

## ***Pseudo-nitzschia* life cycle and the sexual diversity of clones in diatom populations**

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### **Introduction**

The causes of Harmful Algal Blooms (HABs) are diverse, and may involve several different species. Diatoms are common in marine communities and play a significant role as members of HABs (APPENDIX 1). For example, among 25 marine species causing blooms in the Black Sea, diatoms amount to 80 % (Zaitsev & Alexandrov 1998, Table 27). Although not all of them are harmful, several, such as *Pseudo-nitzschia pseudodelicatissima* and *Pseudo-nitzschia seriata*, are known to be deleterious elsewhere in the world (Bates *et al.* 1998).

The principles of cell size diminution during the vegetative phase and the restoration of the original size as a result of auxospore formation during the generative phase of the life cycle are well developed (Pfitzer 1871; Geitler 1932; Drebes 1977). An accepted classification of patterns of sexual reproduction has also been elaborated (Geitler 1935; Mann 1993). Essential developmental work has been carried out to elucidate breeding systems in diatoms (Roshchin & Chepurnov 1999). However, to date the life cycle of an overwhelming majority of diatoms has not been investigated, and information on the life history of most harmful species is fragmentary (Mann, this volume). At least 20,000 species of diatoms are recognised today; with a narrower species concept, this would rise to 200,000 (Mann & Droop 1996), but only slightly more than 200 species have had significant aspects of their life history reported (Mann 1988; Edlund & Stoermer 1997). Thus, there is a requirement to study the reproductive biology of algae, including the following aspects:

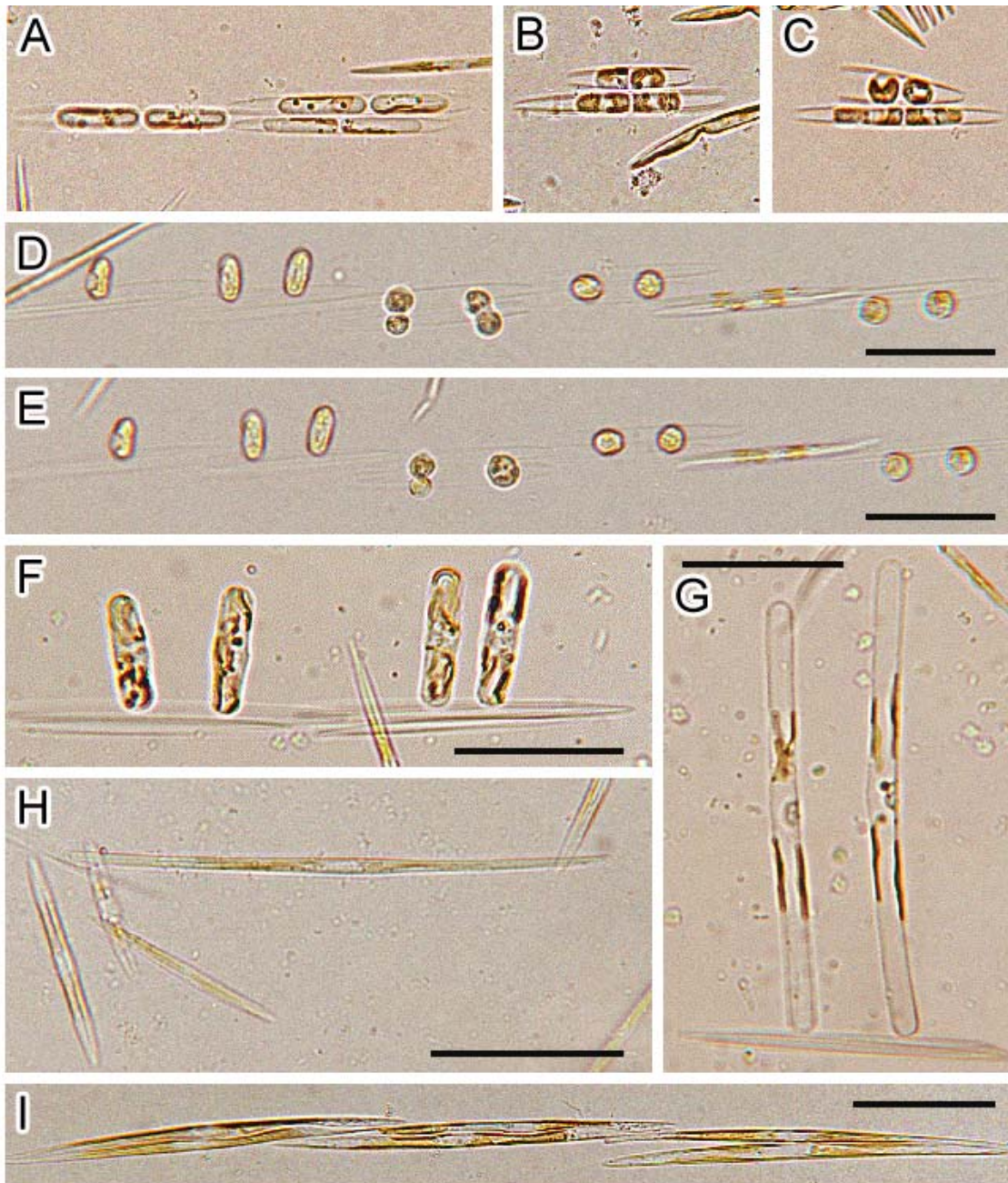
- schemes of the life cycles;
- patterns of the sexual reproduction process (mating behaviour);
- breeding systems.

