IV. Progress since the First Open Science Meeting

Since the first OSM of this CRP, significant progress has been made in evaluating the processes and mechanisms involved in HAB dynamics. Not all of the key questions posed at the previous OSM were reviewed in detail at the subsequent meeting because of partial overlap in the themes with other CRPs and their respective OSMs. For example, nutrients and eutrophication and thin-layer systems within fjords and coastal embayments were not considered for special attention as these issues have been comprehensively addressed at other GEOHAB OSMs. The progress within the CRP on Fjords and Coastal Embayments is described in the following sections through the summaries of oral presentations given by keynote speakers during the second OSM.

A. LIFE HISTORY OF HAB SPECIES

1. Review of Past Programmes on HABs that Have Included Life Cycle Approaches

1.1 Diversity and Complexity of the Life Histories of Harmful Algal Species and the Impact on their Ecology (Outcomes of the SEED Project)

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The SEED project was a collaborative European Union - United States project that aimed to understand the extent to which environmental and physiological factors influence transitions among life cycle stages of harmful microalgal species, thereby contributing to the increase in harmful algal blooms in marine, fresh, and brackish waters. The project focused on the life histories of some of the most relevant HAB taxonomic groups, including examples of freshwater (Raphidophyceae), estuarine (Cyanobacteria) and marine (Dinophyceae and Bacillariophyceae) species, and drew on a wide range of coastal locations (Western Mediterranean, Atlantic Ocean, North Sea, Baltic Sea and Swedish lakes). All of these environments have heavy anthropogenic influences: fisheries, urban development, aquaculture and tourism. All are subject to frequent occurrence of HABs, with a variety of detrimental impacts, including human intoxications, closure of shellfish farms, and water discoloration causing a negative impact on tourism, all with consequent economic impacts.

The SEED research was multifaceted, as the problems in life history transitions are complex and processes occur over a wide range of scales. SEED combined field studies with laboratory experiments. Field work was centred on areas where on-going monitoring programmes and much baseline information about distribution of species and physical-chemical data already exist.

The project allowed a unique *comparative approach*, from species to the ecosystem level (highlighted in the *Deep-Sea Research II* special issue, volume 57 (3-4), February 2010; see also the SEED project Web site: http://www.icm.csic.es/bio/projects/seed/). Our results show that harmful algal events are widely different and that the life cycles of several congeneric species show unexpected complexities. However, some common features among harmful algal events have also been observed, such as the production of benthic resting stages and presence of sexual phases within the life cycle of the concerned species. The results obtained from the SEED project will have an important impact on the understanding of the ecology of bloom events for a range of phylogenetic groups.

Some of the main results obtained during SEED research include:

1. The characterization and quantification of the different stages of the life cycle associated with bloom development for multiple HAB species in laboratory and in field studies, including those of *Alexandrium*, *Kryptoperidinium, Gymnodinium*, *Pseudo-nitzschia, Peridinium, Gonyostomum, Scrippsiella/Woloszynskia* complex, *Peridiniella, Nodularia, Anabaena*, and *Aphanizomenon*. Study areas included the Catalan and Galician coasts (Spain); Sardinia, the Tyrrhenian coast, and Sicily (Italy); Gulf of Finland (Finland); Estonian coast (Estonia); Cork (Ireland) and the British coast (United Kingdom). A main conclusion is that a heteromorphic life stage represents an advantageous survival strategy for a population since it allows the allocation of the species biomass into stages of different size ranges, morphology, and different ecological niches. Based on their life

histories, it is possible to divide phytoplankton species studied into two groups: holoplanktonic (present only as planktonic stages in the water column), and meroplanktonic (with a planktonic stage and documented resting stage that inhabits the benthos). In some cases, the resting stage has been identified, but with no indication of bloom initiation from this benthic resting stage.

