there an endogenous control of life-cycle transitions?; b) role of specific life history stages in avoiding predation, preserving genetic diversity and promoting dispersal; c) single species and life-stage distribution through sampling and observational techniques at the appropriate scale (*e.g.,* microlayers, sediment-water interface); d) identification of key areas to be used as "case studies" and the importance of long-term data sets; and e) species-specific models integrating life cycles.

**D. Anderson** illustrated problematic issues related to the study of cyst germination dynamics *in situ.* Different techniques (emergence traps, changes in cyst fluorescence, laboratory incubation of sediments, repeated quantitative cyst enumeration in core samples through time) were used to estimate *in situ* germination rates of *Alexandrium fundyense* in the ECOHAB-Gulf of Maine program, but none proved successful. Ongoing population dynamic studies have therefore relied on large-scale cyst mapping, parameterization of cyst germination rates using laboratory incubations, and incorporation of these data into a coupled physical/biological model. Model runs indicate that light reaching the sediments is surprisingly *not* a crucial factor in germination success. Cysts from shallow waters germinated at nearly the same rate as those in deeper waters. This is because light is rapidly attenuated in bottom sediments, making attenuation due to water depth a minor factor. Layers of sediment above the cysts as thin as several mm may be enough to inhibit germination. Similarly, germination will likely be inhibited by anoxia even a few mm below the sediment surface. In such cases, resuspension by currents or bioturbation may be important in fostering cyst germination. Overall, this presentation highlighted the difficulties that still exist in estimating *in situ* germination rates.

**B. Dale** introduced a geological time perspective into the debate. Changes in time-scales up to 50 years are being related with El Niño-like events, whereas changes in the order of 100-year periods are related to climatic trends. Climate change could be advantageous to cyst-forming species from cold or warm coastal waters, allowing them to better exploit the time shift in seasonal patterns. He criticized the use of the term "harsh environment," which reflects an anthropocentric point of view.

**C. Bolch** celebrated that probes are solving lots of old problems. Twenty out of 90 attendees to this round table are currently using molecular probes in their research. He emphasized the need to present more risky proposals, and to apply innovations in sampling strategies and molecular designs.

**C. Scholin** noted that probes can be applied to intact cells as well as cell homogenates. It is essential that development of these methods remain tightly integrated with traditional microscopy-based species identification techniques. Systems that enable use of molecular probes for near real-time detection of HAB species, *in situ,* are fast becoming a reality. Many approaches that rely on cell-free detection formats offer incredible sensitivity and speed, and can be packaged in very small platforms such as hand-held devices. However, whole cell and cell-free detection methods can yield different answers as to what species are apparently present in a given sample. Working with natural samples, species identifications based on cell-free methods will likely reveal that target organisms are present with greater frequency than those estimates based on intact cells. Key issues to resolve are creating standards for reporting cell presence/abundance using cell-free formats, and documenting the relationship between molecular signatures as seen in intact versus homogenized material.

**A. Kremp** recalled that, in addition to nucleic acids, cell surface molecules are a group of potential target molecules to improve our understanding of life cycle processes.

Studies of *Chlamydomonas* and ciliate sexual reproduction have shown that cell wall molecules, and changes in their structure and composition, largely mediate gamete recognition and fusion. Thus, looking at cell wall proteins or glycoconjugates may help to characterize sexual cells and the physiological processes involved in the sexual reproduction of HAB species. Immunological techniques and proteomic approaches should be explored to identify specific surface molecules and to characterize proteins. Carbohydrates could be targeted with complementary lectins. Fluorescent probes targeting specific cell surface structures can be developed and optimised for detection of life cycle stages in the field, in conjunction with flow cytometry. Recognition and adhesion molecules on the cell surface can also be useful for inhibition experiments to study the function of signalling, recognition and adhesion of gametes.

## Conclusions

From the general discussion, several commonalities emerged for HAB species research priorities. Complementing those outlined by LIFEHAB, research should be focused on the following knowledge gaps:

- Inadequate knowledge of life cycle events for many of the important HAB species. The present state of knowledge does not allow for the formulation of general paradigms.
- Social behaviour, active aggregation mechanisms.
- Signalling (Quorum Sensing), infochemicals, species-specific features.
- Circadian and circannual clocks. Endogenous regulation of life cycle events. Role of photoperiod or photoperiod fluctuations, temperature or temperature fluctuations, in driving life-cycle transitions.

Major gaps related to the ecological role of life cycles were recognized to be caused by methodological and observational constraints in field studies. The limited time-scale of our data sets is also a problem which should be circumvented by a more extended use of sedimentological record, where possible, and by the support to long-term observational programs. Additional suggestions on new approaches, methodologies and research strategies were as follows:

- Couple traditional techniques with advanced/molecular tools, in a bold and creative fashion.
- Complement/substitute observation with adequate models, to be developed.
- Exploit knowledge and experience from different fields (*e.g.,* microbiology, limnology, genetics of non-microalgal organisms).
- Coordinate efforts among scientists and promote cross-validation of methodologies and results.
- Compare the behaviour of a species over its geographic range through international cooperative research.
- Improve the quality of species identification in field studies through the cooperation of classical taxonomists, molecular biologists and ecologists.

## References

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