Even after over 150 years of observation by microscopy, we still have much to learn about the life strategy and life history of diatoms.

### **Sexual reproduction of *Pseudo-nitzschia***

At the laboratory of Dr. Stephen Bates (Moncton, Canada), we studied the sexual reproduction in two species from the genus *Pseudo-nitzschia*, i.e. *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle and *P. multiseriata* (Hasle) Hasle. Briefly, the pattern of sexual reproduction in these two species is as follows (for details see Davidovich & Bates 1998a, b; Kaczmarek *et al.* 2000; 2001). Two cells belonging to the opposite sex line up side-by-side (either valve-to-valve or valve-to-girdle; the exact configuration is difficult to view by light microscopy) (Fig. 3A-B). Each gametangium produces two gametes, accomplished by the rearrangement of the cellular content (Fig. 3C). Although the gametes in a gametangial pair are morphologically identical, they differ in behaviour; gametes in one gametangium are active, whereas they are passive in the another. Active gametes move by amoeboid action towards the passive ones (Fig. 3D). There is no predetermined direction (cis-

or trans-) of the fusion; as a rule, gametes situated most closely to each other fuse first. Normally, two zygotes arise as a result of gamete fusion (Fig. 3E). The zygotes are weakly connected to one of the frustules of the “mother” cell. Auxospores grow perpendicularly to the frustule if the connection is not lost (Fig. 3F-G), eventually producing a large initial cell (Fig. 3H) which exits the auxospore and divides, forming a chain of large cells (Fig. 3I). The described scheme corresponds to Type IA2 of Geitler's classification (Geitler 1932; 1935).



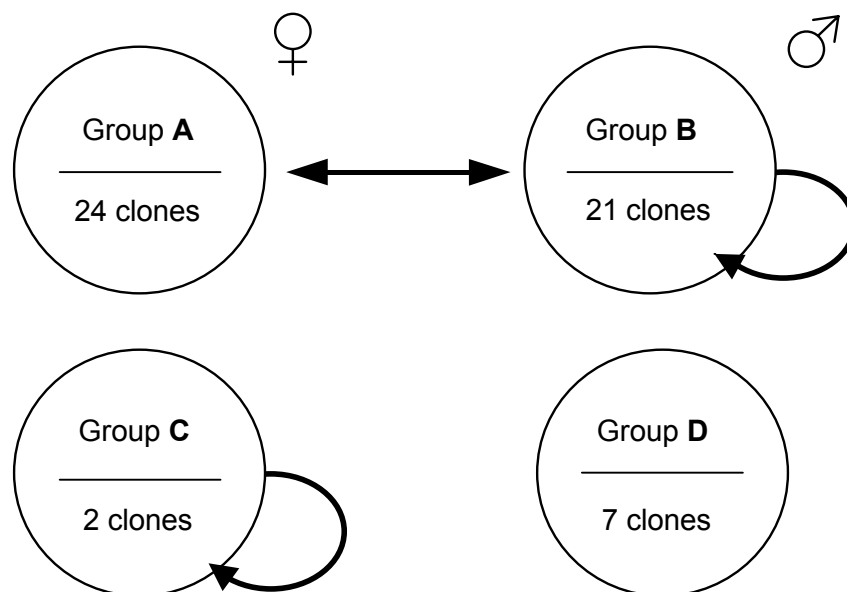
**Fig. 3.** Sexual stages of *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima*. See text for details. Images from Davidovich & Bates (1998a). Scale bars = 100  $\mu\text{m}$ .

The allogamous sexual reproduction that we observed in *P. pseudodelicatissima* and *P. multiseriis* appears to be typical for all pennate diatoms (Rosowski *et al.* 1992). The success of this investigation was based on the presumption that these species have a heterothallic sex distribution (dioecism). This means that two sexually compatible clones of opposite sex, when mixed together, should give rise to auxospores and then initial cells. However, some enigmatic facts were revealed. For example, on one occasion with *P. pseudodelicatissima*, initial cells were produced when a mixture was made of two clones of the same sex (Davidovich & Bates 1998b). There was no proper explanation for this finding, if strict dioecy of the species is believed.

#### Diversity of sexual reproduction behaviour in diatom clones

Meanwhile, a diversity of sexual behaviour was shown to be common in clones of other diatom species derived from natural populations, as well in those resulting from inbred mating (Chepurnov & Mann 1997; 1999).

In addition, some efforts have been undertaken to investigate the variety of sexual behaviour in clones of the diatom *Nitzschia longissima* (Breb.) Ralfs. To date, more than 50 clones have been randomly isolated from field populations near Karadag, Ukraine. Mutual mating gave more than 350 pairs of combinations. According to their mating compatibility, all the clones were arranged into four groups (Fig. 4).