2. The magnitude of pelagic-benthic transitions in microalgae (encystment and excystment in the case of dinoflagellates) was found to be determined by their life cycle features (Fig. 1) and the factors that regulate resting stage formation and germination. The documentation of life cycles in our studies has shown that these processes and their regulatory factors may be very different, depending on which species are considered. Through these resting stage processes, microalgae develop their unique ecological strategies, such as the occurrence of life cycle processes aligned with specific environmental conditions. The possible reversibility of the sexual stage (*e.g.*, of the planozygote in dinoflagellates), which does not always transform into a resting cyst, is a new feature in the life cycle of dinoflagellates confirmed during the project (Figueroa *et al.*, 2006). Moreover, SEED research revealed that some dinoflagellate species may also produce asexual resting cysts that allow survival from one growing season to the next without requiring sex.
3. The control of encystment and excystment was shown to be determined by intrinsic factors unique to each species, whereas the timing and scale of responses appear to be modulated by environmental factors. SEED work regarding the excystment process addressed three main topics: seasonality, the temperature "windows" for germination, and field-determined cyst formation and deposition. Field and laboratory studies provided evidence for seasonality in the germination and/or bloom initiation of meroplanktonic species. Regarding germination, the dormancy period was also found to be species-specific and modulated by temperature. For the first time, the flux of akinetes (resting stages) was reported for the three major bloom-forming genera of cyanobacteria in the Baltic Sea (Suikkanen *et al.*, 2010). Akinetes are produced through asexual differentiation of a vegetative cell that takes place in rapid response to the onset of physiological stress. This finding is significant with respect to the survival strategies and bloom dynamics of cyanobacteria.
4. Mapping the distribution of benthic resting stages of HAB species (dinoflagellates, raphidophytes, haptophytes, cyanobacteria) in superficial sediments is relevant: it allows the establishment of a baseline for monitoring of spreading events, the introduction of new species, and human-assisted dispersal. Some dinoflagellate blooms seem to be largely controlled by the coupling between benthic and pelagic systems. During calm conditions, fluxes towards the sediment are favoured; organic matter, cysts, and other substances accumulate in the uppermost layer of the bottom sediment, which is continually modified by the addition of newly settled particles and by the subsequent degradation of the accumulated material. Two main processes occur under these conditions: (1) the formation of a layer of accumulated resting cells on the sediment surface; and (2) the settling of organic particles that are important for the cycling of nutrients.
5. Molecular and immunological techniques were explored to identify specific life cycle stages of key HAB species (Erdner *et al.*, 2010; Penna *et al.*, 2010a). The rapid development of molecular methods makes it possible to investigate the genetic diversity, phylogenetic relationships and – in the near future – the molecular bases of the life cycles of microorganisms. The real-time polymerase chain reaction (PCR) assay is one of the most promising methods to monitor the presence of harmful species and for risk assessment analysis. This method has also been developed for the detection of dinoflagellate resting stages in sediment samples. Furthermore, it allows the processing of high numbers of samples, and prior knowledge of cyst morphology is not required. This technique can also be developed for an array of different species for which sequence data are available.
6. A reproductive barrier was identified between different toxic and non-toxic genotypes of *A. tamarense* that can explain the population genetic boundaries between them (Brosnahan *et al.*, 2010). Specifically, a method was developed to detect the parentage of hybrids between Group I (toxic) and Group III (non-toxic) genotypes within the *A. tamarense* complex. Hybrid cysts from these matings germinated but did not survive, demonstrating outbreeding lethality. This also suggests a possible mitigation strategy whereby non-toxic strains could be introduced into a region with toxic species, leading to a reduction in the viable cyst population needed to initiate future blooms.
7. Different types of models were explored as useful tools for investigating the relevance of life cycle features in HAB development. The results of the simulations in *Alexandrium minutum* highlighted the importance of knowing not only the magnitude and variability of growth and life-cycle transition rates, but also those of loss rates (both in the water column and in the sediment) due to physical and biological factors (Estrada *et al.*, 2010). Excystment fluxes can enhance population densities of vegetative cells during times of low or negative net growth rate and during the initial phases of a