**Fig. 4.** Sexual compatibility of clones of the diatom *Nitzschia longissima*. See text for details.

Clones of Group A were able to mutually mate with clones of Group B. The latter were also able to reproduce intraclonally, but not as vigorously as in the case of interclonal reproduction. The tendency to reproduce intraclonally may in part be explained by the fact that members of Group B belong to a "male" type, as determined by the specific morphology and activity of the gametes that they produced. The third group (Group C) reproduced exclusively intraclonally. Within Groups B and C, the frequency of intraclonal reproduction varied from clone to

clone. The last group (Group **D**) was unique. We tried to mix those clones with others during almost an entire year. All attempts ended in failure until we isolated two new clones which were able to interbreed each other; one of them was also found to be a sexual partner for all the clones of Group **D**. According to the biological concept of “species”, one should therefore recognise this sexually isolated group as a species separate from other investigated clones of *N. longissima*. The assumption has recently been confirmed by the peculiarities of the frustule structure, as detected by scanning electron microscopy. Based on the above biological concept, one might consider the clones in Group **C** as a separate species, as well. However, the morphology of these clones in essence corresponds to that of Groups **A** and **B**. The sexual characteristics of the descendants of the intraclonal lineage now remain to be determined.

These examples indicate the great complexity of the sexual structure in diatom populations. The clonal variety of sexual behaviour that seems to be under genetic control should be taken into account when the life cycle is considered. It was previously reported that separate phenodemes, which are possibly true species, exhibit different life cycles (Mann & Droop 1996). We are dealing here with the sexual diversity of individuals whose status is lower than species level. Therefore, depending on the sexual structure of a population (clonal composition), one may expect diversity in the way the life cycle is manifested.

In view of the goals of the LIFEHAB meeting, one might consider several questions designed to elevate our knowledge of the reproductive biology and life strategy of diatoms. What is the mechanism of genetic control of the sexual status in a clone? Can the sexual status change in the next generations? What is the ratio of male to female clones in natural populations of dioecious algae? What is the proportion in the population of clones with alternative (bisexual, monoecious) behaviour? And for all that, what is the sexual structure of diatom populations?

Note: The references for this article can be found in the full document, which can be downloaded from: <http://www.icm.csic.es/bio/projects/lifehab/> (an 8 MB file).

**APPENDIX 1.** Life cycle of diatom HAB species that cause or may cause problems in European coastal waters.

Species	Harmful effect	Life history		References
		rs	Auxosp	
<i>Amphora coffeaeformis</i> ?*	Domoic acid	+		Anderson 1975; 1976
<i>Coscinodiscus concinnus</i> *	Bird mortality		n.o.	
<i>C. centralis</i> *	Bird mortality		n.o.	
<i>C. wailesii</i> *	Mucilage; high biomass for nori	+		Nagai & Imai 1999 Nagai <i>et al.</i> 1999
<i>Ceratulina pelagica</i> *	Shellfish and finfish kills		n.o.	
<i>Chaetoceros concavicornis</i> *	Fish kills		n.o.	
<i>C. convolutus</i> *	Fish kills		n.o.	
<i>Corethron</i> sp.*	Fish kills		n.o.	
<i>Guinardia delicatula</i> * (= <i>Rhizosolenia delicatula</i> )	Mucilage clogs fishnets		n.o.	
<i>Leptocylindrus minimus</i> *	Fish kills	+		Hargraves 1990
<i>Minutocellus pseudopolymorphus</i> *	Strong smell in water on beach		n.o.	
<i>Nitzschia navis-varingica</i>	Domoic acid		n.o.	
<i>Pseudo-nitzschia multiseriata</i> *	Domoic acid		+	Davidovich & Bates 1998a, b Hiltz <i>et al.</i> 2000 Kaczmarek <i>et al.</i> 2000
<i>P. australis</i> *	Domoic acid		n.o.	
<i>P. delicatissima</i> *	Domoic acid		n.o.	
<i>P. fraudulenta</i> *	Domoic acid		n.o.	
<i>P. multistriata</i> *	Domoic acid		n.o.	
<i>P. pseudodelicatissima</i> *	Domoic acid		+	Davidovich & Bates 1998a, b
<i>P. pungens</i> *	Domoic acid ?		n.o.	
<i>P. seriata</i> *	Domoic acid		n.o.	
<i>P. turgidula</i> ?	Domoic acid		n.o.	
<i>Rhizosolenia chunii</i>	Shellfish kills; gives bitter taste		n.o.	
<i>Skeletonema costatum</i> *	High biomass	+		Hargraves & French 1975
<i>Tabularia affinis</i> * (= <i>Synedra affinis</i> )	Epiphyte on nori		n.o.	
<i>Thalassiosira mala</i> *	Mucilage on gills kills shellfish		n.o.	

\* Species recorded in European waters, even if harmful events attributable to the species have not been reported in the area. References are given for reports of life cycle events only.

rc = resting spore; Auxosp = auxospore (including gametes and zygotes); n.o. = not observed