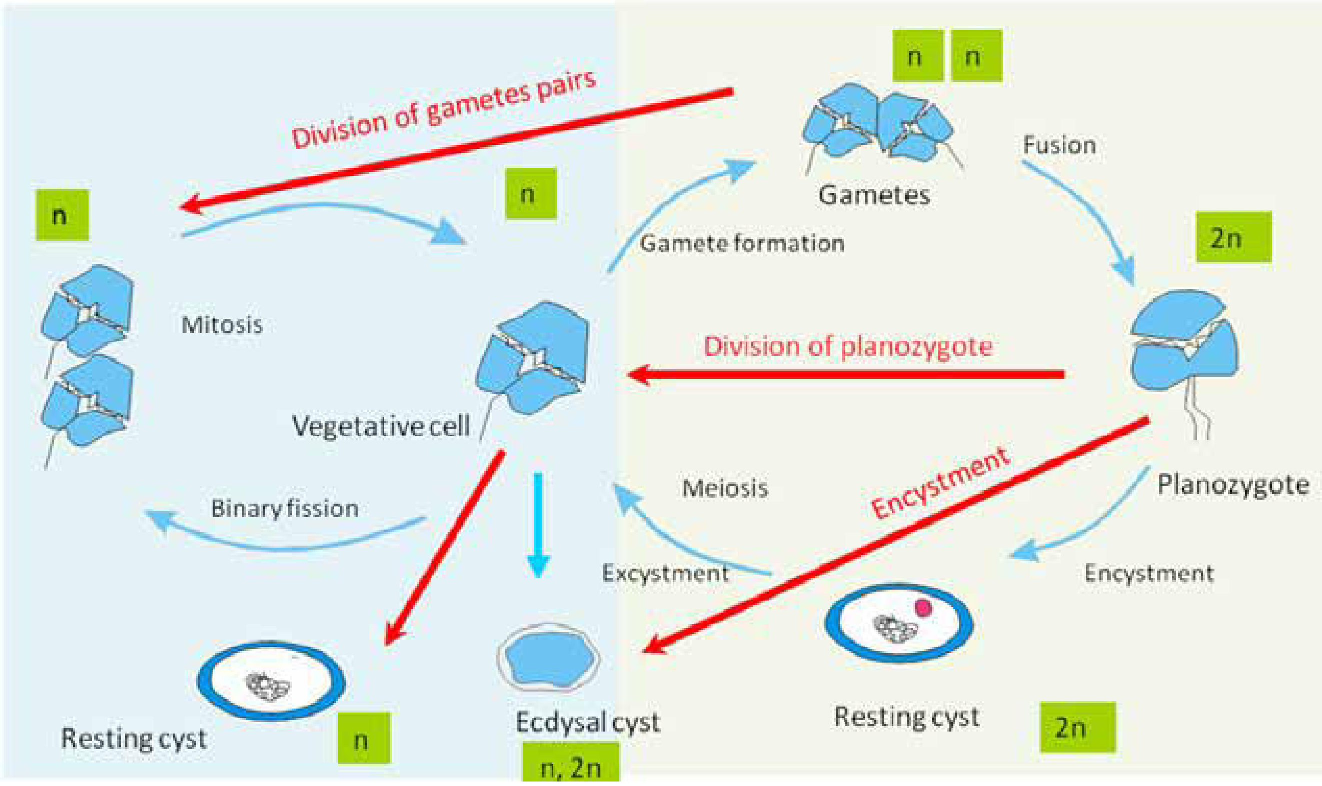

Figure 1. Typical life cycle of dinoflagellates, with new features confirmed during the SEED project (courtesy of R.I. Figueroa, unpublished*).*

bloom, but once exponential growth had started, additional excystment had negligible effect on bloom magnitude. However, even if cysts do not determine the magnitude of larger blooms, they do represent a safety mechanism for reintroduction of the species when the vegetative cell population is no longer present in the water column due to unfavourable environmental conditions.

8. The relative balance between physical and biological forcing controls the timing of microalgal blooms. By choosing a range of sites, from the Mediterranean Sea (*e.g.*, Basterretxea *et al.*, 2007; van Lenning *et al.*, 2007) to cooler latitudes further north (Gulf of Finland, Estonian coast, Ireland; see Kremp *et al.*, 2008; Touzet *et al.*, 2010b), the predictability of blooms was observed to increase with the degree of physical forcing that controls them. *Alexandrium minutum* has been the target species for most investigations during this project and it is a useful organism for comparative studies when considering latitudinal gradients. This organism exhibits plasticity in its growth characteristics. There are, however, some commonalities across Europe relating to this organism. It is indeed a very robust species capable of existing in a wide range of environmental conditions.
9. Diatom species of the genus *Pseudo-nitzschia* also have complex life cycles, including a sexual phase required for the formation of large-sized cells to circumvent the progressive cell size reduction that occurs during mitotic divisions. A simultaneous and massive sexual event involving two *Pseudo-nitzschia* species has been recorded at the LTER station in the Gulf of Naples (Sarno *et al.*, 2010). The recurrent biennial timing of sexual reproduction has been inferred for *P. multistriata* by following cell size over a decade and has been modelled based on experimental parameters and information from natural populations (D'Alelio *et al.*, 2010).

Finally, topics that remain to be addressed were presented and discussed, such as the tools needed to detect and quantify different life stages (gametes and zygotes) in the natural environment, the contribution of cells germinated from surface sediments to the bloom inoculum, and site-specific differences in sexual cycle strategies.